Arch Pharm Res Vol 26, No 9, 768-772, 2003

Archives of Pharmacal Research
http://apr.psk.or.kr

Commonly Used Surfactant, Tween 80, Improves Absorption of P-Glycoprotein Substrate, Digoxin, in Rats

Hongjian Zhang, Ming Yao, Richard A. Morrison[1], and Saeho Chong

Department of Metabolism and Pharmacokinetics, Bristol-Myers Squibb Pharmaceutical Research Institute, P. O. Box 4000, Princeton, NJ 08543 and [1]Schering-Plough Research Institute, 2015 Galloping Hill Road, Kenilworth, NJ 07033

(Received July 15, 2003)

Tween 80 (Polysorbate 80) is a hydrophilic nonionic surfactant commonly used as an ingredient in dosing vehicles for pre-clinical *in vivo* studies (*e.g.*, pharmacokinetic studies, etc.). Tween 80 increased apical to basolateral permeability of digoxin in Caco-2 cells suggesting that Tween 80 is an *in vitro* inhibitor of P-gp. The overall objective of the present study was to investigate whether an inhibition of P-gp by Tween 80 can potentially influence *in vivo* absorption of P-gp substrates by evaluating the effect of Tween 80 on the disposition of digoxin (a model P-gp substrate with minimum metabolism) after oral administration in rats. Rats were dosed orally with digoxin (0.2 mg/kg) formulated in ethanol (40%, v/v) and saline mixture with and without Tween 80 (1 or 10%, v/v). Digoxin oral AUC increased 30 and 61% when dosed in 1% and 10% Tween 80, respectively, compared to control ($P<0.05$). To further examine whether the increase in digoxin AUC after oral administration of Tween 80 is due, in part, to a systemic inhibition of digoxin excretion in addition to an inhibition of P-gp in the GI tract, a separate group of rats received digoxin intravenously (0.2 mg/kg) and Tween 80 (10% v/v) orally. No significant changes in digoxin IV AUC was noted when Tween 80 was administered orally. In conclusion, Tween 80 significantly increased digoxin AUC and Cmax after oral administration, and the increased AUC is likely to be due to an inhibition of P-gp in the gut (i.e., improved absorption). Therefore, Tween 80 is likely to improve systemic exposure of P-gp substrates after oral administration. Comparing AUC after oral administration with and without Tween 80 may be a viable strategy in evaluating whether oral absorption of P-gp substrates is potentially limited by P-gp in the gut.

Key words: P-glycoprotein (P-gp), Digoxin, Tween 80, Pharmacokinetics

INTRODUCTION

P-glycoprotein (P-gp), an ATP-dependent efflux transporter, is a 170 kD membrane protein that is localized in the apical surface (or layer) of epithelial tissues or cells (Gottesman and Pastan, 1993). In humans, P-gp is the product of the *MDR1* gene localized in chromosome locus 7q21.1 (Knutsen *et al.*, 1998). Species differences have been suggested in the gene structure and expression, protein structure, and protein functions as efflux pumps in terms of kinetic characteristics such as Km and Vmax (Borst *et al.*, 1999; Litman *et al.*, 2001; Yamazaki *et al.*, 2001). P-gp is widely distributed throughout the body, particularly in the intestinal mucosa, canalicular membranes of the liver, proximal tubule of the kidney, and endothelial cells of the blood-brain barrier (Silverman, 1999). Increasing evidence have suggested that P-gp plays an important role in drug absorption and disposition (Tanigawara, 2000).

During lead optimization of new drug candidates, preclinical (or animal) pharmacokinetic studies are routinely conducted, and favorable outcomes in animal models are necessary to advance the potential drug candidates to clinical development. Depending on physicochemical properties of lead candidates, surfactants or other adjuvants are often used in the dosing vehicle to achieve adequate systemic exposure for *in vivo* efficacy and safety assessment in preclinical animal models. For example, Tween 80 (or polysorbate 80), a hydrophilic nonionic surfactant,

Correspondence to: Saeho Chong, Ph.D., Department of Metabolism and Pharmacokinetics, Bristol-Myers Squibb Pharmaceutical Research Institute, P. O. Box 4000, Princeton, NJ 08543,
Tel: 609-252-4588
E-mail: saeho.chong@bms.com

is the most commonly used surfactant and it has been shown to enhance solubility of compounds leading to increased absorption of drug candidates (Malingre *et al.*, 2001). Tween 80 is also a common excipient in various human dosage forms (van Zuylen *et al.*, 2001). However, recent reports suggested that Tween 80 is capable of enhancing the permeability of numerous drugs *in vitro* in Caco-2 cells (Hugger, *et al.*, 2002) as well as *ex vivo* tissues such as inverted rat intestinal sac (Cornaire *et al.*, 2000). The enhancement of drug permeability is thought to be due to the inhibition of P-gp by Tween 80. As such, P-gp inhibition by Tween 80 raises concerns that the *in vivo* systemic exposure of P-gp substrates may be significantly higher when Tween 80 is used in the dosing vehicle. An artificial increase in drug exposure due to specific dosing vehicles may lead to an incorrect decision on a particular drug candidate.

The purpose of the present study was to investigate whether an inhibition of P-gp by Tween 80 can potentially improve *in vivo* absorption of P-gp substrates by evaluating the effect of Tween 80 on the disposition of digoxin after oral administration in rats. Digoxin is a cardiac glycoside that is widely used for the treatment of congestive heart failure. It is a well-known substrate of rodent and human P-gps and has been shown to interact with P-gp inhibitors in clinical and pre-clinical studies (Verschraagen *et al.*, 1999; Fromm *et al.*, 1999). In addition, digoxin undergoes minimal metabolism which makes it an ideal probe. In the present study, digoxin was administered to rats both intravenously (IV) and orally (PO) in vehicles with or without Tween 80. PO Tween 80 and IV digoxin were also given to rats to further confirm that the P-gp inhibition by Tween 80 was a local effect in the gastrointestinal (GI) tract.

MATERIALS AND METHODS

Chemicals

Tween 80 was purchased from J.T. Baker (Phillipsburg, NJ). Ethanol (absolute-200 proof) was obtained from AAPER Alcohol and Chemical Co. (Shelbyville, KY). Digoxin and internal standard, oleandrin, were obtained from Sigma Chemical (St. Louis, MO). Methanol (Omnisolve, HPLC grade) was purchased from EM Science (Gibbstown, NJ). Rat plasma was purchased from Bioreclamation Inc. (Hicksville, NY). HPLC grade acetonitrile was obtained from Burdick and Jackson (Muskeson, MI). Water was purified by a Mill-Q-System from Millipore Corp. (Milford, MA). Ammonium formate (Avocado Research Chemicals, Ltd., Wordhill, MA) and formic acid (J.T. Baker, Phillipsburg, NJ) were of analytical-grade. Ammonium chloride was obtained from EM Science (Gibbstown, NJ).

Pharmacokinetic studies in rats

Male Sprague-Dawley rats (Body weight 200-250 g) were obtained from Charles River Lab. (Wilmington, MA). All procedures were approved by the Bristol-Myers Squibb Institutional Animal Care and Use Committee. Rats were fasted overnight and housed individually and water was provided *ad lib* throughout the study. Food was allowed approximately 4-h post dose. Dosing vehicle was consisted of 40% ethanol and 60 to 50% saline in the absence or presence of 1-10% Tween 80 (v/v). A minimum of 40% ethanol was needed to provide a clear dosing solution of digoxin. The final dosing volume was 1 mL/kg for IV containing 0.2 mg/mL digoxin (*via* 10-min constant rate infusion) and 5 mL/kg for PO containing 0.04 mg/mL digoxin (*via* oral gavage). The amount of ethanol given did not produce any abnormal behaviors in these rats. Blood samples were collected for 10 h after the dosing. EDTA was used as anticoagulant and approximately 0.2 mL aliquots of blood samples were collected at each time point. Plasma samples were obtained by centrifugation at 4°C and 3000 rpm for 15 min.

LC/MS/MS quantitation of digoxin in rat plasma

Digoxin concentration in rat plasma was determined using a LC/MS/MS method described elsewhere (Yao *et al.*, 2003). In brief, the HPLC column (50×2.0 mm) was a 3-micron YMC ODS AQ analytical column from Waters Corporation (Milford, MA). The column temperature was held at room temperature. The mobile phase consisted of 50:50 (v/v %) acetonitrile and 5 mM ammonium formate (pH 3.4) and was filtered through a 0.2-micron nylon filter before use. Chromatography was performed isocratically at a flow rate of 0.2 mL/min at room temperature.

For detection, a Sciex API3000 LC/MS/MS system with an atmospheric pressure ionization (API) and electrospray inlet in the positive ion-multiple reaction monitoring mode was used. The ions monitored were: precursor→product ions of m/z 798.6 → 651.6 for digoxin and m/z 577.6 → 433.3 for oleandrin, the internal standard (IS). The API-3000 instrument parameters were (arbitrary units): CAD, 4; DP, 46; FP, 230; EP, -10; CE,19; CXP, 32; IS, 5000 and DF, -400. Additional settings, optimized using the heated nebulizer interface, were as follows (arbitrary units): NEB, 11; CUR, 11; TEMP, 500 and NC, 5.

Data analysis

The area under the curve (AUC) of digoxin plasma concentration vs. time profiles and other pharmacokinetic parameters were calculated using Kinetica™ (Inna Phase Phase Corp.). Cmax was experimentally observed values. Statistical analysis was performed with the two-tailed unpaired *t*-test where appropriate. Significance was set at $P<0.05$.

RESULTS AND DISCUSSION

To investigate the effect of Tween 80 on P-gp efflux *in vivo*, digoxin was selected as the model P-gp substrate. Digoxin is a widely studied P-gp substrate for *in vitro* and *in vivo* studies and it is minimally metabolized *in vitro* and *in vivo* (Hinderling *et al.*, 1991; Salphati and Benet, 1999). The primary clearance pathways *in vivo* for digoxin are renal and biliary secretion of the parent drug, with minimal contributions from phase I and II metabolism (Lisalo, 1977; Hedman *et al.*, 1990). A moderate species difference in digoxin efflux *via* P-gp has been demonstrated in the human *MDR1* and mouse *mdr1a* transfected LLC-PK 1 pig kidney epithelial cells (Yamazaki *et al.*, 2001). In isolated colon tissues from rat and human, however, identical kinetics of digoxin efflux transport was observed (Stephens *et al.*, 2001). Drug-drug interactions involving digoxin *via* P-gp inhibition were reported for many known P-gp inhibitors (Verschraagen *et al.*, 1999; Fromm *et al.*, 1999; Hedman *et al.*, 1990; Troutman *et al.*, 2001). In pre-clinical animal species such as the rat, both ketoconazole and PSC 833 have been shown to affect digoxin absorption and disposition (Song *et al.*, 1999; Salphati and Benet, 1998). Collectively, these evidence suggest that digoxin is an ideal P-gp probe substrate and rodents (such as the rat) can be used as a pre-clinical animal model for the assessment of the potential role of P-gp in drug absorption and disposition.

The first study investigated the effect of Tween 80 on digoxin PK after oral administration in vehicles containing Tween 80, and the results are summarized in Fig. 1 and Table I. 10% Tween 80 increased digoxin Cmax and AUC0-10 h by 161% and 61%, respectively. A similar trend was observed in the dosing vehicle containing 1% Tween 80, where digoxin Cmax and AUC0-10h increased by 163% and 30%, respectively. An improved dissolution

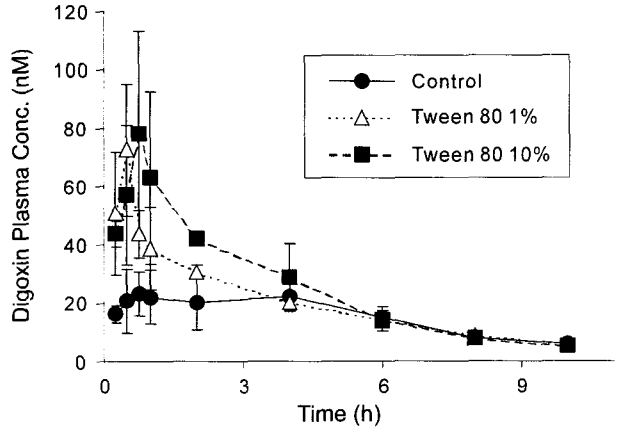

Fig. 1. Concentration *vs.* time profiles of orally administered digoxin (0.2 mg/kg) (mean ± sd, n=3)

Table I. Effect of Tween 80 on the oral pharmacokinetics of digoxin (0.2 mg/kg) in rats (mean ± sd, n=3)

Groups	AUC 0-10 h (nM*h)	Cmax (nM)
No Tween 80 (control)	153.8 ± 18.1	29.9 ± 2.4
Tween 80 (1% v/v)	199.5 ± 18.8*	78.6 ± 15.8**
Tween 80 (10% v/v)	247.9 ± 23.6**	78.1 ± 35.1*

Student *t*-test: * P<0.05, **P<0.01

by adding Tween 80 is not likely because digoxin was completely dissolved in the 40% ethanol and 50% water mixture with or without Tween 80, and the dose of digoxin given was low (0.2 mg/kg). Furthermore, the critical micelle concentration (CMC) of Tween 80 is 0.005% w/v (or ~0.01% v/v). It has been shown that above the CMC, micelles might reduce the thermodynamic activity of drug molecules through solute sequestration, thus reduce the amount of "free" drug molecules for absorption (Nerukar *et al.*, 1997). It is also suggested that above the CMC, the surfactant effect tended to reach a plateau (Batrakova *et al.*, 1998). Although it is difficult to rule out potential *in vivo* "surfactant effect", the results strongly suggest that the increase in digoxin AUC and Cmax is due to P-gp inhibition by Tween 80 in the GI tract, and the inhibitory effect is concentration-dependent.

As a follow up study, the systemic effect of Tween 80 was examined by dosing digoxin in the same vehicle containing Tween 80 intravenously. As shown in Table II, 10% Tween 80 when co-administered intravenously with digoxin increased digoxin IV AUC0-10h by 36% and decreased the clearance of digoxin by 24% from 21.9 to 16.7 mL/min/kg. These results suggest that Tween 80 reduced digoxin clearance systemically, and the reduction is likely due to decreased renal or biliary clearance of digoxin *via* the inhibition of P-gp efflux. The current findings are consistent with previous report that renal or biliary clearance of P-gp substrates (such as vincristine and digoxin) can be significantly reduced by P-gp inhibitors in rats, such as PSC 833 (Song *et al.*, 1999).

Based on the observed systemic effect of Tween 80 on the digoxin clearance, it became unclear whether the

Table II. Effect of Tween 80 on the intravenous pharmacokinetics of digoxin (0.2 mg/kg) in rats (mean ± sd, n=3)

Group	Tween 80 in IV vehicle	Tween 80 in PO vehicle	AUC 0-10h nM*h	CL ml/min/kg
Group 1 (control)	none	none	180.3 ± 18.3	21.9 ± 1.4
Group 2	10%	none	245.3 ± 31.8*	16.7 ± 2.3*
Group 3	none	10%	195.3 ± 56.8	19.7 ± 4.9

Student *t*-test: * P<0.05

effect of Tween 80 on the oral AUC of digoxin is due to inhibition of intestinal P-gp (thereby increasing absorption) or inhibition of P-gp responsible for urinary and biliary clearance (thereby decreasing elimination) of digoxin or in combination of both. To answer this question, the pharmacokinetics of intravenously dosed digoxin was monitored after an oral dose of vehicle containing 10% Tween 80. As summarized in Table II, orally administered Tween 80 did not produced statistically significant difference in digoxin IV AUC. It is generally believed that Tween 80 is not permeable through cell membranes thus its absorption is limited to a minimum. Therefore, the increase in digoxin AUC and Cmax after oral administration is most likely due to P-gp inhibition by Tween 80 in the GI tract.

CONCLUSIONS

The present study demonstrated that Tween 80 improved intestinal absorption of digoxin by inhibiting intestinal P-gp, and it is likely that Tween 80 may produce similar enhancement effect for other P-gp substrates in the GI tract. Consequently, caution should be exercised to interpret pharmacokinetic results when a P-gp substrate is administered in vehicles containing Tween 80. The present study also suggests that comparing AUC after oral administration with and without Tween 80 may be a practical strategy in evaluating whether oral absorption of P-gp substrates is potentially limited by P-gp in the gut.

ACKNOWLEDEGMENTS

The authors are grateful to the Technical Support Unit for their assistance in animal maintenance, dosing and sample collection. We thank Dr. Mingshe Zhu for his support in sample analysis and maintenance of LC/MS/MS.

REFERENCES

Batrakova, E. V., Han, H. Y., Alakhov, V. Y., Miller, D. W., and Kabanov, A. V., Effects of pluronic block copolymers on drug absorption in Caco-2 cell monolayers. *Pharm. Res.*, 15, 850-855 (1998).

Borst, P., Evers, R., Kool, M., and Wijnholds, J., The multidrug resistance protein family. *Biophys. Acta,* 1461, 347-357 (1999).

Cornaire, G., Woodley, J. F., Saivin, S., Legendre, J.Y., Decourt, S. Cloarec, A., and Houin, G., Effect of polyoxyl 35 castor oil and Polysorbate 80 on the intestinal absorption of digoxin *in vitro*. *Arzneimittel-forschung.*, 50, 576-579 (2000).

Fromm, M. F., Kim, R. B., Stein, C. M., Wilkinson, G. R., and Roden, D. M., Inhibition of P-glycoprotein-mediated drug transport: A unifying mechanism to explain the interaction between digoxin and quinidine. *Circulation*, 99, 552-557 (1999).

Gottesman, M. M. and Pastan, I., Biochemistry of multidrug resistance mediated by the multidrug transporter. *Annu. Rev. Biochem.,* 62, 385-427 (1993).

Hedman, A., Angelin, B., Arvidsson, A., Dahlqvist, R., and Nilsson, B.. Interactions in the renal and biliary elimination of digoxin: stereoselective difference between quinine and quinidine. *Clin. Pharmacol. Ther.*, 47, 20-26 (1990).

Hinderling, P. H. and Hartmann, D., Pharmacokinetics of digoxin and main metabolites/ derivatives in healthy humans. *Ther. Drug Monit.*, 13, 381-401 (1991).

Hugger, E. D., Novak, B. L., Burton, P. S., Audus, K. L., and Borchardt, R. T., A comparison of commonly used polyehtoxylated pharmaceutical excipients on their ability to inhibit P-glycoprotein activity *in vitro*. *J. Pharm. Sci.,* 91, 1991-2002 (2002).

Iisalo, E., Clinical pharmacokinetics of digoxin. *Clin. Pharmacokinet.*, 2, 1-16 (1977).

Knutsen, T., Mickley, L. A., Ried, T., Green, E. D., du Manoir, S., Schrock, E., Macville, M., Ning, Y., Robey, R., Polymeropoulos, M., Torres, R., and Fojo, T., Cytogenetic and molecular characterization of random chromosomal rearrangements activating the drug resistance gene, MDR1/P-glycoprotein, in drug-selected cell lines and patients with drug refractory ALL. *Genes Chromosomes Cancer,* 23, 44-54 (1998).

Litman, T., Druley, T. E., Stein, W. D., and Bates, S. E., From MDR to MXR: new understanding of multidrug resistance systems, their properties and clinical significance. *Cell. Mol. Life Sci.* 58, 931-959 (2001).

Malingre, M M., Schellens, J. H., Van Tellingen, O., Ouwehand, M., Bardelmeijer, H. A., Rosing, H., Koopman, F. J., Schot, M. E., Ten Bokkel Huinink, W. W., and Beijnen, J. H., The co-solvent Cremophor EL limits absorption of orally administered paclitaxel in cancer patients. *Br. J. Cancer,* 85, 1472-1477 (2001).

Nerurkar, M. M., Ho, N. F., Burton, P. S., Vidmar, T. J., and Borchardt, R. T., Mechanistic roles of neutral surfactants on concurrent polarized and passive membrane transport a model peptide in Caco-2 cells. *J. Pharm. Sci.*, 86, 813-821 (1997).

Salphati, L. and Benet, L. Z., Effects of ketoconazole on digoxin absorption and disposition in rat. *Pharmacology,* 56, 308-313 (1998).

Salphati, L. and Benet, L. Z., Metabolism of digoxin and digoxigenin digitoxosides in rat liver microsomes: involvement of cytochrome P4503A. *Xenobiotica,* 29, 171-85 (1999).

Silverman, J. A., Multidrug-resistance transporters. *Pharm. Biotechnol.*, 12, 353-86 (1999).

Song, S., Suzuki, H., Kawai, R., and Sugiyama, Y., Effect of PSC 833, a P-glycoprotein modulator, on the disposition of vincristine and digoxin in rats. *Drug Metab. Disp.,* 27, 689-

694 (1999).

Stephens, R. H., O'Neill, C. A., Warhurst, A., Carlson, G. L., Rowland, M., and Warhurst, G., Kinetic profiling of P-glycoprotein-mediated drug efflux in rat and human intestinal epithelia. *J. Pharmacol. Exp. Ther.,* 296, 584-591 (2001).

Tanigawara, Y., Role of P-glycoprotein in drug disposition. *Ther. Drug Monit.,* 22, 137-140 (2000).

Troutman, M. D., Luo, G., Gan, L. S., and Thakker, D. R., The role of P-glycoprotein in drug disposition: significance to drug development. In Rodrigues, A. D. (ed.). Drug-drug interactions. Marcel Dekker, New York. pp. 295-357 (2001).

van Zuylen, L., Verweij, J., and Sparreboom, A., Role of formulation vehicles in taxane pharmacology. *Invest. New Drugs*, 19,125-141 (2001).

Verschraagen, M., Koks, C. H., Schellens, J. H., and Beijnen, J. H., P-glycoprotein system as a determinant of drug interactions: the case of digoxin-verapamil. *Pharmacol. Res.*, 40, 301-306 (1999).

Yamazaki, M., Neway, W. E., Ohe, T., Chen, I., Rowe, J. F., Hochman, J. H., Chiba, M., and Lin, J. H., *In vitro* substrate identification studies for p-glycoprotein-mediated transport: species difference and predictability of *in vivo* results. *J. Pharmacol. Exp. Ther.,* 296, 723-735 (2001).

Yao, M., Zhang, H., Chong, S., Zhu, M., and Morrison, R. A., A rapid and sensitive LC/MS/MS assay for quantitative determination of digoxin in rat plasma. *J. Pharmaceu. Biomed. Anal.*, 32, 1189-1197 (2003)