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Effects of atorvastatin on talinolol absorption: A potential drug-drug interaction

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In this study, we aimed to determine the drug-drug interaction potential between atorvastatin (ATOR), and talinolol (TAL). Concentration-dependent effects of ATOR on the intestinal permeability of TAL were investigated by an *in situ* intestinal perfusion method. Dose-dependent effects of ATOR on TAL exposure were evaluated by measuring plasma concentrations after oral administration in rats. ATOR slightly changed the intestinal secretion of TAL in jejunum but not in colon. Plasma AUC levels of TAL were elevated by co-administration of ATOR at low and high doses whereas medium doses of ATOR resulted in a decrease in TAL bioavailability. However, these changes were not statistically significant. In our study, the pharmacokinetics of TAL were not affected by the concurrent use of ATOR in rats. In conclusion, it should be considered that complex interplay between the efflux and uptake transporters in the tissues and inhibition of these transporters by modulating agents may overshadow individual effects of each other.

1. Introduction

Polypharmacy is increasingly common, especially among elderly patients who often present with multiple chronic diseases and medical conditions. Pharmacokinetic interactions may cause alterations in bioavailability, disposition and elimination of medications resulting in an increase or decrease in the therapeutic effects of drugs. Since many drugs are administered orally, it is of clinical interest to define the mechanisms affecting pharmacokinetics especially intestinal absorption and disposition of drugs. While considering the physiological factors and physicochemical properties of drugs, drug transporters play an important role in the oral absorption and hereby pharmacokinetics of drugs. Transporters can be classified basically as influx and efflux proteins which are responsible for the uptake of drugs into cells and export of drugs or metabolites out of cells, respectively (Konig et al. 2013, Montanari and Ecker 2015). P-glycoprotein (P-gp, MDR1, ABCB1) is well known as an efflux transport protein and a member of ABC superfamily membrane proteins that function as an ATP dependent molecular pump. It is already known that inhibition of P-gp-dependent intestinal secretion may cause increased bioavailability of certain drugs (Hanafy et al. 2001, Wagner et al. 2001). Organic anion-transporting polypeptides (OATPs) are transmembrane transporters that mediate the cellular uptake of a broad range of drugs and conjugates. OATP1A and OATP1B (OATP1a5 homolog in rats) are expressed in the small intestine and liver where they have an important role in oral absorption of drugs (Kalliokoski and Niemi 2009, Tang et al. 2013).

Atorvastatin (ATOR) is a 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor with an oral bioavailability of 14% in humans. ATOR is a well-known CYP3A4 and P-gp substrate and inhibits ATPase activity of P-gp (Holtzman et al. 2006). OATP2B1 is a high-affinity transport protein for ATOR and disposition of ATOR in the liver is affected by OATP proteins (Grube et al. 2006, Higgins et al. 2014).

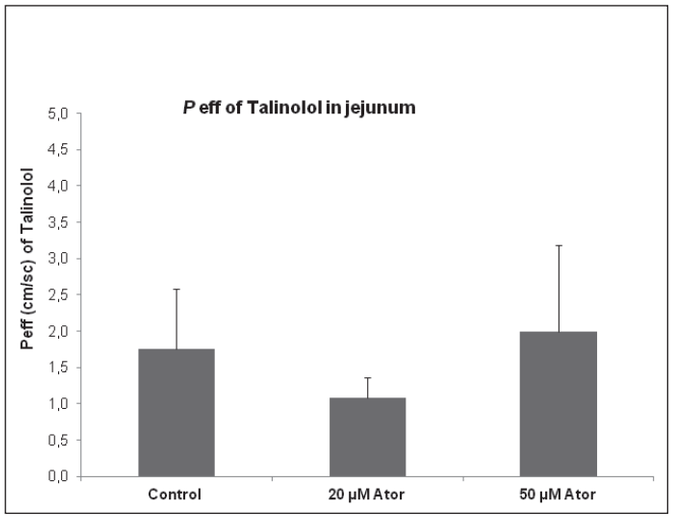
Talinolol (TAL) is a long-acting, highly selective beta-1 blocker and antihypertensive agent (de Mey et al. 1995). TAL is a probe substrate of P-gp and frequently used in the investigational studies on P-gp-related processes because of very low metabolic clearance (<1%; that means negligible metabolism via CYP3A4, mainly unchanged renal and biliary clearance), broad therapeutic range with well tolerability, low protein binding (approximately 25%) and sensitivity of its kinetics for the changes in transporter expression and functionality in rodents (Trausch et al. 1995, Wetterich et al. 1996, Spahn-Langguth et al. 1998, Hanafy et al. 2001). TAL was also shown to be a substrate for rat OATP1a5 like P-gp, which are localized at the apical membrane of enterocytes (Shirasaka et al. 2009).

Based on literature data, it was hypothesized that ATOR present in the intestinal lumen might exhibit interactions with uptake and efflux transporters. Uptake and efflux inhibitory effects of ATOR have not been investigated by using *in situ* intestinal perfusion technique in rats yet and there are limited number of studies evaluating the interactions between ATOR and functional *in vivo* probes of these transporters like TAL. Beta-blockers and other cardiac medications that are transported by P-gp are commonly prescribed together with ATOR in cardiovascular diseases. The aim of the present study was to investigate the effect of ATOR on the exposure of TAL in rats to examine a drug-drug interaction potential between P-gp and OATP1a5 substrate/inhibitor ATOR and TAL.

2. Investigations and results

2.1. In situ studies

Effects of ATOR at different concentrations on the effective intestinal permeability (P_{eff}) of TAL were investigated in the jejunum and colon with *in situ* intestinal perfusion technique. P_{eff} value of TAL was decreased by 39% with ATOR at 20 µM, and in the final concentration of 50 µM ATOR, *Peff* value of TAL was slightly increased by 14.2% in the jejunum. In the colon *Peff* of TAL was almost two times higher than the one in the jejunum. *Peff* of TAL was not affected by ATOR co-administration at both concentrations in the colon (Fig. 1). However, all these increments were not statistically significant.



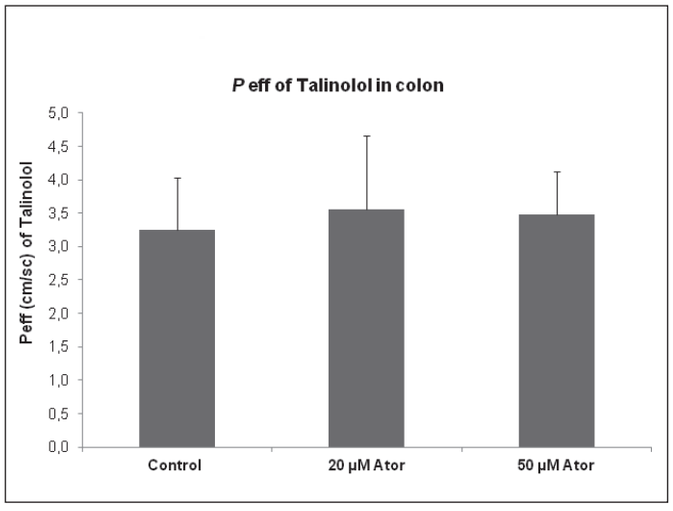


Fig. 1: P eff values as obtained from the average permeabilities of five rats' intestine i.e. jejunal segment (A) and colon (B): Peff (mean ±SEM) in the absence (control) and presence of ATOR (20 and 50 µM) in the perfusion solution. No statistically significant differences was found for both in jejunum and colon in the presence of ATOR.

2.2. In vivo studies

The plasma concentration-time profiles of TAL with and without ATOR are shown in Fig. 2**.** Plasma concentrations of TAL elevated upon all ATOR co-administrations in the samples collected 2 h following administration whereas the influence was no longer obvious after 8 h, e.g. for the control group, 0.45±0.05 µg/ml at 2 h, and 0.05±0.03 µg/ml at 8 h. Generally, plasma levels of TAL were under the quantification limit at 8 h. When compared to the control group, low, medium and high dose ATOR co-administrations lead to the elevated maximum plasma levels of TAL by 44.4%, 13.3%, 60.0% at 2h (i.e. C_{max}), respectively. Nevertheless, these increments were not statistically significant as compared to control group (ANOVA, p= 0.396).

Low and high ATOR co-administrations increased the TAL plasma $AUC_{0\text{-}8h}$ levels by 18.3% and 13.9% respectively; whereas, medium dose ATOR co-administration reduced plasma $AUC_{0\text{-}8h}$ levels by 13.9% when compared with control group. Likewise, with the plasma levels, no significant difference was observed in $AUC_{0\text{-}8h}$ parameters (Table).

Table: Plasma C_{max} and $AUC_{0\text{-}8h}$ levels after oral administration of talinolol (20 mg/kg) alone or with various atorvastatin doses

PK Parameters	TAL	TAL + ATOR (L)	TAL + ATOR (M)	TAL + ATOR (H)
$AUC_{0\text{-}8h}$ (µg·h/mL)	1.8 ± 0.38	2.13 ± 0.53	1.55 ± 0.49	2.05 ± 0.39
C_{max} (µg/mL)	0.45 ± 0.05	0.65 ± 0.15	0.51 ± 0.15	0.72 ± 0.11

Data are expressed means±SEM. L: Low dose atorvastatin (p.o.10 mg/kg), M: Medium dose atorvastatin (p.o. 50 mg/kg) and H: High dose atorvastatin (p.o. 100 mg/kg). Each data point is mean of five Wistar rats.

Plasma $AUC_{0\text{-}8h}$ values which were calculated from the concentrations at the respective time points are shown in Fig. 3.

3. Discussion

The prescription of more than one drug as combination therapy is increasingly common in current medical practice. For example, cholesterol-lowering agents such as HMG-CoA reductase inhibitors may be co-administered with antihypertensive agents for the treatment of hypertension. Considering that drugs used in combination therapy often share their metabolic or cellular transport pathways, there is a high potential for pharmacokinetic and pharmacodynamic drug interactions between antihypertensive agents and HMG-CoA reductase inhibitors including atorvastatin. Changes occurring in the efflux function of P-gp which plays role as a barrier in oral absorption may affect the pharmacokinetics of drugs. In addition to efflux proteins, uptake proteins which are mainly expressed in the small intestine and liver have a pivotal role in the absorption and distribution of many drugs. Efflux and uptake functions of transporter proteins may be changed by drugs, xenobiotics and food constituents. In particular, changes in the transporter functions by drugs seem to be an important problem of cardiovascular drugs which are generally included in polypharmacy especially in the elderly.

In the oral bioavailability of TAL, uptake transporter OATP1a5 mediating absorption (Shirasaka et al. 2009, 2010) is also substantial in addition with the secretion of drugs with P-gp to the small intestine (Wetterich et al. 1996, Gramatte and Oertel 1999). In our study, absorption of TAL was affected by different concentrations

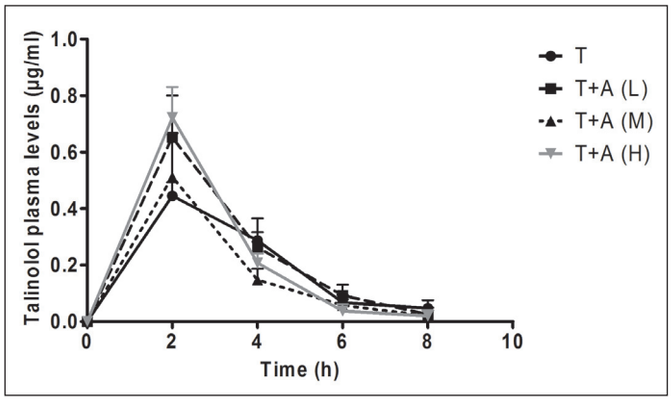


Fig. 2: Plasma concentration-time profiles after oral administration of TAL alone and with ATOR low dose (T+A (L)), medium dose (T+A (M)) and high dose (T+A (H)) coadministration to male Wistar rats. Data are summarized as arithmetical means ±SEM (n= 5).

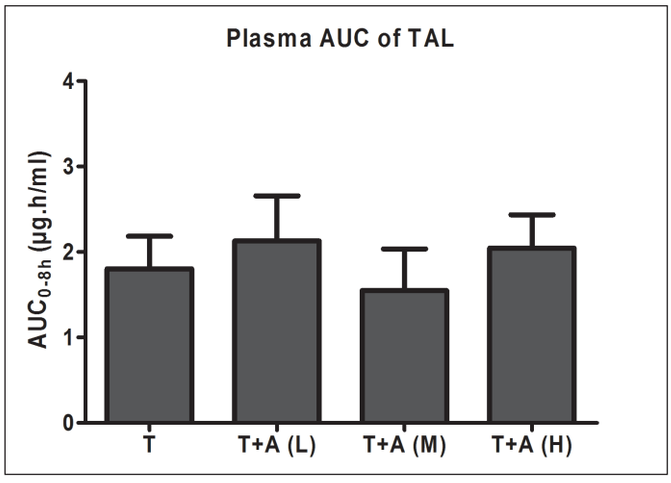


Fig. 3: Average $AUC_{0\text{-}8h}$ values after oral administration of talinolol alone and with ATOR low dose (T+A (L)), medium dose (T+A (M)) and high dose (T+A (H)) in plasma No statistically significant differences was found according to ANOVA, for plasma (p=0,8) $AUC_{0\text{-}8h}$ levels between groups.

of ATOR co-perfusion in the jejunum. While a lower concentration of ATOR leads to the reduced absorption of TAL, higher concentrations caused a slight increase in the *Peff* value of TAL in the jejunum. TAL *Peff* values in the colon were higher than those in the jejunum while no clear effect of ATOR was observed on *Peff* value of TAL in the colon. This difference between TAL *Peff* values in jejunum and colon may be explained by different transporter expressions which are involved in TAL intestinal absorption/secretion throughout the small intestine and colon. P-gp expression is variable along the small intestine from proximal to distal in rats and the highest P-gp expression is shown in the jejunum (Nakayama et al. 2000, Berggren et al. 2007). P-gp expression in the colon is controversial. Some studies show that P-gp expression is lower in the colon compared with the small intestine (Berggren et al. 2007) but some other studies found the opposite (Fojo et al. 1987, Fricker et al. 1996). OATP1a5 mRNA was expressed along the rat intestine with an increasing pattern throughout the small intestine from proximal to distal and a decrease thereafter in the colon (MacLean et al. 2010). Higher TAL *Peff* in the colon than in the jejunum may be explained by a reduced efflux of TAL to lumen due to lower expression of P-gp in colon and no clear ATOR affect were seen on permeability of TAL. ATOR inhibits P-gp ATPase activity in a concentration-dependent manner (Bogman et al. 2001, Yang et al. 2011). It was also shown that human OATP2B1 (homolog of rat OATP1a5) was inhibited by ATOR concentration dependent on *in vitro* conditions (Grube et al. 2006). Higgins et al. (2014) showed that oral exposure of ATOR was significantly increased and liver Kp was significantly decreased in OATP1a/1b-knockout mice relative to wild type mice (Higgins et al. 2014). In the jejunum, OATP1a5 may be inhibited by ATOR at 20 µM concentration dominantly and as a result effective permeability of TAL might be reduced. On the other hand, TAL *Peff* values may be increased predominantly due to P-gp inhibition by ATOR at 50 µM concentration in the jejunum. ATOR is a potent P-gp transport inhibitor *in vitro* despite it has a moderate binding affinity to P-gp (Goard et al. 2010). Shirasaka et al. (2009) has shown that naringin is a potent inhibitor of P-gp and OATP1a5 *in vitro* and effects of naringin on TAL permeability in jejunum was similar with ATOR. In this study, they performed *in situ* intestinal closed loop experiments in rats, showed that TAL *Peff* value was decreased by lower concentration of naringin (200 µM), whereas increased by higher concentration of naringin (2000 µM) in the jejunum. Our results are also similar to the results of Shirasaka et al. (2009) but not statistically significant.

Plasma AUC levels of TAL were elevated when TAL was co-administered with 10 and 100 mg/kg ATOR; however, it was reduced when co-administered with 50 mg/kg ATOR. It is possible that ATOR may have inhibits P-gp in lower doses only and increased plasma AUC level of TAL. On the other hand, 50 mg/kg dose of ATOR may have inhibited P-gp as well as with OATP1a5. TAL AUC value increased when co-administered with ATOR at 50 mg/kg dose. This may be the result of a dominant inhibition or affinity of absorptive transporter OATP1a5. Higher doses of ATOR increased plasma AUC of TAL most probably a dominant inhibition and/or saturation of P-gp. These dose-dependent changes in plasma were correlated with the changes in TAL *Peff* values in jejunum in this study.

There are some studies which are correlated with our *in vivo* results. The mutual drug-drug interaction potential between TAL and verapamil was investigated by Hanafy et al. (2001) and TAL levels were determined in plasma and in some tissues. Verapamil co-administration reduced the AUC values of S- and R-TAL in brain, kidney, liver, and spleen to a different extent, while plasma AUC levels of each enantiomer increased by approx. 50%. The author determined that the interaction potential of TAL with verapamil was fairly complex. According to the author, observed systemic effects may be attributed to the alterations of hemoperfusion, and competitive binding to macromolecules (including P-gp) may also

contribute. Shirasaka et al. (2009) also showed similar results that various doses of naringin affected plasma AUC and C_{max} of TAL differently *in vivo* in rats. Peroral 50 µM dose of naringin reduced the plasma AUC and C_{max} of TAL whereas 200 µM dose increased. These *in vivo* results are compatible with our findings that ATOR and P-gp activity plays a significantly more important role than OATP1a5 activity in the intestinal absorption of TAL in rats. Similar results were seen when digoxin, a P-gp substrate which was employed in P-gp dependent drug-drug interaction studies (clinical), is co-administered with ATOR (MacLean et al. 2010). In this study, concomitant administration of 80 mg ATOR caused an increase in the extent of digoxin absorption. In that context, an interaction between TAL and ATOR through CYP3A4 isoenzyme should be negligible because of the fact that TAL – as opposed to the CYP3A4 substrate ATOR – has a very low metabolic clearance (Trausch et al. 1995).

Our findings show that TAL levels are either decreased or increased by ATOR depending upon the location of the uptake and efflux process within the organism and depending upon the co-administered dose. In general, TAL pharmacokinetics after enteral administration was characterized by a high variability between animals as reported by Kagan et al. (2010) and others (Spahn-Langguth et al. 1998, Konig et al. 2013). P-gp expression shows considerable intra- and interindividual variability, especially at the protein level (MacLean et al. 2008). Therefore, this causes difficulties in demonstrating its direct effect on the pharmacokinetic processes with limited number of experimental animals.

In our study, the exposure of TAL was not affected during co-administration with ATOR in rats. It should be considered that there is a complex interplay between the efflux and uptake transporters in the tissues and inhibition of these transporters by modulating agents may overshadow individual effects of each other. If these results are confirmed in human studies, the cholesterol-lowering HMG-CoA reductase inhibitor ATOR and the beta-blocker TAL may be used as a combination therapy in the cardiovascular diseases without any pharmacokinetic interaction risk at the level of absorption and disposition depending upon uptake and efflux transporters. Whether these pharmacokinetic findings are correlated significantly with the pharmacodynamic effects will be presented with ongoing and future studies.

4. Experimental

4.1. Animals

In vivo and *in situ* experiments were performed on male Wistar rats (180-250 g and 300-350 g, respectively) in overnight fasted state. Animals were accessed to tap water provided *ad libitum*. Light was given by fluorescent lamps and 12/12 light-dark cycle was provided. Animals were kept in cages housing up to four animals. The animal rooms were equipped with temperature control (22±1 °C). All procedures were performed according to the 3R rules i.e. reduction, replacement and refinement accepted by Istanbul University. Preclinical study approvals were obtained from Istanbul University Institute of Health Science Executive Board.

4.2. Drugs and chemical reagents

TAL was kindly provided by the AWD (Arzneimittelwerk Dresden, Radebeul, Germany). ATOR calcium was obtained from Sanovel Pharmaceutical Corporation (Istanbul, Turkey). All other compounds, solvents and reagents were purchased from E. Merck (Darmstadt, Germany) unless stated otherwise. HPLC grade water was obtained from Milli-Q ultrapure water appliance (Millipore, USA).

4.3. In situ studies

Intestinal perfusion studies were performed by using an Ismatec MC-MS CA8/6 peristaltic pump (Ismatec, Switzerland) containing silicone tubes with outer and inner diameters of 4.0 and 2.0mm, respectively.

Male Wistar rats (n= 5, 300-350 g) were fasted overnight prior to surgery, but they had unlimited access to tap water. The surgical procedure was performed according to a method described previously (Doluisio et al. 1969, Hanafy et al. 2001). Anesthesia was induced by intraperitoneal administration of ketamine (40 mg/kg) and xylazine (Rompun™, 10 mg/kg). If necessary, ketamine (10 mg/kg, i.p.) was used to maintain anesthesia during the experiment. The anesthetized rats were placed on 37 °C heating pads to maintain body temperature (C.S., Republic of Korea). Intestinal segments of the rats were exposed to a midline intestinal incision, the selected gut segments (jejunum and colon) were rinsed and cleaned with Tyrode buffer of 37 °C to prevent extensive mucus secretion into the eluate during perfusion, and finally silicon tubes were attached to the two intestinal segments, which were selected for each of the rats (jejunum 5-8 cm, colon 1.5-2 cm).

Tyrode solutions were used as perfusion media as described previously (Hanafy et al. 2001). The perfusate (drug-containing Tyrode buffer) was maintained at 37 °C by keeping it in a water bath and delivered through the intestinal segments simultaneously at a constant flow rate of 0.2 ml/min. At the outlet, perfusate was quantitatively collected at intervals of 5 min and stored at -20 °C until chromatography analysis. Adsorption at the plastic silicon tubes does not occur, it was excluded by blind perfusions (without gut segments), where the respective P_{eff} value was 0 for TAL. A stepwise perfusion was performed, and the total perfusion time of 90 min was divided into two periods, each including a 15 minute-period without monitoring to reach steady-state conditions and a 20 minute-period for TAL *Peff* determination in intervals of 5 min (Hanafy et al. 2001). Perfusions were performed in two steps in each animal at TAL concentration of 10 µM and without and with addition of 20 µM and 50 µM ATOR dose in the sequences given by Hanafy et al. (2001). We did not use an enhancer in perfusion studies at ATOR perfusate concentration. The procedure included the determination of baseline *Peff* and *Peff* upon ATOR co-perfusion in two gut segments of one animal. At the end of the experiment, rats were sacrificed by cervical dislocation.

4.4. In vivo studies

Rats were treated orally via gavage: either with TAL alone (20 mg/kg, control group) or in combination with ATOR at low (10 mg/kg), medium (50 mg/kg), or high (100 mg/kg) doses (n= 5 for each treatment and sampling point). Administered doses of TAL and ATOR were determined according to the literature (Krause and Newton 1995, Spahn-Langguth et al. 1998). Plasma samples were collected just before the experiment and at 2, 4, 6, 8 h after administration. Samples were stored at -20°C until analyses.

4.5. Bioanalytical assays

4.5.1. TAL in Tyrode solution

TAL concentration in perfusate was directly determined by using HPLC system (Waters Alliance® 2690 HPLC module employing Millennium^{32} Chromatography Manager; Waters, USA). HPLC column was Symmetry C_{18} 250x4mm (Waters, USA) and mobile phase contained sodium phosphate buffer, acetonitrile and triethylamine (500 ml and 300 ml and 0.4 m, respectively) at a flow rate of 0.8 ml/min, column temperature at 40 C and double beam UV/VIS detector (2487, Waters, USA) 242 nm as wavelength for eluate monitoring (Wetterich et al. 1996). Retention time of TAL was 7.4 min and the limit of detection was 25 ng/ml. Injection volume of perfusates were 20 µl. Inter-day reproducibilities (RSD) were 1.19%, 2.11%, 2.32% for 5, 1, and 0.25 µg/ml, respectively. Intra-day RSD were 6.43%, 6.66% and 6.07% for 5, 1, and 0.25 µg/ml, respectively. It was shown that ATOR does not interfere with the assay.

4.5.2. TAL in plasma

The method used for determining TAL in plasma was modified from Oertel et al. (1994) and performed with the HPLC system mentioned above. TAL was extracted from plasma by hydrophilic-lipophilic balance type (HLB) solid phase extraction cartridges (Oasis® SPE cartridges 1 cc/30 mg; Waters, USA). The extraction was carried out with VacElut (Varian, USA) sample preparation product and vacuum pump (KNF, Germany). Firstly, cartridges were conditioned with 1 ml methanol and 1 ml HPLC grade water and then 1 ml plasma were passed through the cartridges. Cartridges were rinsed with 1 ml HPLC grade water and methanol (95:5, v/v) for unretained matrix and finally TAL was collected into clean tubes by eluting 1 ml methanol. Phosphate buffer/acetonitrile (73:27, v/v and pH=4) was used as a mobile phase, with a flow rate of 1 ml/min and the column temperature was 40 °C. Retention time of TAL was 9.4 min and the limit of determination was 50 ng/ml. The 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 ATOR.

4.5.3. Calculation of water transport and permeability coefficients

Water transport was quantified by weight and volume measurements. Usually, water transport was in the range of 5 to 10%.

Intestinal permeabilities were calculated on the basis of the mixing tank model using the following equation that was initially described by Sinko et al. (1991):

$$P_{eff} = Q^* [(C_{in}/C_{out})\text{-}1] / 2\pi rl$$

where Q is the flow rate, C_{in} and C_{out} are the respective inlet and outlet concentrations of the drug containing perfusion solution, and r and l are the radius and the length of the corresponding intestinal segment (average radius of the intestinal segments: jejunum 0.21 cm, colon 0.23 cm).

4.6. Pharmacokinetic and statistical analyses

Steady-state average *Peff* values were calculated for each rat and each treatment period (data given as mean±standard error of mean (SEM)). Mean values and their standard deviations for different individuals were calculated from these *Peff* values.

Average concentrations for each time point were used to calculate the area under the curve (AUC) from t= 0 to the last sampling point ($AUC_{0\text{-}8h}$) by the linear trapezoidal rule. The maximum plasma concentrations (C_{max}) were directly determined from the plasma concentration-time curves and expressed as arithmetical mean±SEM.

ANOVA was used for statistical analyses of pharmacokinetic studies. Student t test was used for statistical analyses of *in situ* studies. $P < 0.05$ was considered to be statistically significant.

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