Department of Pharmacology[1], Faculty of Pharmacy, Istanbul University; Department of Pharmacology[2], Faculty of Pharmacy, Bezmialem Vakif University, Istanbul, Turkey

Pharmacokinetics of talinolol is modified by barnidipine: implication of P-glycoprotein modulation

N. Ozturk[1], D. Ozturk[1, 2], Z. Pala-Kara[1], A. Okyar[1,*]

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**Corresponding author: Alper Okyar, Department of Pharmacology, Faculty of Pharmacy, Istanbul University, 34116 Beyazit-Istanbul, Turkey*
aokyar@istanbul.edu.tr

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Concomitant administration of P-glycoprotein substrates and inhibitors may cause pharmacokinetic drug interactions leading to increased concentrations associated with serious side effects and toxicities. Barnidipine is a long-acting calcium-channel blocker and potent inhibitor of P-glycoprotein *in vitro*, and talinolol is a beta-blocker and probe substrate of P-glycoprotein. This study was designed to investigate the effects of single and repeated oral doses of barnidipine on talinolol pharmacokinetics in rats. In the single-dose study, talinolol (20 mg/kg) alone and with barnidipine at low (1 mg/kg) and high doses (10 mg/kg) were orally administered to rats. In the repeated-dose study, rats were treated with barnidipine (1 mg/kg/day) or vehicle only for four days, then with talinolol (20 mg/kg, on day 5). Blood samples were collected at 0.5, 1, 2, 4, 6 h following last dose and plasma talinolol levels were determined by HPLC. Compared to the control, C_{max} of talinolol elevated 10% ($p=0.79$) and 110% ($p<0.05$); plasma AUC_{0-6h} increased 33% ($p=0.41$) and 46% ($p<0.05$) following low and high single doses of barnidipine co-administration, respectively. In the repeated-dose study, C_{max} and AUC_{0-6h} of talinolol increased 131% ($p<0.05$) and 130% ($p<0.05$) respectively, following co-administration of a low barnidipine dose. Double-peaks were observed when single or repeated low doses of barnidipine were co-administered. There may be coupling between occurrence of double-peak phenomenon and P-glycoprotein inhibition. Increment of talinolol bioavailability upon low and high doses of barnidipine co-administration may be due to P-glycoprotein inhibition. The higher increase of talinolol plasma AUC_{0-6h} due to the repeated doses of barnidipine may be explained by downregulation of P-glycoprotein.

1. Introduction

Polypharmacy is a fairly widespread practice in modern medicine due to the multiple chronic diseases especially in the elderly requiring several medications for management. Concomitant administration of multiple medications may contribute to increases in adverse/toxic effects of drugs leading to undesirable drug interactions. Pharmacokinetic interactions which occur at the levels of absorption, distribution, metabolism and elimination may cause alterations in the concentration and effect of duration of the medications resulting in an increase or decrease in the therapeutic effects of drugs. Clinically significant pharmacokinetic interactions mostly include metabolizing enzymes especially cytochrome P450 (CYP450) and drug transporters which play a role in the uptake or efflux of drugs (Lin and Yamazaki 2003; Padowski and Pollack 2010; König et al 2013). Among the transporters involved in clinically significant drug interactions, P-glycoprotein (P-gp, MDR1, ABCB1) is the most extensively studied and best characterized protein of the ABC (ATP-binding cassette) superfamily due to pivotal role on drug pharmacokinetics and its multidrug resistance (Lin and Yamazaki 2003; Takano et al. 2006; Stavrovskaya and Stromskaya 2008; Montanari and Ecker 2015). P-gp is a transmembrane protein acting as an ATP-dependent efflux pump and functions as a biological barrier by extruding drugs and xenobiotics out of cells (Stavrovskaya and Stromskaya 2008). P-gp was first identified by Juliano and Ling in 1976 as a surface phosphoglycoprotein over-expressed in colchicine-resistant Chinese hamster ovary cells (Juliano and Ling 1976). P-gp which has a wide substrate specificity is highly expressed in normal tissues including the intestine, liver, kidney, brain and placenta as a natural defense mechanism. The anatomical localization of P-gp shows that it plays a significant role in the absorption, distribution and excretion of drugs (Lin and Yamazaki 2003; Takano et al. 2006; Cascorbi 2011). P-gp is localized on the columnar epithelial cells of the intestine and in here it may decrease the intestinal absorption and thus the oral bioavailability of drugs via efflux of P-gp substrates. In addition, it may increase the intestinal excretion of substrate drugs from blood to the intestinal lumen by intestinal secretion (Lin and Yamazaki 2003; Takano et al. 2006; Padowski and Pollack 2010). P-gp is also localized on the canalicular surface of hepatocytes in the liver and on the apical surface of epithelial cells of the proximal tubules in the kidneys. In the liver and kidney, it facilitates the excretion of drugs into the bile and urine, respectively, and it may contribute to the drug elimination and detoxification (Schinkel and Jonker 2003; Montanari and Ecker 2015).

Barnidipine (BAR) which is used orally in the treatment of mild to moderate hypertension, is a selective and long-acting new generation dihydropyridine-type calcium channel blocker with high potency and lipophilic character (Malhotrave and Plosker 2001; Liau 2005). *In vitro* studies, carried out so far, have shown that BAR is a substrate and potent inhibitor of CYP3A4 and P-gp (Katoh et al. 2000a, b; Takara et al. 2002; Komoto et al. 2007). Strikingly, in some of the *in vitro* studies it was reported that BAR caused downregulation of P-gp mRNA expression within three days of exposure beside the direct inhibitory effect on P-gp (Takara et al. 2002; Komoto et al. 2007). Inhibitory effects of BAR on P-gp and CYP3A4 may lead to drug interactions when co-administered with the substrates of this transporter and enzyme, and cause changes in the pharmacokinetic profiles of substrate drugs. There is not any study available on the literature regarding the effect of BAR on the oral bioavailability and pharmacokinetic profiles of P-gp substrates *in vivo*. It should be noted that most of the P-gp substrates are also subtsrates of the main metabolizing enzyme CYP3A4, and thus these extensively overlapping substrate specificities make it difficult to distinguish the underlying mechanism of the observed effect when a drug-drug interaction occurred between the substrates of these proteins (Lin and Yamazaki 2003; Takano et al. 2006). Talinolol (TAL) is a long-acting, highly selective β1-adrenoceptor antagonist without intrinsic sympathomimetic and membrane stabilizing activity (Trausch et al. 1995; Wetterich et al. 1996). TAL, a probe substrate of P-gp, is frequently used in the investigational studies on P-gp-related processes with the advantages of very low metabolic clearance (<1%; that means negligible metabolism via CYP3A4), broad therapeutic range with well tolerability, low protein binding (approximately 25%) beside the sensitivity of its kinetics for the changes in P-gp expression and functionality in rodents (Wetterich et al. 1996; Spahn-Langguth et al. 1998; Oswald et al. 2011).

The present *in vivo* study was designed to investigate the effects of both single and repeated doses of BAR on TAL pharmacokinetics depending on efflux pump P-gp in rats. We also aimed to investigate the drug-drug interaction potential between BAR as a P-gp inhibitor and TAL as a model P-gp substrate.

2. Investigations and results

Co-administration of TAL with BAR in all the studied groups resulted in increased bioavailability and maximum plasma concentrations as compared to control groups. The extent of TAL absorption was nearly the same in both control groups. The plasma concentration-time profiles of TAL belong to the single-dose and repeated-dose studies are shown in Fig. 1A and Fig. 1B, respectively. Likewise, $AUC_{0\text{-}6h}$ values and C_{max} of TAL for the single-dose and repeated dose studies are shown in Fig. 2A and Fig. 2B, respectively. In the single-dose study, concomitant administration of TAL with SHB caused a faster absorption, higher C_{max} and $AUC_{0\text{-}6h}$ values of TAL compared with the control (C_S). Co-administration of TAL with SLB and SHB increased the peak plasma TAL concentrations by about 10.3% ($p=0.79$) and 110.3% ($p<0.05$) when compared to C_S, respectively. Concomitant administration of TAL with SLB and SHB increased the $AUC_{0\text{-}6h}$ values of TAL by about 33.3% ($p=0.41$) and 46.2% ($p<0.05$) as compared to C_S, respectively.

In the repeated-dose study, C_{max} and $AUC_{0\text{-}6h}$ values of TAL were significantly higher in the presence of BAR. C_{max} and $AUC_{0\text{-}6h}$ values of TAL increased by 131.0% ($p<0.05$) and 129.8% ($p<0.05$) as compared to the control (C_R) respectively, when co-administered with RLB. C_{max} and $AUC_{0\text{-}6h}$ values of TAL also significantly increased by 125.6% ($p<0.05$) and 71.0% ($p<0.05$) when TAL was co-administered with RLB compared to SLB, respectively. Calculated kinetic parameters $AUC_{0\text{-}6h}$, AUC_{total}, C_{max}, t_{max}, k_{el}, $t_{1/2}$, V_d and CL/F are shown in Table 1.

TAL peak in the plasma concentration-time curve in both control groups of single and repeated-dose studies occurred 2 h after oral TAL administration. Double-peak phenomenon was observed in the concentration-time profiles of TAL when co-administered with SLB and RLB. The double-peaks appeared at 0.5 h (t_{max1}) and 4 h (t_{max2}) following administration. When TAL was co-administered with SLB, the first peak of plasma TAL concentration was 0.37±0.12 μg/ml at 0.5 h, and the second peak was 0.43±0.08 μg/ml at 4 h. In the repeated-dose study delayed TAL absorption was observed. The double-peak phenomenon appeared with a first peak at 0.5 h (0.16±0.03 μg/ml) and a higher second peak at 4 h (0.97±0.17 μg/ml; C_{max}). In contrast, when TAL was co-administered with SHB, the peak of TAL plasma concentration shifted to shorter values of t_{max} (0.5 h) as compared to C_S (2 h) with only one great peak (0.82±0.14 μg/ml; C_{max}).

Concomitant administration of TAL with SHB caused a slower elimination of TAL. Elimination half-life of TAL increased by 23.6% when co-administered with SHB compared to C_S. Nevertheless, the difference was not statistically significant. Total body clearance and volume of distribution of TAL belong to the single-dose and repeated-dose study results are shown in Fig. 3A and Fig. 3B, respectively. In the single-dose study, oral clearance of TAL decreased by 32.8% and 42.9% when co-administered with SLB and SHB as compared to C_S, respectively. In the repeated-dose study, oral clearance reduced by 52.3% when co-administered with RLB compared with C_R. However, these differences did not reach statistical significance because of the high variability of the respective data. Volume of distribution of TAL decreased by 48.5% when co-administered with RLB as compared to C_R without statistically significant difference.

3. Discussion

Drug-drug interactions continue to be a major problem of polypharmacy which is defined as the concurrent use of multiple medications especially in the management of chronic illnesses such as cardiovascular diseases, often with undesirable outcomes. Clinically relevant pharmacokinetic drug interactions mostly occur through the metabolizing enzymes especially CYP450 and drug transporters which play role in the uptake or efflux of drugs (Lin and Yamazaki 2003; Padowski and Pollack 2010; König et al. 2013). The induction or inhibition of CYP450 isoenzymes and transporters by the involved drugs when used concurrently may alter the pharmacokinetic profile of these drugs and may lead to altered pharmacodynamic effects. Thus, drug-drug interactions may cause toxicity and/or enhanced adverse effects due to the increased drug levels or lead to therapeutic failure as a result of decreased drug levels. *In vitro* studies, carried out so far, have shown that BAR is a substrate and potent inhibitor of CYP3A4 and P-gp (Katoh et

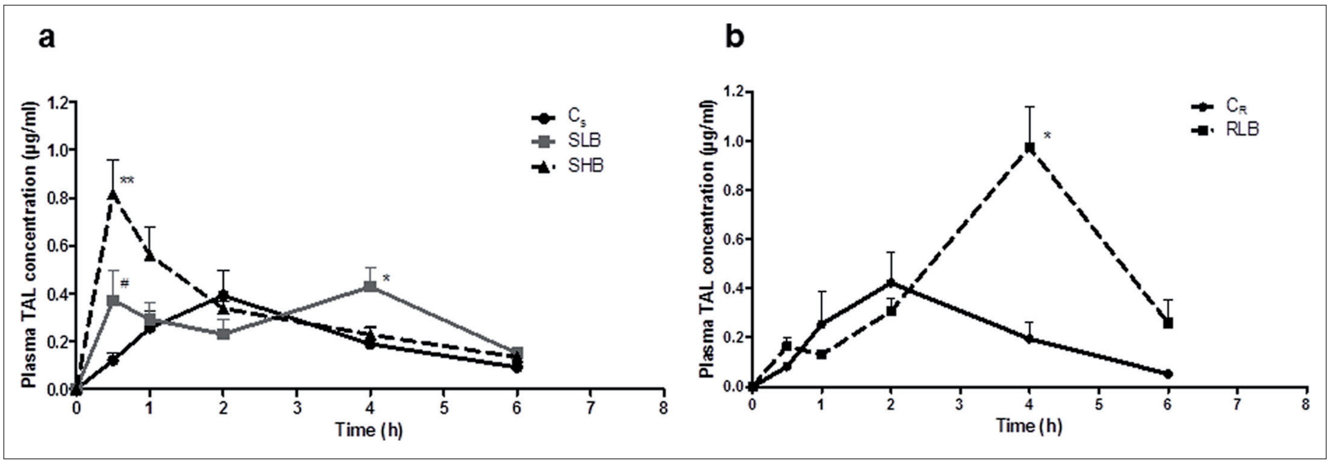

Fig. 1: Mean serum concentration-time profiles of TAL (20 mg/kg, p.o.) **(A)** Single dose study. C_S: Control of single dose study, TAL alone (20 mg/kg, p.o.), SLB: Single Low Dose of BAR (1 mg/kg, p.o.) with TAL (20 mg/kg, p.o.), SHB: Single High Dose of BAR (10 mg/kg, p.o.) with TAL (20 mg/kg, p.o.) **(B)** Repeated dose study. C_R: Control of repeated dose study, TAL alone (20 mg/kg, p.o.), RLB: Repeated Low Dose of BAR (1 mg/kg, p.o.) with TAL (20 mg/kg, p.o.). $^{*,\#}$; $p<0.05$, **; $p<0.01$. *,**; presents the statistically significant difference as compared to control. $^{\#}$; indicates significant difference between SLB and SHB.

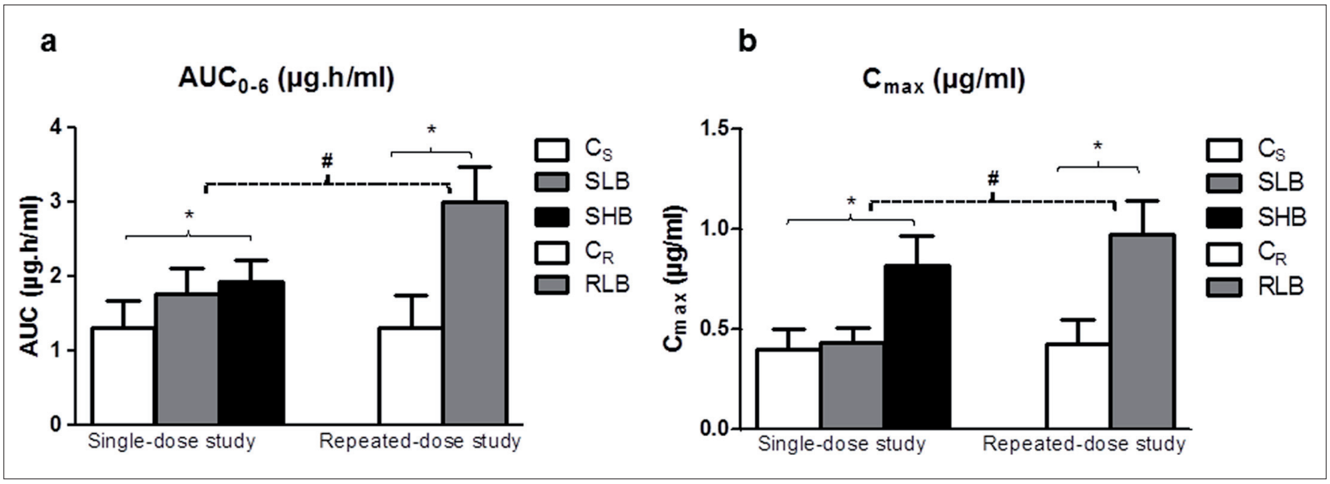

Fig. 2: Plasma $AUC_{0–6h}$ **(A)** and C_{max} **(B)** values of TAL in single-dose and repeated-dose studies. C_S: Control of single dose study, TAL alone (20 mg/kg, p.o.); C_R: Control of repeated dose study, TAL alone (20 mg/kg, p.o.), SLB: Single Low Dose of BAR (1 mg/kg, p.o.) with TAL (20 mg/kg, p.o.), SHB: Single High Dose of BAR (10 mg/kg, p.o.) with TAL (20 mg/kg, p.o.), RLB: Repeated Low Dose of BAR (1 mg/kg, p.o.) with TAL (20 mg/kg, p.o.). $^{*,\#}$; $p<0.05$. *; presents the statistically significant difference as compared to control. $^{\#}$; indicates significant difference between SLB and SHB.

Table 1: Plasma pharmacokinetic parameters calculated after oral administration of TAL (20 mg/kg) alone or with low (1 mg/kg) and high (10 mg/kg) doses of BAR in single or repeated doses

Pharmacokinetic Parameters	Single-Dose Study			Repeated-Dose Study	
	C_S	SLB	SHB	C_R	RLB
$AUC_{0–6h}$ (µg·h/mL)	1.32 ± 0.37	1.76 ± 0.35^{Σ}	1.93 ± 0.28*	1.31 ± 0.43	3.01 ± $0.40^{\#}$
AUC_{total} (µg·h/mL)	1.60 ± 0.44	2.15 ± 0.34^{Σ}	2.44 ± 0.34	1.41 ± 0.45	3.64 ± $0.58^{\#}$
C_{max} (µg/mL)	0.39 ± 0.10	0.43 ± $0.08^{\Sigma, a}$	0.82 ± 0.14*	0.42 ± 0.12	0.97 ± $0.17^{\#,a}$
k_{el} (h^{-1})	0.35 ± 0.06	0.41 ± 0.06	0.26 ± 0.02	0,.50 ± 0.03	0.64 ± 0.09
t_{max}	2	0.5 – 4^{a}	0.5	2	0.5 – 4^{a}
$t_{1/2}$ (h)	2.16 ± 0.40	1.83 ± 0.33	2.67 ± 0.15	1.40 ± 0.09	1.14 ± 0.14
V_d (L)	7.80 ± 1.42	7.25 ± 2.69	8.48 ± 1.50	6.47 ± 1.48	3.33 ± 0.78
CL/F (L/h)	3.78 ± 0.84	2.54 ± 0.45	2.16 ± 0.29	4.89 ± 1.61	2.33 ± 0.86

Data are expressed as means±SEM. TAL: Talinolol, BAR: Barnidipine, F: Bioavailability, C_S: Control of the single-dose study, TAL alone (20 mg/kg) ; C_R: Control of the repeated-dose study, TAL alone (20 mg/kg) ; SLB: Single Low Dose of BAR (1 mg/kg, p.o.) with TAL (20 mg/kg), SHB: Single High Dose of BAR (10 mg/kg; p.o.) with TAL (20 mg/kg), RLB: Repeated Low Dose BAR (1 mg/kg, p.o., 5 days) with TAL (20 mg/kg). n=4 per each group. *; $p<0.05$ C_S vs SHB $^{\Sigma}$; $p<0.05$ C_S vs SLB $^{\#}$; $p<0.05$ C_R vs RLB. a; represent the t_{max} and corresponding C_{max} values.

al. 2000a, b; Takara et al. 2002; Komoto et al. 2007). Strikingly, some *in vitro* studies reported that BAR caused downregulation in P-gp mRNA expression within three days of exposure beside the direct inhibitory effect on P-gp (Takara et al. 2002; Komoto et al. 2007). In our study, concurrent administration of BAR and TAL caused irregular profiles in the plasma concentration-time curves of TAL. Bioavailability of TAL was significantly increased when co-administered with SHB or RLB in spite of the minor changes with the SLB. When the plasma concentration-time curves of the control groups were examined, TAL exhibited similar pharmacokinetic profile with our previous findings which is characterized by a rapid increase and a peak of plasma drug concentration in the second hour after administration (Lennernas and Regardh 1993a). Whereas BAR substantially altered the pharmacokinetic profile of TAL when co-administered with single and repeated dose. When SLB was orally co-administered with single dose of TAL to rats, double-peak phenomenon appeared in the plasma concentration-time curve of TAL. Double-peaks were observed after 0.5 h and 4 h with TAL when co-administered with SLB. Co-administration of TAL with RLB also caused an erratic and delayed absorption of TAL characterized with one small peak at 0.5 h and a quite higher second peak at 4 h in the plasma concentration-time curve.

The rational for the double-peak phenomenon has been attributed to various physiological and physicochemical factors including intestinal pH (Mummaneni et al.1995; Piyapolrungroj et al. 2000), delayed or variable gastric emptying (Orlando et al. 1992), gastrointestinal transit rate or variability in the intestinal motility (Lennernas and Regardh 1993a; Ogiso et al. 2001), inter-segmental differences in transporter expressions and activity along the rat small intestine (Wada et al. 2013), effects of food (Nasiri-Toosi et al. 2012), enterohepatic recycling (Orlando et al. 1992; Wada et al. 2013), bile acids (Lennernas and Regardh 1993b) or failure of dosage forms (Nasiri-Toosi et al. 2012). We performed the current study with over-night fasted rats; therefore, effects of food may be ruled out or minimized. Moreover, the reason of this double-peak phenomenon may not be the enterohepatic recirculation of TAL since only a part of the drug previously absorbed is present for reabsorption. Secondly, TAL undergoes only minor enterohepatic recirculation, namely biliary secretion of the intravenous dose of TAL is less than 10% so it is unlikely to be the cause of this higher second peak (Terhaag et al. 1989; Weitschies et al. 2005; Oswald et al. 2011). A study of Weitschies et al. (2005) documented that TAL absorption was not influenced by fractioned gastric emptying, as well. Therefore this factor was excluded as a cause of double peak phenomenon or erratic and delayed absorption of TAL. Weitschies et al. (2005) also suggested a presystemic storage compartment model to explain the observed double-peak phenomenon of orally administered TAL in their studies. On the other hand, Wada et al. (2013) have reported the role of inter-segmental differences in P-gp expression and activity along the rat small intestine in causing the double-peak phenomenon in the plasma concentration time profiles of substrate drugs. Among the several factors which cause double peak phenomenon or delayed and erratic absorption of drugs, inter-segmental differences in P-gp expression and function along the small intestine must be taken into account seriously. Since TAL is a P-gp substrate, site-dependent absorption

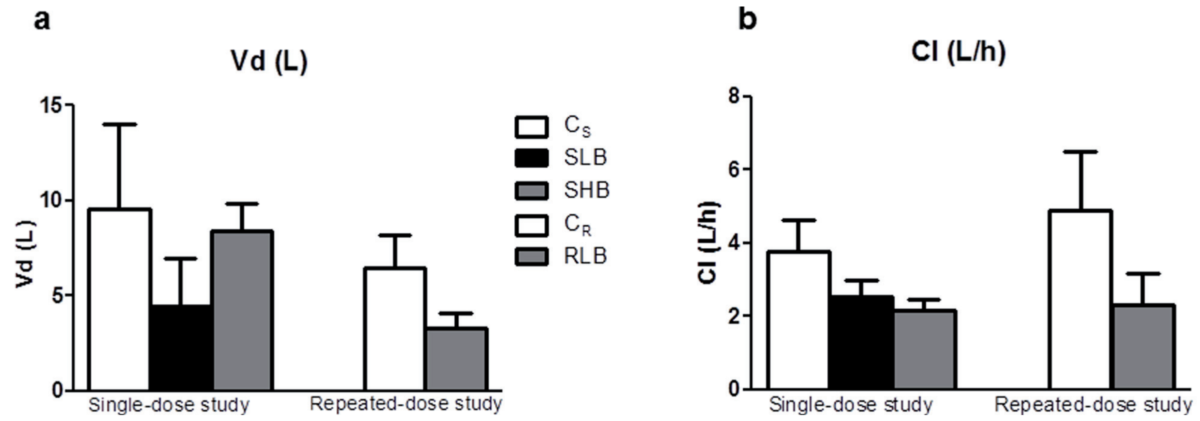

Fig. 3: Volume of distribution, V_d (**A**) and Clearance, Cl (**B**) values of TAL in-single dose and repeated-dose studies. C_S: Control of single dose study, TAL alone (20 mg/kg, p.o.); C_R: Control of repeated dose study, TAL alone (20 mg/kg, p.o.), SLB: Single Low Dose of BAR (1 mg/kg, p.o.) with TAL (20 mg/kg, p.o.), SHB: Single High Dose of BAR (10 mg/kg, p.o.) with TAL (20 mg/kg, p.o.), RLB: Repeated Low Dose of BAR (1 mg/kg, p.o.) with TAL (20 mg/kg, p.o.).

of TAL is a common process. Many studies have been performed so far related to regional expression and activity of P-gp, and in these studies, it has been shown that P-gp expression and/or activity were increased longitudinally throughout the small intestine (Stephens et al. 2002; Mouly and Paine 2003; Ogihara et al. 2006; Lin et al. 2007; Maclean et al. 2008). In a detailed study conducted by Wada et al. (2013), maximum P-gp mRNA expression was also observed at the upper and terminal ileum, and maximum P-gp activity parallel with the P-gp protein level was found in the middle of the ileum in rats. Moreover, Maclean et al. (2008) showed in their studies that P-gp *mdr1a* mRNA expression (one of the P-gp isoforms in rats and mice) and corresponding protein levels increased along the intestine from duodenum to ileum. In this context, intestinal secretion of TAL by P-gp may contribute to the double-peak phenomenon and may be the cause of the decreased oral bioavailability of this drug, as well. Site-dependent absorption and intestinal secretion processes of TAL have been investigated by Gramatte et al. (1996). They reported that TAL absorption was decreased towards the distal segments of the small intestine due to the increasing expression of the efflux pump P-gp. Therefore, in our study, the second peaks observed at 4 h may be the result of the inhibition of P-gp whose expression was much greater in the distal sites of the small intestine than the one in the proximal sites. A possible binding to bile acids may also contribute to the second higher peak of TAL in the concentration-time curve beside site-dependent absorption of TAL associated with the regional differences in P-gp expression and/or function. Lennernas and Regardh (1993b) reported that the beta-blocker agent pafenolol formed micellar complexes with bile acids in the proximal sites of the small intestine that prevented absorption of the drug in these segments and these micellar complexes dissociated in the distal ileum leading to the major second peak of pafenolol in the plasma concentration-time curve. Double peaks were only observed when TAL was co-administered with SLB and RLB, not in the control groups. Therefore it is more likely to explain the rational of this phenomenon through the inhibition of P-gp.

Interestingly, concomitant administration of TAL to rats with SHB shifted TAL plasma concentration peak time to shorter values (t_{max}= 0.5 h) while it was 2 h when TAL was administered alone. Co-administration of TAL with SHB increased both the extent and rate of TAL absorption. The second peak was not existent due to the higher first peak when TAL was co-administered with SHB. It is thought to be related to the faster and enhanced absorption of TAL due to a strong inhibition of the majority of P-gp by high dose of BAR or the saturation of this efflux pump in the gastrointestinal segments. It should be considered that P-gp shows substantial intra- and inter-individual variability in both mRNA and protein levels of P-gp in the rat intestine, especially in the case of protein level (Maclean et al. 2008) and thus make it difficult to generalize the site dependent effects of P-gp on the absorption of substrate drugs and cause great standard deviations in C_{max} and AUC values. The inter-individual variability for P-gp was found higher in the distal segments (Maclean et al. 2008). In one site, inhibition of P-gp in the intestinal segments by BAR causes an increased absorption and bioavailability of TAL, on the other site, BAR may lead to reduced elimination rates of TAL by the inhibition of P-gp in the elimination organs, such as the liver and kidneys. P-gp inhibition may increase the elimination half-lives of the substrate drugs by decreasing hepatobiliary excretion or the clearance of the substrates in the proximal tubules of kidneys. It was reported that about 60% of TAL and metabolites were excreted by kidney and about 40% extrarenally, mainly in faeces (Trausch et al. 1995).

Our results show that elimination half-life of TAL was increased by the concurrent administration of SHB. In addition, oral clearance of TAL was decreased upon BAR co-administration when compared with the control in all the studied groups. Due to the saturability of the efflux process by P-gp, a dose-dependent apparent oral clearance was observed which decreased upon increasing the oral single dose of BAR. Volume of distribution of TAL was also decreased as compared to the control upon co-administration with RLB. The differences between groups were not statistically significant due to the large standard deviation however a marked effect of P-gp inhibition through BAR could be observed on these pharmacokinetic parameters associated with the elimination and distribution processes of TAL. It was clearly observed that in all the studied groups, BAR-mediated P-gp inhibition increased the TAL AUC and C_{max} values to some extent. Increments in the TAL AUC and C_{max} were statistically significant when TAL co-administered with SHB and RLB. TAL bioavailability increased dose-dependently by single oral administration of BAR. As an important point it should be emphasized that repeated low dose of BAR significantly increased C_{max} and AUC values of TAL as compared to the control more than the single low dose of BAR. This dramatic difference between the SLB and RLB effect on TAL bioavailability may be explained by the downregulation of P-gp mRNA through the prolonged exposure to BAR. It should be pointed out that an interaction between TAL and BAR through the CYP3A4 isoenzyme is negligible due to the very low metabolic clearance of TAL. Besides the efflux transporters, uptake transporters have an important role in the determination of orally used drug pharmacokinetics via managing absorption processes and bioavailability. Expressions of these transporters in the intestine and liver affect how much drug will get into the body after an oral dose. It should be considered that the uptake transporter Oatp1a5 (Organic anion-transporting polypeptide (Oatp)-homolog of human OATP2B1) which is responsible for TAL uptake may overshadow the effect of P-gp and thus influence the TAL absorption as reported in recent studies (Schwarz et al. 1999, 2001). Nevertheless, there is no literature data concerned with the possible inhibitory effect of BAR on

Oatp1a5 uptake transporters in the intestine and liver. Therefore the role of Oatp1a5 in the occurrence of double-peak phenomenon is unknown.

In summary, the findings of our study indicated that concurrent administration of TAL with BAR caused different plasma concentration-time profiles and increased bioavailability of TAL in rats. It is likely that site-dependent expression of P-gp throughout the small intestine and inhibition of this transporter by the orally administered P-gp modulating agent BAR may be the reason for different plasma concentration-time profiles of TAL. Increment of TAL bioavailability upon both low and high doses of BAR co-administration may be due to P-gp inhibition. Higher increase in TAL bioavailability due to the repeated doses of BAR may be explained by downregulation of P-gp. It may be suggested that BAR played an important role in the occurrence of double-peaks in the plasma concentration-time profile of TAL. Namely, we could say that there is a coupling between the occurrence of the double-peak phenomenon and the inhibition of P-gp which shows regional differences in the expression and protein levels in the intestine.

In conclusion, our results suggest that BAR-related drug interactions at P-gp level may cause changes in pharmacokinetics, especially in drug absorption from the intestines, and may result in unexpected side effects, especially for the treatment of chronic heart diseases in elderly patients who are often exposed to polypharmacy. In any case, further studies are needed for a better understanding of the altered pharmacokinetic profile and observed double-peak phenomenon of TAL.

4. Experimental

4.1. Animals and housing

All the experiments were conducted in accordance with the guidelines approved for animal experimental procedures by the Istanbul University Animal Experiments Local Ethics Committee (24/09/2009, No. 111). Male Wistar rats (200-250 g) were obtained from Istanbul University Institute of Experimental Medicine. Rats were housed in polystyrene cages up to four animals in the room equipped with temperature control (22±1°C) and humidity (55±5%). Animals were kept under 12/12 h light/dark cycle (LD 12:12) throughout the experiments with water *ad libitum*. Food was also available *ad libitum* prior to any intervention, except for 12 h before and 3 h after the last TAL administration. Drug administrations were performed around 9:00 am to avoid time dependent differences.

4.2. Drugs and reagents

TAL was kindly provided by Arzneimittelwerk Dresden GmbH (Radebeul-Germany) and BAR was purchased from EuroAsisas (USA). TAL and BAR drugs were prepared freshly in 0.5% methyl cellulose (Colorcon, USA) on each study day. Unless otherwise stated, all other chemicals, solvents and reagents were purchased from Merck (Darmstadt, Germany).

4.3. Experimental design

Experiments were conducted in two arms: single-dose study and repeated-dose study. In the single-dose study, rats were divided into three groups each consisting of four animals: Control (C_S), Single Low Dose of BAR (SLB), and Single High Dose of BAR (SHB). In the repeated-dose study, rats were divided into two groups each comprising four animals: Control (C_R) and Repeated Low Dose of BAR (RLB) (Table 2). In the single-dose study, 20 mg/kg TAL (p.o.) alone (C_S) or with BAR at low dose (1 mg/kg, p.o., SLB) and with BAR at high dose (10 mg/kg, p.o., SHB) were administered to rats via oral gavage. In the repeated-dose study, rats were treated with BAR (1 mg/kg/day, p.o., RLB) for four days then with TAL (20 mg/kg, p.o.) on day 5, and rats in the control group (C_R) were treated with vehicle for four days and then TAL alone on day 5. Blood samples were collected from the orbital venous sinus under light inhalation anesthesia at 0, 0.5, 1, 2, 4, and 6 h after administration of TAL, and were stored at -20 °C until analysis. Plasma TAL levels were determined by High Performance Liquid Chromatography (HPLC) as described below.

4.4. Bioanalytical analysis

TAL concentrations in rat plasma were determined by HPLC using the method modified from Oertel et al. (1994). Chromatographic conditions for the determination of TAL in the plasma by HPLC are given in Table 3. Extraction of TAL from rat plasma was performed by using hydrophilic-lipophilic balance type (HLB) Oasis® 1cc/30 mg solid phase extraction cartridges (Waters, USA). Extraction was carried out with VacElut (Varian, USA) sample preparation product and vacuum pump (KNF, Germany). The pressure of the vacuum pump was adjusted to 0.1 bar and it was not allowed to pass the liquid from the cartridges faster than 1 ml/min. First, the cartridges were preconditioned with 1 ml of methanol and followed by 1 ml of HPLC grade distilled water. Rat plasma containing TAL was then passed through the cartridge. Then, cartridges were rinsed with 1 ml of HPLC grade distilled water containing 5% methanol (95:5, v/v). They were allowed to dry for two minutes. The tubes containing the dirty solvents were removed. TAL was taken to clean tubes with 1 ml of methanol and 0.2 ml of this solution was transferred into a glass insert for auto-samples vials. The quantification limit was 50 ng/ml. The standard calibration curve was linear within the range of 50-2000 ng/ml. Variability of the assay was in the range of 3-7%, and no interfering peak was detected during the analysis related to BAR.

Table 2: Experimental design

Study	Experimental groups	Treatment (TAL/BAR)
Single dose of BAR	C_S	20 mg/kg (p.o.) single dose of TAL
	SLB	20 mg/kg (p.o.) single dose of TAL + 1 mg/kg (p.o.) single dose of BAR
	SHB	20 mg/kg (p.o.) single dose of TAL + 10 mg/kg (p.o.) single dose of BAR
Repeated dose of BAR	C_R	20 mg/kg (p.o.) single dose of TAL on day 5 + Vehicle (p.o.) for 5 days
	RLB	20 mg/kg (p.o.) single dose of TAL on day 5 + 1 mg/kg (p.o.) BAR for 5 days

TAL: Talinolol, BAR: Barnidipine, C_S: Control of the single-dose study, C_R: Control of the repeated-dose study, SLB: Single Low Dose of Barnidipine, SHB: Single High Dose of Barnidipine, RLB: Repeated Low Dose of Barnidipine

Table 3: Chromatographic conditions for the determination of TAL from rat plasma

Parameters	Conditions
Column	Symmetry® 250 mm x 4 mm (5 µm) C_{18} reverse phase column
Pre-column	Symmetry® Sentry® 2.1 x 3.9 mm C_{18} reverse phase pre-column
Mobile phase	0.05 M phosphate buffer : acetonitrile = 73:27 (v:v); pH=4
Flow rate	1 ml/min
Column temperature	40ºC
Injection volume	20 µl
Dedector	2487 dual wavelength UV/VIS dedector
Wavelength	242 nm
Retention time	9.5 min
Extraction	Solid phase extraction cartridge (Oasis® 1 cc/30 mg. Waters)

4.5. Pharmacokinetic analysis

Plasma concentrations of TAL were calculated from the standard calibration curve. Peak plasma concentration (C_{max}) and time to peak concentration (t_{max}) values were directly obtained from the TAL plasma concentration-time curve. The double peaks of TAL were expressed as t_{max1} and t_{max2}. The area under the plasma concentration-time curve from 0 to 6h (AUC_{0-6h}) was calculated by the trapezoidal method. $AUC_{0-\infty}$ was obtained by addition of the extrapolated part after the last sampling time using standard techniques. Other pharmacokinetic parameters of TAL were calculated by the non-compartmental method. Elimination rate constant (k_{el}) was calculated from the terminal points of TAL plasma concentration-time plot and the slope of this line was equal to k_{el}. Terminal elimination half-life ($t_{1/2}$) was calculated by log-linear approximation of terminal points of the data. Terminal elimination half-life and elimination rate constant (k_{el}) were interconverted with the following formula: $t_{1/2} = \ln 2 / k_{el}$

The apparent total systemic clearance after per oral dosage (CL/F) was calculated from dose/AUC_{total} and the apparent volume of distribution (V_d) from CL/k_{el}.

4.6. Statistical analysis

Data were expressed as means ± standard error of means (SEM) for each studied variable. Statistical analyses were performed using GraphPad Prism 5.00 for Windows (GraphPad Software, San Diego, California, USA). The statistical significance of differences between groups were validated with Student's t-test and one- or two-way analysis of variance (ANOVA), following Dunnett's or Bonferroni post hoc tests.

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References

Cascorbi I (2011) P-glycoprotein: Tissue distribution, substrates, and functional consequences of genetic variations. In: Fromm MF, Kim RB (eds.) Drug Transporters, Springer Berlin Heidelberg, pp 261-283.

Gramatte T, Oertel R, Terhaag B, Kirch W (1996) Direct demonstration of small intestinal secretion and site-dependent absorption of the beta-blocker talinolol in humans. Clin Pharmacol Ther 59:541-549.

Juliano RL, Ling V (1976) A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. Biochim Biophys Acta 455:152-162.

Katoh M, Nakajima M, Shimada N, Yamazaki H, Yokoi T (2000a) Inhibition of human cytochrome P450 enzymes by 1,4-dihydropyridine calcium antagonists: prediction of in vivo drug-drug interactions. Eur J Clin Pharmacol 55: 843-852.

Katoh M, Nakajima M, Yamazaki H, Yokoi T (2000b) Inhibitory potencies of 1,4-dihydropyridine calcium antagonists to P-glycoprotein-mediated transport: comparison with the effects on CYP3A4. Pharm Res 17: 1189-1197.

Komoto C, Nakamura T, Yamamori M, Ohmoto N, Kobayashi H, Kuwahara A, Nishiguchi K, Takara K, Tanigawara Y, Okamura N, Okumura K, Sakaeda T (2007) Reversal effects of Ca^{2+} antagonists on multidrug resistance via down-regulation of MDR1 mRNA. Kobe J Med Sci 53: 355-363.

König J, Müller F, Fromm MF (2013) Transporters and drug-drug interactions: important determinants of drug disposition and effects. Pharmacol Rev 65: 944–966.

Lennernas H, Regardh CG (1993a) Regional gastrointestinal absorption of the beta-blocker pafenolol in the rat and intestinal transit rate determined by movement of ^{14}C-polyethylene glycol (PEG) 4000. Pharm Res 10: 130–135.

Lennernas H, Regardh CG (1993b) Evidence for an interaction between the beta-blocker pafenolol and bile salts in the intestinal lumen of the rat leading to dose-dependent oral absorption and double peaks in the plasma concentration-time profile. Pharm Res 10: 879–883.

Liau CS (2005) Barnidipine: a new calcium channel blocker for hypertension treatment. Expert Rev Cardiovasc Ther 3: 207-213.

Lin JH, Yamazaki M (2003) Role of P-glycoprotein in pharmacokinetics: clinical implications. Clin Pharmacokinet 42: 59-98.

Lin Y, Shen Q, Katsumi H, Okada N, Fujita T, Jiang X, Yamamoto A (2007) Effects of Labrasol and other pharmaceutical excipients on the intestinal transport and absorption of rhodamine123, a P-glycoprotein substrate, in rats. Biol Pharm Bull 30: 1301-1307.

MacLean C, Moenning U, Reichel A, Fricker G (2008) Closing the gaps: a full scan of the intestinal expression of p-glycoprotein, breast cancer resistance protein, and multidrug resistance-associated protein 2 in male and female rats. Drug Metab Dispos 36: 1249-1254.

Malhotrave HS, Plosker GL (2001) Barnidipine. Drugs 61(7):989-996. doi: 10.2165/00003495-200161070-00007

Montanari F, Ecker GF (2015) Prediction of drug–ABC-transporter interaction - Recent advances and future challenges. Adv Drug Deliver Rev 86: 17–26.

Mouly S, Paine MF (2003) P-Glycoprotein increases from proximal to distal regions of human small intestine. Pharm Res 20: 1595–1599.

Mummaneni V, Amidon GL, Dressman JB (1995) Gastric pH influences the appearance of double-peaks in the plasma concentration time profiles of cimetidine after oral administration in dogs. Pharm Res 12: 780–786.

Nasiri-Toosi Z, Dashti-Khavidaki S, Nasiri-Toosi M, Khalili H, Jafarian A, Irajian H, Abdollahi A, Sadrai S (2012) Clinical pharmacokinetics of oral versus sublingual administration of tacrolimus in adult liver transplant recipients. Exp Clin Transplant 10: 586-591.

Oertel R, Richter K, Trausch B, Berndt A, Gramatte T, Kirch W (1994) Elucidation of the structure of talinolol metabolites in man. Determination of talinolol and hydroxylated talinolol metabolites in urine and analysis of talinolol in serum. J Chromatogr B 660: 353-363.

Ogihara T, Kamiya M, Ozawa M, Fujita T, Yamamoto A, Yamashita S, Ohnishi S, Isomura Y. (2006) What kinds of substrates show P-glycoprotein-dependent intestinal absorption? Comparison of verapamil with vinblastine. Drug Metab Pharmacokinet 21: 238-244.

Ogiso T, Kasutani M, Tanaka H, Iwaki M, Tanino T (2001) Pharmacokinetics of epinastine and a possible mechanism for double peaks in oral plasma concentration profiles. Biol Pharm Bull 24: 790–794.

Orlando R, Sawadogo A, Miglioli PA, Cappellazzo MG, Palatini P (1992) Oral disposition kinetics of ofloxacin in patients with compensated liver cirrhosis. Chemotherapy 38: 1–6.

Oswald S, Terhaag B, Siegmund W (2011) In vivo probes of drug transport: commonly used probe drugs to assess function of intestinal P-glycoprotein (ABCB1) in humans. In: Fromm MF, Kim RB (eds.) Drug Transporters, Springer Berlin Heidelberg, pp 403-447.

Padowski JM, Pollack GM (2010) Pharmacokinetic and pharmacodynamic implications of P-glycoprotein modulation. Methods Mol Biol 596: 359-384.

Piyapolrungroj N, Zhou YS, Li C, Liu G, Zimmermann E, Fleisher D (2000) Cimetidine absorption and elimination in rat small intestine. Drug Metab Dispos 28: 65–72.

Schinkel AH, Jonker JW (2003) Mammalian drug efflux transporters of the ATP binding cassette (ABC) family: an overview. Adv Drug Deliver Rev 55: 3-29.

Spahn-Langguth H, Baktır G, Radschuweit A, Okyar A, Terhaag B, Ader P, Hanafy A, Langguth P (1998) P-glycoprotein transporters and the gastrointestinal tract: evaluation of the potential in vivo relevance of in vitro data employing talinolol as model compound. Inter J Clin Pharm Ther 36: 16-24.

Schwarz UI, Dresser GK, Oertel R, Kim RB (2001) Talinolol–verapamil interaction is not solely due to P-glycoprotein inhibition. Clin Pharmacol Ther 69: P85.

Schwarz UI, Gramatté T, Krappweis J, Berndt A, Oertel R, von Richter O, Kirch W (1999) Unexpected effect of verapamil on oral bioavailability of the beta-blocker talinolol in humans. Clin Pharmacol Ther 65: 283-290.

Stavrovskaya AA, Stromskaya TP (2008) Transport proteins of the ABC family and multidrug resistance of tumor cells. Biochem (Mosc) 73: 592-604.

Stephens RH, Tanianis-Hughes J, Higgs NB, Humphrey M, Warhurst G (2002) Region-dependent modulation of intestinal permeability by drug efflux transporters: In vitro studies in mdr1a(−/−) mouse intestine. J Pharmacol Exp Ther 303: 1095–1101.

Takano M, Yumoto R, Murakami T (2006) Expression and function of efflux drug transporters in the intestine. Pharmacol Ther 109: 137-161.

Takara K, Sakaeda T, Tanigawara Y, Nishiguchi K, Ohmoto N, Horinouchi M, Komada F, Ohnishi N, Yokoyama T, Okumura K (2002) Effects of 12 Ca^{2+} antagonists on multidrug resistance, MDR1-mediated transport and MDR1 mRNA expression. Eur J Pharm Sci 16: 159-165.

Terhaag B, Gramatté T, Richter K, Voss J, Feller K (1989) The biliary elimination of the selective beta-receptor blocking drug talinolol in man. Int J Clin Pharmacol Ther Toxicol 27: 170-172.

Trausch B, Oertel R, Richter K, Gramatte T (1995) Disposition and bioavailability of the β_1-adrenoceptor antagonist talinolol in man. Biopharm Drug Dispos 16: 403-414.

Wada S, Kano T, Mita S, Idota Y, Morimoto K, Yamashita F, Ogihara T (2013) The role of inter-segmental differences in P-glycoprotein expression and activity along the rat small intestine in causing the double-peak phenomenon of substrate plasma concentration. Drug Metab Pharmacokinet 28: 98-103.

Weitschies W, Bernsdorf A, Giessmann T, Zschiesche M, Modess C, Hartmann V, Mrazek C, Wegner D, Nagel S, Siegmund W (2005) The talinolol double-peak phenomenon is likely caused by presystemic processing after uptake from gut lumen. Pharm Res 22: 728-735.

Wetterich U, Spahn-Langguth H, Mutschler E, Terhaag B, Rösch W, Langguth P (1996) Evidence for intestinal secretion as an additional clearance pathway of talinolol enantiomers: concentration- and dose-dependent absorption in vitro and in vivo. Pharm Res 13: 514-522.