ASSESSMENT OF THE ANTIPROTOZOAL ACTIVITY OF SOME TUBULIN INHIBITORS FOLLOWING CYCLODEXTRIN COMPLEXATION.

By

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DECLARATION

I declare that the thesis 'Assessment of the antiprotozoal activity of some tubulin inhibitors following complexation with cyclodextrin' is a true account of my own research. To the best of my knowledge, the work submitted is original and contains no material that has previously been submitted for a degree at any tertiary education institution.

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ABSTRACT

The purpose of the present study was to evaluate the potential usefulness of tubulin inhibitors when complexed with hydroxypropyl-β-cyclodextrin (HPβCD) against a range of protozoan parasites. This approach involved investigations into the complexation of these drugs with HPβCD, and subsequent investigations of these drugs and their complexes in regard to cytotoxicity, pharmacokinetics, *in vitro* efficacy against *Giardia*, *Cryptosporidium* and rodent malaria (*Plasmodium chabaudi*), and their *in vivo* efficacy against *Giardia* and malaria.

Albendazole (ABZ) is a benzimidazole carbamate with a broad anti-parasite spectrum, while the dinitroanilines trifluralin (TF) and oryzalin (OZ) have recently been found to exhibit activity against certain parasites. All three compounds are microtubule antagonists in either nematodes or weeds and have poor aqueous solubility, with the solubility of ABZ and OZ dependent on pH. Cyclodextrins (CD) have a hydrophobic cavity that allows them to form inclusion complexes with hydrophobic drugs, resulting in increased drug aqueous solubility, and often, improved drug dissolution and bioavailability. Thus the complexation of these drugs with HPβCD was investigated. All three compounds exhibited type A_L phase solubility diagrams with HPβCD complexation, with additional increases in ABZ and OZ solubility achieved through the manipulation of temperature and pH. OZ displayed a stronger interaction with HPβCD when ionised over its neutral form. However, insufficient concentrations of the TF/HPβCD complex were achieved for drug efficacy studies.

The cytotoxicity of the drugs and their complexes was assessed using the assay kit Cytotox 96 with human carcinoma cells. This is a colourimetric assay that measures lactate dehydrogenase release as a consequence of compromised cellular and membrane integrity. Both ABZ and OZ are cytotoxic to rapidly proliferating and differentiating cells but are not cytotoxic to cells in the stationary phase. Complexation did not affect drug cytotoxicity. In pharmacokinetic studies, complexation improved ABZ (and metabolites) bioavailability, but had no significant affect on OZ bioavailability. *In vitro* drug assessment studies found ABZ to be highly effective against *Giardia*, and effective

against *Cryptosporidium* and malaria. OZ on the other hand exhibited no activity against *Giardia*, but was effective against *Cryptosporidium* and malaria. Complexation did not improve the antiprotozoal efficacy of either ABZ or OZ. In particular, excess HPβCD decreased the antigiardial effects of ABZ, possibly due to competitive complex formation. In addition, complexation did not improve the antiprotozoal effects of ABZ *in vivo*. However, the cytotoxic effect of the ABZ/HPβCD complex was more evident in the treatment of malaria *in vivo*, resulting in increased anaemia and suppression in weight gain, due to the improved bioavailability of ABZ and metabolites.

HPβCD alone was found to be cytotoxic at greater than 2.5%, and inhibited *Giardia* both *in vitro* and *in vivo* at greater than 1% and 2% respectively. This was attributed to membrane disruption caused by the dissolution and removal of membrane components. In comparison, malaria grew better in the presence of HPβCD *in vitro*, with no detrimental effect observed at up to 8% HPβCD. This was attributed to either the increased solubilization of a necessary media component, or the complexation and removal of an inhibitory compound from the cultivation medium.

Therefore HPβCD complexation did not improve the antiprotozoal activity of the tubulin antagonists ABZ and OZ. However, the results of the pharmacokinetic studies suggest that anthelmintic activity of ABZ, particularly against systemic infections, may be improved with oral administration of the ABZ/HPβCD complex. In addition, the antiparasitic activity of HPβCD alone may be promising, especially against intestinal infections. Finally, the improved *in vitro* cultivation of *P. chabaudi* in the presence of HPβCD presents a promising approach to its potential long term cultivation.

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ABBREVIATIONS

Acetonitrile	CH_3CN
Albendazole	ABZ
Albendazole sulfoxide	ABSO
Albendazole sulfone	$ABSO_2$
Alsevier's solution (Section **7.2.2.4**)	AS
Butylaminobenzoate	BAB
Chloroquine	CHQ
Cyclodextrin	CD
Differential scanning calorimetry –Thermogravimetric analysis	DSC-TGA
2,6-dimethyl-β-cyclodextrin	DMβCD
Dimethyl sulphoxide	DMSO
Distilled water	dH_2O
Foetal calf serum	FCS
Glucose Citrate Solution (Section **7.2.2.2**)	GC
Guanosine triphosphate	GTP
High Performance Liquid Chromatography	HPLC
Hydroxyethyl-cyclodextrin	HECD
2-hydroxypropyl-cyclodextrin	2-HPCD
3-hydroxypropyl-cyclodextrin	3-HPCD
2-hydroxypropyl-β-cyclodextrin	HPβCD
Limit of detection (Section **2.2.2.2**)	LOD
Limit of quantitation (Section **2.2.2.2**)	LOQ
Mebendazole	MBZ
Microsomal flavin monooxygenases	MFMO
New born calf serum	NBCS
Oryzalin	OZ
Phosphate buffered saline (Section **5.2.1.1.4**)	PBS
Randomly methylated-β-cyclodextrin	RMβCD
Red blood cells	RBC
Relative standard deviation (Section **3.2.3.1.5**)	RSD
Stability constant (Section **1.6.1**)	K_C
Sulfobutyl ether-β-cyclodextrin	SBEβCD
Tetrahydrofluran	THF
Thiabendazole	TBZ
Trifluralin	TF
2,3,6-trimethylmethyl-β-cyclodextrin	TMβCD
World Health Organization	WHO

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