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Note

Effect of pluronic F68 block copolymer on P-glycoprotein transport and CYP3A4 metabolism

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Abstract

The aim of this work was to investigate the effects of pluronic F68 block copolymer on the P-gp-mediated transport of celiprolol (CEL) and CYP3A4-mediated formation of midazolam (MDZ) metabolite 1′-hydroxymidazolam. Over a range from 0.03 to 0.3%, pluronic F68 increased apical-to-basolateral permeability (AP-BL) and decreased basolateral-to-apical permeability (BL-AP) of the P-gp substrate CEL in Caco-2 cell monolayer with the efflux ratio values of 2.8 ± 0.3 (0.03%), 2.6 ± 0.3 (0.1%), 2.3 ± 0.2 (0.3%), respectively. CEL transport across the intestinal mucosa in the everted gut sac model was also enhanced by the P-gp inhibitor verapamil and the pharmaceutical excipient pluronic F68. Furthermore, CYP3A4-catalyzed formation of 1′-hydroxymidazolam was inhibited by pluronic F68 with IC_{50} and K_i values of 0.11 and 0.16 mg/ml, respectively. The results indicate that pluronic F68 is a potent *in vitro* inhibitor of both P-gp and CYP3A4, suggesting a potential for pluronic F68 to modify the pharmacokinetics of orally administered drugs that are P-gp and/or CYP3A4 substrates *in vivo*.

Keywords: P-glycoprotein; Pluronic F68 block copolymer; CYP3A4; Caco-2; Everted gut sac

Many drug candidates fail to reach their therapeutic potential due to low solubility, low permeability, and/or high presystemic metabolism. Among numerous factors it is now generally recognized that active efflux of drugs by P-glycoprotein (P-gp) and metabolism of molecules by cytochrome P450 3A (CYP3A) should be considered to optimize oral bioavailability and to decrease variability at the absorption site (Toyobuku et al., 2003). Recently, various types of pharmaceutical excipients have been identified as inhibitors or inducers of P-gp and CYP3A which both can lead to drug interactions (Cornaire et al., 2004; González et al., 2004). Among these excipients, pluronics, poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide) triblock copolymers, represent a class of polymer that is widely used in variety of pharmaceutical formulations and drug delivery systems. Previous studies indicated that pluronic block copolymers interact with multidrug-resistant (MDR) tumors resulting in drastic sensitization of these tumors to various anticancer agents (Kabanov et al., 2002). Furthermore, many of these common excipients may have an effect on the drug metabolism process. However, very few studies have been conducted on the effect of pluronics on CYP3A4. The aims of the current study were to investigate *in vitro* the impact of pluronic F68 on the transport of CEL and metabolism of MDZ.

The transport studies of CEL (100 μM) across Caco-2 cells in the presence or absence of the P-gp inhibitor verapamil (10, 100 μM) and pluronic F68 (0.03, 0.1 and 0.3%) were performed according to previous studies (Lo et al., 2001). To evaluate the potential inhibition effect of pluronic F68 on P-gp, the absorptive and secretory apparent permeation coefficients (P_{app}) of CEL were determined at various concentrations of pluronic F68. The absorptive and secretory P_{app} was estimated to be $(0.34 \pm 0.04) \times 10^{-6}$ cm/s and $(2.16 \pm 0.11) \times 10^{-6}$ cm/s, respectively (Table 1). CEL exhibited a high polarized transport, with the active efflux exceeding the passive influx sixfold. In the presence of pluronic F68, a concentration dependent decrease in the basolateral to apical transport and a slight increase in apical to basalateral transport were observed with the efflux ratio value approaching unity as the dose of inhibitor increases. The ratios of $P_{app\ BL\text{-}AP}/P_{app\ AP\text{-}BL}$ were 2.8, 2.6, 2.3 at different concentrations of pluronic F68 (0.03, 0.1 and 0.3%), whereas that

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Table 1
Bidirectional apparent permeability coefficient ($P_{app} \times 10^{-6}$ cm/s) and net efflux ratio (ER) of P-glycoprotein substrate celiprolol (100 μM) across Caco-2 cell monolayers

	Papp ($\times 10^6$ cm/s)		ER
	AP-BL	BL-AP	
CEL (100 μM)	0.34 ± 0.04	2.16 ± 0.11	6.3 ± 0.4
CEL (100 μM) + verapamil (10 μM)	0.40 ± 0.02*	1.11 ± 0.05**	2.8 ± 0.2**
CEL (100 μM) + verapamil (100 μM)	0.43 ± 0.02*	1.07 ± 0.07**	2.5 ± 0.2**
CEL (100 μM) + pluronic F68 (0.03%)	0.39 ± 0.02*	1.09 ± 0.09**	2.8 ± 0.3**
CEL (100 μM) + pluronic F68 (0.1%)	0.39 ± 0.03*	1.04 ± 0.07**	2.6 ± 0.3**
CEL (100 μM) + pluronic F68 (0.3%)	0.41 ± 0.01*	0.91 ± 0.02**	2.3 ± 0.2**

* $p < 0.05$, significantly different from control group.
** $p < 0.01$, significantly different from control group.

Table 2
CEL concentrations in everted sacs of duoneum, jejunum, and ileum at 120 min in rat intestinal everted-sac experiments

Segment	Control	Verapamil		Pluronic F68		
		10 μM	100 μM	0.03%	0.1%	0.3%
Duodenum	8.42 ± 0.72	11.08 ± 0.74*	18.37 ± 1.06*	9.37 ± 0.61	12.75 ± 0.98*	15.66 ± 1.23*
Jejunum	8.37 ± 0.71	11.94 ± 0.94*	19.67 ± 1.26*	11.28 ± 0.99*	14.59 ± 1.24*	17.15 ± 1.49*
Ileum	8.55 ± 0.82	13.48 ± 1.29*	22.43 ± 1.26*	12.07 ± 0.94*	15.99 ± 1.08*	19.22 ± 1.69*

* $p < 0.01$, significantly different from control group.

without inhibitors averaged 6.3. In the transport study of CEL, TEER values were determined before and after experiments to evaluate the integrity of the monolayers. The results showed that only at high concentration of 0.3%, the TEER values decreased by 10% and after 24 h culture in the media TEER values were equal to the initial TEER data obtained prior to the experiment.

The everted sacs of rat duodenum, jejunum, and ileum were incubated in 40 ml of Tyrode's solution containing CEL (100 μM) using a method described before (Barthe et al., 1998). The transfer of CEL across everted gut sacs under the influence of 10, 100 μM verapamil or 0.03, 0.1, 0.3% pluronic F68 is shown in Table 2. The concentrations of CEL in different intestinal segments at 120 min were significantly elevated by verapamil and pluronic F68, except 0.03% pluronic F68 in duodenum, if compared to the control group ($p < 0.01$, $N = 3$ animals in each group). The lower the intestinal segment, the higher the absorptive amount. With increasing concentrations of verapamil and pluronic F68 cumulative concentration of CEL gradually increased, although to a lesser extent than in the Caco-2 cell model and the enhancement effect of verapamil was superior to that of pluronic F68 in the duodenum, jejunum, and ileum. However, the results of the CEL transport across the intestinal mucosa in the everted gut experiment were consistent with those from the transport studies of Caco-2 cell model. These results implied that pluronic F68 exhibited an inhibitory effect on P-gp and enhance the delivery of a P-gp substrate to the serous membrane in the intestine.

In addition, CYP3A4-mediated biotransformation of MDZ were performed as described previously (Walsky and Scott-

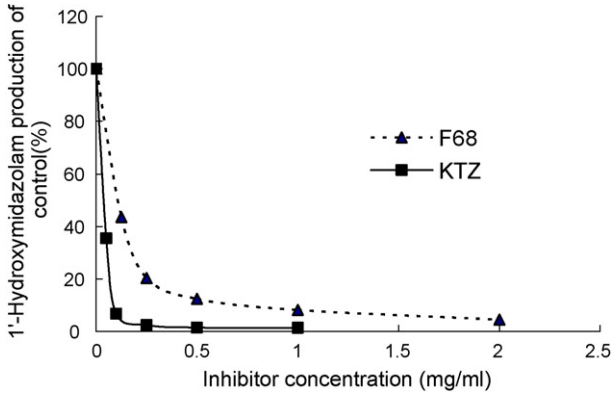

Fig. 1. Effect of increasing concentration of ketoconazole (KTZ) and pluronic F68 (F68) on the metabolism of midazolam to 1′-hydroxymidazolam by recombinant CYP3A4.

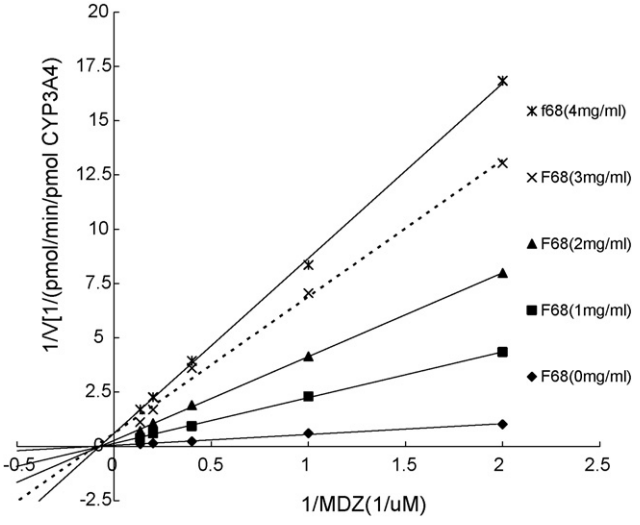

Fig. 2. Double reciprocal (Lineweaver–Burk) plots for inhibition of biotransformation of midazolam to 1′-hydroxymidazolam by pluronic F68.

Obach, 2004). The IC_{50} and K_i values were determined to evaluate the inhibitory activity on CYP3A4 in presence of pluronic F68 using a Lineweaver–Burk plot and the Hill equation, respectively (Mountfield et al., 2000). The effect of pluronic F68 on formation of 1′-hydroxymidazolam by recombinant CYP3A4 is illustrated in Figs. 1 and 2. It is apparent that both pluronic F68 and KTZ inhibited CYP3A4 activity and the excipient showed a dose-dependent inhibition of 1′-hydroxymidazolam formation. The IC_{50} and K_i values for pluronic F68 averaged 0.11 and 0.16 mg/ml, respectively. However, the mechanisms of this inhibition remain unclear. The literature reported that the inhibitory mechanisms of surface-active excipients on the metabolism of MDZ in rat liver microsomes might include direct interaction between the surfactants and metabolizing enzymes, alteration of cell function and disruption of cell membrane (González et al., 2004).

In conclusion, pluronic F68 inhibits P-gp-mediated CEL transport and acts as a potent inhibitor of CYP3A4 activity *in vitro*. Due to the benign and non-irritating nature of the pluronic F68, its addition in the formulation for oral delivery of P-gp/CYP3A4 substrates appears to be extremely promising.

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