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Induction of rat intestinal p-glycoprotein by spironolactone and its effect on absorption of orally administered digoxin.

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ABBREVIATIONS

P-gp - P-glycoprotein; Mdr1 - multidrug resistance protein 1; MDR1 - human multidrug resistance protein 1; SL - spironolactone; Mrp2 - Multidrug resistance-associated protein 2; BBM - brush border membrane.

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ABSTRACT

The effect of the diuretic spironolactone (SL) on expression and function of intestinal P-glycoprotein (P-gp), and its impact on intestinal absorption of digoxin, were explored. Rats were treated with daily doses of 200 μmol/kg b.w. of SL, i.p., for 3 consecutive days. The small intestine was divided into 4 equal segments of ~25 cm, with segment I being the most proximal. Brush border membranes were isolated and used in analysis of P-gp expression by western blotting. P-gp content increased in SL group by 526, 292, 210 and 622% over Controls for segments I, II, III and IV, respectively. Up-regulation of apical P-gp was confirmed by immunofluorescence microscopy. P-gp transport activity was explored in intestinal sacs prepared from segment IV, using two different model substrates. Serosal to mucosal transport (efflux) of rhodamine 123 was 140% higher and mucosal to serosal transport (absorption) of digoxin was 40% lower in SL group, both indicating increased P-gp function. *In vivo* experiments demonstrated that intestinal absorption of a single dose of digoxin administered orally was attenuated by SL pretreatment. Thus, concentration of digoxin in portal and peripheral blood was lower in SL vs Control groups, as well as its accumulation in kidney and liver. Urinary excretion of digoxin was significantly decreased in SL group, likely reflecting decreased systemic availability of digoxin for subsequent urinary elimination. We conclude that SL induces P-gp expression with potential impact on intestinal absorption of substrates with therapeutic application.

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INTRODUCTION

P-glycoprotein (P-gp) is a versatile xenobiotic pump, which was first described in cancer cells, decreasing accumulation of chemotherapeutic agents such as the vinca alkaloids. It is constitutively expressed in a variety of normal human and rodent tissues, including liver, brain, adrenal gland, kidney and intestinal tract epithelia. In the small intestine and colon, P-gp is one of the most important efflux proteins (Sun et al., 2004). Localized to the apical membrane of the mature epithelial cells, it is responsible for pumping a wide range of xenobiotics, mostly hydrophobic, into the intestinal lumen. P-gp is encoded by the multidrug resistance gene *MDR1* in humans and by genes *Mdr1a/b* in rats, and because of its high expression in intestine, P-gp likely plays a physiologic role as a defense mechanism against toxic substances present in the diet.

Several studies report on the role of intestinal P-gp in modulating oral drug bioavailability (Fromm, 2003;Lin and Yamazaki, 2003). In addition, P-gp is known to be induced *in vitro* and *in vivo* by a wide range of xenobiotics and therapeutic drugs, hormones, and classical inducers, either in experimental animals or humans (Schuetz et al., 1996;Takano et al., 2006). Among these compounds, the effect of rifampicin has been well documented by different groups. During rifampicin treatment, plasma concentrations of several orally administered therapeutic drugs, all demonstrated to be P-gp substrates, are decreased (Takano, et. al., 2006). As a consequence, a decreased pharmacological potency

of these drugs is expected. Clearly, potential drug interactions should be considered when P-gp inducers/substrates are co-administered clinically.

Spironolactone (SL), an aldosterone antagonist, is a widely used diuretic, indicated in patients with edema or ascites produced by cirrhosis or congestive heart failure (Ochs et al., 1978). It has been found that SL induces hepatic and intestinal microsomal drug-metabolism enzymes in experimental animals (Stripp et al., 1971;Feller and Gerald, 1971a;Hamrick et al., 1973;Feller and Gerald, 1971b;Catania et al., 2003) and humans (Miguet et al., 1980;Wirth et al., 1976). SL has been demonstrated to induce also specific membrane transport systems involved in organic anion uptake or secretion such as the organic anion transporter polypeptide 2 (Oatp2) and multidrug resistance associated protein 2 (Mrp2) in the rat (Guo et al., 2002;Ruiz et al., 2005). The effect of SL on expression and activity of P-gp has not been explored. An eventual induction of intestinal P-gp by SL would lead to altered bioavailability of drugs co-administered orally with the diuretic. Interestingly, SL is usually co-administered with digoxin in patients at late stages of congestive heart failure (Ochs et.al., 1978;Pitt et al., 1999) and it has been described that digoxin is transported by P-gp in the intestine and liver (Drescher et al., 2003).

The aim of this study was to evaluate the effect of SL on the expression and activity of intestinal P-gp in the rat. The data indicate that SL up-regulated P-gp protein all along the small intestine, with concomitant increase in its transport activity. As a consequence, intestinal absorption of orally administered digoxin was found to be significantly decreased.

MATERIALS AND METHODS

Chemicals. [^{3}H] digoxin (37.0 Ci/mmol) and OptiPhase liquid scintillation cocktail were purchased from Perkin Elmer Life Science Products (Boston, MA). Unlabeled digoxin was from ICN Biomedicals Inc. (Costa Mesa, CA), whereas SL, verapamil, leupeptin, phenylmethylsulfonyl fluoride, pepstatin A and bovine serum albumin were from Sigma Chemical Company (St. Louis, MO). All other chemicals were of analytical grade purity.

Animals and treatment. Male Wistar rats (250-290 g) were used throughout. They were maintained *ad libitum* on a standard laboratory pellet diet and were allowed free access to water and saline during treatment. A group of animals was treated with SL (200 µmol/kg i.p.) in propylene glycol (60 mM), or vehicle alone (3.3 ml/Kg), for 3 consecutive days. This dose was demonstrated to maximally induce microsomal UDP-glucuronosyltransferase (Mottino et al., 1989) and canalicular Mrp2 in rat liver (Ruiz et al., unpublished results). Studies were performed 18 hr after the last injection of SL or vehicle. All procedures were conducted in accordance with NIH guidelines for the Care and Use of Laboratory Animals.

Specimen collection and plasma membrane preparation. Rats were anesthetized with sodium pentobarbital (50 mg/Kg, i.p.) and the whole small intestine was removed and divided into four equal segments, which were

carefully rinsed with ice-cold saline. The proximal segment, starting from the pylorus, was given the number I, whereas the most distal segment, close to the ileocecal valve, was given the number IV. These segments were immediately used in preparation of mucosal homogenates. For brush-border membrane (BBM) isolation, the mucosal tissue was obtained from each segment by scraping (Catania et al., 1998) and homogenates and BBM were prepared as described (Mottino et al., 2000). Livers were perfused with ice-cold saline through the portal vein, removed, and immediately used in crude plasma membrane preparation as described (Meier et al., 1984). Protein concentration in membrane preparations was measured using bovine serum albumin as a standard (Lowry et al., 1951).

Western blot studies. Immunoblotting for detection of P-gp was performed using a rabbit polyclonal antibody to human MDR-1 (Santa Cruz Biotechnologies, Santa Cruz, CA), as previously described (Ghanem et al., 2004). Densitometry was performed using the Gel Pro Analyzer (Media Cybernetics, Inc., Silver Spring, MD) software.

Immunofluorescence microscopy studies. Slices (5 µm) from distal ileum were prepared with a Zeiss Microm HM500 microtome cryostat, air dried for 2 h, and fixed for 10 min with cold acetone (-20ºC). For labeling, tissue sections were incubated overnight with the polyclonal anti-human MDR-1 (1:100) antibody. Sections were then washed 5 times with PBS, and incubated with Cy3-

conjugated donkey anti-rabbit IgG (Jackson ImmunoResearch Laboratory, Inc., West Grove, PA) (1:200) for 2 h. After washing 3 times with PBS and once with distilled water, slices were air dried and mounted. The images were captured on a Zeiss Axiovert 25 CFL inverted microscope. To ensure comparable staining and image capture performance for SL and control groups, intestine slices were prepared the same day, mounted on the same glass slide, and subjected to the staining procedure and microscopy analysis simultaneously.

P-gp activity in intestinal sacs. Intestinal sacs were prepared from segment IV, which was removed as described above. P-gp activity was examined analyzing the serosal to mucosal transport (efflux) of rhodamine 123, a well known P-gp substrate. Four-cm segments were filled (mucosal compartment) with Krebs-Henseleit buffer (40 mM glucose, pH 7.4) previously gassed with 95% O_2-5% CO_2. After 15 min of pre-incubation in 100 ml of the same buffer at 37°C (serosal compartment), with or without 100 µM of the P-gp inhibitor verapamil (Zhang et al., 2005), rhodamine 123 was added to this external medium to reach a final concentration of 50 µM. The sacs were incubated for 0, 5, 10, 20, or 40 min. The external medium was continuously gassed with 95% O_2-5% CO_2. At the end of incubation, the exterior of the sacs was carefully rinsed with drug free buffer, and the entire mucosal solution was collected. Intestinal tissue was homogenized in 2.5 ml of the same buffer. Rhodamine 123 concentration was determined spectrofluorometrically (Efferth et al., 1989) in aliquots of serosal and mucosal buffers, and in intestinal homogenates. The fluorescence intensity of

rhodamine123 was measured at an excitation wavelength of 488 nm and emission wavelength of 550 nm.

Intestinal absorption of digoxin.

Interference of intestinal P-gp induction on intestinal absorption of the P-gp substrate digoxin was evaluated *in vitro* and *in vivo.*

Absorption in intestinal sacs: Four-cm segments isolated from distal ileum (segment IV) were everted and filled with Krebs-Henseleit buffer (serosal compartment). Everted sacs were preincubated in 100 ml of buffer (mucosal compartment) for 15 min, in the presence or absence of 100 µM verapamil. Digoxin was then added to the external medium to reach a final concentration of 10 µM (10.6 µmol [^{3}H] digoxin/mol unlabeled digoxin). Incubation was performed as described above. Radioactivity was determined in serosal and mucosal samples, and in intestinal homogenates, by liquid scintillation analysis (Wallac counter 1409, Turku, Finland).

Absorption and distribution of digoxin in vivo: Digoxin absorption and subsequent distribution into blood, liver and kidney were examined by assessing its portal concentration as well as its systemic blood concentration and hepatic and renal content and disposition. Rats were anesthetized with sodium pentobarbital (50 mg/Kg, i.p.), and thus maintained throughout the experiment. Body temperature was measured with a rectal probe, and maintained at 37ºC with a heating lamp. Appropriate volumes of 5% bovine serum albumin in saline were administered intravenously throughout the experiment to replenish body fluids. To study

digoxin systemic distribution, the jugular vein, and the carotid artery and common bile duct were cannulated with polyethylene tubing (PE50 and PE10, respectively). The urinary bladder was exteriorized through an abdominal midline incision and cannulated with polyethylene tubing (PE75). The animals received an oral dose of digoxin of 25.6 nmol/Kg (17.0 µmol [^{3}H] digoxin/mol of unlabeled digoxin) by gavage, and arterial blood, bile and urine were sampled at 15-min periods for 135 min. At the end of the experiment, the liver and kidneys were removed and homogenized in 20 and 6 ml of saline, respectively. Bile and urinary flow were determined gravimetrically. Samples of serum, bile, urine and homogenates were used for assessment of digoxin concentration by liquid scintillation analysis. To study the portal content of digoxin, a PE10 tubing was inserted into the portal vein and secured with an adhesive agent in a different set of animals. Portal blood flow was not altered by this procedure. The jugular vein was cannulated to replenish body fluids as described above. A dose of 25.6 nmol/kg b.w. of digoxin (17.0 µmol [^{3}H] digoxin/mol of unlabeled digoxin) was administered orally by gavage, and blood from portal vein was sampled at 15, 20, 25, and 30 min after digoxin administration. Serum samples were used for determination of digoxin concentration by liquid scintillation analysis.

Statistical analysis. Data are presented as the means ± SD. Comparison between groups was performed using the Student's *t* test or one way ANOVA followed by Bonferroni's test (when more than two groups were compared). Values of $P<0.05$ were considered to be statistically significant.

RESULTS

Effect of SL on P-gp expression. Because P-gp expression varies along the small intestine (Doherty and Charman, 2002), we divided the whole organ in four segments of same length. Panel A in Fig 1 shows western blot detection of P-gp in BBMs isolated from SL and Control groups in these different regions. SL induced an increase in P-gp expression of 526, 292, 210 and 622% over Controls for segments I, II, III and IV, respectively. To confirm the localization of P-gp *in situ*, we performed an immunofluorescence microscopy study. Fig 1B shows that P-gp is mainly localized to the surface of the intestinal villus and that increased fluorescent detection of this transporter is seen in SL group when compared to Controls. In some regions of the villus, detection of P-gp is clearly evidenced at the apical membrane, particularly in the SL group (see arrowheads).

P-gp is widely expressed in different tissues. To evaluate if SL induction of P-gp was restricted to the small intestine, we analyzed its expression in crude plasma membranes from liver. Fig 1C shows that P-gp expression was increased in liver of SL animals, though to a lower extent than in intestine. By densitometry, detection of P-gp protein was increased by 40% in SL group.

Effect of SL on P-gp transport activity.

To determine the functional impact of increased P-gp protein levels by SL, we studied the secretion of rhodamine 123, a typical P-gp substrate, into the

mucosal compartment of intestinal sacs prepared from distal ileum. Addition of rhodamine 123 to the serosal compartment resulted in progressive recovery of the dye in the mucosal side. Fig 2A shows that basolateral to apical transport of rhodamine 123 was increased by SL. Efflux of the dye was 140% higher in SL group at 40 min. It is known that rhodamine 123 is also a substrate for breast cancer resistance protein (Bcrp or Abcg2) (Alqawi et al., 2004), which is expressed in intestine from rodents (Han and Sugiyama, 2006). To further confirm participation of P-gp in rhodamine 123 transport, we repeated these same experiments, but in the presence of 10 μM verapamil, a P-gp but not a Bcrp inhibitor (Zhang et al., 2005). The results demonstrate that the efflux of rhodamine 123 was decreased by verapamil by 10- and 15-fold in SL and Control groups, respectively, by the end of the incubation period. This indicates that the dye was mainly transported by P-gp both under constitutive and SL induction conditions. Additionally, no difference was observed between groups in the tissue content of rhodamine 123 (see Fig 2B), thus suggesting that the difference in the secretory rate of the dye produced by SL pretreatment was due to a higher transport efficiency at the apical membrane, and not to a different uptake at the basolateral membrane.

Effect of P-gp induction on intestinal absorption of digoxin.

P-gp in intestine functions as a membrane barrier against absorption of xenobiotics and drugs available intraluminally. To test whether SL also enhances this particular function, we first evaluated the mucosal to serosal transport of

digoxin, a typical substrate of P-gp (Drescher et. al., 2003), in everted intestinal sacs. Fig 3A shows that intestinal absorption of digoxin was 40% lower in SL vs Controls after 40 min of incubation. In the presence of verapamil, both groups showed an increase in the absorption of digoxin (2.6- and 2.0-fold for SL and Control groups, respectively) with respect to incubations without inhibitor. These results clearly implicated P-gp as a membrane barrier for restriction of digoxin absorption. The intestinal content of digoxin in SL group was significantly lower than in Control group (see Fig 3B). However, when P-gp activity was assayed in the presence of verapamil, the intestinal concentration of digoxin was similar in both groups. Taken together, these data indicate that induction of apical P-gp has led to decreased intracellular availability of digoxin for subsequent secretion into the serosal compartment, thus explaining the lower rate of mucosal to serosal transport shown in Fig 3A.

Intestinal P-gp was described to be one of the factors that modulate digoxin steady state concentrations (Nakamura et al., 2001). Variations of serum concentration of digoxin with time after its oral administration are depicted in Fig 4A. Whereas digoxin concentration increased with time in Control group, this measure remained constant in SL group; a statistically significant difference was observed between groups from 90 min onwards. SL was found to induce hepatic P-gp in addition to intestinal P-gp. To confirm that the difference observed in digoxin serum concentration between groups was due to a decrease in the intestinal absorption of the drug and not to an increase in hepatic first-pass extraction and elimination, we evaluated the concentration of the drug in portal

vein. The results shown in Fig 4D demonstrate a significant reduction in digoxin concentration 25 and 30 min after drug administration.

As a consequence of its decreased intestinal absorption, digoxin exhibited reduced accumulation in liver and kidney from SL rats (-65 and -88% respectively, see Fig 4C) 135 min after drug administration. Fig 4B shows that cumulative biliary excretion of digoxin was preserved in SL animals, and that cumulative urinary excretion was decreased by 88%. The first-pass effect in which the liver extracts most of the drug absorbed likely contributed to the decreased urinary excretion of digoxin, while biliary excretion was maintained.

DISCUSSION

Due to the central role that intestinal P-gp plays in the absorption and presystemic elimination of many chemicals including medicines, understanding the factors that regulate its expression is relevant both from a clinical and toxicological point of view. In this study we evaluated the effect of pretreatment with SL on the expression and activity of intestinal P-gp in the rat. We chose this known inducer of multiple enzyme systems because it presents a therapeutic application as a diuretic and is often co-administered with digoxin, a typical P-gp substrate, in congestive heart failure. Thus, drug-drug interactions may occur. The current data indicate significant induction of intestinal P-gp by SL. Though the mechanism by which SL modulates expression of this protein is unknown, because SL is a well known ligand for PXR (Schuetz et al., 1998) it is possible that binding of SL to this nuclear receptor represents the initial step in P-gp induction.

We demonstrated that the increase in expression of P-gp at the apical membrane of the enterocyte correlated well with increased transport activity detected in *in vitro* experiments using two different P-gp substrates: rhodamine 123 and digoxin, the latter of therapeutic application. Digoxin is widely used in the treatment of congestive heart failure and it is important to note that this drug presents a narrow therapeutic index. Increased plasma levels could result in dangerous toxicity to the heart, whereas a decrease in its blood concentration would produce an ineffective treatment. For example, co-administration of

quinidine (Fromm et al., 1999;Verschraagen et al., 1999), verapamil (Verschraagen et.al., 1999) or diltiazem (Takara et al., 2002) with digoxin, results in an increased digoxin plasma levels, most likely as a consequence of inhibitory action on intestinal P-gp. Similarly, it is expected that modulation of P-gp expression affects digoxin absorption and disposition. To test the possibility that SL induction of intestinal P-gp affects digoxin absorption, we further performed experiments *in vivo.* Analysis of digoxin portal concentration vs time demonstrated a decrease in intestinal absorption of this drug after its oral administration in SL group. This likely resulted in decreased systemic concentration of digoxin, as revealed by detection of lower plasma, renal and liver contents. Renal elimination of digoxin depends on a balance between filtered and tubular processes. Though the mechanism for impaired urinary excretion of digoxin in SL group was not explored, the data on decreased systemic and portal plasma concentrations strongly suggest decreased systemic availability for subsequent renal elimination. Interestingly, we found no change in biliary elimination of digoxin in spite of decreased plasma and intrahepatic concentration of the drug. This result can be tentatively explained by a compensatory increase in expression of liver P-gp as detected by western blotting, additionally contributing to decrease digoxin intracellular levels. Taken together, these data suggest lower tissue digoxin exposure in SL rats, mainly as a result of increased intestinal barrier function against absorption of selective P-gp substrates.

Previous studies in experimental animals demonstrated that treatment with SL decreases toxicity of digitoxin (Buck and Lage, 1971;Solymoss et al., 1971), another P-gp substrate with similar therapeutic application as digoxin. Digitoxin is partially metabolized to digoxin by hepatic microsomal enzymes (Eberhart et al., 1992), undergoes efficient enterohepatic recirculation, and only a small fraction of unchanged drug is eliminated in feces either in humans or rats (Rietbrock and Vohringer, 1974;Vohringer and Rietbrock, 1974). Protection against digitoxin toxicity by SL has been ascribed to hepatic SL-induced metabolism of the drug (Selye et al., 1969;Buck and Lage, 1971;Solymoss et al., 1971). According to the current results, it is also possible that modulation of digitoxin toxicity by SL results from increased elimination of the intact drug or its metabolite digoxin. Our western blot study demonstrated a substantial increase in expression or P-gp not only in distal but also in proximal intestine, where P-gp constitutive expression is normally low. In consequence, the extended induction of the transporter, demonstrated in this work, may critically affect absorption of digitoxin or its metabolite digoxin, either from oral or biliary source, once entero-hepatic recirculation is completed. This could result in shortening the drug half-life and decreasing the toxicity, as previously observed.

Wirth et al. (1976) reported on the effect of SL on the metabolism of intravenously administered ^{3}H-digitoxin in patients receiving SL chronically. SL caused a 20% reduction in the half-life of serum radioactivity and a 16% reduction in the volume of distribution. Induction of phase II (e.g. UDP-glucuronosyltransferase) enzymes by SL was proposed to explain these

alterations, which appear to contribute to the reduction in half-life. The dose of SL administered to these patients (100-200 mg per day) is proportionally far below the dose administered to rats in the current study. However, the therapeutic protocol requires chronic administration and could represent a much more sustained action leading to alterations not only in metabolism but also in transport of drugs co-administered with SL.

In conclusion, the present study demonstrates that SL increases the expression and activity of P-gp throughout the small intestine in the rat. These modifications have pronounced effects on *in vivo* intestinal absorption of digoxin when administered orally. The possibility that simultaneous treatment with SL and digoxin results in clinical drug-drug interactions is suggested.

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FOOTNOTES

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Figure 1. Western blotting and immunofluorescence detection of P-gp.

Western blot study of P-gp was performed in BBM from enterocytes throughout the small intestine (panel A) and in hepatic mixed plasma membranes (panel C). Segment I is the most proximal and segment IV the most distal. Equal amounts of total protein (20 µg) were loaded in all lanes. This amount of protein gave a densitometric signal in the linear range of the response curve for P-gp antibody. Uniformity of loading and transfer from gel to nitrocellulose membrane was controlled with Ponceau S. Data on densitometric analysis represent means ± SD of 3 rats per group. P-gp was additionally detected by immunofluorescence microscopy (panel B). Similar pattern of staining was observed in two additional preparations per group. White arrowheads indicate regions with high detection of P-gp in SL group.

a: SL different from Ctrl ($P<0.05$).

Figure 2. Efflux of rhodamine 123 in intestinal sacs.

A: Sacs from distal ileum (segment IV) were incubated in the presence of rhodamine 123, with of without verapamil, in the serosal side. Rhodamine 123 secreted into the mucosal compartment was analyzed at different times. Data are mean ± SD of 3 rats per group.

a: SL different from Ctrl, Ctrl+Ver and SL+Ver ($P<0.05$).

b: SL different from Ctrl, Ctr+Ver and SL+Ver ($P<0.01$).

c: Ctrl different from SL, and Ctrl+Ver ($P<0.05$), and SL different from Ctrl+Ver and SL+Ver ($P<0.001$).

B: Content of rhodamine 123 in intestinal tissue by the end of the experiment. Data are mean ± SD of 3 rats per group.

Figure 3. Absorption of digoxin in intestinal sacs.

A: Everted intestinal sacs were incubated in the presence of 10 µM digoxin (10.6 µmol [^{3}H] digoxin/mol unlabeled digoxin), with or without verapamil, in the mucosal side. Digoxin accumulated in the serosal side was analyzed at different times. Data are mean ± SD of 4-7 rats per group.

a: SL different from Ctrl+Ver and SL+Ver ($P<0.05$).

b: Ctrl different from SL, Ctrl+Ver and SL+Ver ($P<0.05$), and SL different from Ctrl+Ver and SL+Ver ($P<0.001$).

B: Content of digoxin in intestinal tissue by the end of the experiment. Data are mean ± SD of 4-7 rats per group.

a: Ctrl different from SL and Ctrl+Ver ($P<0.05$).

b: SL different from Ctrl+Ver and SL+Ver ($P<0.05$).

Figure 4. *In vivo* intestinal absorption of digoxin.

The animals received an oral dose of digoxin of 25.6 nmol/Kg (17.0 µmol [^{3}H] digoxin/mol of unlabeled digoxin) by gavage. A: Changes in concentration of digoxin in systemic blood with time. B: Cumulative biliary and urinary excretion of digoxin by 135 min. C: Tissue concentration of digoxin at 135 min after drug

administration. D: Changes in concentration of digoxin in portal blood with time. Data are mean ± SD of 4 rats per group.

a: SL different from Ctrl ($P<0.05$).

Figure 1

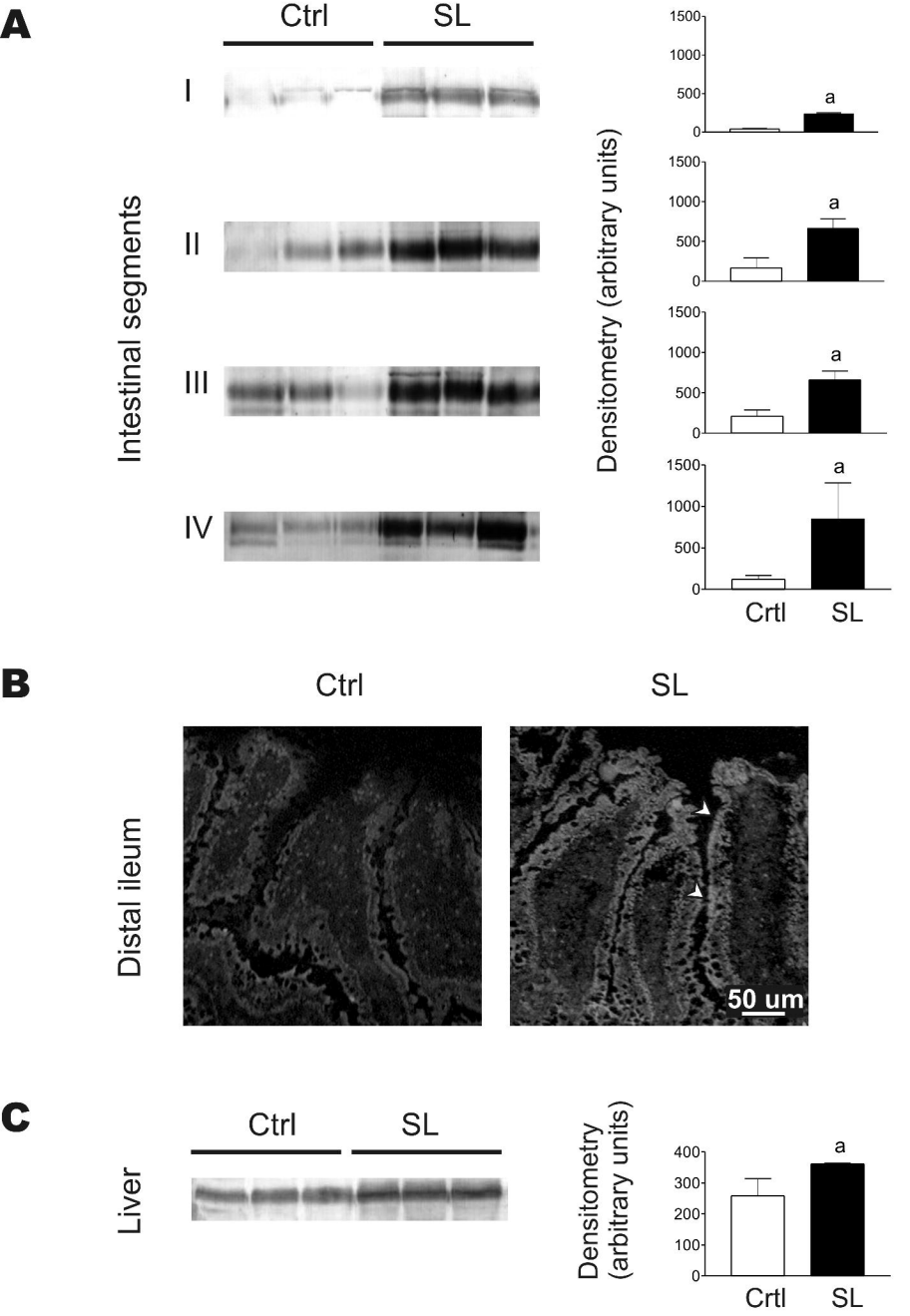

A
Ctrl
SL
I
II
III
IV
Intestinal segments
Densitometry (arbitrary units)
1500
1000
500
0
a
Crtl
SL
B
Ctrl
SL
Distal ileum
50 um
C
Liver
Ctrl
SL
Densitometry (arbitrary units)
400
300
200
100
0
a
Crtl
SL

Figure 2

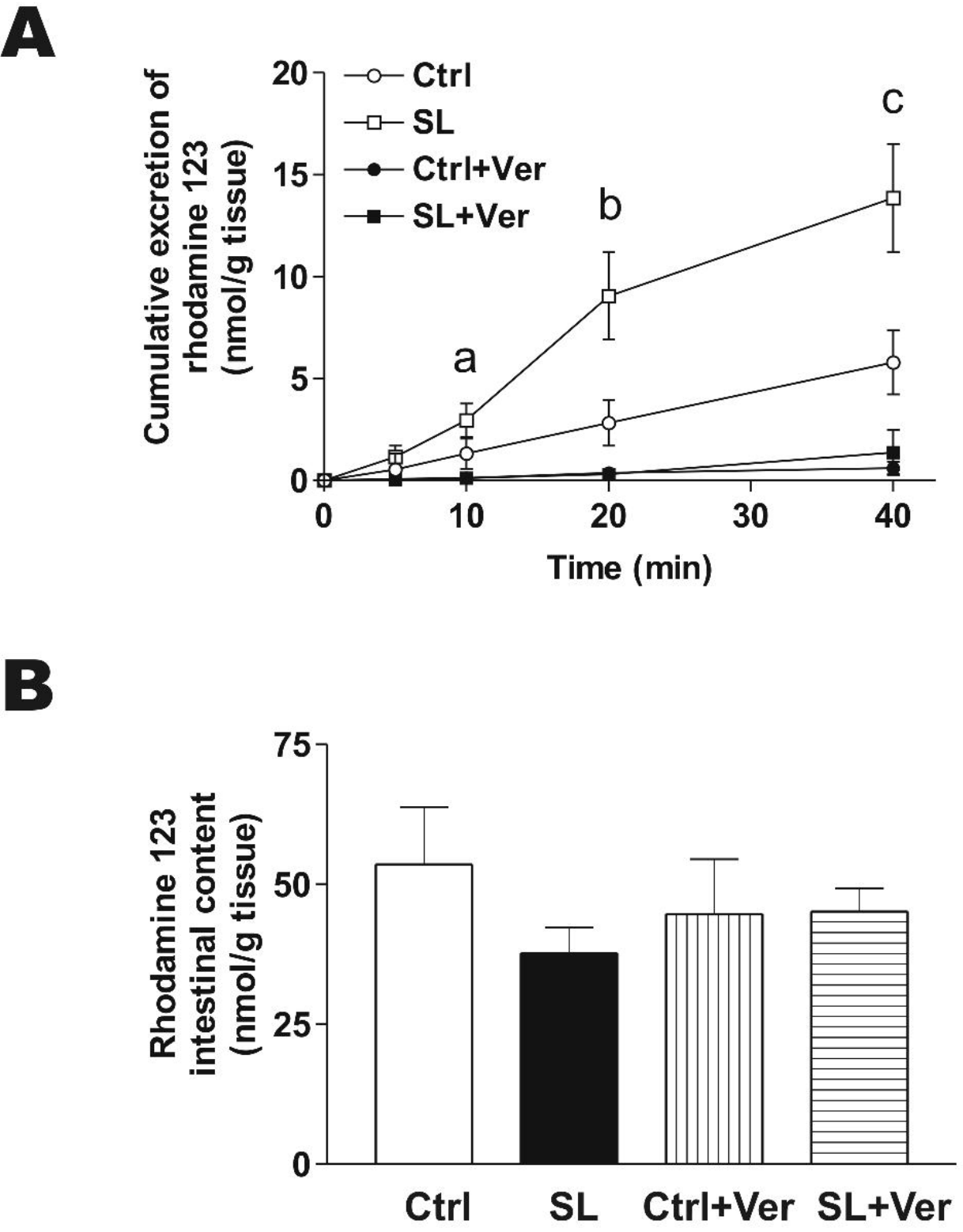
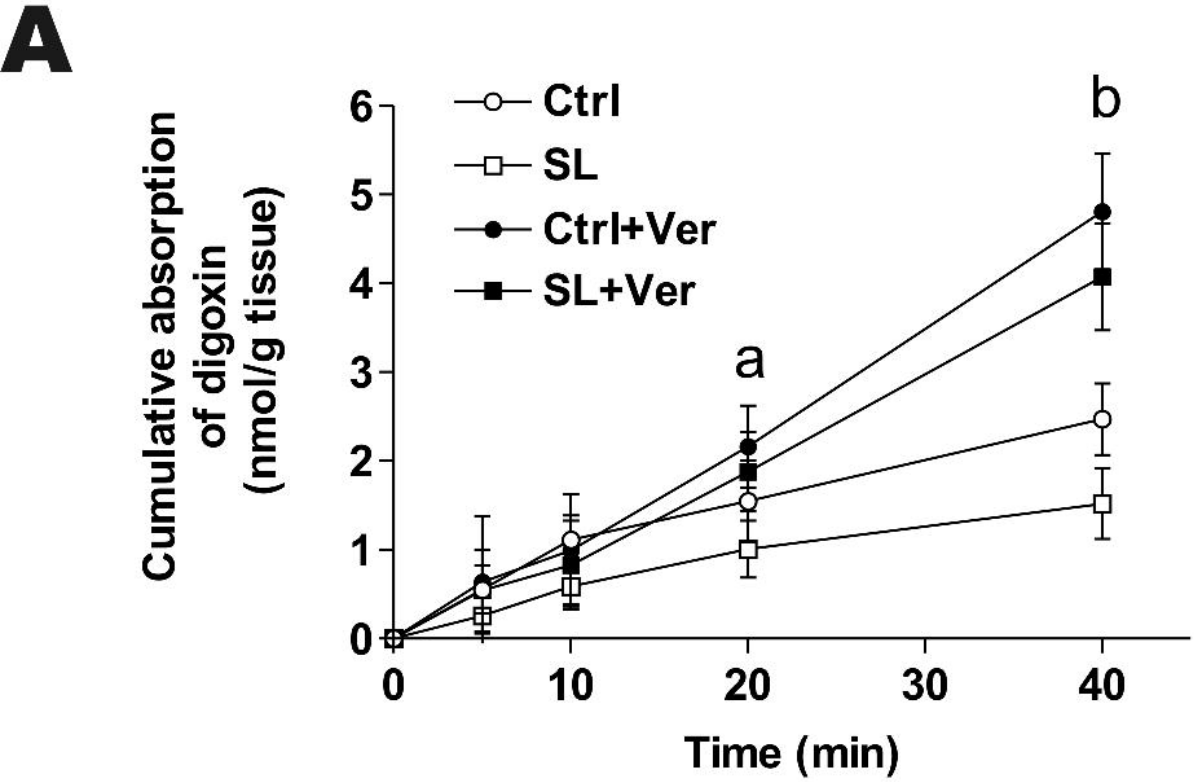

A
Cumulative excretion of rhodamine 123 (nmol/g tissue)
20
15
10
5
0
Ctrl
SL
Ctrl+Ver
SL+Ver
a
b
c
0
10
20
30
40
Time (min)
B
Rhodamine 123 intestinal content (nmol/g tissue)
75
50
25
0
Ctrl
SL
Ctrl+Ver
SL+Ver

Figure 3


A
Cumulative absorption of digoxin (nmol/g tissue)
Ctrl
SL
Ctrl+Ver
SL+Ver
a
b
0
1
2
3
4
5
6
0
10
20
30
40
Time (min)

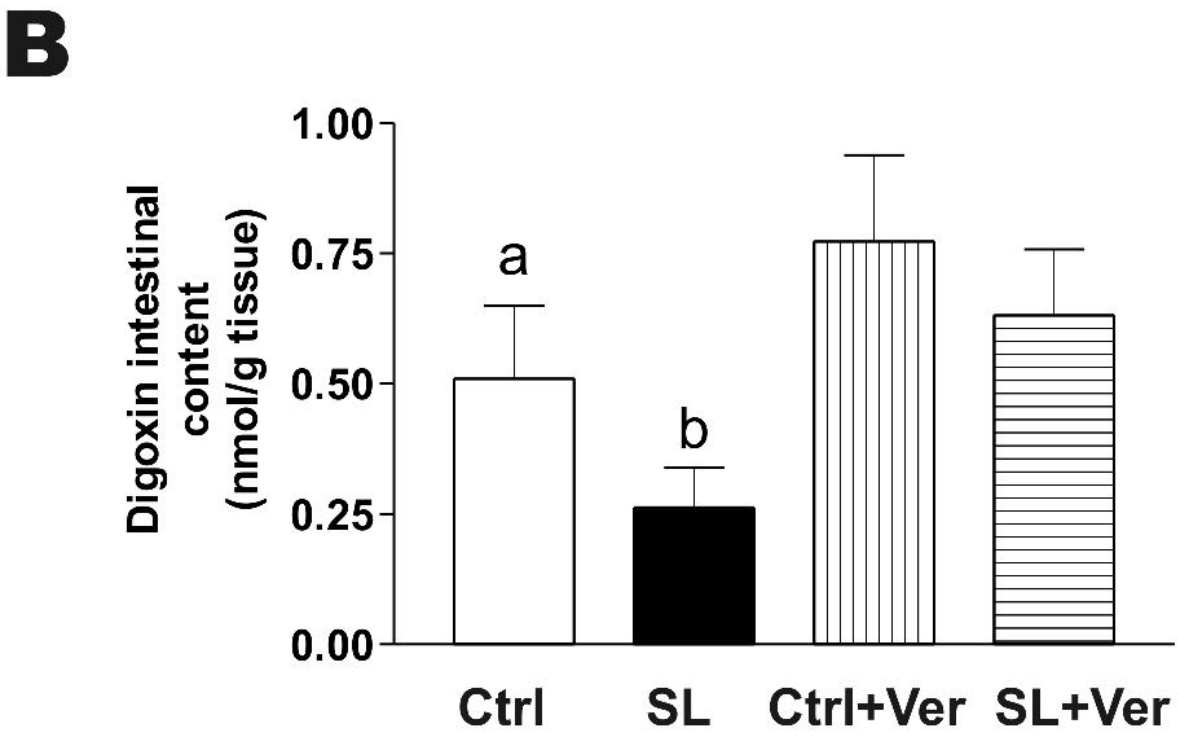
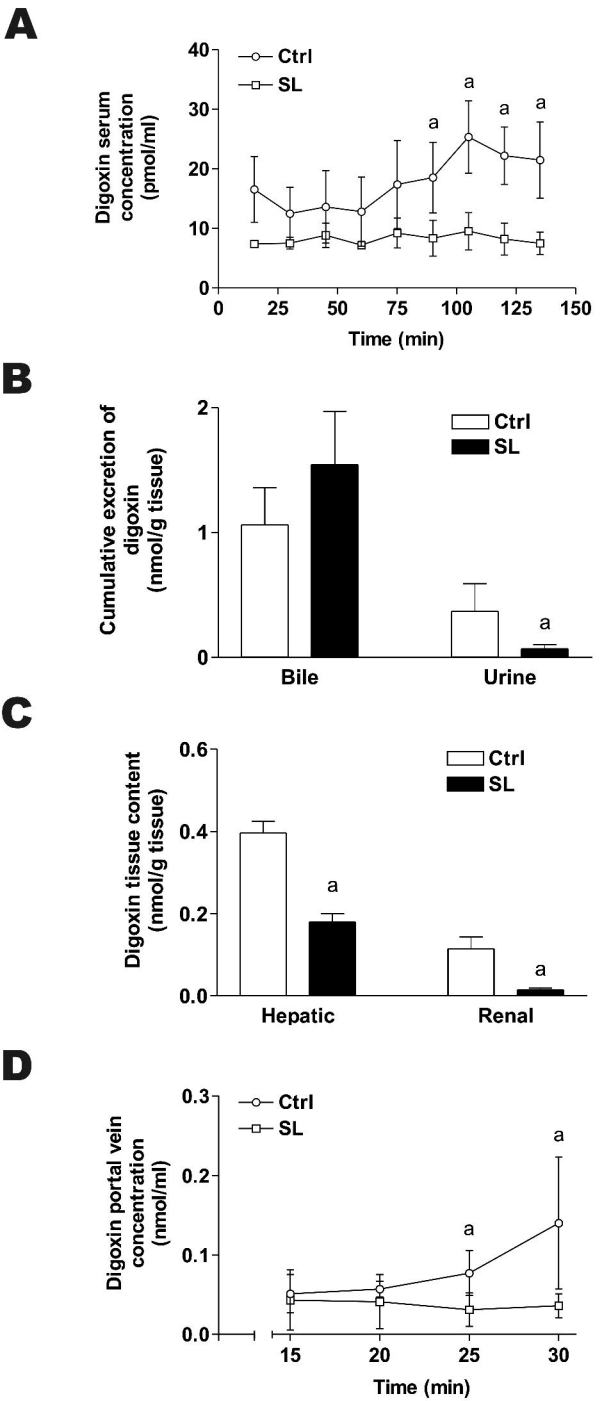

B
Digoxin intestinal content (nmol/g tissue)
1.00
0.75
0.50
0.25
0.00
a
b
Ctrl
SL
Ctrl+Ver
SL+Ver

Figure 4


A
Digoxin serum concentration (pmol/ml)
Ctrl
SL
a
Time (min)
B
Cumulative excretion of digoxin (nmol/g tissue)
Ctrl
SL
a
Bile
Urine
C
Digoxin tissue content (nmol/g tissue)
Ctrl
SL
a
Hepatic
Renal
D
Digoxin portal vein concentration (nmol/ml)
Ctrl
SL
a
Time (min)