Supplementary files

Preclinical and clinical characteristics of the trichuricidal drug oxantel pamoate and clinical development plans: a review

Running heading: Oxantel pamoate for Trichuris trichiura infections

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Supplementary file 1. Results from an experiment assessing the binding affinity of oxantel pamoate to the human and rat α7 nicotinic acetylcholine receptor (nAChR) at different concentrations of the oxantel pamoate.

Experiments were conducted at Eurofins according to their in-house standard procedures https://www.eurofinsdiscoveryservices.com/catalogmanagement/viewitem/nAChR-alpha7-Human-Ion-Channel-Binding-Antagonist-Radioligand-Assay-Panlabs/299032; https://www.eurofinsdiscoveryservices.com/catalogmanagement/viewitem//258630.

Human recombinant nicotinic acetylcholine α7 receptor were expressed in SH-SY5Y cells. A 20 µg aliquot of membranes was incubated in modified phosphate buffer pH 7.4 with 0.4 nM [125I]α-Bungarotoxin for 120 minutes at 37ºC. Non-specific binding was estimated in the presence of 1 µM α-Bungarotoxin. Membranes were filtered and washed, and the filters were counted to determine specifically-bound [125I] α-Bungarotoxin.

For the studies on the rat ion channel whole brain (minus cerebellum) membranes of male Wistar derived rats weighing 175 ± 25 g were prepared in modified phosphate buffer at pH 7.4 using standard techniques. A 0.6 mg aliquot of membrane was incubated with 0.6 nM [125I]a-Bungarotoxin for 150 minutes at 37ºC. Non-specific binding was estimated in the presence of 1 µM a-Bungarotoxin. Membranes were filtered and washed three times and the filters were counted to determine specifically bound [125I]a-Bungarotoxin.

Below are the results from an experiment assessing the binding affinity of oxantel pamoate to the human and rat α7 nicotinic acetylcholine receptor (nAChR) at different concentrations of the oxantel pamoate; estimated half maximal inhibitory concentration (IC_{50}), inhibition constant (K_i) and Hill coefficient (n_H).

	Concentration	% Inhibition	IC_{50}	K_i	n_H
Rat	1.65 mM	-120	33.0 µM	25.6 µM	1.34
	165 µM	90			
	16.5 µM	28			
	1.65 µM	2			
	0.165 µM	-2			
	16.5 nM	-8			
	1.65 nM	-6			
	0.165 nM	3			
Human	1.65 mM	-117	3.48 µM	1.60 µM	0.9
	165 µM	103			
	16.5 µM	83			
	1.65 µM	28			
	0.165 µM	18			
	16.5 nM	6			
	1.65 nM	-3			
	0.165 nM	0			

Note: IC_{50}, half maximal inhibitory concentration; K_i, inhibition constants calculated using the equation of Cheng and Prusoff (Cheng, Y., Prusoff, W.H., Biochem. Pharmacol. 22:3099-3108, 1973) using the observed IC_{50} of the tested compound, the concentration of radioligand employed in the assay, and the historical values for the dissociation constant (K_D) of the ligand (obtained experimentally at Eurofins Panlabs, Inc.); n_H, Hill coefficient defining the slope of the competitive binding curve, was calculated using MathIQTM. Data are presented without Standard Error of the Mean because they are insufficient to be quantitative, and the values presented (Ki, IC50, nH) should be interpreted with caution.

Supplementary file 2. *In vitro* experiment to assess the intestinal epithelial permeability of oxantel pamoate using Caco-2 cells.

Experiments were conducted at Eurofins based on in house methods (https://www.eurofinsdiscoveryservices.com/catalogmanagement/viewitem/Bidirectional-permeability-Caco-2-pH-6.5-7.4/G235)

Supplementary file 3. *In vitro* experiment to assess metabolism in human and rat microsomes. Experiments were conducted at Eurofins based on in house methods. Each experiment is performed with four compound concentration(s) and each concentration tested using 48 replicate by means of HPLC-MS/MS - https://www.eurofinsdiscoveryservices.com/catalogmanagement/viewitem/Intrinsic-clearance-intestinal-microsomes-human/1422.

				% Compound remaining			Half-life (min)			
	Compound	Concentration	Incubation time (min)	First	Second	Mean	First	Second	Mean	Intrinsic clearance (µL/min/mg)
Rat	Oxantel pamoate	0.1 µM	0	100.0	100.0	100	36049.6	435.8	>120	<57.8
			30	102.0	93.3	102				
			60	107.2	90.5	99				
			90	98.1	87.5	93				
			120	116.4	82.2	82				
Human			0	100.0	100.0	100	3064.6	696.1	>120	<57.8
			30	102.9	97.6	100				
			60	98.7	89.7	99				
			90	100.6	90.2	95				
			120	97.7	89.6	94				
Reference compounds										
Rat	Imipramine	0.1 µM	-	-	-	-	566.6	568.1	>120	<57.8
	Midazolam		-	-	-	-	792.9	1118.0	>120	<57.8
	Propanolol		-	-	-	-	16.6	14.6	16	447.3
Human	Imipramine		-	-	-	-	205.0	277.3	>120	<57.8
	Midazolam		-	-	-	-	41.9	39.0	40	171.7
	Propanolol		-	-	-	-	931.5	646.2	>120	<57.8