UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
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Effects of aspartame on the blood coagulation system of the rabbit

by

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Submitted in partial fulfilment of the requirements for the degree

Philosophiae Doctor

(PhD)

In the Faculty of Health Sciences,

Department of Anatomy

University of Pretoria

South Africa

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July 2007

ABSTRACT

Aspartame is a dipeptide sweetener that can be found in most of the sugar-free products available on the market today. The FDA approved the use of aspartame, but ever since the safety of the consumption of aspartame has been questioned. Thus the aim of this thesis was to determine the effects of aspartame ingestion on the blood coagulation system and the blood filtering organs (liver and kidneys) of the rabbit.

The protocol for obtaining blood from a rabbit as well as successful administration of aspartame was perfected. The rabbit was proven as best experimental model, when compared to a mouse, for studying the effects of aspartame on coagulation and haemostasis. The effects of aspartame were determined by: 1.) measuring the factors from the different coagulation pathways, namely the common pathway (factors II, V, X and fibrinogen); factors in the intrinsic pathway (factors VIII, IX), as well as factor VII, found in the extrinsic pathway. The *prothrombin time* (PT; measures how long blood takes to form a clot) and activated *partial thromboplastin time* (aPTT; measures recalcification time of plasma) was also measured; 2.) The ultrastructure of the fibrin networks, platelet morphology and endothelial lining were studied; 3.) The histological morphology of the leukocytes, liver and kidney were examined.

Results obtained indicated that F VII, X and VIII were decreased with a prolonged prothrombin time. The concentration of circulating fibrinogen increased significantly, which corroborated with results obtained for the ultrastructure of the fibrin networks. The degree of fibrin fibre formation increased the higher the concentration of aspartame and the degree of platelet aggregation occurring, decreased with the increase of aspartame concentration. It is hypothesized that the amount of circulating serotonin decreased. The endothelial lining of the rabbits were damaged with the nuclei appearing apoptotic. The endothelial lining and their tight junctions play an integral part in the functioning of the BBB, in synchronization with cAMP (complexity of tight junctions, decreased due to decreased amount of serotonin), thus it appeared as though the BBB was compromised. The morphology of the leukocytes were altered, specifically that of the eosinophils and heterophils. The granules inside the eosinophils of the aspartame treated rabbit appeared to have increased and were more clearly visible, while the granules in the heterophils appeared to have decreased. The total number of leukocytes also decreased. The

normal histological morphology of both the liver and kidney were affected by aspartame. Damage to the hepatocytes and their subsequent arrangement were noted. The visceral layer of the capsule of Bowman appeared thickened and the cuboidal epithelium lining the proximal convoluted tubule was also damaged.

The final judgment and conclusion of the results obtained in this thesis regarding the consumption of abuse doses of aspartame, was that aspartame could lead to bleeding disorders (especially in genetically predisposed individuals), suppressed immunity and a compromised BBB. Trouble can occur with formation of the glomerular filtrate and absorption of fluid from the proximal convoluted tubule, which could result in high blood pressure and an increased probability of dehydration respectively.

DECLARATION

I, Petro Humphries hereby declare that this thesis entitled:

"Effects of aspartame on the coagulation system of the rabbit"

which I herewith submit to the University of Pretoria for the Degree of Philosophiae Doctor in Anatomy, is my own original work and has never been submitted for any academic award to any other tertiary institution for any degree.

_____ _____

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ACKNOWLEDGEMENTS

I would like to acknowledge with gratitude the following people and institutions:

Firstly and foremost I would like to thank the Lord my God for the patience to see this project through and for the knowledge needed for interpretation of the results.

The Anatomy Department of the University of Pretoria, and Prof Meiring (Head of the department), for granting me the opportunity to undertake and complete my study. Also for the funding and hosting of the project.

The Department of Anatomy, University of Limpopo, Medunsa campus, and Mr Human (Head of the department) for granting me study leave and the opportunity to complete my research and my thesis.

All the lecturers in the Anatomy department at the University of Limpopo, Medunsa campus, who helped by taking my lectures during the study leave period.

Prof. Etheresia Pretorius, my supervisor, for the academic and intellectual input and her support through the darker times in the project. Many thanks for the great mentoring during the project and for the guidance and assistance for completion of this thesis.

Anette deFreitas, animal technologist at the University of Limpopo, for all her help in taking care of the rabbits and when blood had to be drawn.

The Unit of Microscopy and Micro-analysis of the University of Pretoria, for being able to use the facilities of the unit and their expert knowledge. A special thanks for all your friendliness and to Nanette for preparing the sections for the TEM studies on the ultramicrotome.

My deepest gratitude and very special thanks is addressed to Eureke Smit, one of the greatest people and friends in my life. Thanks for all the help with the coagulation studies and all the hours in front of the SEM,TEM and light microscope. Thanks for all the laughs and for being able to cry on your shoulder when things did not always go as planned. You were an inspiration to complete this thesis.

To all my friends, especially Lucinda and Desire, for moral support and just being my friends.

Last, but most definitely not least, my family. A very special thanks to my husband, James, for granting me the opportunity to further my studies while being a mother to two very bouncy babies. To Saskia and JP, I love you and I am very sorry for all the times I had to work on my thesis when you would rather have had me play with you two. To my mother and father, thanks for all your support through out my studies, not just this thesis. Thanks for looking after the twins when I had to work on my thesis. I love you all very much!

DEDICATION

I would like to dedicate this thesis to my sister, Ciska, who passed away at a too early stage in my life. I miss you with all my heart and will love you forever!

LIST OF PUBLICATIONS

Full articles:

Pretorius, E., Humphries, P. 2007. Ultrastructural changes to rabbit fibrin and platelets due to aspartame. *Ultrastructural Pathology*; 31: 77-83.

Pretorius E., Humphries P., Ekpo O.E., Smit E., van der Merwe C.F. 2007a. Comparative ultrastructural analyses of mouse, rabbit and human platelets and fibrin networks research. *Microscopy and Technique;* (*In Press*).

Review articles:

Humphries P., Pretorius E., Naude H. Direct and indirect cellular effects of aspartame on the brain. *European Journal of Clinical nutrition*; (*In Press)*.

Sumbitted for publication:

Humphries P., Smit E., Pretorius E. Effects of aspartame on certain coagulation factors of the rabbit model. *British Journal of Clinical Nutrition*; (*Submitted for publication).*

Humphries P., Smit E., Pretorius E. Ultrastructural morphology of platelets and fibrin networks of lactating and non-pregnant rabbits. *Anatomica Histologica Embryologica*; (*Sumbitted for publication)*

Humphries P., Smit E., Pretorius E. Report on the changes found in the ultrastructure of the fibrin network, platelet aggregates, endothelial lining and leucocyte counts of the rabbit after treatment with aspartame. *Cell and Tissue Research*; (*Submitted for publication)*

Articles in preparation:

Humphries P., Smit E., Pretorius E. Ultrastructural changes in the aorta of the rabbit after treatment with aspartame.

Humphries P., Smit E., Pretorius E. Changes in the histological morphology of the liver and kidney of the rabbit after long-term ingestion of abuse doses of aspartame.

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Appenix B: Pretorius E., Humphries P. Ultrastructural changes to rabbit fibrin and platelets due to aspartame. *Ultrastructural Pathology*; 31: 77-83

Appendix C: Pretorius E, Humphries P, Ekpo OE, Smit E, van der Merwe CF. (2007a) Comparative ultrastructural analyses of mouse, rabbit and human platelets and fibrin networks research. *Microscopy and Technique* (*In Press*).

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LIST OF ABBREVIATIONS AND SYMBOLS

ADP	-	Adenosine diphosphate
α	-	Alpha
α_2-PI	-	α_2-plasmin inhibitor
ANOVA	-	Analysis of variance
APM	-	Aspartame
aPTT	-	Activated partial thromboplastin time
Arg-Gly	-	Arginine-glycine
AT III	-	Antithrombin III
ATP	-	Adenosine triphosphate
β	-	Beta
BBB	-	Blood brain barrier
Ca^{2+}	-	Calcium
$(Ca^{2+})i$	-	Intracellular calcium
$CaCl_2$	-	Calcium chloride
CARR	-	Carrageenan
cAMP	-	Cyclic adenosine 3,5-monophosphate
CNS	-	Central nervous system
CO_2	-	Carbon dioxide
Cl^-	-	Chlorine ion
^{o}	-	Degree
^oC	-	Degrees Celsius

DIC	-	Disturbed intravascular coagulation
DKP	-	Diketopiperazine
DPBS	-	Dulbecco's phosphate buffered saline
F IIa	-	Activated F II
F IXa	-	Activated F IX
F Va	-	Activated F V
F VIIa	-	Activated F VII
F VIIIa	-	Activated F VIII
F Xa	-	Activated F X
F XIIIa	-	Activated F XIII
FDA	-	Food and Drug Administration
FPA	-	Fibrinopeptide A
FPB	-	Fibrinopeptide B
14C	-	14 Carbon
5-FU	-	5-Fluorouracil
g	-	Gram
g/L	-	Gram per litre
H^+	-	Hydrogen
HfX	-	Human factor X
HMWK	-	High molecular weight kininogen
INR	-	International Normalized Ratio
ISI	-	International Sensitivity Index

K^+	-	Potassium
L-aspartyl-L-phenylalanine methyl ester	-	Aspartame
M	-	Molar
mg	-	Milligram
mg/kg	-	Milligram per kilogram
ml	-	Millilitre
mM	-	Millimolar
mmol/L	-	Millimolar per litre
MS	-	Mass spectrometry
µl	-	Microlitres
µm	-	Micrometre
n	-	Number of values used to obtain mean
Na/K	-	Sodium/Potassium
Na^+	-	Sodium
NaOH	-	Sodium hydroxide
NMR	-	Nuclear magnetic resonance
OIT	-	Optimal incubation time
OsO_4	-	Osmium tetraoxide
P	-	Level of significance
P/T-Ph	-	Platelet or tissue phospholipids
PAI-1	-	Plasminogen activator inhibitor 1
PBS	-	Phosphate buffered saline

PK	-	Prekallikrein
PKU	-	Phenylketonuria
PO_4	-	Phosphate buffer
PRP	-	Platelet rich plasma
PT	-	Prothrombin time
PTT	-	Partial prothrombin time
RafX	-	Rabbit factor X
RafXa	-	Activated rabbit factor X
RNA	-	Ribonucleic acid
rpm	-	Resolutions per minute
RuO_4	-	Ruthenium oxide vapour
SEM	-	Scanning electron microscope
TAFI	-	Thrombin-activatible fibrinolysis inhibitor
TEM	-	Transmission electron microscope
TF	-	Tissue factor
TFPI	-	Tissue factor pathway inhibitor
tPA	-	Tissue plasminogen activator
U/ml	-	Units per millilitre
uPA	-	Urokinase plasminogen activator
vWF	-	von Willebrand factor
γ	-	Gamma