Basic & Clinical Pharmacology & Toxicology, **107**, 847–852 Doi: 10.1111/j.1742-7843.2010.00574.x

Interactions between Verapamil and Digoxin in Langendorff-Perfused Rat Hearts: The Role of Inhibition of P-glycoprotein in the Heart

Mualla Aylin Arici[1], Emrah Kilinc[2], Omer Demir[1], Mehmet Ates[3], Alaattin Yesilyurt[2] and Ayse Gelal[1]

[1]Dokuz Eylul University, School of Medicine, Department of Pharmacology, Izmir, Turkey, [2]Ege University, Faculty of Pharmacy, Department of Analytical Chemistry, Izmir, Turkey, and [3]Dokuz Eylul University, Advanced Professional School of Health Sciences, Izmir, Turkey

(Received 9 October 2009; Accepted 11 February 2010)

Abstract: P-glycoprotein (P-gp) is expressed in tumour cells as well as normal tissues including heart. Modulation of P-gp transport *in vivo* may lead to increased drug penetrance to tissues with resulting increases in toxicity. We aimed to investigate the effects of P-gp on the isolated heart by digoxin infusion in the absence and presence of verapamil. The study was performed in Langendorff isolated perfused rat hearts. After a 20 min. stabilisation period with Tyrode Buffer, digoxin (125 μg/5 mL) was infused for 10 min. in the control group (n = 7). The same dose of digoxin was infused during perfusion with verapamil (1 nM) containing Tyrode Buffer (n = 8) in the study group. Outflow concentration and cardiac parameters of digoxin were measured at frequent intervals for 40 min. $AUEC_{(0–40\ min)}$ for left ventricular developed pressure was significantly increased in the presence of verapamil (4260 ± 39.37 mmHg min *versus* 4607 ± 98.09 mmHg min; 95% CI -587.7 to −105.8; $p = 0.0083$). The significant increases in left ventricular developed pressure were at 20, 25, 30, 35 and 40 min. $AUC_{(0–40\ min)}$ value for outflow digoxin concentration-time curve was significantly lower in the presence of verapamil. Verapamil increased the positive inotropic effect of digoxin, probably through the inhibition of P-gp, which effluxes digoxin out of cardiac cells.

P-glycoprotein (P-gp) is an important ATP-dependent transmembrane protein which acts as an efflux transporter for a wide variety of chemicals [1,2]. Its expression was first described in multidrug resistant tumour cells [3]. Now, it is well-known to be expressed in numerous human and rodent normal tissues including heart [4]. Modulation of P-gp transport *in vivo* may lead to increased drug plasma and tissue concentration with resulting increases in toxicity due to reduced drug elimination. The functional role of P-gp in heart tissue and clinical implication of its modulation have not been well understood. It was proposed that P-gp serves as a functional barrier between blood and cardiac myocytes and it may control intracellular access of drugs to their binding sites and then modulate drug efficacy or toxicity [5]. The absence of P-gp in *mdr1a(−/−)* mice resulted in increased cardiac levels of doxorubicin and doxorubicinol [6]. Combination of doxorubicin, daunorubicin and idarubicin with P-gp inhibitors increased their accumulation and toxicity in the heart [5,6]. Cardiotoxicity in patients was observed with the concomitant administration of verapamil and erythromycin or clarithromycin which are well-known substrates of P-gp [5]. Therefore, a possible mechanism of action would be the increase of drug concentrations in cardiac tissues producing cardiotoxicity due to inhibition of heart P-gp. According to these findings, it was suggested that P-gp might have a protective effect in the heart.

Despite the introduction of a variety of new classes of drugs for the management of heart failure, digoxin continues to have an important role in long-term outpatient management [7]. Digoxin is a well-known probe for investigating the activity of P-gp in humans and rodents [8,9]. It has a narrow therapeutic index. Certain inhibitors of P-gp have been demonstrated to increase digoxin levels due to a decreased renal tubular elimination [10–12]. In some cases of atrial fibrillation, digoxin is used in combination with verapamil [13]. Verapamil is a P-gp inhibitor demonstrating effects on digoxin kinetics and clinical experience suggests that it may increase digoxin toxicity [14]. In Weinhouse's study, digoxin levels were significantly higher in the plasma, heart, liver and muscle of the verapamil pre-treated rats [15].

The aim of the present study was to test the hypothesis that increased effects of digoxin, when used in combination with verapamil, is due to digoxin-verapamil interaction through the inhibition of P-gp-mediated transport in heart tissue. The study was performed in Langendorff isolated perfused rat hearts. If verapamil inhibits cardiac P-gp, pharmacokinetic and pharmacodynamic parameters of digoxin would be altered in isolated heart tissues.

Author for correspondence: Ayse Gelal, Professor of Pharmacology, Dokuz Eylul University Medical Faculty, Department of Pharmacology, 35340 Narlidere, Izmir, Turkey (fax +90 232 2590541, e-mail ayse.gelal@deu.edu.tr).

© 2010 The Authors
Basic & Clinical Pharmacology & Toxicology © 2010 Nordic Pharmacological Society

Materials and Methods

The experimental protocol was approved by the Dokuz Eylul University, School of Medicine Animal Use Committee with Guide for the Care and Use of Laboratory Animals (NIH: Publication no.85–23, revised 1996). The experimental study was performed with isolated hearts of 28 adult female Wistar rats weighing between 200–250 g (232.5 ± 3.7). Rats were fasted but allowed free access to water overnight before the onset of the experiments.

Chemicals Digoxin-Sandoz Ampoule (0.5 mg/2 mL; Novartis, Turkey) and Isoptin Ampoule (5 mg/2 mL; Abbot, Turkey) were purchased from a local pharmacy store. Verapamil was prepared as stock solutions of 10^{-6} M in distilled water. Solvents used for chromatography were methanol and phosphoric acid (HPLC ultra gradient grade) supplied by J.T. Baker and Riedel-de Haen AG, respectively.

Perfused rat heart Rats were stunned by a blow on the neck and performed cervical dislocation to avoid any anaesthetic medication effect. Hearts were excised and immersed immediately into an iced, heparinised modified Tyrode Buffer Solution (in mM; NaCl 128, KCl 4.7, $CaCl_2$ 1.36, $NaHCO_3$ 20, NaH_2PO_4 0.36, $MgCl_2$ 1 and glucose 10), pH 7.4. The heart was cannulated (within 60 sec.) via the ascending aorta and retrograde perfusion was started with an oxygenated (95% O_2 and 5% CO_2) Tyrode Buffer Solution at 37°C. The perfusion was in constant flow condition maintaining a coronary flow 10 mL/min. (Ismatec® SA Labortechnic Analytic, CH-8152 Glattbrugg, Zürich). Tyrode Buffer Solution was filtered (40 μm Microaggregate filter, Bicakcilar, Turkey) to prevent particulate matter from entering the coronary circulation.

A balloon filled with distilled water and connected to a pressure transducer (BPT 300, Commat Ltd, Ankara, Turkey) was inserted into the left ventricle cavity for measurement of left ventricular developed pressure (LVDP=LVSP-LVEDP). The balloon volume was adjusted to maintain a left ventricular end-diastolic pressure (LVEDP) of 10 mmHg. The coronary perfusion pressure was measured by a pressure transducer connected to the aortic cannula. Maximum rates of left ventricular developed pressure development (dp/dt_{max}) were calculated from the left ventricular developed pressure automatically. Heart rate was also calculated from the LVEDP trace. Data were transferred to a Data Acquisition System (BIOPAC, MP30B-CE, 206B1564; USA). Every parameter was recorded continuously during the course of the study.

Experimental protocol Hearts were allowed to equilibrate for 20 min. with Tyrode Buffer Solution. After the stabilisation period, 25 μg/mL digoxin was infused for 10 min. with an infusion pump (Braun Perfusor® compact S, Melsungen, Germany) by a rate of 0.5 mL/min. Hearts were perfused with only Tyrode Buffer Solution or with Tyrode Buffer Solution containing 1 nM verapamil for 40 min., where the same dose of digoxin was infused after the equilibration period.

Coronary perfusion pressure, left ventricular developed pressure, dp/dt_{max} and heart rate were recorded and outflow samples were collected every 1 min. for the first 10 min. and every 5 min. for the remaining 30 min. (total collection period of 40 min.). The outflow samples were kept frozen at −20°C until analysis of digoxin. High performance liquid chromatography (HPLC) was used for the analysis.

Verapamil dose (1 nM) was tested in the preliminary study (n = 3) [6]. The concentration of verapamil did not change the measured cardiac parameters.

Hearts were excluded from the study if they were mechanically unstable or had left ventricular developed pressure <70 mmHg, or arrhythmic.

Determination of digoxin: Mobile phase preparation. The entire mobile phase used for overall liquid chromatographic determinations was a mixture of methanol and 0.01 M *o*-phosphoric acid (H_3PO_4) (50–50%, v/v) with a pH of 2.5. Prepared mobile phase was degassed with an ultrasonication process of 45 min.

Sample preparation Stock solution of digoxin was prepared by dilution of Digoxin-Sandoz Ampoule (0.5 mg/2 mL; Novartis, Turkey) with mobile phase to the desired final concentration value. A series of standard solutions with the final concentration of 0.5 μg/mL (6.40×10^{-7} M), 1, 2, 5, 10, 50, 100 and 200 μg/mL (2.56×10^{-4} M) were used for calibration curve. The relationship between response and analyte concentration was linear in the considered calibration range ($y = 15.955x^{0.9283}$, $R^2 = 0.9989$). Samples collected at various times from perfusion solutions were injected directly into the sample loop after filtration from the 0.45 μm pore size membranes.

Apparatus The liquid chromatographic system (Agilent 1100 series) supplied by SEM Company (Izmir, Turkey) was equipped with a pump (G1310A isocratic pump), a thermostatted column unit (G1316A thermostatted column compartment), a DAD detector (G1315D diode-array detector), a manual injector (G1328A Rheodyne 7725İ) with 20 μL loop and a chromatographic data processing software (Agilent ChemStation for LC Rev. A. 06. 03 [509]). The separation was performed using an octadecyl (C18) analytical column (Hichrom 5 C18, 7.75 × 300 mm, 5 μm particle size).

Chromatographic conditions and detection For the chromatographic detection, a portion (≈ 100 μL) of digoxin standard or perfusion sample was injected into HPLC-DAD system through the 20 μL constant volume sample loop at 21 ± 0.5°C. Separation of digoxin was performed with a flow rate of 1 mL/min. for 20 min. and UV–Vis detection was carried out in accordance with the literature by recording 190–400 nm spectrums [16]. Both solvents were degassed (ELMA LC 30/H ultrasonic bath) for 45 min. before use. Each compound was tentatively identified by its unique retention time under the same conditions. Quantitative determinations were carried out by the external standard method based on peak area. The retention time of digoxin was 13.2 min. The limit of detection and quantification values of the proposed method was calculated as 3.45×10^{-4} μg/L and 1.27×10^{-3} μg/L, respectively.

Pharmacokinetics and pharmacodynamics The time course of amount of digoxin recovered in outflow perfusate (R) was calculated from the outflow concentration *versus* time data, $C(t)$ and perfusate flow (Q), using the equation:

$$R(t) = Q \int_0^{40} C(t)\mathrm{d}t$$

The area under effect curve which measures the changes of digoxin effects on coronary perfusion pressure, left ventricular developed pressure, dp/dt_{max} with time during the first 40 min. was calculated ($AUEC_{0\text{-}40\ min}$) using the equation:

$$AUEC = \int_0^{40} E(t)\mathrm{d}t$$

Statistical analyses Statistical analyses were performed using percent changes in coronary perfusion pressure, left ventricular developed pressure, dp/dt_{max} and heart rate. Data within groups were evaluated by Student's t-test for paired data. Comparisons of differences between the control group and the verapamil group at any particular time period were performed by Student's t-test for unpaired determinations. Mean values (mean ± S.E.M.) and 95% confidence intervals (95% CIs) were presented in the text (GraphPad Instat™, 1990–1994, GraphPad Software V2.05a 9342, San Diego, CA, USA). For all studies, p values of <0.05 were considered to be statistically significant.

© 2010 The Authors

Table 1.

Baseline values of coronary perfusion pressure (CPP), left ventricular developed pressure (LVDP), dp/dt_{max} and heart rate in control and verapamil (1 nM) groups. Control group is the digoxin-infused group; verapamil group is the digoxin-infused group in the presence of verapamil.

Baseline values	Control group (n = 7)	Verapamil group (n = 8)
CPP (mmHg)	89.3 ± 7.2	91.0 ± 8.8
LVDP (mmHg)	88.2 ± 3.9	87.6 ± 4.4
dp/dt_{max} (mmHg)	2000.0 ± 110.8	1846.0 ± 125.4
Heart rate (bpm)	257.1 ± 11.1	258.8 ± 8.3

Results

Pharmacodynamic response of experimental groups

One of the isolated rat hearts from the control group was removed from the study since baseline value of left ventricular developed pressure was low (59.3 mmHg). There were no significant differences among the baseline measurements of both groups in coronary perfusion pressure, left ventricular developed pressure, dp/dt_{max} and heart rate (table 1).

The maximum increase in left ventricular developed pressure after treatment with digoxin was 9.9% ±1.9 for the control group but 21.8% ±3.5 in the verapamil group (fig. 1). In the presence of verapamil, digoxin infusion increased left ventricular developed pressure at 20, 25, 30, 35 and 40 min., significantly when compared to the control group (table 2).

The increase in the coronary perfusion pressure was only significant in the third minute compared to the control groups (119.4 ± 2.7 *versus* 129.1 ± 3.3%, 95% CI-19.04 to −0.34, $p < 0.05$) (fig. 2).

The increase in the dp/dt_{max} was only significant in the 40th min compared to the control groups (98.9 ± 3.7 *versus* 116.0 ± 6.6%, 95% CI -34.2 to −0.2, $p < 0.05$) (fig. 3).

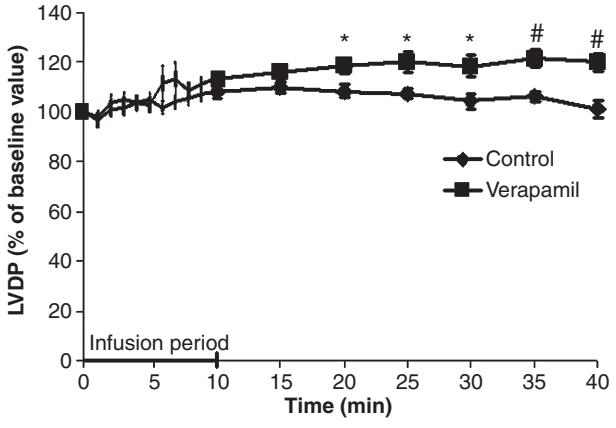

Fig. 1. The effects of digoxin (◆) infusion in Tyrode Buffer Solution and Tyrode Buffer Solution containing verapamil (■ 1 nM) on left ventricular developed pressure (LVDP) (mean±S.E.M.; n = 7, n = 8, respectively). (*), $p < 0.05$; (#), $p < 0.01$. Control group is the digoxin-infused group; verapamil group is the digoxin-infused group in the presence of verapamil.

Table 2.

The effects of digoxin infusion in Tyrode Buffer Solution and Tyrode Buffer Solution containing verapamil (1 nM) on left ventricular developed pressure (LVDP) (mean ± S.E.M.; n = 7, n = 8, respectively). (*), p < 0.05; (**), p < 0.01. Control group is the digoxin-infused group; verapamil group is the digoxin-infused group in the presence of verapamil.

LVDP (mmHg)	Control group (n = 7)	Verapamil group (n = 8)	95% CI
0 min.	88.2 ± 3.9	87.6 ± 4.4	−13.5 to 12.2
10 min. (end of infusion)	95.4 ± 4.9	98.9 ± 4.9	−13.3 to 3.1
15 min.	96.7 ± 3.2	101.5 ± 4.7	−13.6 to 3.4
20 min.	95.4 ± 3.7	103.5 ± 4.6*	−18.6 to −1.9
25 min.	94.8 ± 4.2	105.0 ± 4.9*	−23.2 to −2.5
30 min.	92.3 ± 5.2	103.3 ± 4.9*	−25.8 to −2.2
35 min.	93.6 ± 3.7	106.0 ± 3.9**	−24.6 to −6.4
40 min.	89.5 ± 5.5	104.5 ± 3.9**	−28.9 to −8.7

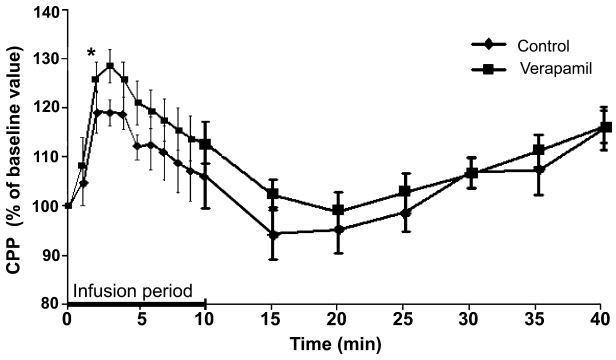

Fig. 2. The effects of digoxin (◆) infusion in Tyrode Buffer Solution and Tyrode Buffer Solution containing verapamil (■ 1 nM) on coronary perfusion pressure (CPP) (mean ± S.E.M.; n = 7, n = 8, respectively). (*), $p < 0.05$. Control group is the digoxin-infused group; verapamil group is the digoxin-infused group in the presence of verapamil.

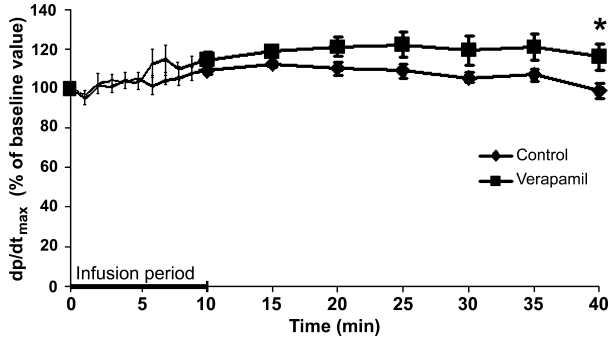

Fig. 3. The effects of digoxin (◆) infusion in Tyrode Buffer Solution and Tyrode Buffer Solution containing verapamil (■ 1 nM) on dp/dt_{max} (mean ± S.E.M.; n = 7, n = 8, respectively). (*), $p < 0.05$. Control group is the digoxin-infused group; verapamil group is the digoxin-infused group in the presence of verapamil.

© 2010 The Authors

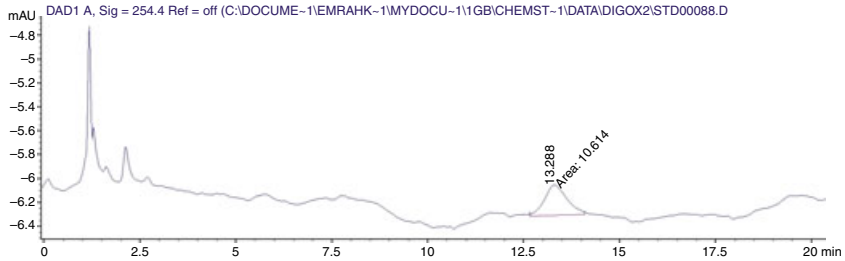

Fig. 4. Typical chromatogram of outflow sample of perfusion solution, experimental conditions as in Materials and Method section.

Heart rates were not different between the groups at any time during the study.

$AUEC_{(0–40\ min)}$ for left ventricular developed pressure was significantly increased in the presence of verapamil (4260 ± 39.37 mmHg.min *versus* 4607 ± 98.09 mmHg.min; 95% CI -587.7 to −105.8; $p = 0.0083$), whereas for coronary perfusion pressure, dp/dt_{max}, and heart rate, there were no differences between the groups.

Pharmacokinetic results

Fig. 4 shows the typical chromatogram of outflow sample of perfusion solution and fig. 5 shows the average outflow concentration-time profiles in the rat hearts for a 10 min. infusion of digoxin. Compared with control, the outflow concentration curve was shifted downward during the infusion period in the presence of verapamil ($p < 0.05$), indicating increased uptake of digoxin. $AUC_{(0\text{-}40\ min)}$ values for digoxin was also significantly lower in the presence of verapamil (19.9 ± 2.3 μg min/mL *versus* 28.5 ± 2.1 μg min/mL; 95% CI 1.7–15.6; $p = 0.0185$).

Recoveries of digoxin in the outflow perfusate (up to 40 min.) were 23.9 ± 1.8 and 16.8 ± 1.9% of the dose in the absence and presence of verapamil, respectively. The difference between the groups was statistically significant ($p < 0.05$).

Discussion

It is suggested that P-gp can protect an organism against toxic xenobiotic compounds and prevent their accumulation in critical organs, such as brain or testis [13]. Analogous to the function of P-gp in the brain, cardiac P-gp has been suggested to serve as a functional barrier between blood and cardiomyocytes, limiting the entry of certain molecules into the heart [17]. There are many studies demonstrating that alteration of P-gp function may explain the variability in treatment response to certain drugs. One prominent example is the variability in treatment effects of digoxin, actively transported by the P-gp, caused by interaction with a second drug that alters transporter activity and thereby affecting bioavailability of digoxin [17,18].

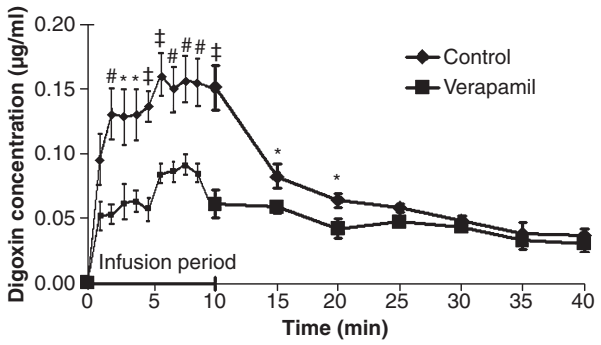

Fig. 5. Outflow concentrations of digoxin. Control group (◆) is the digoxin-infused group; verapamil group (■) is the digoxin-infused group in the presence of verapamil (1 nM)) (n = 7, n = 8, respectively). (*), $p < 0.05$; (†), $p < 0.01$; (‡), $p < 0.001$.

In our study, approximately 24% of the digoxin dose was recovered in the outflow perfusate at the end of the 40 min. perfusion after the 10 min. infusion of digoxin (125 μg), whereas nearly complete recovery of infused doses of digoxin (0.1 μmol, 0.2 μmol, 0.3 μmol; infused for 1 min.) were measured by Kang *et al.* They suggested that the infusion time in their study was not long enough for the cellular accumulation of digoxin [19]. The considerable difference between the percentage of digoxin recoveries in the outflow perfusate of the two studies could be due to two reasons: Firstly, in our study, infusion time was ten times longer than in the study of Kang *et al.* Secondly, our lower recovery level could be explained by the possibility that digoxin might have been converted to unidentified metabolites such as digitoxigenin, which we might have been unable to measure with our current HPLC hardware. To perform detection of such metabolites, mass detector facilities for liquid chromatography are essential and a possible preferred configuration would be LC-MS/MS. Identification of the molecular structures of unidentified peaks of possible metabolites could be the subject of another study [16,20].

On the other hand, recoveries of digoxin in the outflow perfusate were lower in the verapamil group than in the control group in our study. This result supports our hypothesis that verapamil increases the uptake of digoxin in the isolated perfused rat heart through the inhibition of P-gp. In the study of Kang and Weiss, increased accumulation of doxorubicin in mice and rat myocardium was explained by the inhibition of P-gp in the heart tissue [6].

There are some controversial results in *mdr1a* (-/-) knockout mice on tissue distribution of digoxin in the heart compared with wild-type mice. Schinkel *et al.* reported that the absence of *mdr1a* P-gp in mice affects tissue distribution and pharmacokinetics of P-gp substrates including

© 2010 The Authors

dexamethasone, digoxin and cyclosporine A. In their study, *mdr1a* knockout mice had higher cardiac digoxin concentrations compared with wild-type animals, but this enhancement was accompanied by a similar increase in the plasma levels [21]. In the other study, the heart-to-plasma ratio of digoxin was even lower in *mdr1a*-deficient animals compared with wild-type mice [22]. The above findings could be due to the use of knockout animals, since this may mask effects in organs with lower transporter abundance such as the heart [23].

Other studies showed that pre-treatment of the rats with verapamil increases digoxin concentration in the plasma, heart, liver and muscle [14,15]. In these studies, increased digoxin might be due to the inhibition of P-gp at all sites, including kidneys and intestines. In our study, as opposed to the previous studies using *in vivo* methods, we used the isolated Langendorff-perfused method to examine the effects of P-gp specifically on the heart. This approach has the advantage in that the pharmacokinetics and pharmacodynamics can be investigated at the organ level.

In the verapamil group, the increase in left ventricular developed pressure values at the end of infusion was not significantly higher than that of the control group, but the increase reached a significant level at 20th min and lasted until 40th min. At the end of 40th min, the curve did not recover in the verapamil group whereas it returned to baseline value in the control group. The AUEC value for left ventricular developed pressure in the verapamil group was significantly higher than that of the control group. These findings may show that in the verapamil group, positive inotropic effect was increased due to digoxin accumulation in heart tissue. Digoxin exerts its positive inotropic effect through an inhibition of Na^+-K^+ ATPase, leading to a rise in intracellular Na^+ concentration which causes intracellular Ca^{2+} overload via the Na^+-Ca^{2+} exchanger. Digoxin also promotes Ca^{2+} release from sarcoplasmic reticulum by increasing single channel activity of ryanodine receptors (RyR2) [24]. This is an additional action which contributes to its positive inotropic effects. Furthermore, digoxin-induced arrhythmia is also linked to a direct action on RyR2 [25]. The RyR2 receptors are located in the intracellular site of the cardiac cells. Consequently, factors such as P-gp transporters that regulate intracellular concentrations of RyR2 binding drugs could modulate risk of cardiac adverse effects. In our study, we obtained indirect evidence that digoxin concentration in heart tissue in the verapamil group was higher than that in the control group and this increased tissue concentration of digoxin might have activated RyR2 receptors leading to increased positive inotropic effect. The limitation of our study is that we deduced the information regarding cardiac digoxin levels from outflow perfusate concentrations; we could not measure the levels of digoxin in heart tissue directly.

In summary, we evaluated the effect of verapamil on the pharmacokinetics and pharmacodynamics of digoxin in the isolated perfused rat heart. The present study indicates that while verapamil increases the accumulation of digoxin in the rat heart, it potentiates the positive inotropic effects of digoxin. It is probable that the inhibition of P-gp in the heart tissue also contributes to development of adverse effects when digoxin and verapamil are used together.

Acknowledgements

Pharmacodynamic results of this work were previously published as an abstract (Arici A *et al.*, *Basic & Clinical Pharmacology & Toxicology*, 105, p73, 2009); presented at 9th Congress of the European Association for Clinical Pharmacology and Therapeutics, 12–15 July 2009, Edinburgh, UK. The study was presented as a poster in 20th National Congress of Pharmacology, p270, Antalya, Turkey, 4–7 November 2009.

The authors would like to thank Izmir Province Control Laboratory of Ministry of Agriculture and Rural Affairs (Director Ibrahim OZGENC), Turkey, for partial temporary LC hardware support (DAD detector) during the analysis of outflow samples.

This work was supported by Dokuz Eylul University Research Foundation (2009. KB. SAG. 080).

References

1 Matheny CJ, Lamb MW, Brouwer KR, Pollack GM. Pharmacokinetic and pharmacodynamic implications of P-glycoprotein modulation. Pharmacotherapy 2001;**21**:778–96.
2 DuBuske LM. The role of P-glycoprotein and organic anion transporting polypeptides in drug interactions. Drug Saf 2005;**28**: 789–801.
3 Lin LH, Yamazaki M. Role of P-Glycoprotein in Pharmacokinetics. Clinical implications. Clin Pharmacokinet 2003;**42**:59–98.
4 Balayssac D, Authier N, Cayre A, Coudore F.. Does inhibition of P-glycoprotein lead to drug-drug interactions?. Toxicol Lett 2005;**156**:319–29.
5 Couture L, Nash JA, Turgeon J. The ATP-Binding Cassette Transporters and their implication in drug disposition: a special look at the heart. Pharmacol Rev 2006;**58**:244–58.
6 Kang W, Weiss M. Influence of P-glycoprotein modulators on cardiac uptake, metabolism, and effects of idarubicin. Pharm Res 2001;**18**:1535–41.
7 Dec GW. Digoxin remains useful in the management of chronic heart failure. Med Clin North Am 2003;**87**:317–37.
8 De Lannoy IA, Silverman M. The MDR1 gene product, P-glycoprotein, mediates the transport of the cardiac glycoside, digoxin. Biochem Biophys Res Commun 1992;**189**:551–7.
9 Fenner KS, Troutman MD, Kempshall S, Cook JA, Ware JA, Smith DA *et al.* Drug-drug interactions mediated through P-glycoprotein: clinical relevance and in vitro-in vivo correlation using digoxin as a probe drug. Clin Pharmacol Ther 2009;**85**:173–81.
10 Su SF, Huang JD. Inhibition of the intestinal digoxin absorption and exsorption by quinidine. Drug Metab Dispos 1996;**24**:142–7.
11 Fromm MF, Kim RB, Stein CM, Wilkinson GR, Roden DM. Inhibition of P-glycoprotein-mediated drug transport: a unifying mechanism to explain the interaction between digoxin and quinidine. Circulation 1999;**99**:552–7.
12 Ding R, Tayrouz Y, Riedel KD, Burhenne J, Weiss J, Mikus G *et al.* Substantial pharmacokinetic interaction between digoxin and ritonavir in healthy volunteers. Clin Pharmacol Ther 2004;**76**:73–84.
13 Verschraagen M, Koks CH, Schellens JH, Beijnen JH. P-glycoprotein system as a determinant of drug interactions: the case of digoxin-verapamil. Pharmacol Res 1999;**40**:301–6.

© 2010 The Authors

14 Kaplanski J, Weinhouse E, Topaz M, Genchik G. Verapamil and digoxin: interactions in the rat. Res Commun Chem Pathol Pharmacol 1983;**42**:377–88.
15 Weinhouse E, Kaplanski J, Genchik G. Plasma and tissue levels of digoxin in the rat following pretreatment with verapamil. Res Commun Chem Pathol Pharmacol 1985;**47**:469–72.
16 Jedlicka A, Grafnetterova T, Miller V. HPLC method with UV detection for evaluation of digoxin tablet dissolution in acidic medium after solid-phase extraction. J Pharm Biochem Anal 2003;**33**:109–115.
17 Solbach TF, König J, Fromm MF, Zolk O. ATP-binding cassette transporters in the heart. Trends Cardiovasc Med 2006;**16**:7–15.
18 Eberl S, Renner B, Neubert A, Reisig M, Bachmakov I, König J *et al.* Role of p-glycoprotein inhibition for drug interactions: evidence from in vitro and pharmacoepidemiological studies. Clin Pharmacokinet 2007;**46**:1039–49.
19 Kang W, Weiss M. Digoxin uptake, receptor heterogeneity, and inotropic response in the isolated rat heart: a comprehensive kinetic model. J Pharmacol Exp Ther 2002;**302**:577–83.
20 Sudergat H, Unger KK, Emmert J, Wendt J, Mandel F. Determination of Digoxin in human serum by LC/MS with online sample preparation, Application Note 2001;**15**: 5988–4364EN.
21 Schinkel AH, Wagenaar E, van Deemter L, Mol CA, Borst P. Absence of the mdr1a P-Glycoprotein in mice affects tissue distribution and pharmacokinetics of dexamethasone, digoxin, and cyclosporin A. J Clin Invest 1995;**96**:1698–705.
22 Kawahara M, Sakata A, Miyashita T, Tamai I, Tsuji A. Physiologically based pharmacokinetics of digoxin in mdr1a knockout mice. J Pharm Sci 1999;**88**:1281–7.
23 Oswald S, Grube M, Siegmund W, Kroemer HK. Transporter-mediated uptake into cellular compartments. Xenobiotica 2007;**37**:1171–95.
24 Sagawa T, Sagawa K, Kelly JE, Tsushima RG, Wasserstrom JA. Activation of cardiac ryanodine receptors by cardiac glycosides. Am J Physiol Heart Circ Physiol 2002;**282**:H1118–6.
25 Demiryurek AT, Demiryurek S. Cardiotoxicity of digitalis glycosides: roles of autonomic pathways, autacoids and ion channels. Auton Autacoid Pharmacol 2005;**25**:35–52.

© 2010 The Authors
Basic & Clinical Pharmacology & Toxicology © 2010 Nordic Pharmacological Society.