UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Effect of multiple doses of imidocarb dipropionate on renal and hepatic function of ponies

By

C Meyer, BVSc

Submitted to the Faculty of Veterinary Science, University of Pretoria, in partial fulfillment of the requirements for the degree M Med Vet (Med)

© University of Pretoria

Dedicated to my family and friends through whom all things are possible, and especially to Sunday
- some dogs are better than others

Acknowledgement

I would like to express my sincere appreciation to the following people:

Professor Alan Guthrie, my promotor, for his patience and invaluable assistance. Thank you for your time, effort and insight.

Professor Koos van den Berg, my co-promotor, for his much appreciated input and guidance. Thank you for your support and encouragement.

Kim Stevens, for her friendship and assistance.

The staff and students at the Equine Research Centre, for their patience and assistance, without which such trials would not be possible.

I would also like to express my gratitude to the following organisations whose generous financial assistance enabled this experimental work to be undertaken and completed: The **Thoroughbred Breeders Association**, **Schering-Plough Pharmaceuticals**, the **Equine Research Centre** and the **University of Pretoria**.

Declaration

I, Carla Meyer, do hereby declare that the experiments presented in this dissertation were conceived and executed by myself and, apart from the normal guidance from my supervisor, I have received no assistance.

Neither the substance, nor any part of this dissertation has been submitted in the past, or is to be submitted for a degree at this University or any other university.

This dissertation is presented in partial fulfillment of the requirements for the degree M Med Vet in Equine Medicine.

I hereby grant the University of Pretoria free license to reproduce this dissertation in part or as a whole, for the purpose of research or continuing education.

Signed:..

Date:..

Abstract

Previous studies have shown that four intramuscular doses of imidocarb dipropionate administered at 72-hour treatment intervals are effective in sterilising experimental *Babesia equi* infections in horses. It has also been documented that imidocarb dipropionate has dose dependent hepato- and nephrotoxic effects in a number of species. The purpose of this study was to examine the clinical and clinicopathological effects of this multiple treatment regime of imidocarb dipropionate in healthy ponies. Specific emphasis was placed on the potential adverse effects on hepatic and renal function in this species. Serum bile acids and serum gamma glutamyltransferase activity were measured to evaluate the effect of this treatment regime on hepatic function. The diffuse hepatocellular necrosis and pronounced periportal hepatocellular swelling and degeneration previously reported as the most consistent hepatic lesions noted in equines following imidocarb treatment were not evident at the dose and dosage interval used in this study. Urinary gamma glutamyltransferase: creatinine ratios (IU/g) and fractional clearance of sodium, potassium and phosphate (%) were calculated as a measure of renal function. Urinary GGT and urinary GGT: creatinine ratios were significantly elevated on Day 5 of the trial and were considered indicative of transient changes in renal function. The rapid return to previous baseline values supported reported observations that changes between 25 and 100 IU/g may be a function of drug excretion and are not necessarily indicative of significant nephrotoxicity. It was concluded that four intramuscular treatments of imidocarb dipropionate at a dose of 4 mg/kg every 72 hours may be a relatively safe method whereby persistent *Babesia equi* infections can be sterilised.

Samevatting

Vorige navorsing het aangetoon dat vier dosisse imidokarb dipropionaat wat binnespiers in 72-uur intervalle toegedien is, effektief is in die sterilisering van eksperimentele *Babesia equi* infeksies in perde. Daar is ook aangeteken dat imidokarb dipropionaat dosis-afhanklike hepatotoksiese en nephrotoksiese effekte in ’n aantal spesies het. Die doel van die studie was om die kliniese en klinies-patologiese effekte van herhaalde behandelings met imidokarb dipropionaat in gesonde ponies te ondersoek. Spesifieke klem was gelê op die potensiële nadelige effekte op hepatiese en nierfunksies in dié spesies. Serum galsuur en serum GGT aktiwieteit was gemeet om die effek van die medikasie op hepatiese funksie te bepaal. Die verspreide hepatosellulêre nekrose en die duidelike periportale hepatosellulêre swelling en degenerasie wat voorheen opgeteken is in perde na imidokarb toediening, kon nie gedemonstreer word met die dosis en dosis interval wat in dié studie gebruik is nie. Urinêre GGT: kreatinien verhouding (IU/g) en fraksionele uitskeiding van natrium, kalium en fosfaat (%) is bereken as ’n maatstaf van nier funksie. Urinêre GGT asook die urinêre GGT: kreatinien verhouding was betekenisvol verhoog op Dag 5 van die eksperiment en is beskou as ’n aanduiding van kortstondige verandering in nier funksie. Die skielike terugkeer na die vorige basislyn waardes ondersteun aangetekende bevindinge dat veranderings tussen 25 en 100 IU/g dalk ’n funksie van middel uitskeiding is en nie noodwendig ’n aanduiding is van betekenisvolle nefrotoksisiteit nie. Daar is tot die gevolgtrekking gekom dat vier binnespierse behandelings met ’n dosis van 4 mg/kg imidokarb dipropionaat elke 72 uur ’n veilige metode mag wees waarmee *Babesia equi* draers gesteriliseer kan word.

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Table of Contents

Acknowledgement........ ii

Declaration........ iii

Abstract........ iv

Samevatting........ v

List of Tables........ viii

List of Figures........ ix

Chapter 1

General introduction........ 1

Chapter 2

Literature review........ 3

Equine piroplasmosis........ 3

Introduction........ 3

Toxicity of imidocarb dipropionate........ 6

Assessment of renal and hepatic function in horses........ 12

Renal function........ 12

Fractional clearance of electrolytes........ 12

Gamma glutamyl transpeptidase in equine urine........ 17

Hepatic function........ 19

Serum bile acids........ 19

Serum gamma glutamyl transpeptidase........ 21

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Chapter 3

Clinical and clinicopathological changes in six healthy ponies following intramuscular administration of multiple doses of imidocarb dipropionate

Abstract... 22

Introduction.. 23

Materials and methods.. 25

Experimental animals... 25

Experimental design.. 25

Experimental procedure.. 26

Statistical methods.. 29

Results.. 29

Clinical manifestations and observations.................................. 29

Clinicopathological results... 32

Discussion... 48

Chapter 4

General conclusions.. 53

References... 54

Appendix- Analysis of variance tables... 66

List of Tables

Table 1 Fractional electrolyte clearance values of sodium, potassium and phosphorus for ponies and horses.....16

Table 2 Summary of the incidence and duration of systemic signs observed following intramuscular administration of four doses of 4 mg/kg imidocarb dipropionate every 72 hours to 6 healthy ponies.....30

Table 3 Summary of the incidence and nature of the local reactions that were noted at the injection site following four intramuscular treatments of 4 mg/kg imidocarb dipropionate to six healthy ponies.....31

Table 4 Mean and standard error of the urine specific gravity and urine pH and incidence of glucosuria measured on Days 0, 2, 5, 8, 11 and 18.....47

List of Figures

Figure 1. Temporal changes of mean and standard error of the total red cell count and haematocrit and haemoglobin concentrations of 6 ponies following intramuscular administration of 4 mg/kg imidocarb dipropionate on Days 1, 4, 7 and 10. 34

Figure 2. Temporal changes of mean and standard error of the total white cell count of 6 ponies following intramuscular administration of 4 mg/kg imidocarb dipropionate on Days 1, 4, 7 and 10...35

Figure 3. Temporal changes in mean and standard error of mature and immature neutrophil counts ($x10^9/l$) of 6 ponies following intramuscular administration of 4 mg/kg imidocarb dipropionate on Days 1, 4, 7 and 10... 36

Figure 4. Temporal changes of mean and standard error of lymphocyte, eosinophil and monocyte counts ($x10^9/l$) of 6 ponies following intramuscular administration of 4 mg/kg imidocarb dipropionate on Days 1, 4, 7 and 10...37

Figure 5. Temporal changes of mean and standard error of the thrombocyte count ($x10^9/l$) of 6 ponies following intramuscular administration of 4 mg/kg imidocarb dipropionate on Days 1, 4, 7 and 10...38

Figure 6. Temporal changes of mean and standard error of the total serum protein concentration (g/*l*) of 6 ponies following intramuscular administration of 4 mg/kg imidocarb dipropionate on Days 1, 4, 7 and 10..39

Figure 7. Temporal changes of mean and standard errôr of aspartate aminotransferase activity (IU/*l*) of 6 ponies following intramuscular administration of 4 mg/kg imidocarb dipropionate on Days 1, 4, 7 and 10..40

Figure 8. Temporal changes of mean and standard error of serum creatinine (mg/d*l*) and urine creatinine concentrations (mg/d*l*) of 6 ponies following intramuscular administration of 4 mg/kg imidocarb dipropionate on Days 1, 4, 7 and 10.......................................41

Figure 9. Temporal variation in mean and standard error of serum sodium concentrations (mmol/*l*) and fractional clearance of sodium (%) of 6 ponies following intramuscular administration of 4 mg/kg imidocarb dipropionate on Days 1, 4, 7 and 10.....................42

Figure 10. Temporal changes in mean and standard error of serum potassium concentrations (mmol/*l*) and the fractional clearance of potassium (%) of 6 ponies following intramuscular administration of 4 mg/kg imidocarb dipropionate on Days 1, 4, 7 and 10.....................43

Figure 11. Temporal changes in mean and standard error of serum inorganic phosphate (mmol/*l*) and the fractional clearance of phosphate (%) of 6 ponies following intramuscular administration of 4 mg/kg imidocarb dipropionate on Days 1, 4, 7 and 10. 44

Figure 12. Temporal variation of mean and standard error of serum gamma glutamyl transpeptidase (GGT) (IU/*l*), urine GGT (IU/*l*) and the urine GGT: urine creatinine ratios (IU/g) of 6 ponies following intramuscular administration of 4 mg/kg imidocarb dipropionate on Days 1, 4, 7 and 10.. 45

Figure 13. Temporal changes in mean and standard error of serum bile acids (µmol/*l*) of 6 ponies following intramuscular administration of 4 mg/kg imidocarb dipropionate on Days 1, 4, 7 and 10... 46

Chapter 1

General introduction

Equine piroplasmosis is a tick-transmitted disease caused by *Babesia equi* and *Babesia caballi* protozoa. The prevalence of this disease corresponds to the pattern of vector distribution with infections by both *Babesia* species endemic in equids throughout the tropics and subtropics[32]. Equine babesiosis presents a global problem in equestrian sports and trade, and is particularly significant in those regions where the tick vectors responsible for transmission of the disease are present. Introduction of infected equids into these areas may lead to transmission of the disease to the vector population, with subsequent spread to the naïve, indigenous equids of the region. This would naturally have a considerable economical impact on the region.

The development of a more sensitive test, or a routine method for elimination of the infection in suspected carrier animals prior to exportation, becomes necessary. Some success in eliminating piroplasmosis infections in horses has been demonstrated with intramuscular administration of 4 mg/kg imidocarb dipropionate every 72 hours for four treatments[42]. The reported nephrotoxic and hepatotoxic effects of imidocarb dipropionate and the potential risk of using this treatment protocol in valuable animals has not been evaluated. This study examined the clinical and clinicopathological effects of multiple doses of imidocarb dipropionate administered intramuscularly to clinically healthy ponies. The specific objectives were as follows:

1. To evaluate the effect of four intramuscular doses of 4 mg/kg imidocarb dipropionate administered every 72 hours on haematological variables in six healthy ponies.

2. To evaluate the clinical and clinicopathological effects of a multiple dose treatment regime of imidocarb dipropionate in healthy ponies with particular emphasis on evaluation of renal and hepatic function.

Chapter 2

Literature review

Equine piroplasmosis

Introduction

Imidocarb dipropionate is used for the treatment of piroplasmosis, a tick-borne disease affecting a wide range of domestic and wild animals[25,42,58]. Clinical signs of this disease include anaemia, anorexia, icterus, listlessness, depression and if left untreated, death from multiple organ failure. Occasionally the infection may become chronic resulting in inappetence, weight loss and poor performance. Increased mortality rates may be seen when the parasites are introduced into a susceptible population[32,72,80]

Babesia equi and *Babesia caballi* have been isolated as the causative agents of piroplasmosis in equids. *Babesia caballi* is an intraerythrocytic protozoa that exhibits characteristic features of the genus *Babesia*. *B. caballi* infections usually cause extremely low parasitaemias and are rarely associated with clinical signs in equids[32,72,80]. In contrast, *Babesia equi* parasites are more virulent and undergo initial multiplication in the lymphocytes of the host, followed later by the characteristic intraerythrocytic stages. The lymphocytic stage, or schizonts, of *B. equi* are morphologically similar to those of the *Theileria* genus, hence the discrepancies commonly encountered in the classification of these parasites[72,80]. There is also no cross-immunity between *B. equi* and *B. caballi*[32].

Intrauterine infections may occur, but transmission is predominantly through tick vectors of the *Dermacentor, Rhipicephalus* and *Hyalomma* genera[35,80]. *Rhipicephalus evertsi evertsi*, a two-host tick, is the only confirmed tick vector of *B. equi* in South Africa. The occurrence of clinical babesiosis is more common during the summer months and coincides with the seasonal activity of the adult stages of the tick vectors[32]. A carrier status, in which antibodies are maintained for a minimum period of two years, is recognised in animals born and reared in endemic areas[35]. Although many carriers remain asymptomatic lifelong, periods of stress or concurrent disease may precipitate clinical infections.

Equine piroplasmosis is endemic to many regions throughout the world including Africa, central Russia, central Mongolia, southern Europe, Madagascar and South America[10,34,47,52,82,87]. A single case of *Babesia equi* has also been documented in Australia, but it is considered to be an exotic disease[65]. Similarly, equine piroplasmosis has been described in the United States of America, but is confined to Florida, and is also considered an exotic disease[59]. Countries in which the indigenous horses have not been exposed to this disease include the United Kingdom, New Zealand, Germany, Switzerland, Austria, North America and Japan[35,87]. Carrier horses, donkeys, mules and zebras are infective to ticks and introduction of the disease via importation of subclinical carrier animals is therefore of particular importance in those non-endemic areas where suitable tick vectors exist[25,32,35,47,82].

The possibility of sterilising the infection prior to exportation from endemic areas may provide an acceptable method for preventing the spread of infection without restricting the movement of horses. While a single treatment of imidocarb dipropionate is sufficient to sterilise *B. caballi* infections in equids, *B. equi* infections are more difficult to

eradicate[25,60,91]. In a trial performed by Frerichs *et al* using imidocarb dihydrochloride, promising results were obtained with regards to the elimination of experimentally induced *B.equi* infections from treated horses. Four injections of imidocarb dihydrochloride at a dose of 4 mg/kg every 72 hours, eliminated *B.equi* from 13 of 14 experimentally infected horses[42].

The majority of horses that are exported from one country to another are often valuable in terms of breeding or athletic potential and therefore, any treatment aimed at sterilisation of carrier animals should pose minimal threat of irreversible or long-term damage to organs. In addition, there is an implication that the parasites in horses infected naturally by tick vectors are more drug resistant than those organisms in horses infected by sub-inoculation of infected blood, questioning the probability of successful sterilisation[25].

Toxicity of imidocarb dipropionate

A wide variety of drugs including arsenicals, quinolone derivatives, trypan-blue, acridine derivatives and aromatic diamidines have been advocated for the treatment of piroplasmosis in domestic animals. Of the chemotherapeutic agents, aromatic diamidines have been shown to be the most efficient and least toxic[42,58,60]. Imidocarb dipropionate, an aromatic diamidine, [3, 3' bis-(2-imidazolin-2-yl) carbanilide dipropionate], is a weak, lipophilic base, and has a large volume of distribution throughout the body[1,8]. The precise mode of action against piroplasms is not clearly understood, but diamidine derivatives are thought to produce a partial denaturation of the DNA double helix with subsequent dilatation of nuclear cisternae, karyorrhexis, cytoplasmic vacuolation, ribosomal damage and inhibition of food vacuole formation[58,82,83].

Although safer than many of the earlier chemotherapeutic agents used, toxic effects of aromatic diamidines have been documented in cattle, sheep, goats, dogs, cats and horses[3-5,7,8,29,30]. These have been ascribed to the anticholinesterase properties of the drug and to direct nephrotoxic and hepatotoxic effects[3-5,7,8,29,30]. Neurological symptoms have also been described and although likely attributable to the anticholinesterase effects of the drug, the presence of imidocarb dipropionate in tissues of the brain at post mortem may suggest an additional direct effect on the central nervous system[3,7,8]. In a pharmacokinetic study of imidocarb dipropionate in sheep performed by Aliu *et al*, brain concentrations of imidocarb did not decrease greatly over the 32 day period of the trial. The slow rate at which imidocarb entered the brain, as evidenced by the low values at 6 hours post-injection relative to concurrent plasma levels, and the subsequent slow elimination from these tissues were attributed to the blood-brain barrier[8].

Imidocarb dipropionate, like most diamidines, is poorly absorbed when administered orally. However, the low variability of plasma concentration of imidocarb dipropionate obtained in sheep indicates a high uniformity in the rates of absorption and diffusion to various tissues in this species[8]. The maximal *in vivo* plasma protein binding capacity of the drug in these sheep was estimated to be 68%[8]. The degree of protein binding following intravenous administration appears to be concentration dependent and is partially responsible for the persistence of the drug in the body following an initial rapid elimination phase. The majority of the drug however, binds tightly to components of liver, kidney, muscle and brain resulting in more significant prolonged release from these tissue depots[8].

There appear to be significant differences in the absorption rate after intramuscular injection of this drug in various species. In horses, two intramuscular injections of 6 mg/kg, administered at 24-hour intervals, reached a maximal plasma concentration of 0.25 μg/ml on Days 3 and 6, while the same dose in cattle gave peak plasma values of 4.5 to 8.5 μg/ml within 20-30 minutes post-injection[42]. This variation may provide some insight into the success with which piroplasm infections can be sterilised in cattle when compared to horses. The concentration and dosage interval required for the drug to be effective in eliminating piroplasm infections from various domestic species remains inconclusive.

The severity of the adverse reactions appear to be dose and species dependent, with a single intravenous injection of imidocarb dipropionate (1 mg/kg) reported as being fatal in cattle[3,5,29,42]. Very low resting levels of plasma cholinesterase activity in cattle combined with their potential for excessive release of acetylcholine are responsible for the increased susceptibility in this species[3,5]. In contrast, in a chemoprophylactic study using dogs infected with *B.canis*, 6 mg/kg

of imidocarb administered subcutaneously had no reported toxic side effects. Death due to acute renal failure was, however, reported in a dog eight days after intravenous administration of 4 mg/kg imidocarb dipropionate[3]. In dogs, the pathological changes reported resemble those seen in goats, with the normally high resting level of plasma cholinesterase activity in these species responsible for reducing the potential adverse effects of the drug[29]. The rapidly fatal effects seen in cattle may therefore be directly related to severe bronchoconstriction and pulmonary oedema rather than the more prolonged nephrotoxic effects seen in other species[3,4,29,30,42].

In adult goats the lethal dose is approximately 6.75 mg/kg by intramuscular injection with deaths due to acute tubular nephrosis occurring between four and eight days post-injection[29,30]. In these animals the absence of extra-renal lesions at the time that the kidneys were undergoing acute tubular nephrosis suggested that such lesions, including cardiac and pulmonary pathology, were a consequence of renal failure and uremia, rather than related to the direct toxic effect of the drug[29,30]. The presence of mild reversible hepatocellular lesions in goats also indicated that the direct hepatotoxic effects of imidocarb dipropionate in this species were minimal[8,29]. In horses, the LD_{50} of intramuscularly administered imidocarb dipropionate for 21 days was reported to be two doses of 15.99 ± 1.49 mg/kg at a 24-hour interval with mortalities due to hepatorenal failure occurring within six days after the first injection[4,42]. Hepatic changes in these animals were evident in those subjects that received in excess of 8 mg/kg imidocarb dipropionate, with diffuse hepatocellular necrosis and pronounced periportal hepatocellular swelling and degeneration the most consistent hepatic lesions noted[4,8,42]. Extensive periportal haemorrhage and bile stasis, the severity of which appeared directly proportional to the dosage received, were also evident[4]. In horses, imidocarb concentrations ranging between 50-275 µg/g

non-ionized state and can be reabsorbed[8]. It can be speculated that due to the herbivorous nature and subsequent alkaline urine of horses, similar excretion profiles may be expected. Urinary excretion in sheep is rapid during the first 24 hours, whereafter it becomes closely correlated to the plasma concentrations. These concentrations are dependent on the slow rate of absorption from tissue depots[8]. In sheep, thin-layer chromatography failed to reveal the presence of metabolites in the urine, bile, liver and kidneys of treated animals, suggesting that the drug is not substantially metabolised, contributing to the prolonged plasma levels in treated animals[5,8]. The apparent concentration dependent nephrotoxic effects were further verified by the absence of microscopic lesions in animals treated with lower doses of the drug[3-5,8].

Additional factors accounting for the susceptibility of the renal tubular epithelium to drug or other toxic insults include high renal blood flow and detoxification of certain substances by the renal tubular epithelium, with subsequent increased exposure to toxins[33]. Toxins may also become concentrated during the routine concentration of the glomerular filtrate within the renal tubules[28,61,81]. The high metabolic rate and rapid epithelial turnover of renal tubular cells also contribute to the susceptibility of renal cells to external toxic insults[28,61,81]. The prognosis for total recovery is dependent on the presence of an intact basement membrane and sufficient viable epithelial cells to allow regeneration of the epithelium with at least partial return of renal function[17]. In general the prognosis is much better when the toxin has not caused serious damage to other organs[81].

Although imidocarb dipropionate is potentially both nephrotoxic and hepatotoxic, treatment with this drug at the recommended dose rate of 2.4 mg/kg intramuscularly in horses, only results in mild transient effects such as salivation, diarrhoea and abdominal discomfort. Similar effects

Assessment of renal and hepatic function in horses

Renal function

Fractional clearance of electrolytes

Clearance ratios of urinary substances relative to plasma substances indicate renal ability to filter or reabsorb those substances and is an accurate reflection of the rate at which a substance is removed from the blood into the urine[89]. Levels of serum urea and serum creatinine are used as standard indices to assess renal function[23,46,54]. As creatinine production by the body is constant and minimal renal tubular excretion and re-absorption occurs, fractional clearance of creatinine can be used as an estimate of glomerular filtration rate[23,69]. Serum urea and serum creatinine on their own are not very sensitive or specific measurements, as these do not differentiate between prerenal and renal azotemia[21,46,55,69]. It has been reported in man and other animals, and extrapolated to horses, that an increase in serum creatinine due to renal dysfunction is only evident when at least 70% of the renal tubules are compromised[13,55,57,69]. Serum urea levels are similarly influenced by non-renal factors and may rise or fall independently of renal function. More specific and sensitive tests are therefore required to assess early renal damage[13,57].

Fractional clearances of electrolytes provide useful diagnostic and prognostic information and are expressed as a percentage of endogenous creatinine clearance[27,31,55,88]:

$$\%\ Clearance\ Ratio\ [x] = \frac{urine\ [x]\ X\ plasma\ [creatinine]}{plasma\ [x]\ X\ urine\ [creatinine]}\ X\ 100$$

The ability of the kidney to conserve water can be estimated by the ratio of urine osmolality to plasma osmolality which can be measured using fractional clearance of filtered sodium (FC_{Na})[46,69,73]. Clearance ratios are also practical methods for differentiating between prerenal, renal and postrenal azotemia as tubular capability for re-absorption of electrolytes differs between horses with prerenal azotemia and those with tubular damage[21,46]. The fractional clearance of sodium, supported by changes in urinalyses is considered an accurate diagnostic test for renal tubular dysfunction. Normally, in the presence of a functional aldosterone homeostatic mechanism, hyponatremia should elicit low FC_{Na}, normal serum sodium should have normal FC_{Na} and in response to hypernatremia, FC_{Na} values should increase[27,69,88]. Only an increase in FC_{Na} is considered of real diagnostic significance, but may occur due to excessive dietary sodium, dehydration, Addison's disease or renal tubular insufficiency[26,36,46,88]. Urine specific gravity in animals suffering from dehydration or excessive dietary intake would however be increased in contrast to those with Addison's disease or renal tubular insufficiency[26,27,36,69,88]. In the latter conditions sodium regulation is lost, and naturesis may occur in the presence of hyponatremia. In horses with Addison's disease, FC_{Na} is increased but fractional clearance of potassium (FC_K), fractional clearance of phosphate (FC_P) and other urine variables are normal and additional renal function tests can be used to differentiate between these conditions[27,36,53,88]. Typically, horses with renal azotemia have FC_{Na} values $> 1.0\%$, while those with prerenal azotemia have a $FC_{Na} < 0.5\%$[46,53,55]. Some investigators feel however, that due to an overlap between healthy horses, horses with prerenal azotemia and horses with renal azotemia, diagnosis of renal disease should probably not be based on $FC_{Na} < 3\%$[46,53,69,89]. Postrenal azotemia is uncommon in the horse and may not become apparent until prolonged lower urinary tract

obstruction has led to a significantly increased back-pressure with secondary renal parenchymal damage[21,46].

An increase in the fractional clearance of phosphate has been described as an early indicator of renal disease in horses[15,21,37,53,55,88]. Other conditions besides renal damage may be associated with an increased clearance of phosphate and include primary or pseudohyperparathyroidism, nutritional secondary hyperparathyroidism and an obscure condition, phosphate wasting nephropathy, affecting mature horses and causing chronic, multifocal lameness[27,53,67,75,88]. Intravenous administration of crystalloid solutions, particularly glucose, also has a phospha-uric effect in rats, people and horses and may affect interpretation of results[78].

The fractional clearance of potassium, is extremely variable and affected considerably by type of feed[27,53,69]. High FC_K values are most likely due to dietary intake of potassium. This ion is present in abundance in most forages with subsequent absorption of excessive amounts from the gastrointestinal tract[27,31,56]. Horses fed green feed may have a normal FC_K as high as 150%[27]. Although in renal disease the FC_K would be expected to increase in an effort to retain sodium, the extreme range of normal values obscures this response and decreases the diagnostic sensitivity of this variable[27,56].

It can clearly be seen that fractional clearance values are related to electrolyte status, and can vary considerably according to the type of diet fed and the clinical condition of the animal[24,31,67]. Similar effects may be expected in disease conditions where intravenous fluid therapy is instituted, or where feed and water deprivation has occurred. In addition, values

for fractional clearances of each electrolyte vary markedly during a 24-hour period within an individual, necessitating a wide range of normal reference values[27,31,78,88].

Although the fractional clearance ratios of a substance are normally variable, they usually fall within a definable range[88]. Studies comparing single measures with 24-hour clearances have found that the total daily urine output volume can be excluded, provided that the urine and plasma samples are obtained at the same time (within 30 minutes of each other)[24,56,62,69]. The wide diversity, however, decreases the sensitivity of single sample fractional clearance values and extremes of normal ranges should be interpreted with care[27,31,55]. It should also be emphasized that fractional clearance values should always be evaluated concurrently with serum electrolyte concentrations and samples should be repeated if necessary, allowing the clinician to establish a baseline reference range, or to obtain repeatable abnormalities for an individual animal[27,37,53,69]. Table 1 summarizes the reference ranges for sodium, potassium and phosphorus clearance reported in previous studies.

Sodium	Potassium	Phosphorus	References
Adults			
0.16 ± 0.24	27.0 ± 14.6	NR	73
0.02-1.00	15-65	0.00-0.50	88
0.11-0.87	10.8-28.5	0.07-0.74	89
0.01-0.70	NR	NR	46
0.27 ± 0.02	38.52 ± 7.26	NR	69
0.00-0.46	23.9-75.1	0.04-0.16	56
0.032-0.52	23.3-48.1	0-20	22
0.034 ± 0.095	42.4 ± 9.8	0.710 ± 0.250	44
0.04-0.52	35-80	0.00-0.20	48
0.0002-2.43	1.0-42.7	0.023-2.77	37
NR	NR	0.115-0.302	62
NR	NR	0.08-5.53	24
NR	NR	0.61-0.75	31
Foals			
0.31 ± 0.18	13.26 ± 4.49	3.11 ± 3.81	19

Table 1. Fractional electrolyte clearance values of sodium, potassium and phosphorus for ponies and horses.

NR- not reported.

Gamma glutamyl transpeptidase in equine urine

Measurement of urinary enzyme activities is used extensively in man as a non-invasive method of evaluating renal integrity. Enzymuria occurs significantly earlier than other indicators of renal dysfunction[14,20]. A number of urinary enzymes, including *N*-acetyl-ß-D-glucosaminidase (NAG), alanine aminopeptidase (AAP), alkaline phosphatase (AP), leucine aminopeptidase (LAP), gamma glutamyl transpeptidase and kallikrein have been documented as sensitive indicators of early renal damage in humans and dogs[15]. To be diagnostically relevant these enzymes need to be renal specific, stable during collection and relatively easily assayed. This often proves difficult as the varying composition and volume of urine presents an environment which may be responsible for either decreased or enhanced enzyme activity.

Due to inconsistent results, many enzymes have been discarded as diagnostic or prognostic indicators of renal injury[14,15,61]. Measurement of urine gamma glutamyl transpeptidase (GGT) is currently receiving attention and has provided promising results in equine medicine which may result in it being used more extensively in the future for early diagnosis and therapeutic monitoring of acute renal disease in horses[14,43,49]. In a study by Bayly *et al*[14], urinary GGT activities increased rapidly following the administration of the first of 5 daily doses of nephrotoxins, reaching peak levels 5-8 days after the onset of the trial. The observed changes in the urine GGT activities preceded the appearance of proteinuria by 1-2 days, glucosuria by at least 2 days and the detection of azotemia by up to 6 days[14]. A gradual decline in urine GGT levels in the second week of the trial suggested that these levels are primarily indicative of acute proximal tubular damage and may return to normal in chronic disease[14].

Gamma glutamyl transpeptidase (GGT) is an enzyme found in high concentrations in the liver, pancreas and brush border of the proximal tubular epithelium of the kidney[79]. The high molecular weight of GGT prevents its filtration from the blood by the normal glomerulus and elevations in urine GGT enzyme activity are considered to be of renal origin[6,79]. No correlation between serum GGT and urine GGT has been observed. Although mild diurnal variation occurs, these changes are not significant[15,20]. Similarly, neither the sex nor the age of the horse influences urinary enzyme activity[20,79]. In an alkaline environment, the urine GGT enzyme shifts from a membrane bound form to a more soluble form. The alkaline nature of horse urine may thus contribute to an increase in urine GGT activity[79]. To eliminate external influences such as temperature variations and storage on results, assays should be performed as soon after collection as possible[6,15]. If this is not practical, samples for measurement of GGT enzyme activity should be stored at ±4°C and analysed within 72 hours of collection. Freezing of samples should be avoided as this has a deleterious effect on enzyme activity[6,15].

The absolute concentration of urinary enzymes is of limited value as diuresis or dehydration causes variations in these concentrations. A 24-hour measurement of urinary enzymes while more accurate, is not practical. The measurement of urinary enzymes has however been shown to be clinically significant when expressed as a ratio relative to urine creatinine concentration[6,20]. Although urine GGT and urine creatinine concentrations vary, the ratio remains constant and single random urine samples in which these fractions are measured may provide the clinician with valuable and practical information on the health status of the kidneys[6]. In a trial by Fuentes *et al*, the toxic effects of neomycin on the equine kidney were studied. The urinary excretion of GGT was measured, and increased significantly above

pretreatment levels by the third day of treatment. The urine GGT: urine Cr ratios of animals receiving neomycin, were calculated with a significant increase of this ratio evident from the 12th until the last day of treatment (15th day), and persisting for an additional 6 days after treatment was discontinued[43]. In spite of this, there was little change in the absorption and disposition of neomycin and the authors concluded that although urine GGT: urine Cr ratios were elevated, the equine kidney was capable of handling the dose of neomycin recommended by the manufacturers. This was confirmed at post mortem where the histopathological appearances of the kidney and liver samples were normal[43]. It would therefore appear that certain drugs enhance urinary enzyme concentrations with minimal or no renal damage[43]. On the other hand, the massive and rapid regenerative capabilities of healthy kidneys may be responsible for the limited effects seen[43]. With values exceeding 100 IU/g urine enzyme ratios remain a sensitive method for detecting early tubular damage both in primary renal disease as well as other disease states where renal perfusion is decreased due to dehydration or hypovolaemia[15,49].

Hepatic function

Serum bile acids

Bile consists of several components of which bile acids make up 90% of the organic portion[12]. These bile acids act as detergents which facilitate the uptake of lipid soluble compounds from the gastrointestinal tract as well as excretion of cholesterol and phospholipids from the liver[12,71]. Bile acids are produced solely by the liver and elevation of total plasma bile acid concentration is considered a highly sensitive and very specific indicator of hepatic disease and provides a measure of hepatobiliary transport function[39,50,68].

The majority of bile acids are restricted to the enterohepatic circulation resulting in normally low plasma bile acid concentrations[39,92]. A study by Hoffman *et al* determined reference values for serum bile acid concentrations from 15 fasted horses to be 6.06 $\pm$ 2.56 µmol/*l*, from 5 ponies to be 5.64 $\pm$ 3.09 µmol/*l* , and for all equids, 5.94 $\pm$ 2.27 µmol/*l*[50]. There was no significant difference between the mean bile acids of ponies when compared with values for horses[50]. It has been estimated that bile acids are circulated approximately 38 times per day between the gastrointestinal tract and the liver in healthy ponies[50,71]. The age and sex of animals does not influence bile acid concentrations, nor is there a significant diurnal variation in these concentrations[92]. There is also no significant difference in results of bile acid concentrations obtained from serum or plasma samples and examination of either blood component is acceptable[92]. An additional analytical advantage is that plasma bile acids remain stable during storage at -20°C for at least one month enhancing the practicality of this test[12,92].

Measurement of bile acids is less specific for the type of hepatobiliary disease, although studies in man have indicated that there may be some value in determining the cholic: chenodeoxycholic acid ratio for the differential diagnosis of the region of hepatic insult[92]. Cholate and chenodeoxycholate are also the predominant bile acids in equines and may have similar diagnostic advantages[12,39,71]. Serum bile acids can be expected to increase approximately 17 fold above base-line values in ponies with cholestasis and approximately 10 fold with hepatocellular necrosis[68,69]. These increases are relatively small when compared to other species, because the pool size of bile acids in ponies is smaller[39,68].

Serum gamma glutamyl transpeptidase

Serum gamma glutamyl transpeptidase occurs as a membrane-bound enzyme on the canalicular surface of hepatocytes and on the biliary epithelial cell membrane. The only other potential source of serum GGT is the pancreas, but pancreatic disease in equines is rare and an elevated serum GGT is more likely to be of hepatic origin[12,18]. There is no correlation between serum bile acid concentrations and serum GGT activity[71]. In contrast to serum bile acids, which may increase significantly within 24-48 hours following acute hepatic damage, increases in serum GGT are more consistent in chronic disease[18,70,71]. Similarly, fasting for 2 to 4 days results in elevations of serum bile acids to approximately three times base-line concentrations, with little or no effect on serum GGT activity[12]. Thus, although not as sensitive as serum bile acids, serum GGT activity may be used in combination with measurement of bile acids and a particular diagnosis is made only if all the tests performed are positive[11,45,64]. This method of testing increases the specificity or number of animals without a disease that test negative. The positive predictive value, or probability of a disease being present in an animal that tests positive, is also increased[11,45,64,74] Normal serum GGT activity may thus rule out suspected hepatic damage in animals with elevated serum bile acid concentrations which have fasted for longer than 3 days[18,50].

b. 14442292
i. 14745239

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Chapter 3

Clinical and clinicopathological changes in six healthy ponies following intramuscular administration of multiple doses of imidocarb dipropionate

Abstract

Haematological variables and select serum indices, particularly those affected by changes in renal and hepatic function, were examined in six healthy ponies following four intramuscular doses of 4 mg/kg imidocarb dipropionate administered every 72 hours. This treatment regime has been reported to sterilise experimental *Babesia equi* infections in horses and may have value in preventing the spread of this disease during exportation of possible carrier horses to non-endemic countries. Serum bile acids and serum gamma glutamyltransferase activity were measured to evaluate the effect of this treatment regime on hepatic function. The diffuse hepatocellular necrosis and pronounced periportal hepatocellular swelling and degeneration previously reported as the most consistent hepatic lesions noted in equines following imidocarb treatment were not evident at the dose and dosage interval used in this study. Urinary gamma glutamyltransferase: creatinine ratios (IU/g) and fractional clearance of sodium, potassium and phosphate (%) were calculated as a measure of renal function. These variables were used to examine the reported nephrotoxicity of imidocarb dipropionate. Urinary GGT and urinary GGT: creatinine ratios were significantly elevated on Day 5 of the trial and were considered indicative of transient changes in renal function. The rapid return to previous baseline values supported reported observations that changes between 25 and 100 IU/g may be a function of drug excretion and are not necessarily indicative of significant nephrotoxicity. It was concluded that the renal effects in healthy animals following this treatment regime were

minimal. The changes in urine GGT: urine creatinine ratios observed in this study provides further evidence of the value of this ratio for the early detection of renal involvement following exposure to nephrotoxic agents.

Key words: equine, imidocarb dipropionate, gamma glutamyl transpeptidase, creatinine, fractional clearance, bile acids

Introduction

Equine piroplasmosis is a tick-transmitted, disease caused by the intra-erythrocytic protozoa *Babesia equi* and *Babesia caballi*. The geographical distribution of this disease is limited to regions in which the tick vectors exist[32,80,90]. To prevent the spread of infection to unexposed, susceptible equids, endemic countries are subject to rigid exportation restrictions with substantial economic implications for equine industries. The spread of infection to non-endemic regions could be contained if a suitable and safe sterilisation protocol became instituted to eliminate infection in equids destined for exportation. A study performed by Frerichs *et al* described experiments in which imidocarb dihydrochloride was used to sterilise *Babesia equi* infections in horses and donkeys[42]. Twenty-four horses and eight donkeys, all initially seronegative for *B. equi* on complement fixation testing were acquired from an area free of piroplasmosis. These animals were sub-inoculated with *B. equi* to establish an infection and then treated according to different protocols in an attempt to sterilise the infection[41,42]. Only those animals treated with four doses of 4 mg/kg imidocarb dihydrochloride at 72-hour dosage intervals were successfully sterilised. The absence of infection was confirmed when blood from treated horses inoculated into susceptible, splenectomized ponies at a rate of 1.0 ml/kg failed to establish an infection. Animals treated

with the same dose and number of treatments, but at different dosage intervals failed to eliminate the infection. These animals were isolated for three months and re-treated successfully using the 72-hour treatment regime. This trial provided evidence that timing of administration is crucial when attempting to sterilise piroplasmosis although the precise mechanism of action remains elusive[25,41,42,58].

Dose dependent nephrotoxic and hepatotoxic effects of imidocarb have been documented in sheep, goats, cattle, dogs and horses[3-5,7,8,29,30]. Although in the study by Frerichs *et al* the anticholinesterase effects of imidocarb, including salivation, restlessness, moderate colic and hypermotility of the gastro-intestinal tract as well as local reactions at the injection sites were described, clinicopathological tests addressing the potential nephro- and hepatotoxic effects of the drug were not performed[4,42].

The primary aim of this study was to examine the clinical and clinicopathological effects of a multiple dose treatment regime of imidocarb dipropionate in healthy ponies with particular emphasis on evaluation of renal and hepatic function. Renal tubular function was evaluated using urinary gamma glutamyl transpeptidase (GGT): creatinine ratios (IU/g) and fractional clearance of phosphate (%) as sensitive indicators of early tubular disease. Serum gamma glutamyl transpeptidase (GGT) and serum bile acids were measured to determine the effects of the drug on hepatic function.

Materials and methods

Experimental animals

Three healthy Nooitgedacht ponies and three part-bred Welsh Mountain ponies between 2 and 4 years of age and weighing 244-294 kg (mean 272.5 kg) were used for the trial. These animals were maintained in a tick-free isolation unit at the Faculty of Veterinary Science, University of Pretoria, Onderstepoort, for the duration of the trial. The three Nooitgedacht ponies and two of the part-bred Welsh Mountain ponies were mares. The remaining part-bred Welsh Mountain pony was a gelding. The limited space available in the isolation unit precluded the inclusion of control ponies in this experiment.

Experimental design

The six ponies selected for the trial were introduced into their new environment approximately one week prior to the commencement of treatment. Two of the ponies were stabled individually, but due to a limited number of stalls available the remaining ponies were housed in pairs in large 4 X 5 m^2 box stalls. The stalls were in an insect proof, artificially ventilated building with continuous air filtration. The ponies were maintained in these stalls for most of the day and at night, but were turned out into a sand enclosure for a short period of time during the day to allow them to exercise. Diets were standardised and a routine was established to eliminate the potential effects of nutrition and stress on clinicopathological testing. The animals received mixed grass hay and water *ad libitum*, as well as 500g of a 12% protein pelleted ration (Epol Rider Cubes, Epol (Pty) LTD, Pretoria, RSA) twice daily. Clean pine shavings were used for bedding.

A complete physical examination, including assessment of vital signs and body weight, established that all of the ponies were healthy at the onset of the trial. Ethylene diaminetetraacetic acid (EDTA) and serum blood samples from each pony were submitted to the Clinical Pathology Laboratory (Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa), for complete blood counts and select serum indices. Due to the potential nephrotoxic and hepatotoxic effects of imidocarb dipropionate, serum and urinary concentrations of creatinine, sodium, potassium, inorganic phosphate, total serum proteins and serum bile acids were measured. Serum and urine activities of the enzymes GGT and serum aspartate aminotransferase (AST) were also measured.

Although in the trial performed by Frerichs *et al*, imidocarb dihydrochloride was used, the locally available aromatic diamidine, imidocarb dipropionate was substituted for the less readily available dihydrochloride derivative. This may however be preferable, because while these two compounds have the same active ingredient the dipropionate salt [3, 3'-bis-(2-imidazol-2 yl) carbanilide dipropionate] is reported to be less irritant[42,58].

Experimental procedure

The six ponies were given four intramuscular injections of 4 mg/kg imidocarb dipropionate (Forray 65, Schering-Plough, Isando, RSA) at 72-hour dosage intervals. Immediately prior to each treatment the ponies were pre-medicated with 0.02 mg/kg atropine sulphate (Atropine 10, Centaur Laboratories, Bryanston, South Africa) administered intravenously to minimise adverse reactions caused by the anticholinesterase effects of imidocarb. The local reactions observed were examined daily by the same investigator and were subjectively classified as mild, moderate, severe or not observed according to the amount of heat present, pain on

palpation and the extent and consistency of the raised areas. A reaction was classified as mild if the affected area was less than 5 cm in diameter, required firm palpation to elicit pain or resulted in a barely discernable elevation in skin temperature over the affected area. A reaction was termed moderate if the affected area was between 5 and 10 cm in diameter, if pain was evident with gentle palpation or if heat was easily discernible over the affected area. A reaction was classified as severe if the local reaction was larger than 10 cm in diameter, the animal resisted or avoided palpation or heat was evident with the hand raised above, but almost touching, the affected area. Oedema was recorded if these areas were seen to 'pit on pressure' while more persistent swellings were firm and were noted as such. The moderate to severe local reactions observed following the first intramuscular injection, prompted the investigators to divide the remaining doses in half and administer each half at a different site.

At the onset of the trial and prior to each treatment, blood samples were collected from the jugular vein of each animal into a serum and an EDTA tube. At the same time a urine sample was collected via sterile urinary catheterisation. To facilitate urinary catheterisation of the gelding, 15 mg acepromazine maleate (Aceprom, Centaur Laboratories, Bryanston, South Africa) was administered intravenously half an hour prior to collection. Complete blood counts and urinalyses were performed on each of the relevant samples. Blood cell counts were measured using an automated cell counter (Cell-Dyn 3500, Abbott Diagnostics, Abbott Park, USA). Blood smear examinations were done manually using thin blood smears stained with Cam's Quick-Stain (Milsch, Krugersdorp, South Africa). Urinalyses consisted of macroscopic examination of the urine, urine dipstick analysis (Combur 9 Test, Boehringer, Mannheim, Germany), measurement of urine specific gravity and microscopic evaluation of urine sediment.

Serum and urine creatinine analysis was based on the Jaffe alkaline picrate reaction as described by Rossignol[77] using a Technicon RA-1000 analyser. Sodium and potassium were measured using a NOVA Biomedical (Nova Biomedical, Waltham, USA) electrolyte analyser 1 with sodium and potassium ion selective electrodes[38]. Serum and urine inorganic phosphate was measured using the phospho-molybdate reaction as described by Amador and Urban[9] using a Technicon RA-1000 analyser. Serum and urine GGT were analysed by the optimised, kinetic method of Szasz[86] using a Technicon RA-1000 analyser.

Total serum protein analyses was based on the biuret reaction of Weichselbaum as described by Skeggs and Hochstrasser[84] using a Technicon RA-1000 analyser. AST analyses was performed using an optimised, kinetic assay based on the Karmen method described by Bergmeyer *et al*[16] using a Technicon RA-1000 analyser. A Sigma Diagnostics bile acid reagent kit was used to measure serum bile acid concentrations enzymatically using the method described by Mashige *et al*[66] on a Technicon RA-1000 analyser. Glucose concentrations were measured using the hexokinase procedure for glucose determination as described by Léon *et al*[63] using an automated analyser (Technicon RA-1000, Technicon Instrument Corporation, Tarrytown, USA).

Indices previously reported as being useful in assessing equine renal function were calculated and values obtained were compared to previously established reference ranges[27]. These included calculation of the fractional clearance of creatinine, sodium, potassium and phosphate (%) as well as urine GGT: urine creatinine ratios (IU/g). The fractional clearance of electrolytes were calculated using the following equation:

$$\%\ \text{Clearance Ratio}\ [x] = \frac{\text{urine}\ [x]\ \times\ \text{plasma (creatinine)}}{\text{plasma}\ [x]\ \times\ \text{urine (creatinine)}} \times 100$$

Statistical methods

The data in this study was analysed using Sigma Stat 2.0 statistical software (Jandel Scientific Software, San Rafael, USA). Significant changes with time within the treatment group were analysed using one way analysis of variance (ANOVA) for repeated measures followed by Dunnett's test to examine deviation from pre-treatment values. Where the data was either not normally distributed or the Equal Variance Test failed, the data was analysed using Friedman Repeated Measures Analysis of Variance (ANOVA) on Ranks followed by Dunnett's method to examine deviations from pre-treatment values. $P < 0.05$ was considered significant. Data were reported as mean ± standard error.

Results

Clinical manifestations and observations

The type and duration of the systemic signs observed after each imidocarb dipropionate treatment is summarised in Table 2. One of the ponies became severely depressed and anorectic following the administration of the third intramuscular injection. This behaviour coincided with a serum glucose and elevated serum creatinine level of 5.4 mmol/*l* and 195 µmol/*l*, respectively. These variables had returned to normal by the following day. The incidence of the systemic reactions following the first injection did not differ from the incidence of reactions following subsequent injections. Local reactions were noted at the injection site in all of the animals following the first treatment. Distribution of the drug

across various sites, as well as the administration of a smaller amount per site, resulted in a drastic reduction in the local tissue reactions seen. The characteristics of the local reactions are summarised in Table 3.

	Treatment				Duration
	1	2	3	4	
Mild colic	3/6	3/6	3/6	3/6	±30 minutes
Depression	2/6	2/6	2/6	0/6	2-6 hours
Anorexia	0/6	2/6	2/6	0/6	2-6 hours

Table 2. Summary of the incidence and duration of systemic signs observed following intramuscular administration of four doses of 4 mg/kg imidocarb dipropionate every 72 hours to 6 healthy ponies.

	Number of animals	Size	Heat	Pain	Oedema	Firm swelling
Day 1	6/6	10 x 10 cm	+++	++	-	-
Day 2	6/6	15 x 10 cm	++	+++	+++	-
Day 3	5/6	5 x 8 cm	+	+	++	-
Day 5	2/6	5 x 5 cm	-	+	-	-
Day 15	2/6	3 x 5 cm	-	-	-	+
Day 30	2/6	2 x 3 cm	-	-	-	+
Day 38	0/6	-	-	-	-	-

Table 3. Summary of the incidence and nature of the local reactions that were noted at the injection site following four intramuscular treatments of 4 mg/kg imidocarb dipropionate to six healthy ponies (+ mild, ++ moderate, +++ severe, - not observed).

Clinicopathological results

The red cell count and haematocrit were significantly lower than their respective pre-treatment values on Days 2, 5 and 18 of the study. No significant changes were observed in the haemoglobin concentration (Figure 1). No significant changes were observed in the total white cell count (Figure 2) or in the mature and immature neutrophil counts (Figure 3) at any time during the trial. The lymphocyte count was significantly lower than the pre-treatment value on Day 5 while the eosinophil count was significantly reduced on Day 18. No significant changes were observed in monocyte counts (Figure 4). The thrombocyte count was significantly higher than the pre-treatment value on Day 11 and 18 of the study (Figure 5). The total serum protein concentration was significantly lower than the pre-treatment values on Day 11 and 18 (Figure 6). Serum aspartate aminotransferase activity was significantly elevated above pre-treatment values on Days 2, 5, 8, 11 and 18, but had returned to pre-treatment levels by Day 38 of the trial (Figure 7). Serum and urine creatinine concentrations did not differ significantly from the pre-treatment value at any time during the trial (Figure 8). The serum sodium concentration was significantly lower than the pre-treatment value on Day 5 of the trial (Figure 9). There were no significant differences between the fractional clearance of sodium (Figure 9), the serum potassium concentration, the fractional clearance of potassium (Figure 10), the serum inorganic phosphate or the fractional clearance of phosphate (Figure 11) at any time during the trial and their respective pre-treatment values. The serum and urine GGT activities and urine GGT: urine creatinine ratios were all significantly elevated above pre-treatment values on Day 5. The serum GGT activity was also elevated on Day 18 of the trial (Figure 12). The total bile acid concentration was significantly lower than the pre-treatment values on Day 5 of the trial (Figure 13). No significant changes were observed in urine specific gravity during this trial. In contrast, urine

pH was significantly lower than the pre-treatment value on Day 18 of the trial (Table 4). No abnormalities or changes were detected on macro- or microscopic examination of the urine and urine sediment. Glucosuria was present in two of the animals following the third intramuscular injection. The concurrent serum glucose and serum creatinine levels in these animals were 5.2 μmol/*l* and 5.4 μmol/*l*, and 168 μmol/*l* and 195 μmol/*l*, respectively (reference ranges 3.5-6.3 μmol/*l*; 76.8-174.5 μmol/*l*)[40].

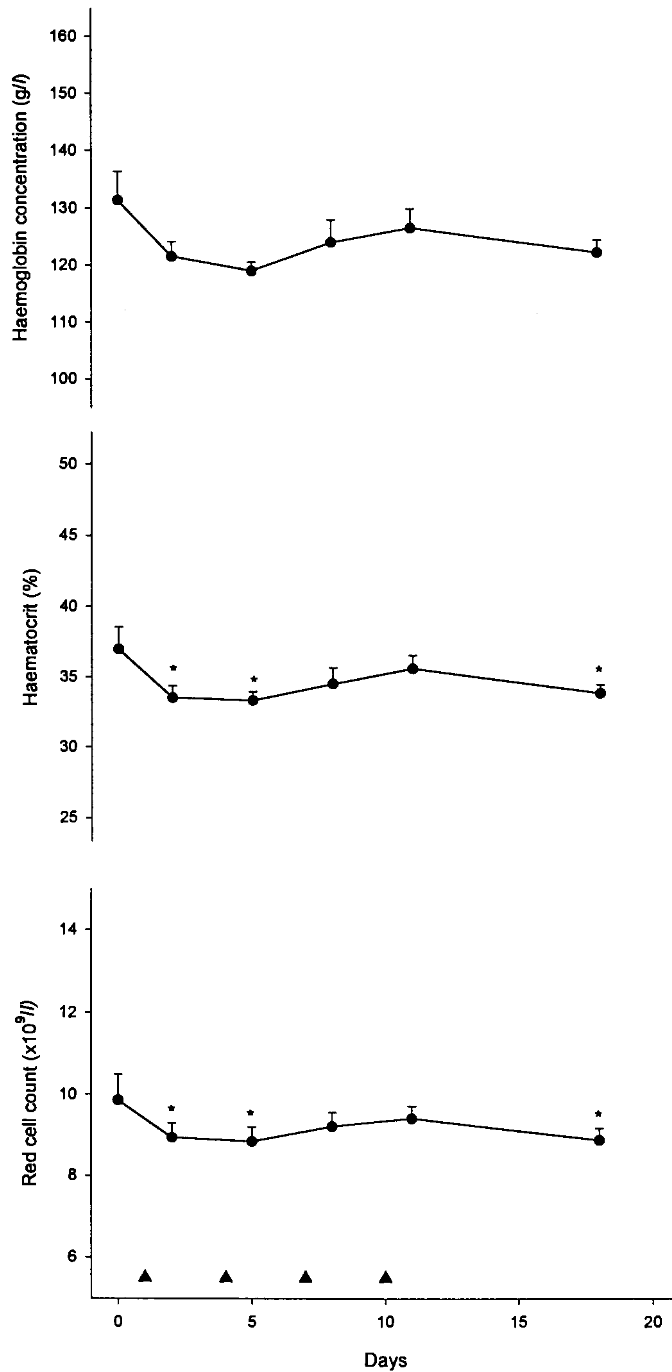

Figure 1. Temporal changes of mean and standard error of the total red cell count and haematocrit and haemoglobin concentrations of 6 ponies following intramuscular administration of 4 mg/kg imidocarb dipropionate on Days 1, 4, 7 and 10. ▲ - indicates days on which imidocarb was administered. *- mean at time indicated was significantly different ($P < 0.05$) from the pre-treatment value.

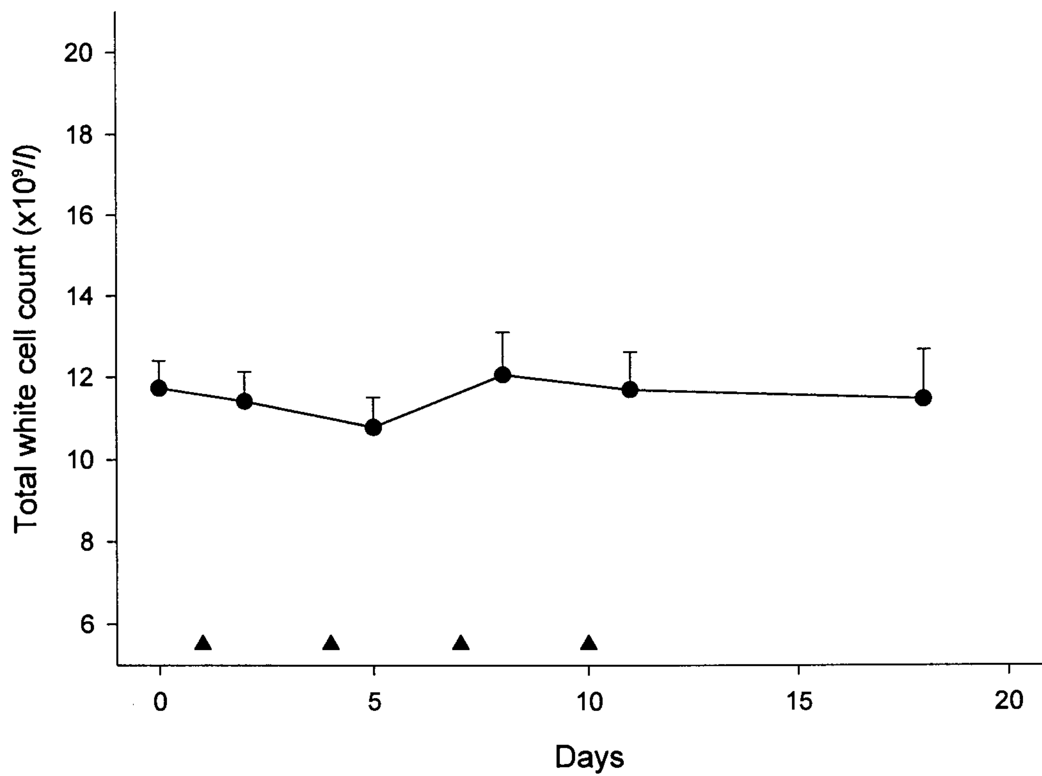

Figure 2. Temporal changes of mean and standard error of the total white cell count of 6 ponies following intramuscular administration of 4 mg/kg imidocarb dipropionate on Days 1, 4, 7 and 10. ▲ - indicates days on which imidocarb was administered.

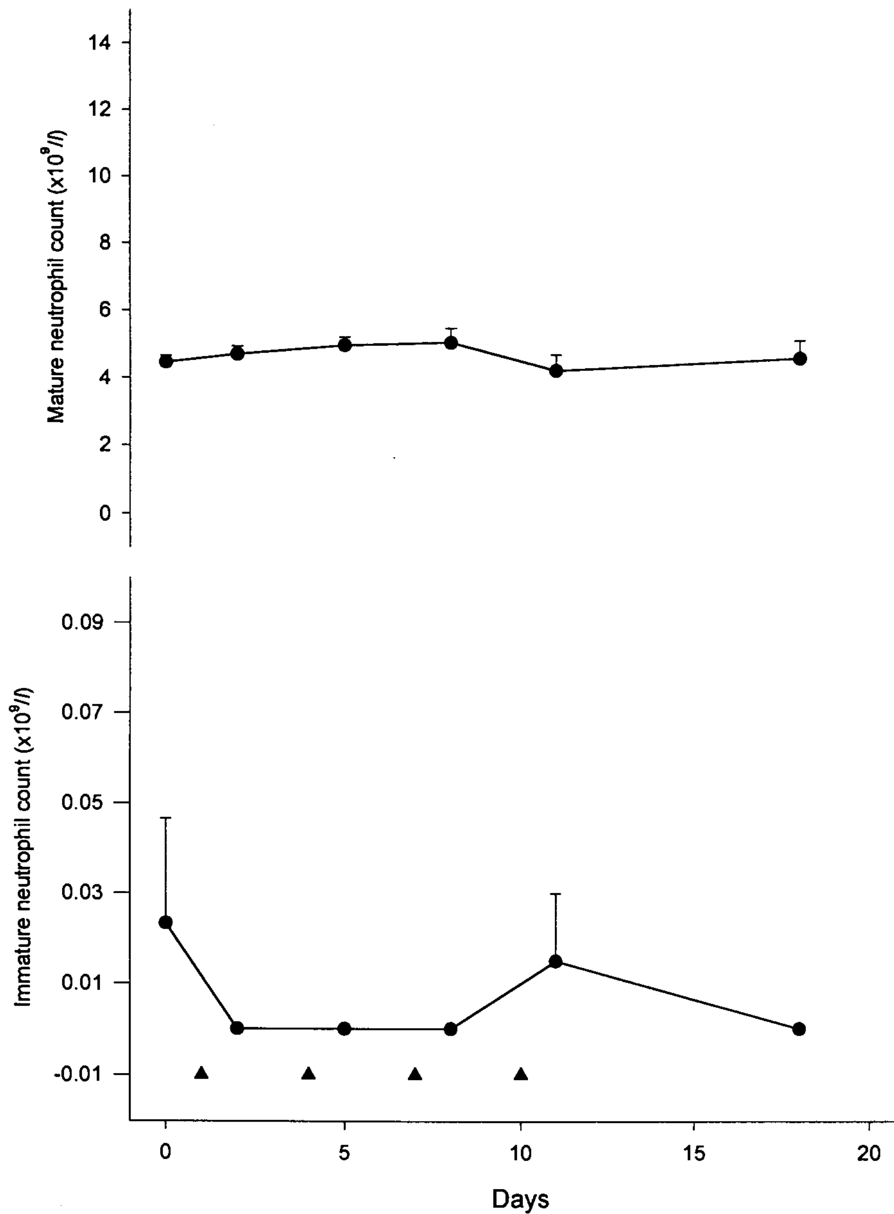

Figure 3. Temporal changes in mean and standard error of mature and immature neutrophil counts (x10^9/*l*) of 6 ponies following intramuscular administration of 4 mg/kg imidocarb dipropionate on Days 1, 4, 7 and 10. ▲- indicates days on which imidocarb was administered.

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

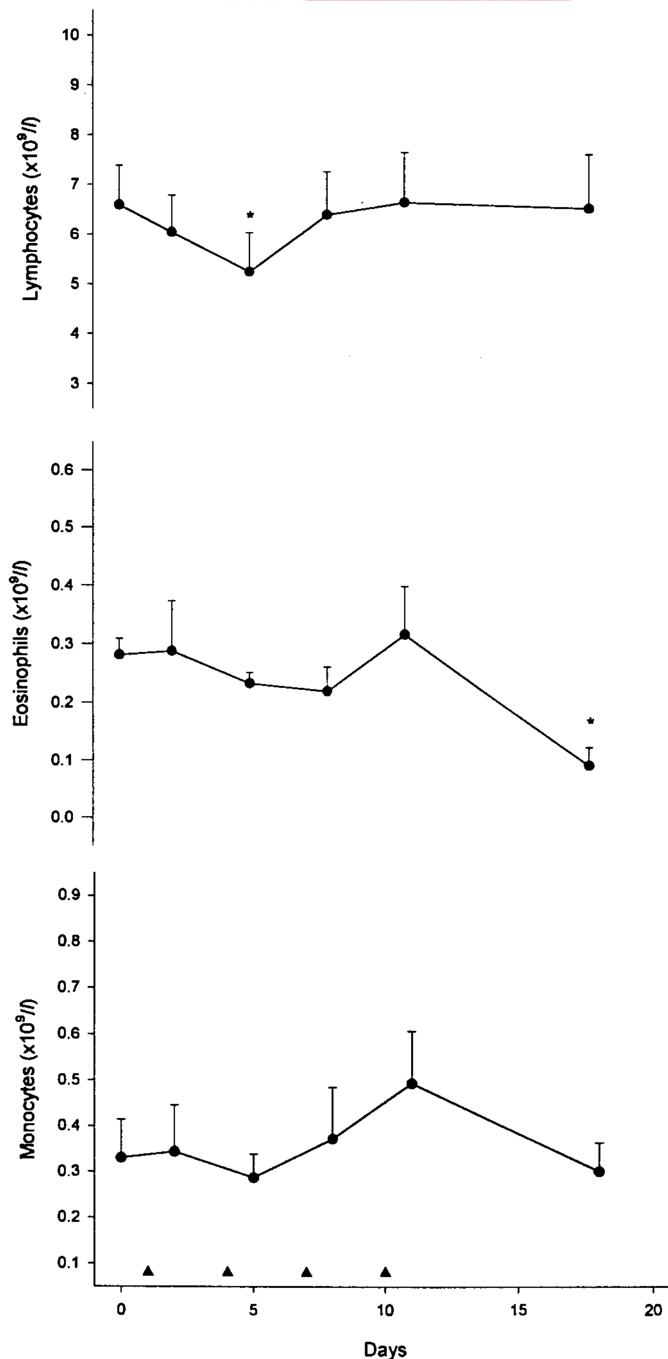

Figure 4. Temporal changes of mean and standard error of lymphocyte, eosinophil and monocyte counts ($x10^9/l$) of 6 ponies following intramuscular administration of 4 mg/kg imidocarb dipropionate on Days 1, 4, 7 and 10. ▲- indicates days on which imidocarb was administered. *- mean at time indicated was significantly different ($P < 0.05$) from the pre-treatment value.

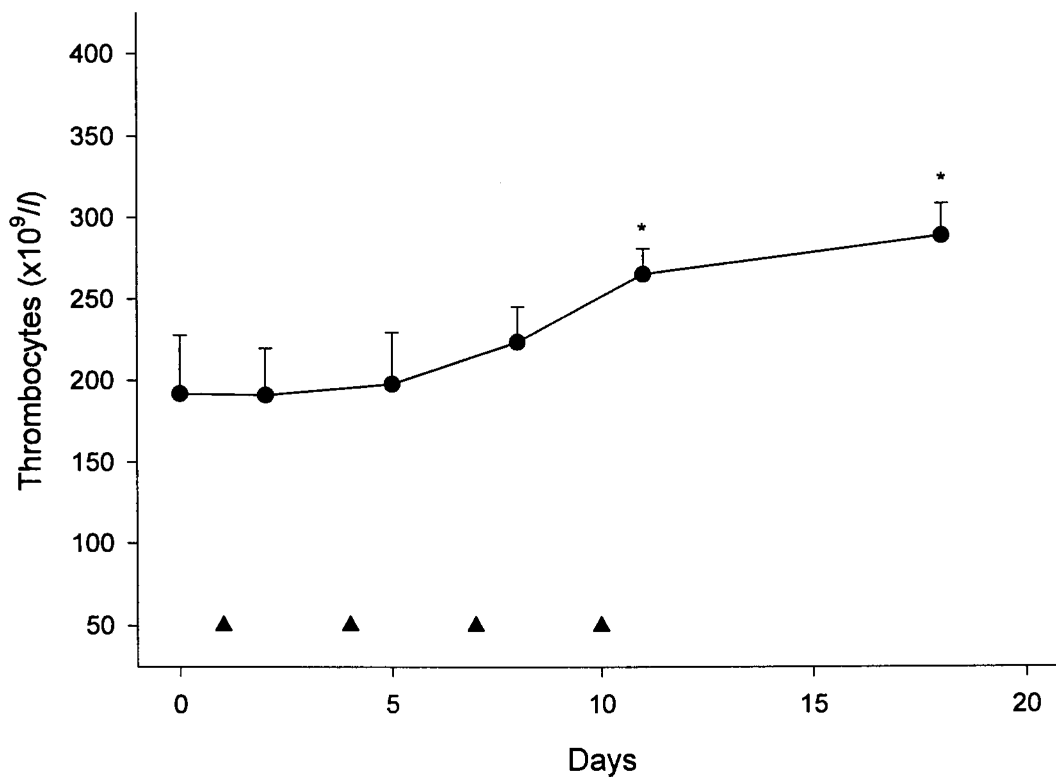

Figure 5. Temporal changes of mean and standard error of the thrombocyte count ($x10^9/l$) of 6 ponies following intramuscular administration of 4 mg/kg imidocarb dipropionate on Days 1, 4, 7 and 10. ▲- indicates days on which imidocarb was administered. *- mean at time indicated was significantly different ($P < 0.05$) from the pre-treatment value.

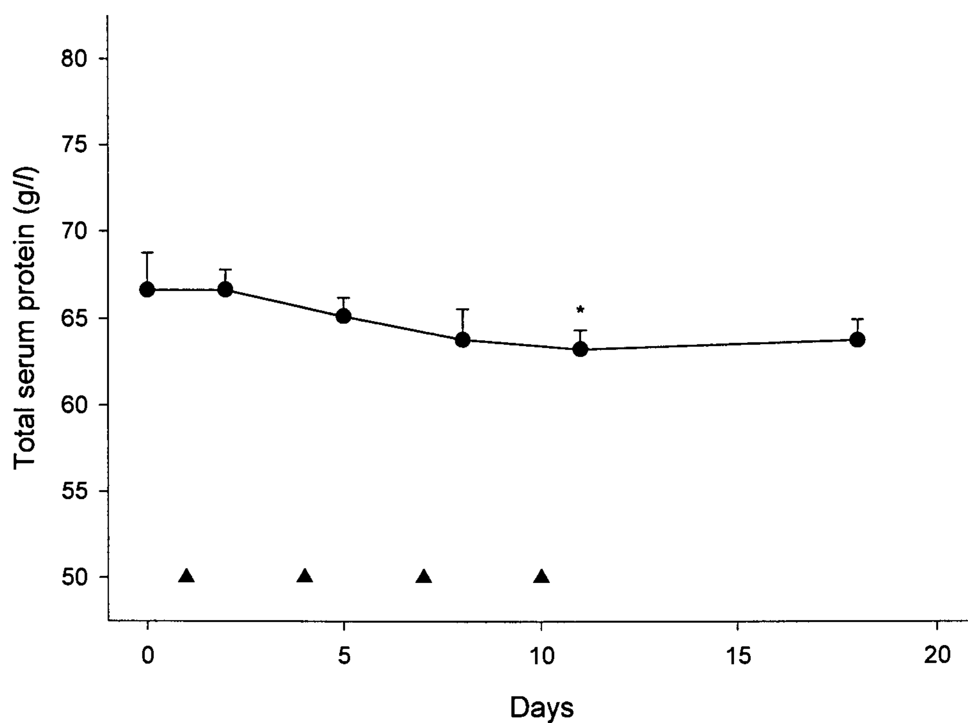

Figure 6. Temporal changes of mean and standard error of the total serum protein concentration (g/*l*) of 6 ponies following intramuscular administration of 4 mg/kg imidocarb dipropionate on Days 1, 4, 7 and 10. ▲- indicates days on which imidocarb was administered. *- mean at time indicated was significantly different ($P < 0.05$) from the pre-treatment value.

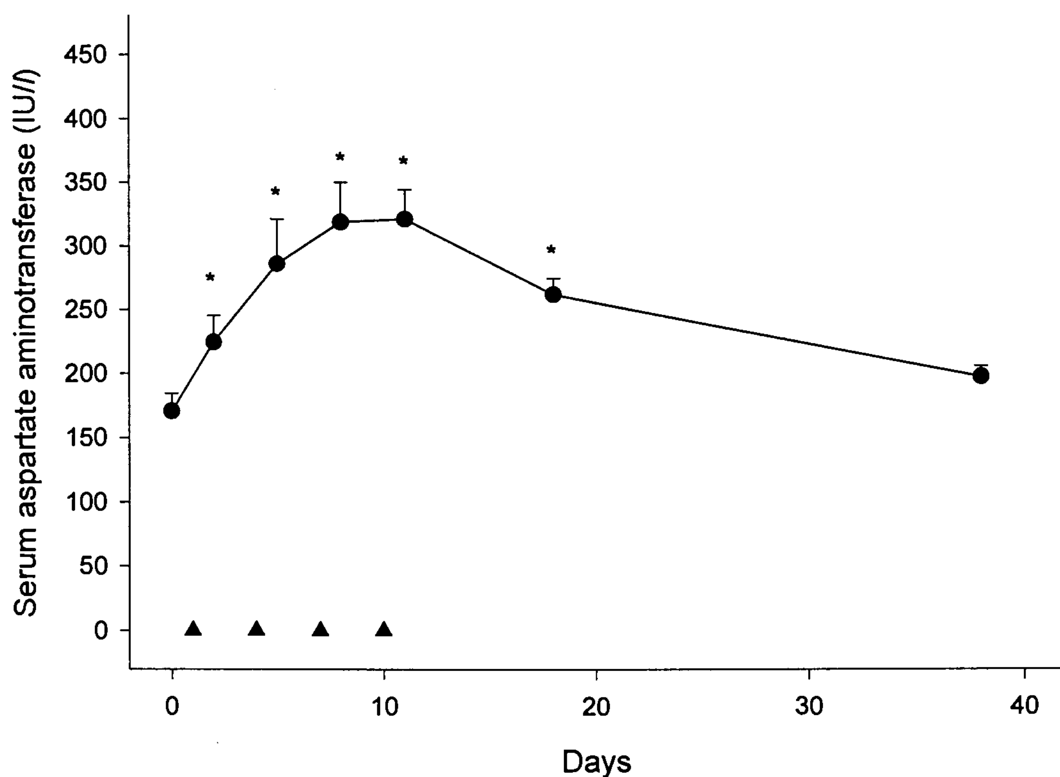

Figure 7. Temporal changes of mean and standard error of aspartate aminotransferase activity (IU/*l*) of 6 ponies following intramuscular administration of 4 mg/kg imidocarb dipropionate on Days 1, 4, 7 and 10. ▲- indicates days on which imidocarb was administered. *- mean at time indicated was significantly different ($P < 0.05$) from the pre-treatment value.

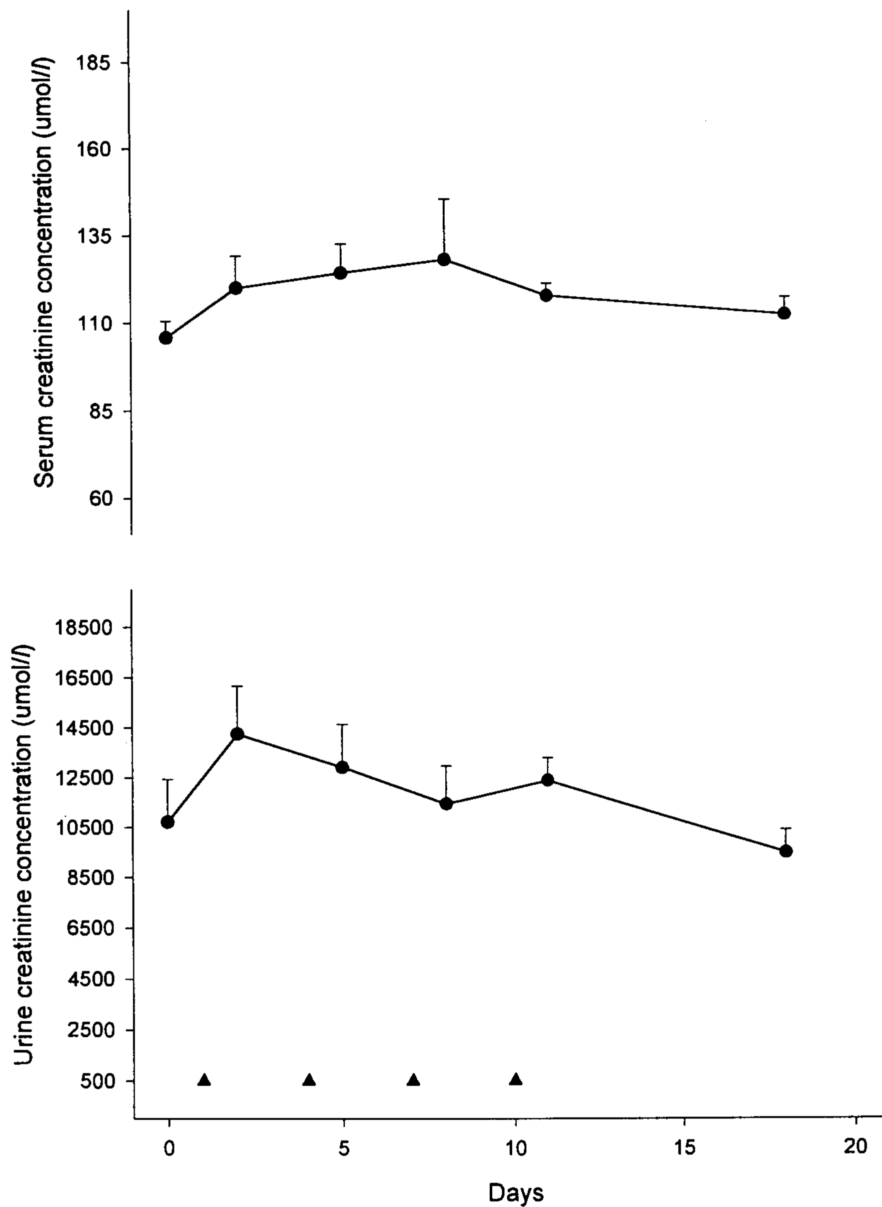

Figure 8. Temporal changes of mean and standard error of serum creatinine (mg/d*l*) and urine creatinine concentration (mg/d*l*) of 6 ponies following intramuscular administration of 4 mg/kg imidocarb dipropionate on Days 1, 4, 7 and 10. ▲- indicates days on which imidocarb was administered.

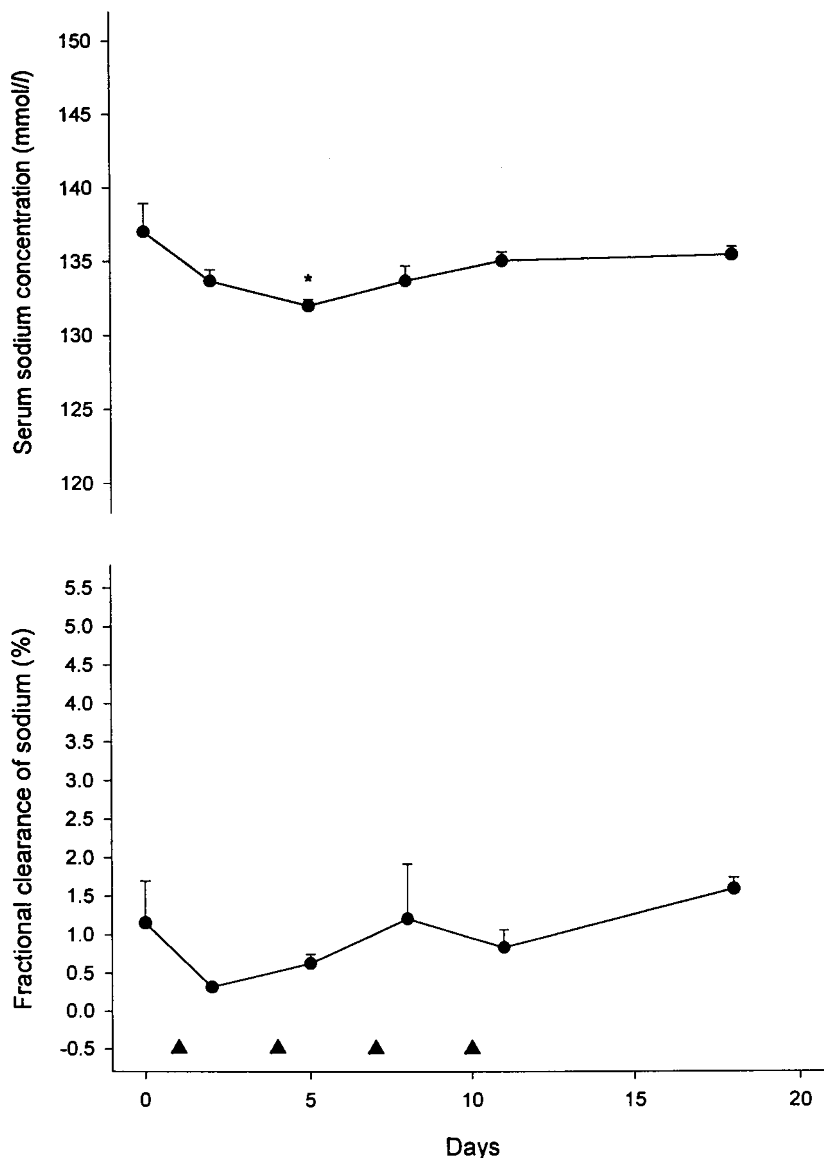

Figure 9. Temporal variation in mean and standard error of serum sodium concentrations (mmol/*l*) and fractional clearance of sodium (%) of 6 ponies following intramuscular administration of 4 mg/kg imidocarb dipropionate on Days 1, 4, 7 and 10. ▲ - indicates days on which imidocarb was administered. *- mean at time indicated was significantly different ($P < 0.05$) from the pre-treatment value.

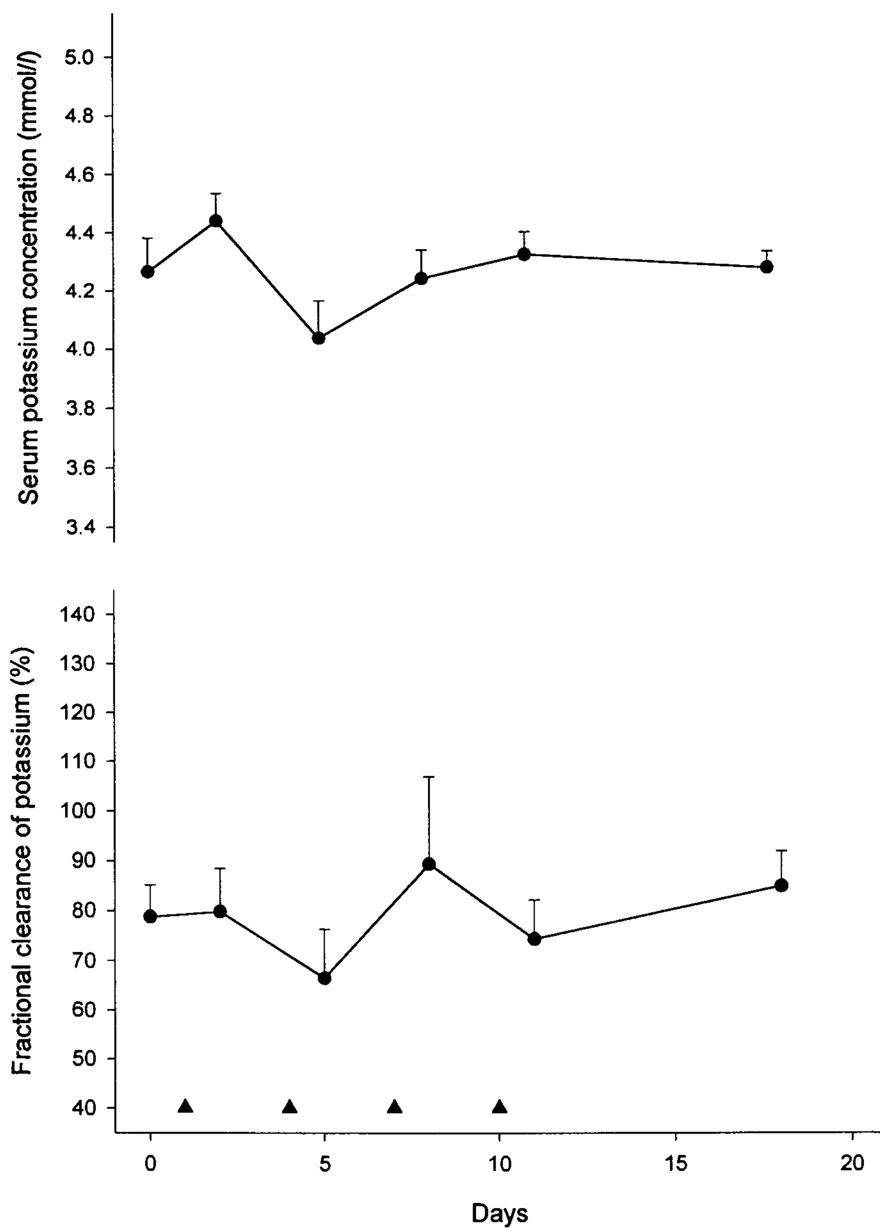

Figure 10. Temporal changes in mean and standard error of serum potassium concentrations (mmol/*l*) and the fractional clearance of potassium (%) of 6 ponies following intramuscular administration of 4 mg/kg imidocarb dipropionate on Days 1, 4, 7 and 10. ▲ - indicates days on which imidocarb was administered.

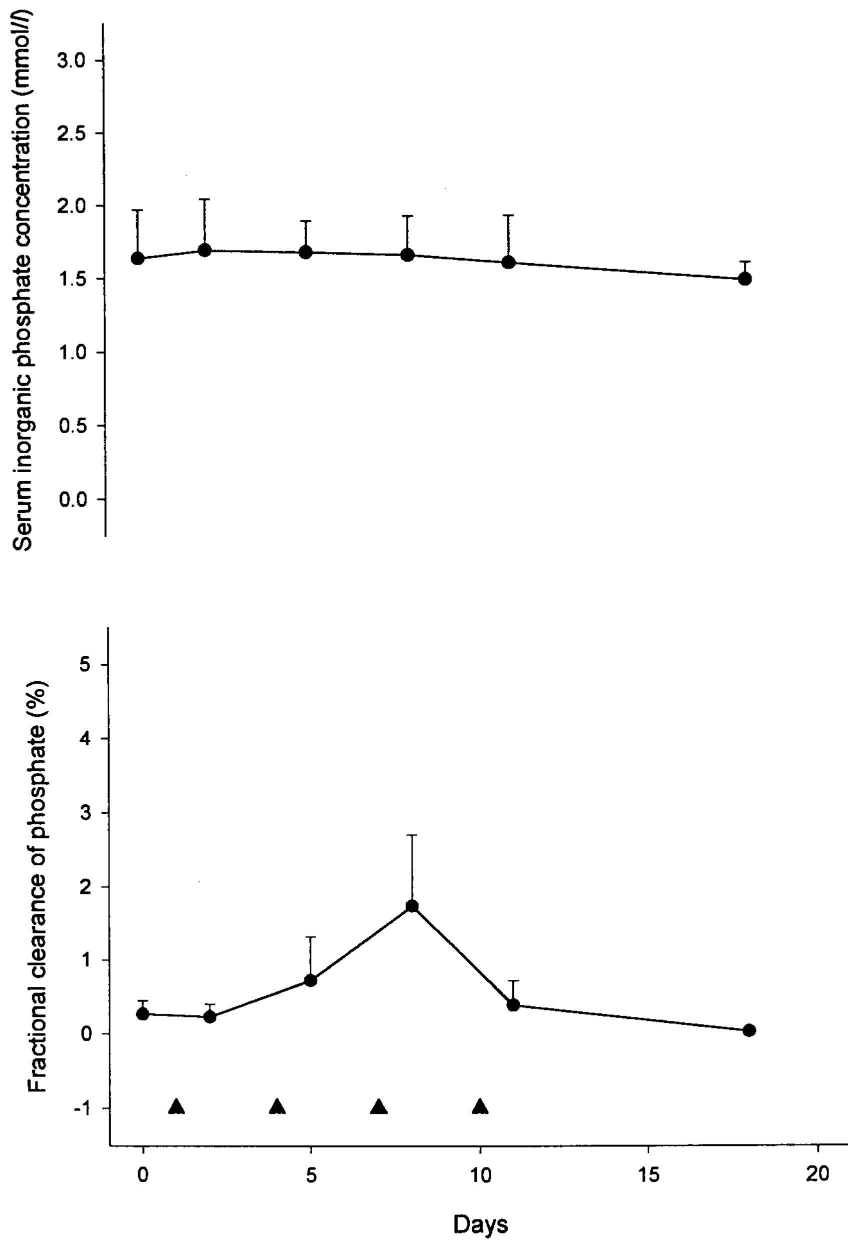

Figure 11. Temporal changes in mean and standard error of serum inorganic phosphate (mmol/*l*) and the fractional clearance of phosphate (%) of 6 ponies following intramuscular administration of 4 mg/kg imidocarb dipropionate on Days 1, 4, 7 and 10. ▲ - indicates days on which imidocarb was administered.

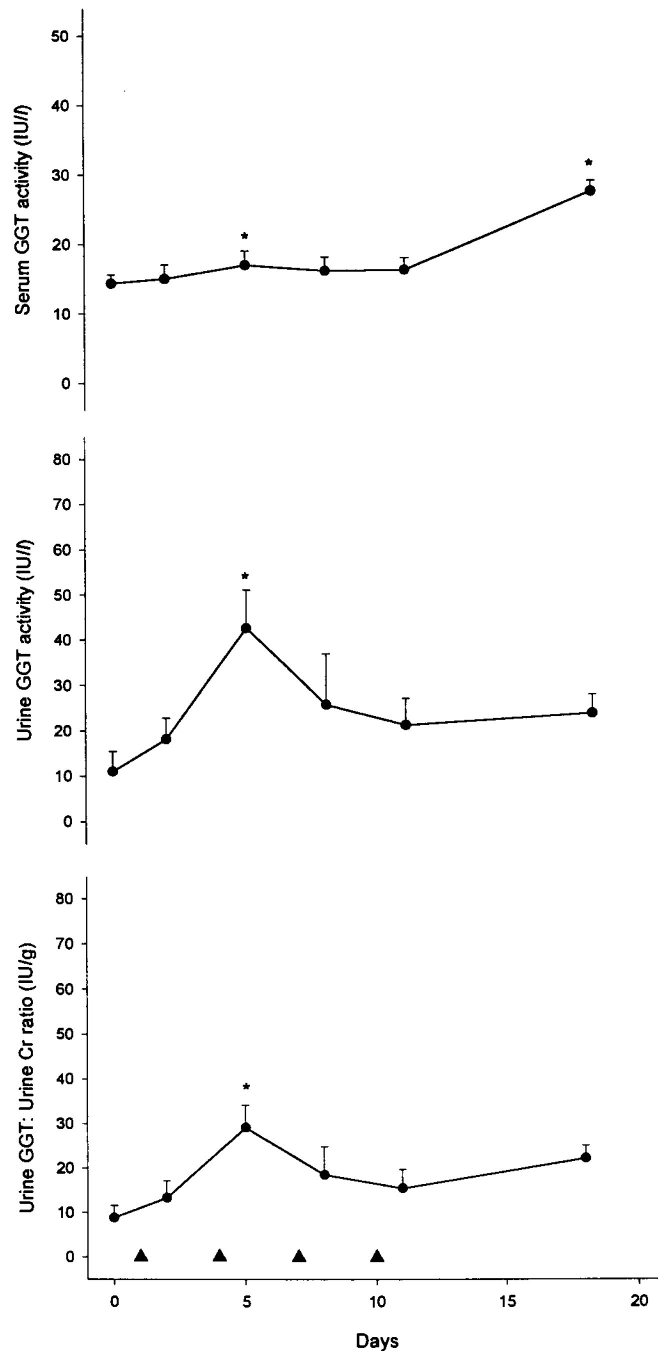

Figure 12. Temporal variation of mean and standard error of serum gamma glutamyl transpeptidase (GGT) (IU/*l*), urine GGT (IU/*l*) and the urine GGT: urine creatinine ratios (IU/g) of 6 ponies following intramuscular administration of 4 mg/kg imidocarb dipropionate on Days 1, 4, 7 and 10. ▲ - indicates days on which imidocarb was administered. *- mean at time indicated was significantly different ($P < 0.05$) from the pre-treatment value.

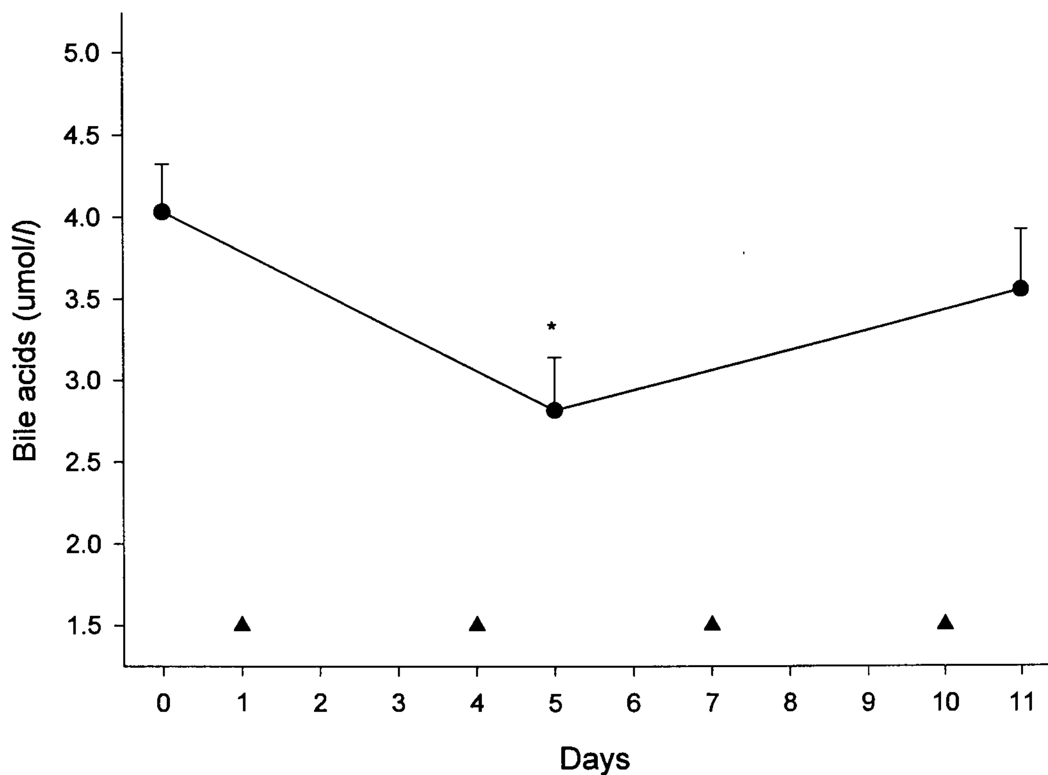

Figure 13. Temporal changes in mean and standard error of serum bile acids (µmol/*l*) of 6 ponies following intramuscular administration of 4 mg/kg imidocarb dipropionate on Days 1, 4, 7 and 10. ▲- indicates days on which imidocarb was administered. *- mean at time indicated was significantly different ($P < 0.05$) from the pre-treatment value.

	Urine specific gravity	Urine pH	Glucosuria
	mean ± standard error	mean ± standard error	
Day 0	1.030 ± 0.00258	8.17± 0.31	0/6
Day 2	1.030 ± 0.00258	8.83± 0.17	0/6
Day 5	1.030 ± 0.00307	8.83± 0.21	0/6
Day 8	1.040 ± 0.00333	7.83± 0.17	2/6
Day 11	1.040 ± 0.00211	7.67± 0.33	0/6
Day 18	1.040 ± 0.00167	7.33± 0.33*	0/6

Table 4. Mean and standard error of the urine specific gravity and urine pH and incidence of glucosuria measured on Days 0, 2, 5, 8, 11 and 18. *- mean at time indicated was significantly different ($P < 0.05$) from the pre-treatment value.

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Discussion

Imidocarb dipropionate [3, 3' bis-(2-imidazolin-2-yl) carbanilide dipropionate] is an aromatic diamidine used as a chemotherapeutic agent for the treatment of *Babesia equi* and the less pathogenic *Babesia caballi* infections in equids[25,41,58,60]. Although this drug is routinely associated with adverse effects such as salivation, restlessness, moderate colic and hypermotility of the gastro-intestinal tract the results of this trial, in concurrence with previous reports[25,58,60], showed that administration of parasympatholytics such as atropine sulphate significantly reduced these effects. In addition, varying injection sites and administering smaller amounts of drug per site substantially reduced the mild to moderate local reactions previously reported with this drug [25].

The ponies in this study showed a uniform local response at the site of injection following the administration of multiple doses of imidocarb dipropionate. A focal myonecrosis induced by repeated intramuscular injections of imidocarb dipropionate may explain the elevation in aspartate aminotransferase (AST) activity seen in this trial. AST is also present in liver and heart tissue and examination of sera for enzyme activity in the absence of additional serum biochemistry, is not a specific indicator of tissue source[15]. The plasma half-life of AST in the horse is 7-10 days with persistent elevations indicative of ongoing damage and a continuous release of isoenzymes[15]. The absence of an ongoing possible myonecrosis in this study could be confirmed by the return of AST levels to within normal limits (reference range: 10- 240 U/*l*)[40] approximately 2 weeks after administration of the final intramuscular injection.

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

The haematological results obtained in this study indicated that the dosage regime of imidocarb dipropionate used had minimal effect on cellular blood components. The changes in haematological variables, including the mild leukocytosis and concurrent lymphocytosis observed were considered a normal response for healthy, young and excited animals and often occur when a struggle develops during restraint for sample collection[51]. This physiological leukocytosis occurs in response to adrenaline release during which the marginal pools of neutrophils and/or lymphocytes are mobilized into the general circulation raising the absolute neutrophil and/or lymphocyte counts[51]. Similarly, increases in red cell variables were likely due to splenic contraction in response to circulating catecholamines[51]. The decrease in total eosinophils over time was interesting and may have been due to the limited exposure of the animals to insects or allergens while housed in an insect proof, artificially ventilated building with continuous air filtration. This observation may be supported by a concurrent significant decrease in total serum proteins, which in turn may have resulted from a decrease in antigenically stimulated immunoglobulin production[51].

FC_P is reportedly a sensitive indicator of early renal dysfunction in horses and elevations in this variable precede increases in fractional clearance of sodium (FC_{Na}) in this species[15,21,37,53,55,88]. The absence of significant increases above pretreatment concentrations for either variable suggests that the nephrotoxic effects of imidocarb dipropionate may be limited at this dose and dosage interval.

The use of enzymuria in screening for renal disease has been actively pursued in human medicine and is considered a non-invasive, sensitive indicator of early renal dysfunction[14,43,49]. Elevations in urine GGT activity are considered to be renal in origin, as

the high molecular weight of this enzyme prevents its filtration from the blood by the normal glomerulus[14]. There is therefore no correlation between increased serum GGT activity originating from the liver, or more rarely the pancreas, and urine GGT originating from the brush border of the proximal renal tubular epithelium[14]. The significant increase in serum GGT in conjunction with a similar decrease in serum bile acid concentrations observed on Day 5 may have been due to drug induced hepatic enzyme induction. Clearly, further studies would be required to elucidate such speculation.

In equines, urine GGT: urine creatinine ratios less than 25 IU/g are considered normal, but the small sample sizes on which these estimates are based may not be representative of the larger population and there is some indication that ratios between 25 and 100 IU/g should be interpreted with care[49,76]. In this study, the serial determination of this variable allowed comparison with individual baseline activity with the significant elevation observed on Day 5 confirming renal involvement. However, the rapid return to previous baseline values supported reported observations that changes between 25 and 100 IU/g may be a function of drug excretion and are not necessarily indicative of significant nephrotoxicity[14,43,49,76]. The lack of evidence of significant renal damage may, however, in part be due to the extensive regenerative capacity of the kidney[43]. Consequently, in response to these changes, reduction of dose or frequency of drug administration may prevent manifestation of clinical nephrotoxicity. In concurrence with previous studies, this ratio appears to be one of the earliest practical indicators of renal involvement and may thus have clinical value in the therapeutic monitoring of potentially nephrotoxic substances, including imidocarb[14,20]. Serial monitoring of renal function may be particularly significant in diseased animals as it has previously been shown that significant alterations in the disposition kinetics of imidocarb

were evident in diseased goats[2]. In these goats, changes in the volume of distribution and drug clearance, related to the pathophysiology and febrile reactions of various experimentally induced disease states, were reported[2]. Altered drug disposition increases the susceptibility of treated animals to the potential toxic effects of various substances[2]. This has been extensively documented in drug efficacy trials in neonates[85].

Elevated serum creatinine levels outside the normal reference range were observed in two of the ponies on Day 11 of the trial. Changes in concentrations of this variable from pre-treatment values for the group were, however, not significant and supported the observation that the renal effects of this treatment regime were limited, although individual variation may occur. In horses elevations above normal, or an increase in serum creatinine within the normal reference range of greater than 26.5 µmol/*l* in the absence of an alternative underlying cause of renal damage, is considered an indicator of nephrotoxicity[49]. Nephrotoxicity in man has similarly been associated with elevations in serum creatinine concentration greater than 44.0 µmol/*l*[49]. Glucosuria has also been documented as a nonspecific indicator of renal disease[14]. There should be no glucose present in normal urine, although in the presence of serum hyperglycemia the renal threshold for glucose is exceeded resulting in glucosuria in the absence of underlying renal disease[28]. In this study the glucosuria, in the presence of normal serum glucose levels and elevated serum creatinine levels, was likely a consequence of the drug-induced renal changes. As shown in this study and previously reported, the poor sensitivity and specificity of these two variables render them inappropriate as individual spot tests for detection of early renal dysfunction[28,46,49].

The effect of this treatment regime on liver function was examined by sequential analysis of serum bile acid and serum GGT concentrations. Total plasma bile acids have been shown to be the most sensitive indicator of hepatic disease when compared to other liver function tests[39,45,68,92]. Due to the absence of any increase in this variable, it was concluded that this treatment regime had no detectable deleterious effect on hepatic function in healthy ponies.

In conclusion, the haematological changes observed were considered the result of intramuscular drug administration to excitable, young animals, rather than a functional property of the drug itself. Mild changes in indicators of renal function observed in this study were indicative of renal involvement. The nephrotoxic properties of imidocarb have been reported in previous studies and although the renal changes were mild and transient, the potentially harmful effects of imidocarb dipropionate should not be disregarded as individual variation may occur[4]. These effects may also be of particular significance when prior renal or generalised cardiovascular compromise is evident. The severe hepatotoxic effects of imidocarb previously reported[4] were not evident at the dose and dosage interval used in the present study.

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Chapter 4

General conclusions

The following conclusions were drawn from this study:

1. The haematological changes evident were considered a normal physiological response for healthy, young and excited animals induced by restraint during sample collection.

2. The mild transient changes in renal function were not indicative of significant nephrotoxicity and may have been a function of drug excretion. The potentially harmful effects of imidocarb dipropionate previously reported should however not be disregarded as individual variation may occur. Altered drug disposition in animals suffering from underlying disease may also be significant.

3. The diffuse hepatocellular necrosis and pronounced periportal hepatocellular swelling and degeneration previously reported as the most consistent hepatic lesions noted in equines following imidocarb treatment, were not evident at the dose and dosage interval used.

4. Four intramuscular treatments of imidocarb dipropionate at a dose of 4 mg/kg every 72 hours may be a relatively safe method whereby persistent *Babesia equi* infections can be sterilised.

Reference list

1. Abdullah A S, Baggot J D 1983 Pharmacokinetics of imidocarb in normal dogs and goats. *Journal of Veterinary Pharmacology and Therapeutics* 6: 195-199

2. Abdullah A S, Baggot J D 1986 Influence of induced disease states on the disposition kinetics of imidocarb in goats. *Journal of Veterinary Pharmacological Therapies* 9: 192-197

3. Abdullah A S, Sheikh-Omar A R, Baggot J D, Zamri M 1984 Adverse effects of imidocarb dipropionate (Imizol®) in a dog. *Veterinary Research Communications* 8: 55-59

4. Adams L G 1981 Clinicopathological aspects of imidocarb dipropionate toxicity in horses. *Research in Veterinary Science* 31: 54-61

5. Adams L G, Corrier D E 1980 A study of the toxicity of imidocarb dipropionate in cattle. *Research in Veterinary Science* 28: 172-177

6. Adams R, McClure J J, Gossett K A, Koonce K L, Ezigbo C 1985 Evaluation of a technique for measurement of γ–glutamyltranspeptidase in equine urine. *American Journal of Veterinary Research* 46: 147-150

7. Ali B H, Hassan T, Suliman H B, Abdelsalam E B 1985 Some effects of imidocarb in goats. *Veterinary and Human Toxicology* 27: 477-480

8. Aliu Y O, Davis R H, Camp B J, Kuttler K L 1977 Absorption, distribution, and excretion of imidocarb dipropionate in sheep. *American Journal of Veterinary Research* 38: 2001-2007

9. Amador E, Urban J 1972 Simplified serum phosphorous analysis by continuous flow spectrophotometer. *Clinical Chemistry* 18: 604

10. Avarzed A, de Waal D T, Igarashi I, Saito A, Oyamada T, Toyoda Y, Suzuki N 1997 Prevalence of equine piroplasmosis in Central Mongolia. *Onderstepoort Journal of Veterinary Research* 64: 141-145

11. Baldock F C 1987 Operating characteristics of diagnostic tests, interpretation of results and criteria for selecting a test. *Australian Veterinary Practice* 17: 126-130

12. Barton M H, Morris D D 1998 Diseases of the Liver. In Reed S M, Bayly W M (eds) *Equine Internal Medicine.* W.B.Saunders Company, Philadelphia: 707-738

13. Bayly W M 1991 A practitioner's approach to the diagnosis and treatment of renal failure in horses. *Veterinary Medicine* 6: 632-639

14. Bayly W M, Brobst D F, Elfers R S, Reed S M 1986 Serum and urinary biochemistry and enzyme changes in ponies with acute renal failure. *Cornell Veterinarian* 76: 306-316

15. Bayly W M, Schott H C 1998 The Urinary System. In Reed S M, Bayly W M (eds) *Equine Internal Medicine.* W.B.Saunders Company, Philadelphia: 830-855

16. Bergmeyer H U, Scheibe P, Wahlefeld A W 1978 Optimization of methods for aspartate aminotransferase and alanine aminotransferase. *Clinical Chemistry* 24: 58-73

25. Carbrey E A, Avery R J, Knowles R C, Sash S C 1971 Chemotherapy of equine babesiosis. *Journal of the American Veterinary Medical Association* 159: 1538-1545

26. Carlson G P, Rumbaugh G E, Harrold D 1979 Physiologic alterations in the horse produced by feed and water deprivation during periods of high environmental temperatures. *American Journal of Veterinary Research* 4: 982-985

27. Coffman J 1980 Percent creatinine clearance ratios. *Veterinary Medicine* 12: 673-676

28. Coles E H 1986 Kidney Function. In Pedersen D, Dick E (eds) *Veterinary Clinical Pathology*. W.B. Saunders Company, Philadelphia: 171-202

29. Corrier D E, Adams L G 1976 Clinical, histologic, and histochemical study of imidocarb dipropionate toxicosis in goats. *American Journal of Veterinary Research* 37: 811-816

30. Corrier D E, Adams L G 1977 Ultrastructural renal lesions in goats given a lethal dose of imidocarb dipropionate. *American Journal of Veterinary Research* 38: 217-222

31. Cuddeford D, Woodhead A, Muirhead R 1990 Potential of alfalfa as a source of calcium for calcium deficient horses. *The Veterinary Record* 126: 425-429

32. de Waal D T, van Heerden J 1994 Equine babesiosis. In Coetzer J A W, Thomson G R, Tustin R C (eds) *Infectious Diseases of Livestock*. Oxford University Press, Oxford, United Kingdom: 295-303

33. Divers T J 1996 Diseases of the Renal System. In Smith B P (eds) *Large Animal Internal Medicine.* Mosby, Baltimore: 953-974

34. Donnelly J, Joyner L P, Frank C 1980 Quantitative epidemiological studies on the prevalence of babesiosis in horses in Kuwait. *Tropical Animal Health and Production* 12: 253-258

35. Donnelly J, Joyner L P, Graham-Jones O, Ellis C P 1980 A comparison of the complement fixation and immunofluorescent antibody tests in a survey of the prevalence of *Babesia equi* and *Babesia caballi* in horses in the Sultanate of Oman. *Tropical Animal Health and Production* 12: 50-60

36. Dowling P M, Williams M A, Clark T P 1993 Adrenal insufficiency associated with long-term anabolic steroid administration in a horse. *Journal of the American Veterinary Medical Association* 203: 1166-1169

37. Edwards D J, Brownlow M A, Hutchins D R 1989 Indices of renal function: reference values in normal horses. *Australian Veterinary Journal* 66: 60-63

38. Eisenmann G 1967 Particular properties of cation-selective glass electrodes containing Al_2O_3. In Eisenmann G (eds) *Glass Electrodes for Hydrogen and Other Cations: Principles and Practice.* Marcel Dekker, Inc, New York: 268-283

39. Engelking L R 1989 Evaluation of equine bilirubin and bile acid metabolism. *Compendium on Continuing Education for the Practicing Veterinarian* 11: 328-336

40. Fraser C M, Bergeron J A, Mays A, Aiello S E 1991 Clinical Values and Procedures. *The Merck Veterinary Manual.* Merck & Co.,Inc., New Jersey,U.S.A.: 933-975

41. Frerichs W M 1977 Effect of imidocarb dipropionate and hemicastration on spermatogenesis in pony stallions. *American Journal of Veterinary Research* 38: 139-141

42. Frerichs W M, Allen P C, Holbrook A A 1973 Equine piroplasmosis *(Babesia equi)*: Therapeutic trials of imidocarb dihydrochloride in horses and donkeys. *Veterinary Record* 93: 73-75

43. Fuentes V O, Gonzalez H, Sanchez V, Fuentes P, Rosiles R 1997 The effect of neomycin on the kidney function of the horse. *Journal of Veterinary Medicine* 44: 201-205

44. Genetzky R M, Lopanco F V, Ledet A E 1987 Clincal pathologic alterations in horses during a water deprivation test. *American Journal of Veterinary Research* 48: 1007

45. Gerstman B B, Cappucci D T 1986 Evaluating the reliability of diagnostic test results. *Journal of the American Veterinary Medical Association* 188: 248-251

46. Grossman B S, Brobst D F, Kramer J W, Bayly W M, Reed S M 1982 Urinary indices for differentiation of prerenal azotemia and renal azotemia in horses. *Journal of the American Veterinary Medical Association* 180: 284-288

47. Gummow B, de Wet C S, de Waal D T 1996 A sero-epidemiological survey of equine piroplasmosis in the Northern and Eastern Cape Provinces of South Africa. *Journal of the South African Veterinary Association* 67: 204-208

48. Harris P, Colles C 1988 The use of creatinine clearance ratios in the prevention of equine rhabdomyolysis: A report of four cases. *Equine Veterinary Journal* 20: 459-463

49. Hinchcliff K W, McGuirk S M, MacWilliams P S 1987 Gentamycin nephrotoxicity. *Proceedings of the Annual Convention of the American Association of Equine Practitioners* 33: 67-75

50. Hoffman W E, Baker G, Reiser S, Dorner J L 1987 Alterations in selected serum biochemical constituents in equids after induced hepatic disease. *American Journal of Veterinary Research* 48: 1343-1347

51. Jain N 1993 Interpretation of leukocyte parameters. In Mundorff G H, Stead L (eds) *Essentials of Veterinary Hematology*. Lea & Febiger, Philadelphia: 295-306

52. Joyner L P, Donnelly J, Huck R A 1981 Complement fixation tests for equine piroplasmosis (*Babesia equi* and *B caballi*) performed in the UK during 1976 to 1979. *Equine Veterinary Journal* 13: 103-106

53. King C 1994 Practical use of urinary fractional excretion. *Journal of Equine Veterinary Science* 14: 464-468

54. Knudsen E 1959 Renal clearance studies on the horse 1.) Inulin, endogenous creatinine and urea. *Acta Veterinaria Scandinavica* 1: 52-66

55. Kohn C W, Chew D J 1987 Laboratory diagnosis and characterization of renal disease in horses. *The Veterinary Clinics of North America: Equine Practice* 3: 585-615

56. Kohn C W, Strasser S L 1986 24-Hour renal clearance and excretion of endogenous substances in the mare. *American Journal of Veterinary Research* 47: 1332-1337

57. Koterba A M, Coffman J R 1981 Acute and chronic renal disease in the horse. *Compendium on Continuing Education for the Practicing Veterinarian* 3: S461-S469

58. Kuttler K L 1980 Pharmacotherapeutics of drugs used in treatment of anaplasmosis and babesiosis. *Journal of the American Veterinary Medical Association* 176: 1103-1108

59. Kuttler K L, Goff W L, Gipson C A, Blackburn B O 1988 Serologic response of *Babesia equi*-infected horses as measured by complement-fixation and indirect fluorescent antibody tests. *Veterinary Parasitology* 26: 199-205

60. Kuttler K L, Zaugg J L, Gipson C A 1987 Imidocarb and parvaquone in the treatment of piroplasmosis *(Babesia equi)* in equids. *American Journal of Veterinary Research* 48: 1613-1616

61. Lane I F, Grauer G F, Fettman M J 1994 Acute renal failure. Part I. Risk factors, prevention, and strategies for protection. *Compendium on Continuing Education for the Practicing Veterinarian* 16: 15-29

62. Lane V M, Merritt A M 1983 Reliability of single-sample phosphorous fractional excretion determination as a measure of daily phosphorous renal clearance in equids. *American Journal of Veterinary Research* 44: 500-502

63. Leon L P, Chu D K, Statsw R O, Snyder L R 1976 Advances in automated analysis. *Technicon International Congress* 1: 152-156

64. MacWilliams P S, Thomas C B 1992 Basic principles of laboratory medicine. *Seminars In Veterinary Medicine and Surgery* 7: 253-261

65. Mahoney D F, Wright I G, Frerichs W M, Groenedyk S, O'Sullivan B M, Roberts M C, Waddell A H 1977 The identification of *Babesia equi* in Australia. *Australian Veterinary Journal* 53: 461-464

66. Mashige F, Osuga T, Tanaka N, Yamanaka M 1978 Continuous flow determination of bile acids in serum and its clinical application. *Clinical Chemistry* 24: 1150

67. Mason D K, Watkins K L, McNie J T 1988 Diagnosis, treatment and prevention of nutritional secondary hyperparathyroidism in Thoroughbred race horses in Hong Kong. *Equine Practice* 10: 10-16

68. Mendel V E, Witt M R, Gitchell B S, Gribble D N, Rogers Q R, Segall H J, Knight H D 1988 Pyrrolozidine alkaloid-induced liver disease in horses: An early diagnosis. *American Journal of Veterinary Research* 49: 572-578

69. Morris D D, Divers T J, Whitlock R H 1984 Renal clearance and fractional excretion of electrolytes over a 24-hour period in horses. *American Journal of Veterinary Research* 45: 2431-2435

70. Noonan N E 1980 Variations of plasma enzymes in the pony and the dog after carbon tetrachloride administration. *American Journal of Veterinary Research* 42: 674-678

71. Pearson E G, Craig A M 1986 Serum bile acids for the diagnosis of chronic liver disease in horses. *Proceedings of the Fourth Annual Veterinary Medical Forum* II: 10/71-10/75

72. Phipps L P 1996 Equine piroplasmosis. *Equine Veterinary Education* 8: 33-36

73. Rawlings C A, Bisgard G E 1975 Renal clearance and excretion of endogenous substances in the small pony. *American Journal of Veterinary Research* 36: 45-48

74. Ris D R 1985 Prevalence and predictive value. *New Zealand Veterinary Journal* 34: 12-13

75. Ronen N, van Heerden J, van Amstel S R 1992 Clinical and biochemistry findings, and parathyroid hormone concentrations in three horses with secondary hyperparathyroidism. *Journal of the South African Veterinary Association* 63: 134-136

76. Rossier Y, Divers T J, Sweeney R W 1995 Variations in urinary gamma glutamyl transferase/urinary creatinine ratio in horses with or without pleuropneumonia treated with gentamicin. *Equine Veterinary Journal* 27: 217-220

77. Rossignol B, Rossignol D, PetitClerk C 1984 Improvement of creatinine measurement on RA-1000. *Clinical Biochemistry* 17: 521-531

78. Roussel A J, Cohen N D, Ruoff W W, Brumbaugh G W, Schmitz D G, Kuesis B S 1993 Urinary indices of horses after administration of crystalloid solutions. *Journal of Veterinary Internal Medicine* 7: 241-246

79. Rudolph W G, Corvalan E O 1992 Urinary and serum gamma glutamyl transpeptidase in relation to urinary pH and proteinuria in healthy and Thoroughbred horses in training. *Equine Veterinary Journal* 24: 316-317

80. Schein E 1988 Equine Babesiosis. In Ristic M (eds) *Babesiosis of Domestic Animals and Man.* CRC Press Inc., Florida: 197-208

81. Schmitz D G 1988 Toxic nephropathy in horses. *Compendium on Continuing Education for the Practicing Veterinarian* 10: 104-111

82. Simpson C F, Neal F C 1980 Ultrastructure of *Babesia equi* in ponies treated with imidocarb. *American Journal of Veterinary Research* 41: 267-271

83. Simpson C F, Taylor W J, Kitchen H 1980 Crystalline inclusions in erythrocytes parasitized with *Babesia equi* following treatment of ponies with imidocarb. *American Journal of Veterinary Research* 41: 1336-1340

84. Skeggs L T, Hochstrasser H 1964 Multiple automatic sequential analysis. *Clinical Chemistry* 10: 918-936

85. Sojka J E, Brown S A 1986 Pharmacokinetic adjustment of gentamicin dosing in horses with sepsis. *Journal of the American Veterinary Medical Association* 189: 784-789

86. Szazs G 1976 Reaction rate method for serum gamma glutamyl transpeptidase. *Clinical Chemistry* 22: 2051-2055

87. Tenter A M, Friedhoff K T 1986 Serodiagnosis of experimental and natural *Babesia equi* and *B.caballi* infections. *Veterinary Parasitology* 20: 49-61

88. Traver D S, Coffman J, Moore J N, Salem C A, Garner H E, Johnson J H, Tritschler L G 1976 Urine clearance ratios as a diagnostic aid in equine metabolic disease. *Proceedings of the Annual Convention of the American Association of Equine Practitioners* 22: 177-183

89. Traver D S, Salem C A, Coffman J, Garner H E, Moore J N, Johnson J H, Tritschler L G, Amend J F 1977 Renal metabolism of endogenous substances in the horse: volumetric vs. clearance ratio. *Journal of Equine Medicine and Surgery* 1: 378-382

90. van Heerden J 1996 Equine babesiosis in South Africa: a report of two cases. *Equine Veterinary Education* 8: 3-5

91. Weiland G 1986 Species-specific serodiagnosis of equine piroplasma infections by means of complement fixation test (CFT), immunofluorescence (IIF), and enzyme-linked immunosorbent assay (ELISA). *Veterinary Parasitology* 20: 43-48

92. West H J 1989 Evaluation of total plasma bile acid concentrations for the diagnosis of hepatobiliary disease in horses. *Research in Veterinary Science* 46: 264-270

Appendix – Analysis of variance tables

Haemoglobin concentration

One Way Repeated Measures Analysis of Variance

Normality Test: Failed (P = 0.042)

Test execution ended by user request, RM ANOVA on Ranks begun

Friedman Repeated Measures Analysis of Variance on Ranks

Group	N	Missing
0.000	6	0
2.000	6	0
5.000	6	0
8.000	6	0
11.000	6	0
18.000	6	0
Tested	6	0

Group	Median	25%	75%
0.000	130.500	123.000	142.000
2.000	122.000	117.000	126.000
5.000	119.000	117.000	122.000
8.000	126.000	121.000	128.000
11.000	128.500	123.000	132.000
18.000	121.500	117.000	128.000

Chi-square= 10.771 with 5 degrees of freedom. (P = 0.056)

The differences in the median values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.056)

Haematocrit

One Way Repeated Measures Analysis of Variance

Normality Test: Passed (P = 0.140)

Equal Variance Test: Passed (P = 0.433)

Group	N	Missing
0.000	6	0
2.000	6	0
5.000	6	0
8.000	6	0
11.000	6	0
18.000	6	0

Group	Mean	Std Dev	SEM
0.000	36.967	3.839	1.567
2.000	33.517	2.059	0.840
5.000	33.333	1.547	0.632
8.000	34.517	2.771	1.131
11.000	35.600	2.301	0.940
18.000	33.833	1.485	0.606

Power of performed test with alpha = 0.050: 0.693

The power of the performed test (0.693) is below the desired power of 0.800.
You should interpret the negative findings cautiously.

Source of Variation	DF	SS	MS	F	P
Between Subjects	5	98.656	19.731		
Between Treatments	5	59.816	11.963	3.556	0.014
Residual	25	84.101	3.364		
Total	35	242.572			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.014). To isolate the group or groups that differ from the others use a multiple comparison procedure.

Multiple Comparisons versus Control Group (Dunnett's Method) :

Comparisons for factor: days

Comparison	Diff of Means	p	q'	P<0.05
0.000 vs. 11.000	-1.367	6.000	1.291	No
0.000 vs. 8.000	-2.450	5.000	2.314	No
0.000 vs. 18.000	-3.133	4.000	2.959	Yes
0.000 vs. 2.000	-3.450	3.000	3.258	Yes
0.000 vs. 5.000	-3.633	2.000	3.431	Yes

Total red cell count

One Way Repeated Measures Analysis of Variance

Normality Test: Passed (P = 0.381)

Equal Variance Test: Passed (P = 0.189)

Group	N	Missing
0.000	6	0
2.000	6	0
5.000	6	0
8.000	6	0
11.000	6	0
18.000	6	0

Group	Mean	Std Dev	SEM
0.000	9.852	1.552	0.634
2.000	8.933	0.882	0.360
5.000	8.840	0.881	0.360
8.000	9.213	0.858	0.350
11.000	9.408	0.750	0.306
18.000	8.875	0.715	0.292

Power of performed test with alpha = 0.050: 0.742

The power of the performed test (0.742) is below the desired power of 0.800.
You should interpret the negative findings cautiously.

Source of Variation	DF	SS	MS	F	P
Between Subjects	5	22.779	4.556		
Between Treatments	5	4.641	0.928	3.815	0.011
Residual	25	6.083	0.243		
Total	35	33.503			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.011). To isolate the group or groups that differ from the others use a multiple comparison procedure.

Multiple Comparisons versus Control Group (Dunnett's Method) :

Comparisons for factor: days

Comparison	Diff of Means	p	q'	P<0.05
0.000 vs. 11.000	-0.443	6.000	1.557	No
0.000 vs. 8.000	-0.638	5.000	2.241	No
0.000 vs. 2.000	-0.918	4.000	3.224	Yes
0.000 vs. 18.000	-0.977	3.000	3.429	Yes
0.000 vs. 5.000	-1.012	2.000	3.552	Yes

Total white cell count

One Way Repeated Measures Analysis of Variance

Normality Test: Passed (P = 0.102)

Equal Variance Test: Passed (P = 0.242)

Group	N	Missing
0.000	6	0
2.000	6	0
5.000	6	0
8.000	6	0
11.000	6	0
18.000	6	0

Group	Mean	Std Dev	SEM
0.000	11.733	1.640	0.670
2.000	11.417	1.768	0.722
5.000	10.783	1.800	0.735
8.000	12.067	2.541	1.038
11.000	11.700	2.265	0.925
18.000	11.467	2.930	1.196

Power of performed test with alpha = 0.050: 0.328

The power of the performed test (0.328) is below the desired power of 0.800.
You should interpret the negative findings cautiously.

Source of Variation	DF	SS	MS	F	P
Between Subjects	5	133.019	26.604		
Between Treatments	5	5.596	1.119	2.130	0.095
Residual	25	13.138	0.526		
Total	35	151.752			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.095).

Mature neutrophil count

One Way Repeated Measures Analysis of Variance

Normality Test: Passed (P = 0.291)

Equal Variance Test: Passed (P = 0.973)

Group	N	Missing
0.000	6	0
2.000	6	0
5.000	6	0
8.000	6	0
11.000	6	0
18.000	6	0

Group	Mean	Std Dev	SEM
0.000	4.455	0.483	0.197
2.000	4.695	0.595	0.243
5.000	4.962	0.597	0.244
8.000	5.043	1.047	0.427
11.000	4.205	1.158	0.473
18.000	4.550	1.304	0.533

Power of performed test with alpha = 0.050: 0.050

The power of the performed test (0.050) is below the desired power of 0.800.
You should interpret the negative findings cautiously.

Source of Variation	DF	SS	MS	F	P
Between Subjects	5	7.804	1.561		
Between Treatments	5	2.999	0.600	0.852	0.527
Residual	25	17.605	0.704		
Total	35	28.409			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.527).

Immature neutrophil count

One Way Repeated Measures Analysis of Variance

Normality Test: Failed (P = <0.001)

Test execution ended by user request, RM ANOVA on Ranks begun

Friedman Repeated Measures Analysis of Variance on Ranks

Group	N	Missing
0.000	6	0
2.000	6	0
5.000	6	0
8.000	6	0
11.000	6	0
18.000	6	0
Tested	6	0

Group	Median	25%	75%
0.000	0.000	0.000	0.000
2.000	0.000	0.000	0.000
5.000	0.000	0.000	0.000
8.000	0.000	0.000	0.000
11.000	0.000	0.000	0.000
18.000	0.000	0.000	0.000

Chi-square= 4.000 with 5 degrees of freedom. (P = 0.549)

The differences in the median values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.549)

Total thrombocyte count

One Way Repeated Measures Analysis of Variance

Normality Test: Passed (P = 0.065)

Equal Variance Test: Passed (P = 0.969)

Group	N	Missing
0.000	6	0
2.000	6	0
5.000	6	0
8.000	6	0
11.000	6	0
18.000	6	0

Group	Mean	Std Dev	SEM
0.000	191.917	88.180	35.999
2.000	191.167	70.187	28.654
5.000	197.850	77.664	31.706
8.000	223.667	52.576	21.464
11.000	265.167	38.696	15.798
18.000	288.500	47.853	19.536

Power of performed test with alpha = 0.050: 0.932

Source of Variation	DF	SS	MS	F	P
Between Subjects	5	79216.089	15843.218		
Between Treatments	5	51673.939	10334.788	5.473	0.002
Residual	25	47209.294	1888.372		
Total	35	178099.322			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.002). To isolate the group or groups that differ from the others use a multiple comparison procedure.

Multiple Comparisons versus Control Group (Dunnett's Method) :

Comparisons for factor: days

Comparison	Diff of Means	p	q'	P<0.05
0.000 vs. 18.000	96.583	6.000	3.850	Yes
0.000 vs. 11.000	73.250	5.000	2.920	Yes
0.000 vs. 8.000	31.750	4.000	1.265	No
0.000 vs. 5.000	5.933	3.000	0.236	No
0.000 vs. 2.000	-0.750	2.000	0.0299	No

Total lymphocyte count

One Way Repeated Measures Analysis of Variance

Normality Test: Passed (P = 0.315)

Equal Variance Test: Passed (P = 0.647)

Group	N	Missing
0.000	6	0
2.000	6	0
5.000	6	0
8.000	6	0
11.000	6	0
18.000	6	0

Group	Mean	Std Dev	SEM
0.000	6.592	1.950	0.796
2.000	6.043	1.820	0.743
5.000	5.250	1.924	0.785
8.000	6.397	2.126	0.868
11.000	6.647	2.476	1.011
18.000	6.510	2.671	1.090

Power of performed test with alpha = 0.050: 0.569

The power of the performed test (0.569) is below the desired power of 0.800.
You should interpret the negative findings cautiously.

Source of Variation	DF	SS	MS	F	P
Between Subjects	5	128.993	25.799		
Between Treatments	5	8.432	1.686	3.012	0.029
Residual	25	13.995	0.560		
Total	35	151.420			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.029). To isolate the group or groups that differ from the others use a multiple comparison procedure.

Multiple Comparisons versus Control Group (Dunnett's Method) :

Comparisons for factor: days

Comparison	Diff of Means	p	q'	P<0.05
0.000 vs. 11.000	0.0550	6.000	0.127	No
0.000 vs. 18.000	-0.0817	5.000	0.189	No
0.000 vs. 8.000	-0.195	4.000	0.451	No
0.000 vs. 2.000	-0.548	3.000	1.269	No
0.000 vs. 5.000	-1.342	2.000	3.106	Yes

Total eosinophil count

One Way Repeated Measures Analysis of Variance

Normality Test: Passed (P = 0.061)

Equal Variance Test: Passed (P = 0.896)

Group	N	Missing
0.000	6	0
2.000	6	0
5.000	6	0
8.000	6	0
11.000	6	0
18.000	6	0

Group	Mean	Std Dev	SEM
0.000	0.282	0.0677	0.0276
2.000	0.288	0.209	0.0854
5.000	0.233	0.0455	0.0186
8.000	0.220	0.101	0.0414
11.000	0.317	0.202	0.0823
18.000	0.090	0.0767	0.0313

Power of performed test with alpha = 0.050: 0.537

The power of the performed test (0.537) is below the desired power of 0.800.
You should interpret the negative findings cautiously.

Source of Variation	DF	SS	MS	F	P
Between Subjects	5	0.0856	0.0171		
Between Treatments	5	0.252	0.0420	2.664	0.034
Residual	25	0.473	0.0158		
Total	35	0.811			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.034). To isolate the group or groups that differ from the others use a multiple comparison procedure.

Multiple Comparisons versus Control Group (Dunnett's Method) :

Comparisons for factor: days

Comparison	Diff of Means	p	q'	P<0.05
0.000 vs. 11.000	0.0350	7.000	0.483	No
0.000 vs. 2.000	0.00667	6.000	0.0919	No
0.000 vs. 5.000	-0.0483	5.000	0.666	No
0.000 vs. 8.000	-0.0617	4.000	0.850	No
0.000 vs. 18.000	-0.192	2.000	2.643	Yes

Total monocyte count

One Way Repeated Measures Analysis of Variance

Normality Test: Failed (P = 0.009)

Test execution ended by user request, RM ANOVA on Ranks begun

Friedman Repeated Measures Analysis of Variance on Ranks

Group	N	Missing
0.000	6	0
2.000	6	0
5.000	6	0
8.000	6	0
11.000	6	0
18.000	6	0
Tested	6	0

Group	Median	25%	75%
0.000	0.250	0.220	0.470
2.000	0.305	0.140	0.430
5.000	0.250	0.230	0.400
8.000	0.265	0.230	0.340
11.000	0.390	0.340	0.740
18.000	0.345	0.120	0.430

Chi-square= 1.481 with 5 degrees of freedom. (P = 0.915)

The differences in the median values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.915)

Total serum protein concentration

One Way Repeated Measures Analysis of Variance

Normality Test: Passed (P = 0.172)

Equal Variance Test: Passed (P = 0.164)

Group	N	Missing
0.000	6	0
2.000	6	0
5.000	6	0
8.000	6	0
11.000	6	0
18.000	6	0

Group	Mean	Std Dev	SEM
0.000	66.633	5.165	2.109
2.000	66.650	2.783	1.136
5.000	65.133	2.601	1.062
8.000	63.800	4.281	1.748
11.000	63.250	2.654	1.083
18.000	63.733	2.928	1.195

Power of performed test with alpha = 0.050: 0.660

The power of the performed test (0.660) is below the desired power of 0.800.
You should interpret the negative findings cautiously.

Source of Variation	DF	SS	MS	F	P
Between Subjects	5	274.997	54.999		
Between Treatments	5	68.450	13.690	3.400	0.018
Residual	25	100.673	4.027		
Total	35	444.120			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.018). To isolate the group or groups that differ from the others use a multiple comparison procedure.

Multiple Comparisons versus Control Group (Dunnett's Method) :

Comparisons for factor: days

Comparison	Diff of Means	p	q'	P<0.05
0.000 vs. 2.000	0.0167	6.000	0.0144	No
0.000 vs. 5.000	-1.500	5.000	1.295	No
0.000 vs. 8.000	-2.833	4.000	2.446	No
0.000 vs. 18.000	-2.900	3.000	2.503	Yes
0.000 vs. 11.000	-3.383	2.000	2.920	Yes

Serum aspartate aminotransferase activity

One Way Repeated Measures Analysis of Variance

Normality Test: Passed (P = 0.332)

Equal Variance Test: Passed (P = 0.111)

Group	N	Missing
0.000	6	0
2.000	6	0
5.000	6	0
8.000	6	0
11.000	6	0
18.000	6	0
38.000	6	0

Group	Mean	Std Dev	SEM
0.000	170.667	33.315	13.601
2.000	224.500	50.552	20.638
5.000	286.000	85.807	35.030
8.000	318.833	76.159	31.092
11.000	321.167	56.623	23.116
18.000	261.833	30.961	12.640
38.000	197.000	20.582	8.402

Power of performed test with alpha = 0.050: 1.000

Source of Variation	DF	SS	MS	F	P
Between Subjects	5	77825.714	15565.143		
Between Treatments	6	125179.238	20863.206	21.393	<0.001
Residual	30	29257.619	975.254		
Total	41	232262.571			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001). To isolate the group or groups that differ from the others use a multiple comparison procedure.

Multiple Comparisons versus Control Group (Dunnett's Method) :

Comparisons for factor: days

Comparison	Diff of Means	p	q'	P<0.05
0.000 vs. 11.00	150.500	7.000	8.347	Yes
0.000 vs. 8.00	148.167	6.000	8.218	Yes
0.000 vs. 5.00	115.333	5.000	6.397	Yes
0.000 vs. 18.00	91.167	4.000	5.056	Yes
0.000 vs. 2.00	53.833	3.000	2.986	Yes
0.000 vs. 38.00	26.333	2.000	1.461	No

Serum creatinine concentration

One Way Repeated Measures Analysis of Variance

Normality Test: Failed (P = 0.012)

Test execution ended by user request, RM ANOVA on Ranks begun

Friedman Repeated Measures Analysis of Variance on Ranks

Group	N	Missing
0.000	6	0
2.000	6	0
5.000	6	0
8.000	6	0
11.000	6	0
18.000	6	0
Tested	6	0

Group	Median	25%	75%
0.000	106.000	96.000	117.000
2.000	108.000	105.000	143.000
5.000	121.500	107.000	146.000
8.000	107.000	99.000	168.000
11.000	119.000	111.000	125.000
18.000	110.000	104.000	119.000

Chi-square= 9.019 with 5 degrees of freedom. (P = 0.108)

The differences in the median values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.108)

Urine creatinine concentration

One Way Repeated Measures Analysis of Variance

Normality Test: Passed (P = 0.413)

Equal Variance Test: Passed (P = 0.849)

Group	N	Missing
0.000	6	0
2.000	6	0
5.000	6	0
8.000	6	0
11.000	6	0
18.000	6	0

Group	Mean	Std Dev	SEM
0.000	10720.000	4208.715	1718.201
2.000	14246.667	4713.515	1924.285
5.000	12920.000	4209.779	1718.635
8.000	11446.667	3739.982	1526.841
11.000	12386.667	2257.739	921.718
18.000	9466.667	2248.650	918.008

Power of performed test with alpha = 0.050: 0.212

The power of the performed test (0.212) is below the desired power of 0.800.
You should interpret the negative findings cautiously.

Source of Variation	DF	SS	MS	F	P
Between Subjects	5	158775288.889	31755057.778		
Between Treatments	5	85773155.556	17154631.111	1.714	0.168
Residual	25	250194844.444	10007793.778		
Total	35	494743288.889			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.168).

Serum sodium concentration

One Way Repeated Measures Analysis of Variance

Normality Test: Failed (P = 0.009)

Test execution ended by user request, RM ANOVA on Ranks begun

Friedman Repeated Measures Analysis of Variance on Ranks

Group	N	Missing
0.000	6	0
2.000	6	0
5.000	6	0
8.000	6	0
11.000	6	0
18.000	6	0
Tested	6	0

Group	Median	25%	75%
0.000	136.000	134.000	137.000
2.000	134.000	132.000	135.000
5.000	132.000	131.000	132.000
8.000	133.000	132.000	136.000
11.000	135.000	134.000	136.000
18.000	135.500	135.000	136.000

Chi-square= 11.709 with 5 degrees of freedom. (P = 0.039)

The differences in the median values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.039)

To isolate the group or groups that differ from the others use a multiple comparison procedure.

All Pairwise Multiple Comparison Procedures (Dunnett's Method) :

Comparison	Diff of Ranks	p	q'	P<0.05
5 vs 0	18.000	6	2.777	Yes
2 vs 0	9.000	5	1.643	No
8 vs 0	6.000	4	1.342	No Test Needed
11 vs 0	1.500	3	0.433	No Test Needed
18 vs 0	1.500	2	0.612	No Test Needed

Fractional clearance of sodium

One Way Repeated Measures Analysis of Variance

Normality Test: Failed (P = <0.001)

Test execution ended by user request, RM ANOVA on Ranks begun

Friedman Repeated Measures Analysis of Variance on Ranks

Group	N	Missing
0.000	6	0
2.000	6	0
5.000	6	0
8.000	6	0
11.000	6	0
18.000	6	0
Tested	6	0

Group	Median	25%	75%
0.000	0.615	0.480	1.180
2.000	0.320	0.230	0.410
5.000	0.575	0.350	0.950
8.000	0.510	0.260	1.000
11.000	0.670	0.360	1.160
18.000	1.452	1.310	1.710

Chi-square= 15.905 with 5 degrees of freedom. (P = 0.007)

The differences in the median values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.007)

To isolate the group or groups that differ from the others use a multiple comparison procedure.

All Pairwise Multiple Comparison Procedures (Dunnett's Method) :

Comparison	Diff of Ranks	p	q'	P<0.05
2 vs 0	15.000	6	2.315	No
18 vs 0	10.000	5	1.826	No Test Needed
5 vs 0	4.000	4	0.894	No Test Needed
8 vs 0	4.000	3	1.155	No Test Needed
11 vs 0	1.000	2	0.408	No Test Needed

Serum potassium concentration

One Way Repeated Measures Analysis of Variance

Normality Test: Passed (P = 0.386)

Equal Variance Test: Passed (P = 0.693)

Group	N	Missing
0.000	6	0
2.000	6	0
5.000	6	0
8.000	6	0
11.000	6	0
18.000	6	0

Group	Mean	Std Dev	SEM
0.000	4.265	0.286	0.117
2.000	4.442	0.233	0.0951
5.000	4.038	0.315	0.129
8.000	4.243	0.239	0.0974
11.000	4.325	0.193	0.0787
18.000	4.278	0.135	0.0553

Power of performed test with alpha = 0.050: 0.298

The power of the performed test (0.298) is below the desired power of 0.800.
You should interpret the negative findings cautiously.

Source of Variation	DF	SS	MS	F	P
Between Subjects	5	0.452	0.0905		
Between Treatments	5	0.521	0.104	2.026	0.109
Residual	25	1.286	0.0514		
Total	35	2.259			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.109).

Fractional clearance of potassium

One Way Repeated Measures Analysis of Variance

Normality Test: Passed (P = 0.216)

Equal Variance Test: Passed (P = 0.231)

Group	N	Missing
0.000	6	0
2.000	6	0
5.000	6	0
8.000	6	0
11.000	6	0
18.000	6	0

Group	Mean	Std Dev	SEM
0.000	78.760	15.671	6.397
2.000	79.768	21.318	8.703
5.000	66.528	23.964	9.783
8.000	89.350	42.951	17.535
11.000	74.332	19.257	7.862
18.000	84.853	16.952	6.921

Power of performed test with alpha = 0.050: 0.050

The power of the performed test (0.050) is below the desired power of 0.800.
You should interpret the negative findings cautiously.

Source of Variation	DF	SS	MS	F	P
Between Subjects	5	6146.511	1229.302		
Between Treatments	5	1916.040	383.208	0.752	0.593
Residual	25	12740.280	509.611		
Total	35	20802.830			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.593).

Serum inorganic phosphate concentration

One Way Repeated Measures Analysis of Variance

Normality Test: Passed (P = 0.529)

Equal Variance Test: Passed (P = 0.416)

Group	N	Missing
0.000	6	0
2.000	6	0
5.000	6	0
8.000	6	0
11.000	6	0
18.000	6	0

Group	Mean	Std Dev	SEM
0.000	1.638	0.332	0.135
2.000	1.695	0.349	0.143
5.000	1.680	0.214	0.0876
8.000	1.663	0.265	0.108
11.000	1.610	0.323	0.132
18.000	1.488	0.118	0.0480

Power of performed test with alpha = 0.050: 0.084

The power of the performed test (0.084) is below the desired power of 0.800.
You should interpret the negative findings cautiously.

Source of Variation	DF	SS	MS	F	P
Between Subjects	5	1.611	0.322		
Between Treatments	5	0.170	0.0340	1.179	0.347
Residual	25	0.722	0.0289		
Total	35	2.502			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.347).

Fractional clearance of phosphate

One Way Repeated Measures Analysis of Variance

Normality Test: Failed (P = 0.007)

Test execution ended by user request, RM ANOVA on Ranks begun

Friedman Repeated Measures Analysis of Variance on Ranks

Group	N	Missing
0.000	6	0
2.000	6	0
5.000	6	0
8.000	6	0
11.000	6	0
18.000	6	0
Tested	6	0

Group	Median	25%	75%
0.000	0.0681	0.0623	0.208
2.000	0.0550	0.0200	0.130
5.000	0.165	0.000	0.320
8.000	1.035	0.0200	2.260
11.000	0.0700	0.000	0.170
18.000	0.0350	0.000	0.050

Chi-square= 5.057 with 5 degrees of freedom. (P = 0.409)

The differences in the median values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.409)

Serum gamma glutamyl transpeptidase activity

One Way Repeated Measures Analysis of Variance

Normality Test: Failed (P = 0.013)

Test execution ended by user request, RM ANOVA on Ranks begun

Friedman Repeated Measures Analysis of Variance on Ranks

Group	N	Missing
0.000	6	0
2.000	6	0
5.000	6	0
8.000	6	0
11.000	6	0
18.000	6	0
Tested	6	0

Group	Median	25%	75%
0.000	13.000	12.000	16.000
2.000	13.500	12.000	14.000
5.000	15.000	15.000	17.000
8.000	14.500	14.000	15.000
11.000	15.000	14.000	15.000
18.000	26.500	25.000	27.000

Chi-square= 22.436 with 5 degrees of freedom. (P = <0.001)

The differences in the median values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001)

To isolate the group or groups that differ from the others use a multiple comparison procedure.

All Pairwise Multiple Comparison Procedures (Dunnett's Method) :

Comparison	Diff of Ranks	p	q'	P<0.05
18 vs 0	24.000	6	3.703	Yes
5 vs 0	14.500	5	2.647	Yes
11 vs 0	8.500	4	1.901	No
8 vs 0	8.000	3	2.309	No Test Needed
2 vs 0	1.000	2	0.408	No Test Needed

Urine gamma glutamyl transpeptidase activity

One Way Repeated Measures Analysis of Variance

Normality Test: Passed (P = 0.142)

Equal Variance Test: Passed (P = 0.163)

Group	N	Missing
0.000	6	0
2.000	6	0
5.000	6	0
8.000	6	0
11.000	6	0
18.000	6	0

Group	Mean	Std Dev	SEM
0.000	11.000	10.826	4.420
2.000	18.167	11.392	4.651
5.000	42.667	20.781	8.484
8.000	25.833	27.440	11.202
11.000	21.333	14.487	5.914
18.000	23.833	10.187	4.159

Power of performed test with alpha = 0.050: 0.649

The power of the performed test (0.649) is below the desired power of 0.800.
You should interpret the negative findings cautiously.

Source of Variation	DF	SS	MS	F	P
Between Subjects	5	2126.405	425.281		
Between Treatments	5	5680.667	946.778	3.066	0.018
Residual	25	9264.762	308.825		
Total	35	17071.833			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.018). To isolate the group or groups that differ from the others use a multiple comparison procedure.

Multiple Comparisons versus Control Group (Dunnett's Method) :

Comparisons for factor: days

Comparison	Diff of Means	p	q'	P<0.05
0.000 vs. 5.000	31.667	6.000	3.121	Yes
0.000 vs. 8.000	14.833	5.000	1.462	No
0.000 vs. 18.000	12.833	4.000	1.265	No
0.000 vs. 11.000	10.333	3.000	1.018	No
0.000 vs. 2.000	7.167	2.000	0.706	No

Urine gamma glutamyl transpeptidase: urine creatinine ratio

One Way Repeated Measures Analysis of Variance

Normality Test: Passed (P = 0.529)

Equal Variance Test: Passed (P = 0.582)

Group	N	Missing
0.000	6	0
2.000	6	0
5.000	6	0
8.000	6	0
11.000	6	0
18.000	6	0

Group	Mean	Std Dev	SEM
0.000	8.786	6.738	2.751
2.000	13.242	9.407	3.841
5.000	29.105	12.327	5.033
8.000	18.472	15.631	6.381
11.000	15.412	10.548	4.306
18.000	22.128	7.263	2.965

Power of performed test with alpha = 0.050: 0.472

The power of the performed test (0.472) is below the desired power of 0.800.
You should interpret the negative findings cautiously.

Source of Variation	DF	SS	MS	F	P
Between Subjects	5	580.800	116.160		
Between Treatments	5	1528.199	305.640	2.644	0.047
Residual	25	2890.182	115.607		
Total	35	4999.181			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.047). To isolate the group or groups that differ from the others use a multiple comparison procedure.

Multiple Comparisons versus Control Group (Dunnett's Method) :

Comparisons for factor: days

Comparison	Diff of Means	p	q'	P<0.05
0.000 vs. 5.000	20.319	6.000	3.273	Yes
0.000 vs. 18.000	13.342	5.000	2.149	No
0.000 vs. 8.000	9.685	4.000	1.560	No
0.000 vs. 11.000	6.625	3.000	1.067	No
0.000 vs. 2.000	4.455	2.000	0.718	No

Total bile acid concentration

One Way Repeated Measures Analysis of Variance

Normality Test: Passed (P = 0.568)

Equal Variance Test: Passed (P = 0.107)

Group	N	Missing
0.000	6	0
5.000	6	0
11.000	6	0

Group	Mean	Std Dev	SEM
0.000	4.033	0.712	0.291
5.000	2.817	0.796	0.325
11.000	3.550	0.901	0.368

Power of performed test with alpha = 0.050: 0.679

The power of the performed test (0.679) is below the desired power of 0.800.
You should interpret the negative findings cautiously.

Source of Variation	DF	SS	MS	F	P
Between Subjects	5	6.007	1.201		
Between Treatments	2	4.503	2.252	6.004	0.019
Residual	10	3.750	0.375		
Total	17	14.260			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.019). To isolate the group or groups that differ from the others use a multiple comparison procedure.

Multiple Comparisons versus Control Group (Dunnett's Method) :

Comparisons for factor: days

Comparison	Diff of Means	p	q'	P<0.05
0.000 vs. 11.000	-0.483	3.000	1.367	No
0.000 vs. 5.000	-1.217	2.000	3.441	Yes

Urine specific gravity

One Way Repeated Measures Analysis of Variance

Normality Test: Failed (P = 0.007)

Test execution ended by user request, RM ANOVA on Ranks begun

Friedman Repeated Measures Analysis of Variance on Ranks

Group	N	Missing
0.000	6	0
2.000	6	0
5.000	6	0
8.000	6	0
11.000	6	0
18.000	6	0
Tested	6	0

Group	Median	25%	75%
0.000	1.030	1.030	1.030
2.000	1.030	1.030	1.030
5.000	1.030	1.020	1.030
8.000	1.040	1.040	1.040
11.000	1.040	1.030	1.040
18.000	1.040	1.040	1.040

Chi-square= 12.911 with 5 degrees of freedom. (P = 0.024)

The differences in the median values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.024)

To isolate the group or groups that differ from the others use a multiple comparison procedure.

All Pairwise Multiple Comparison Procedures (Dunnett's Method) :

Comparison	Diff of Ranks	p	q'	P<0.05
18 vs 0	11.500	6	1.774	No
11 vs 0	9.000	5	1.643	No Test Needed
8 vs 0	8.500	4	1.901	No Test Needed
5 vs 0	3.500	3	1.010	No Test Needed
2 vs 0	1.500	2	0.612	No Test Needed

Urine pH

One Way Repeated Measures Analysis of Variance

Normality Test: Passed (P = 0.178)

Equal Variance Test: Passed (P = 0.734)

Group	N	Missing
0.000	6	0
2.000	6	0
5.000	6	0
8.000	6	0
11.000	6	0
18.000	6	0

Group	Mean	Std Dev	SEM
0.000	8.167	0.753	0.307
2.000	8.833	0.408	0.167
5.000	8.333	0.516	0.211
8.000	7.833	0.408	0.167
11.000	7.667	0.816	0.333
18.000	7.333	0.816	0.333

Power of performed test with alpha = 0.050: 0.867

Source of Variation	DF	SS	MS	F	P
Between Subjects	5	3.472	0.694		
Between Treatments	5	8.472	1.694	4.692	0.004
Residual	25	9.028	0.361		
Total	35	20.972			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.004). To isolate the group or groups that differ from the others use a multiple comparison procedure.

Multiple Comparisons versus Control Group (Dunnett's Method) :

Comparisons for factor: days

Comparison	Diff of Means	p	q'	P<0.05
0.000 vs. 2.000	0.667	6.000	1.922	No
0.000 vs. 5.000	0.167	5.000	0.480	No
0.000 vs. 8.000	-0.333	4.000	0.961	No
0.000 vs. 11.000	-0.500	3.000	1.441	No
0.000 vs. 18.000	-0.833	2.000	2.402	Yes