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In vitro Metabolic Modulation of Aryl Sulfotransferases by Pharmaceutical Excipients

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Pharmaceutical excipients (PEs) are an essential part of drug products to make them have desirable characteristics. Most pharmaceutical excipients have been considered as biologically inert. However, recent studies showed that PEs affect pharmacokinetic behaviors of drugs in several manners when absorbed through gastrointestinal (GI) tract. For example, oral administration of the excipients such as non-ionic surfactants could modulate cytochrome P450 enzymes, in GI tract and liver, and p-glycoprotein (p-gp) leading to changes in pharmacokinetic characteristics *in vitro* and *in vivo*.[1-4]

Sulfotransferase (SULT) is one of the phase II metabolism enzymes mainly found in the liver and small intestine and catalyzes the transfer of sulfuryl groups from 3'-phospho-adenosine 5'-phosphosulfate (PAPS) to hydroxyl or amino groups of the compounds. The biological functions of SULT include metabolism and detoxification of xenobiotics by sulfation.[5-9]

It can therefore be anticipated that inhibitors or enhancers of SULT alter the metabolic profiles of substrate drugs susceptive to the enzymes which may decrease or increase the bioavailability of the drug. For example, SULT1A1 and SULT1A3 play important roles in the pre-systemic inactivation of β2 agonists in the liver and intestine. Thus, inhibition of SULTs can lead to an increase in the bioavailability of β2 agonists.[10]

In this study, we attempted to investigate the possibility of the modulation of SULT by a wide range of PEs frequently used in the formulation of oral drug delivery systems to improve the bioavailability of drugs that are extensively degraded by SULTs.

Result and Discussion

Eleven commonly used excipients in solid and liquid oral dosage forms were selected for this study. Nonionic surfactants including Tween 20, Tween 80, Cremophor EL, Cremophor ELP, Cremophor RH40 and Cremophor RH60, and anionic surfactant, sodium lauryl sulfate (SLS) were selected. And polymeric excipients such as Lutrol F68, PVP K30, PEG 4000 and PEG 6000 that might be used as diluents or fillers were investigated. The action of PEs on sulfation was evaluated by the enzyme assay using SULTs obtained from the rat liver cytosol and 4-methylumbelliferone (4-MU) was used as substrate.[11]

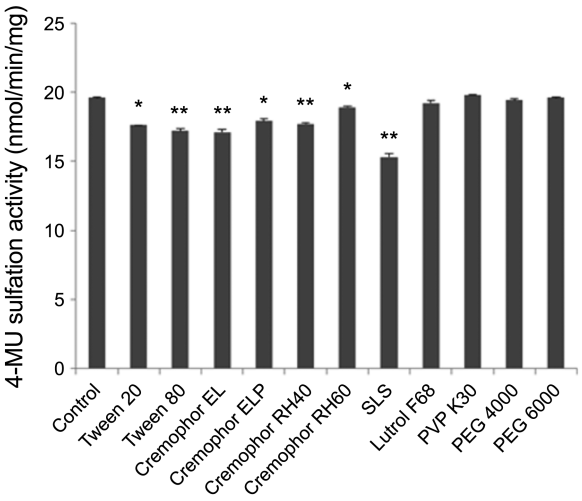

Figure 1. Metabolic inhibition effect of 2 mg/mL PEs (120 μg/mL for SLS) on aryl SULT activity (*$p < 0.1$, **$p < 0.05$).

The effect of various PEs on the activity of aryl SULT is shown in Figure 1. Of the 11 tested excipients, Tween 20, Tween 80, Cremophor EL, Cremophor ELP, Cremophor RH40, Cremophor RH60 and SLS significantly inhibited the activity of aryl SULT as compared with control, suggesting that the surfactants are effective in inhibiting enzyme activity. In contrast, the polymeric excipients such as Lutrol F68, PVP K30, PEG 4000 and PEG 6000 did not considerably change the sulfation of 4-MU.

The enzyme kinetics of aryl SULT in the presence of PEs was further investigated with Lineweaver-Burk plot and secondary plot. As Tween 80, Cremophor EL and SLS concentration increased the metabolic inhibition toward the sulfation of 4-MU seemed to increase in a concentration-dependent manner (Figure 2). The inhibition constants, K_i values, of Tween 80, Cremophor EL and SLS were measured to be 6.43, 3.24 and 0.22 mg/mL, respectively (Figure 3,

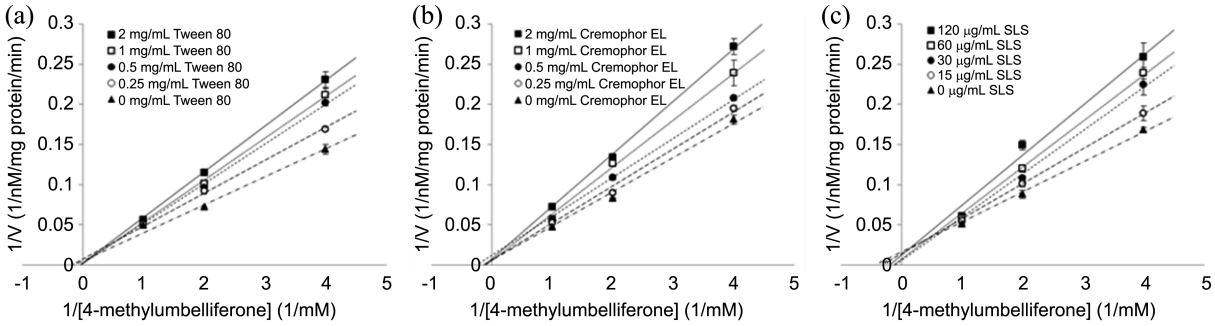

Figure 2. Lineweaver-Burk plots of Tween 80 (a), Cremophor EL (b) and SLS (c).

Table 1). Based on K_i values, the greatest inhibitory effect on aryl SULT could be obtained with SLS.

The activity profiles of aryl SULT after incubation with 2 mg/mL Tween 80, Cremophor EL or 120 μg/mL SLS showed that the enzyme activity was diminished as a function of time (Figure 4). However, when comparing slopes of each PE profile with that of control profile, no significant differences could be observed. This indicates that the aryl SULT is not denatured irreversibly by PEs at concentrations tested in this study although other factors such as temperature and humidity might decrease the activity of enzyme. Therefore, the results from this study may indicate that the surface-active agents can prevent the interaction between the aryl SULT and substrate without irreversible inactivation of the enzymes.

Recently, the inhibitory effects of PEs on metabolic enzymes and cell membrane embedded transporters have been explored when absorbed through GI tract. For example, it has been reported that PEs such as non-ionic surfactants including Tween 20 and Cremophor EL could modulate the activity of cytochrome P450 enzymes located in GI tract and liver when administered orally.[12]

And, in several studies, it was reported that the surfactants act as inhibitors of intestinal efflux transporter, p-gp. These inhibitory activities of surfactants seem to mainly be caused by membrane fluidization. The change of membrane physical state has been known to lead to disruption of membrane-bound p-gp.[13]

However, membrane fluidization effect might not be proper to explain the inhibitory activity of the surfactants on SULT in our study because SULT is not a membrane-bound protein. Since enzyme inhibition or modulation can be affected by multiple factors rather than one single mechanism, thus to elucidate the exact mechanism of inhibition remains quite problematic.[14]

Table 1. The K_i values of PEs obtained by analysis of secondary plot

Enzyme	Pharmaceutical excipient (Inhibitor)	K_i (mg/mL)
Aryl sulfotransferase	Tween® 80	6.43
	Cremophor® EL	3.24
	SLS	0.22

Alternatively, the reason for the SULT inhibition might be attributed to the binding of the surfactants to the enzymes, probably resulting in the prevention of proper interaction between the substrate and the SULT.[15] It has been reported that at low concentrations, SLS molecules bind to specific sites of proteins such as albumin by ionic and hydrophobic interactions.[16] However, higher levels of SLS caused the denaturation of the protein in the same study. As demonstrated in Figure 4, because significant inactivation of SULT was not observed, the surfactant binding may be responsible for one of the possible causes of the inhibitory effect of the surfactants. This hypothesis is also considered to be supported by the result that the anionic surfactant SLS was much stronger inhibitor of enzymatic metabolism than non-ionic surfactants such as Tween 80 and Cremophor EL. To identify the mechanism of inhibition on the enzyme, more research works to characterize the interaction of surfactants with SULT at molecular level are required.

In conclusion, it was demonstrated that PEs noticeably inhibited aryl sulfotransferase activity. Although we examined the activity of the sulfotransferases obtained from rat liver, the sulfotransferases are also found in the small intestine at higher levels.[17-19] Therefore, it might be expected that the inhibition of sulfotransferases by PEs can modify the absorption profiles of drugs administered *via* the oral route. This suggests that the selection of appropriate PEs when designing oral dosage forms would provide a chance to modulate or improve the bioavailability of the drugs metabolized mainly by SULT. These *in vitro* behaviors could be considered at the early formulation stages. Although, in this study, *in vitro* effect of PEs was identified, the action of PEs on absorption should be confirmed with appropriate *in vivo* models.

Experimental

4-Methylumbelliferone (4-MU), *p*-nitrophenyl sulfate (PNPS) and 3'-phosphoadenosine 5'-phosphosulfate (PAPS) were purchased from Sigma-Aldrich Company (St. Louis, USA). Polyoxyl 35 castor oil (Cremophor EL), purified polyoxyl 35 castor oil (Cremophor ELP), polyoxyl 40 hydrogenated castor oil (Cremophor RH40), polyoxyl 60 hydrogenated castor oil (Cremophor RH60) and poloxamer 188 (Lutrol F68) were obtained from BASF (Ludwigshafen, Germany).

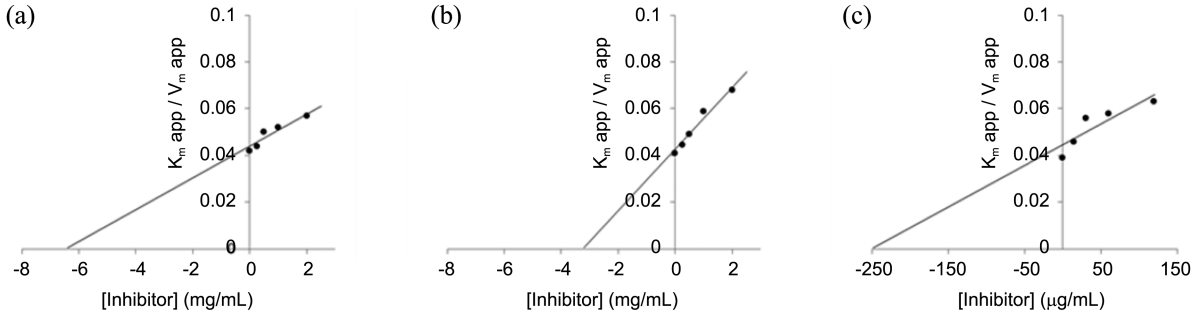

Figure 3. Secondary conversion plots of Tween 80 (a), Cremophor EL (b) and SLS (c).

Polysorbate 20 (Tween 20), and polysorbate 80 (Tween 80) were supplied from Sigma-Aldrich Company (St. Louis, USA). Sodium lauryl sulfate (SLS) was purchased from Daejung Chemicals (Kyungki-do, Korea). Polyvinylpyrrolidone K-30 (PVP K-30) was purchased from Junsei Chemical Co., Ltd. (Kyoto, Japan). Polyethylene glycol 4000 (PEG 4000) and polyethylene glycol 6000 (PEG 6000) were obtained from Duksan Pure Chemical Co., Ltd. (Kyungki-do, Korea). All other chemicals were of reagent grade and were used without further purification.

To extract the rat liver cytosol, rats were expired under deep surgical anesthesia with ether. The livers were then removed and homogenized in ice-cold 50 mM Tris-HCl buffer (pH 7.4) containing 0.25 M sucrose. All homogenates were centrifuged at 100,000 g for 1 h at 4 °C.[20] Cytosol aliquots were then collected and preserved at −80 °C for enzymatic assay.

Enzyme activity of SULT was determined by *p*-nitrophenyl sulfate (PNPS) assay method with little modification as described previously.[21,22] For the estimation of SULT activity, a reaction mixture was prepared containing 50 mM Tris buffer (pH 6.2), 5 mM PNPS, 20 μM PAPS and 1 mM 4-MU. The rat liver cytosol (50 μg) was added and the total volume of reaction mixture was set to 500 μL. The SULT from the rat liver cytosol catalyzed the sulfation of 4-MU while incubating for 20 min at 38 °C. After the incubation, 500 μL of 0.25 M Tris buffer (pH 8.7) was added to terminate the reaction. Finally, absorbance was measured with a spectrophotometer at 401 nm. To study the effect of PEs on the activity of aryl sulfotransferase, the excipients at a concentration of 2 mg/mL except SLS (120 μg/mL) were added to the reaction mixtures together with 4-MU.

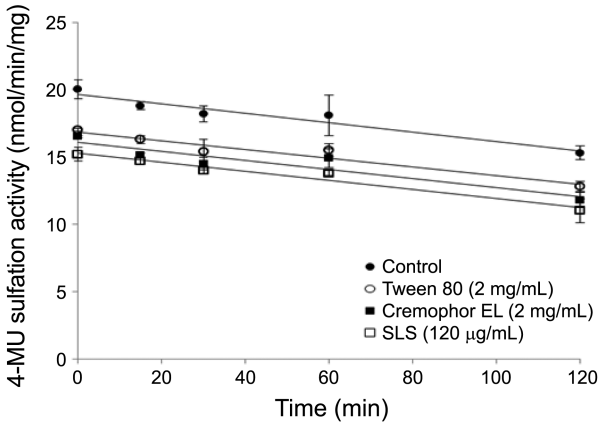

Figure 4. Stability profiles of aryl SULT in the presence of selected pharmaceutical excipients.

Enzyme reaction kinetics as a function of excipient concentrations was studied for Tween 80, Cremophor EL and SLS. 4-MU was incubated in the presence of PEs at different concentrations. Incubation concentration range of Tween 80 and Cremophor EL was 0.25-2 mg/mL while the range of SLS was 15-120 μg/mL because of low solubility in ethanol used as a solvent for PEs. The kinetic parameters such as K_m and V_{max} were estimated by Lineweaver-Burk plot:[23]

$$\frac{1}{V} = \frac{K_m + [S]}{V_{max}[S]} = \frac{K_m}{V_{max}[S]} + \frac{1}{V_{max}} \quad (1)$$

where, V is the reaction velocity and K_m, the Michaelis-Menten constant, is the concentration of substrate at which the halves of active sites of enzyme are filled. V_{max} is the maximum rate of enzyme catalysis reaction, and [S] is the substrate concentration. This equation shows that a plot of 1/V *versus* 1/[S] yields a straight line with an intercept on the vertical axis of $1/V_{max}$ and a slope of K_m/V_{max}. V_{max} can be obtained from $1/V_{max}$, the intercept on Y-axis line and K_m/V_{max} is known as slope. Therefore, K_m can be calculated. Based on the V_{max} and K_m, K_i values, the values of inhibitor constants, were determined by regression analysis of secondary plots:

$$\frac{K_{m\,app}}{V_{max\,app}} = \frac{K_m[I]}{K_i \cdot V_{max}} + \frac{K_m}{V_{max}}$$

where, the values of $K_{m\,app}$ and $V_{max\,app}$ represent the values of K_m and V_{max} in the presence of PEs, [I]. K_i value was determined from the intercept on the X-axis line.[24-26]

The stability of aryl sulfotransferase was evaluated by incubating at 0 °C in the presence of Tween 80, Cremophor EL and SLS for 15, 30, 60 and 120 min. After each incubation time, the sulfation was carried out to assay the residual activity with the incubated enzyme samples.

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