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Note

Potential inhibitory effects of formulation ingredients on intestinal cytochrome P450

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Abstract

The use of common formulation ingredients (categorized into six groups) for preclinical animal studies has been assessed with respect to cytochrome P450 (CYP) inhibition, specifically CYP3A inhibition, in expressed human CYP3A4, human liver microsomes, dog- and cynomolgus monkey intestinal microsomes. Results indicated a wide range of inhibition potentials and there appeared to be species differences with inhibition of CYP3A activity. Generally, greater inhibition of CYP3A activity was observed with amphiphilic ingredients (for example mixed micellar solutions, Tween 80, and oleic acid). From the data presented, it can be predicted that the majority of the ingredients tested would not have a significant impact on the potential inhibition, by the formulation, on any apparent first pass metabolism in the intestinal tract for new drug entities being tested in the preclinical environment. However a number of common ingredients will require further investigation based on the estimated concentration within the gastrointestinal tract.

Keywords: Formulations; Cytochrome P450; Inhibition; Microsomes

Critical to the development of a NDE (New Drug Entity) is an understanding of it's drug metabolism and pharmacokinetic properties. This is achieved, in part, by dosing of preclinical species with various formulations of the test compound, but unfortunately many NDEs are lipophilic and insoluble, and as a consequence a variety of different formulations are often utilized in order to obtain an oral dosage form. However, many of these common formulations contain excipients that may have an effect on the metabolism and absorption process. Thus the aims of the current study were to investigate 16 commonly used formulation ingredients (categorized into six groups), used for preclinical animal experiments, with respect to the inhibition of cy-

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tochrome P450 (CYP) activity, specifically CYP3A in man and two large preclinical species (dog and Cynomolgus monkey).

Formulation ingredients were: (1) co-solvents, which included *N*-methylpyrrolidinone (Fluka), dimethylacetamide (Fluka), propylene glycol (Fluka) and PEG 400 (Fluka); (2) oils, which included soybean oil (Merck) and miglyol (Merck); (3) surfactants, which included Tween 20 (Merck), Tween 80 (Merck) and oleic acid (Merck); (4) mixed micelles, which included taurocholic acid:lecithin (1:1 [v/v]) (Sigma) and glycocholic acid:lecithin (1:1 [v/v]) (Sigma) at 200 mM (in water); (5) hydroxypropyl-β-cyclodextrin (Fluka); and (6) mixtures, which included cremophor:PEG 400 (1:9 [v/v]), Cremophor (Sigma): water (1:9 [v/v]), Vit E-TPGS (Eastman): PEG 400 (1:9 [v/v]), and Vit E-TPGS: water (1:9 [v/v]). Initially, each excipient was incubated at different concentrations with cDNA expressed human CYP3A4 (Supersomes, product No. P202 [Gentest corporation, USA]) to determine the IC50 values for the inhibition of the metabolism of the probe CYP3A4 substrate 7-benzyloxy-4-(trifluoromethyl)-coumarin (BFC) metabolism to 7-hydroxy-4-(trifluoromethyl)-coumarin (7-HFC), as described by Crespi et al. (1997), and subsequent modifications. Based on initial results, selected formulations were incubated in the presence of human liver microsomes (Lot no 1032), dog- and Cynomolgus monkey intestinal microsomes (Lot no X02201 and X02311, respectively), supplied by In Vitro Technologies, USA, in order to determine the inhibition constant (Ki) for the hydroxylation of midazolam (F. Hoffmann-La Roche, Switzerland). The formation of 1-hydroxymidazolam (a typical specific CYP3A4 reaction) was quantified using LC-MS-MS (Psiex Inc, USA) and K_i determined using GraFit Version 4 (Leatherbarrow, 1998).

There have been several reports on the effects of assay conditions, ionic strength and common organic solvents on in vitro cytochrome P450 mediated metabolic activities (Schenkmen et al., 1994; Chauret et al., 1998; Mäenpää et al., 1998). From Table 1 and Fig. 1 it is apparent that the use of different formulation ingredients can also have an impact on CYP3A4 activity, the principal P450 in the intestinal tract (Watkins et al., 1987). Initially expressed enzymes were selected since if these were unaffected by the formulation ingredients in vitro, then an in vivo effect would be

Table 1
IC50 and K_i determination for selected excipients

Formulation	Source of CYP	Method of determination	Determined parameter	Value (mM)
N-Methylpyrrolidone	Supersomes (CYP3A4)	Inhibition of BFC metabolism	IC_{50}	5.3
Dimethylacetamide	Supersomes (CYP3A4)	Inhibition of BFC metabolism	IC_{50}	29.1
Propylene glycol	Supersomes (CYP3A4)	Inhibition of BFC metabolism	IC_{50}	>50
PEG400	Supersomes (CYP3A4)	Inhibition of BFC metabolism	IC_{50}	16.5
Soybean oil	Supersomes (CYP3A4)	Inhibition of BFC metabolism	IC_{50}	>50
Miglyol	Supersomes (CYP3A4)	Inhibition of BFC metabolism	IC_{50}	>50
Glycocholic acid/lecithin	Supersomes (CYP3A4)	Inhibition of BFC metabolism	IC_{50}	0.0194
Tween 20	Supersomes (CYP3A4)	Inhibition of BFC metabolism	IC_{50}	0.0038
Oleic acid	Supersomes (CYP3A4)	Inhibition of BFC metabolism	IC_{50}	2.0
Taurocholic acid/lecithin	Supersomes (CYP3A4)	Inhibition of BFC metabolism	Ki	0.075
	Dog intestinal microsomes	Inhibition of midazolam metabolism	Ki	>0.3
	Monkey intestinal microsomes	Inhibition of midazolam metabolism	Ki	>0.3
	Human intestinal microsomes	Inhibition of midazolam metabolism	Ki	0.030

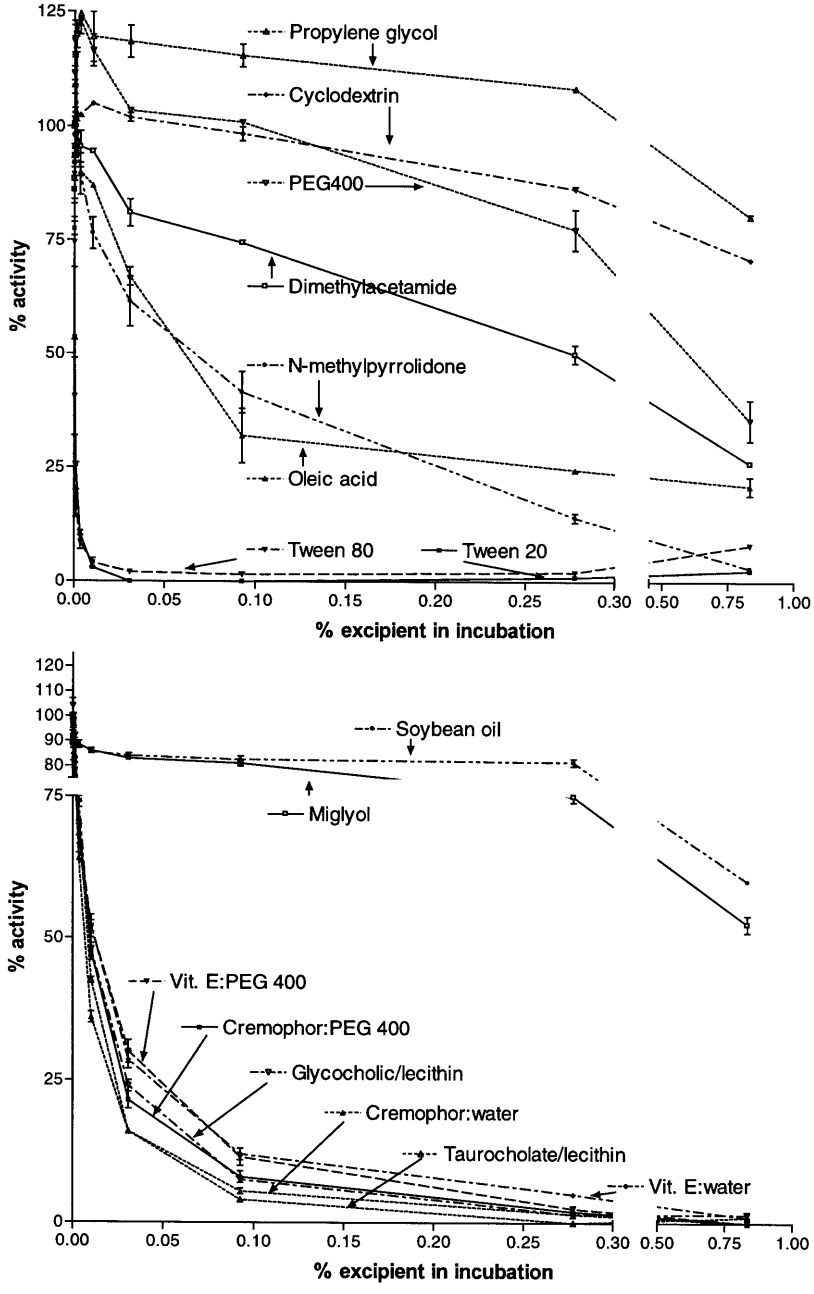

Fig. 1. Effect of increasing concentrations of various excipients on the activity of cDNA expressed human CYP3A4. Inhibition of CYP3A4 activity by the various excipients was calculated based on the inhibition of the conversion of the probe CYP3A4 substrate 7-benzyloxy-4-(trifluoromethyl)-coumarin (BFC) to the fluorescent metabolite 7-hydroxy-4-(trifluoromethyl)-coumarin (7-HFC), as described by Crespi et al. (1997).

unlikely (due to limited access of the ingredient to the enzyme within the intestinal epithelial). As shown in Table 1 (IC50 and K_i values for selected excipients), for the majority of the ingredients tested, the high IC50 values for CYP3A4 inhibition would suggest that there would be no significant clinical or pre-clinical interaction when used for NDE administration. Ingredients that produced dramatic inhibition curves, for example Tween 20 and 80 (Fig. 1A for Tween 20 and Tween 80, and Table 1 for Tween 20), could have been predicted since this class of formulation, i.e. surfactant, are known to disrupt enzyme activity. The preclinical consequences of this inhibition require further investigation with respect to exposure of the enzyme to the inhibitor since this would rely on uptake of the excipient into the cells of the intestine.

Of particular interest was CYP3A4 inhibition by mixed micelles, since these excipients produced the second lowest IC50 values for the compounds tested. Therefore taurocholic acid/lecithin was investigated further with the use of liver microsomes from human, and intestinal microsomes from dog and monkey. Human liver microsomes were selected as being representative of human intestinal microsomes, with respect to CYP3A4, since the availability of human intestinal microsomes was limited and previous results have demonstrated that the sequences of intestinal and hepatic cytochrome P450 3A4 cDNAs are identical (Lown et al., 1998). Interestingly there was no significant inhibition of midazolam metabolism by taurocholic acid/lecithin in dog- and monkey intestinal microsomes (Table 1), however this may be related to species differences with respect to CYP3A structure, P450 content and distribution in the intestine. The K_i for inhibition of midazolam and BFC metabolism, assuming competitive inhibition, was calculated as 75 and 30 μM for human liver microsomes and expressed CYP3A4, respectively, and would therefore be predicted not to be significant with respect to drug–drug interaction at the intestinal wall. Further, when assessing the use of formulation ingredients it is pertinent to consider the exposure of the ingredient at the active enzyme site, since in many cases uptake of the excipient across the intestinal wall is unlikely.

In conclusion, inhibition of CYP3A activity by common formulation ingredients has been assessed, and although there was inhibition of enzyme activity, in many instances this was in the mM range and would therefore be considered insignificant. However, inhibition of CYP3A4 activity by surfactants and mixed micelles should be investigated further, especially since there appeared to be species differences with respect to inhibition of midazolam hydroxylation in human liver microsomes, dog- and Cynomolgus monkey intestinal microsomes by mixed micelles. Future work will focus on different ingredients and the effect on other absorption parameters such as inhibition of the efflux pump P-glycoprotein.

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