Chapter 10 - References

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SUMMARY

The artificial dipeptide sweetener aspartame (APM; L-aspartyl-L-phenylalanine methyl ester) is present in many products, especially unsweetened or sugar free products. These products are frequently utilised by people trying to lose weight or patients with diabetes. Concern relating to the possible adverse effects have been raised due to aspartame's metabolic components, which is produced during its breakdown, namely phenylalanine, aspartic acid (aspartate), diketopiperazine (DKP) and methanol. Great controversy surrounds the usage of this product, even up to today.

Animal models are being used more frequently these days for determining the effects of pharmaceutical products, still in their preclinical trial periods. Animal models are more readily available and their environment can be controlled, unlike that of humans, especially in the case of their diets. A new approach has been established, which makes use of system biology. Systems biology used the idea of testing pharmaceutical product *in vivo*, and then determining its effects on the whole system of the experimental animal. Thus, a systems biology approach was also followed in this thesis, where proteins where quantified (coagulation factors; metabolomics).

Thus, the purpose of this study was to put a little light on the controversies relating to the effects of the dipeptide aspartame, and to focus on the effects of aspartame on the blood coagulation system of the New Zealand white rabbit.

Thus, the attention of this study was centred on determining whether the rabbit could be used and implemented successfully as an experimental animal model in obtaining blood samples to determine the effects of aspartame, and if so, how aspartame would influence the ultra-structural morphology of the fibrin networks, platelet aggregates and the endothelial lining. Would the coagulation profile of the rabbit be altered due to treatment with aspartame and could an immune response be activated by change in the morphology and counts of the different leukocytes present in blood? And if all of the above were affected by the intake of aspartame, would it cause changes in the normal histological morphology of the liver and kidney?

The hypothesis of this study was therefore that the morphology of both the platelets and the fibrin fibres would be altered by the presence of aspartame and that the concentration of the different coagulation factors would be changed. It was also thought that the morphology of the endothelial cells lining the blood vessels would be modified and if all of the above mentioned was true, the liver and kidneys which filter and detoxify the blood, would most certainly also be affected.

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, as the rabbit exhibited similar fibrin fibre morphology and fibre thickness as that of the human. The effects of aspartame were determined by 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. The ultrastructure of the fibrin fibre networks and platelet morphology was determined by utilizing the scanning electron microscope. The endothelial lining of the aorta was also studied by utilizing both scanning and transmission electron microscopy. Lastly the histological morphology of the leukocytes, liver and kidney were examined by means of light microscopy. The number of leukocytes was also counted after long-term treatment with aspartame to determine the accumulative effects of aspartame.

Results obtained indicated certain of the factors tested, were more than that found in humans, but the amount of circulating fibrinogen compare well with that found in humans, thus the rabbit makes a good candidate for studying coagulation. After treatment with aspartame, the results indicated that factors II, V, IX and the recalcification time was not adversely affected by ingestion of aspartame. But, F VII, X and VIII were decreased with a prolonged prothrombin time. All three these factors plays an integral part in the conversion of prothrombin to thrombin, which in turn s needed for a.) conversion of fibrinogen to fibrin; b.) conversion of F XIII to its active form, needed for stabilization of the fibrin fibres; c.) thrombin is needed for degranulation of the platelets, so that platelet aggregation can occur. 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, with the minor fibres becoming more pronounced and starting to form a mat-like structure over the major fibres. The degree of platelet aggregation occurring, decreased with the increase of aspartame concentration, and it was hypothesized that it was due to the decreased amounts of thrombin present. It was hypothesized that the amount of circulating serotonin decreased due to the inability of the platelets to de-granulise (desne granules secrete serotonin) and the high amounts of phenylalanine from the aspartame, which inhibits the conversion of tryptophan to serotonin. The endothelial lining of the rabbits were damaged with the nuclei appearing apoptotic (chromatin marginalization against nuclear envelope; damage to nuclear envelope). The endothelial lining and their tight junctions play an integral part in the functioning of the blood brain barrier, in synchronization with cAMP which controls the complexity of tight junctions. The activity of cAMP is enhanced by the presence of serotonin, thus a decrease in serotonin causes lowered activity of cAMP, causing decreased complexity of the tight junctions between the endothelial cells. Thus apoptotic cells and less complex tight junctions could indicate a compromised blood brain barrier. 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 become less. The granules contained within the eosinophils inhibit the degranulation of mast cells, which secrete heparin and histamine. Thus it appeared as though an immune response was triggered to prevent further decreases in coagulation. The total number of leukocytes also decreased, which indicated a level of suppressed immunity. The normal histological morphology of both the liver and kidney were affected by aspartame. Damage to the hepatocytes, as seen in the cytoplasm and the nuclei, and the subsequent arrangement of the hepatocytes into cords, which was also damaged, were noted. Thus, supplying corroborating evidence as to why a number of the coagulation factors were lowered. The visceral layer of the capsule of Bowman appeared thickened and the cuboidal epithelium lining the proximal convoluted tubule was also damaged. The visceral layer of the capsule of Bowman plays a key role in formation of the glomerular filtrate, thus a thickening of this layer could results in decreased formation of the filtrate. The proximal convoluted tubule forms part of the reabsorption apparatus of the kidney, thus damage to this part could lead to inability of reabsorption (dehydration).

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 may also occur with formation of the glomerular filtrate and absorption of fluid from the proximal convoluted tubule, which could result in high blood pressure and dehydration respectively.

Further suggested studies were to determine the effects of the aspartame on the ultra-structure of the renal corpuscle and the proximal convoluted tubule. Further experiments to determine the reason why only certain of the coagulation factors were influenced by the intake of aspartame should also be undertaken.

APPENDICES

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

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*).

Appendix A:

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

Appendix B:

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

Appendix C:

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*).