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Pharmacokinetics of radiolabelled quinlukast in rats

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Abstract

Pharmacokinetics together with in vivo metabolism and elimination of quinlukast, a potential anti-asthmatic and anti-inflammatory drug, were designed in rats. For this purpose, bile duct cannulated rats and an in situ perfused rat liver preparation were employed. ^{3}H-radiolabelled compound was administered i.v. or loaded to the perfusion medium, respectively. Quinlukast represented the main form of radioactivity determined in plasma; in comparison with the parent drug metabolites were present in lower levels in the systemic circulation. The pharmacokinetic parameters related to the whole animal were calculated from quinlukast rat plasma concentration–time course. The distribution of quinlukast in the body was relatively fast (distribution half-life was approx. 6 min), the elimination half-life exceeded 2 h. Binding of quinlukast to rat plasma proteins was very high (approx. 99.7%) and this binding influenced distribution volumes of quinlukast. Both the volume of the central compartment and the volume at a steady state were approx. 115 and 430 ml, respectively. The experiments showed that the biliary clearance was the major route of elimination of this compound from the systemic circulation of rats. In agreement with the determined elimination half-life approx. 42% of the radioactivity was found in the bile, with <0.5% appearing in the urine. The majority of the eliminated radioactivity in the bile was in the form of polar metabolites; only a small part of the parent compound was determined. Two hours after intravenous administration, polar metabolites – but no parent drug – were detected in the urine.

Keywords: Quinlukast; Pharmacokinetics; Liver perfusion; HPLC

1. Introduction

Quinlukast (QL), 4-(4-(quinoline-2′-yl-methoxy) phenylsulphanyl) benzoic acid, (VUFB 19363, Leciva, Prague, The Czech Republic), is a new promising drug with anti-inflammatory and anti-asthmatic effects. The compound is in a preclinical development at present. Leukotrienes (LT) are pathophysiological mediators of asthma and various inflammatory diseases. LT originate as the products of arachidonic acid biotransformation [1,2]. Eosinophil chemoattractant cysteinyl LT (LTC_4, LTD_4, LTE_4) cause bronchial constriction, induce an anaphylactic reaction, stimulate mucus secretion and increase vascular permeability, leading to oedema formation [1,2]. Neutrophil LTB_4, a mediator in the release of lysosomal enzymes, has degranulating properties [2,3]. Compounds interfered with LT synthesis, and/or characterized by an antagonistic effect towards LT, are intensively investigated. In comparison to the specific effect of other antileukotrienics, e.g., montelukast [4], zafirlukast [5] or pranlukast [6], QL displays multiple antileukotrienic effects [7]. QL is characterized by a significant inhibitory activity for LT biosynthesis combined with a high affinity to LT receptors. QL exhibits a low toxicity including the absence of an ulcerogenic effect. The first published work, dealing with QL, was presented in 2003 and describes the SPE-SIA method for the determination of the parent drug in serum [8]. The chemical structure of QL is shown in Fig. 1. Due to its chemical structure, QL probably undergoes an intensive metabolism and elimination in the form of polar metabolites. The incubations of this compound in a rat microsomal fraction and in a primary culture of rat hepatocytes showed, that the main metabolic pathway of QL was the liver metabolism, especially oxidation of the sulphide bridge to its S-oxide [9], which is usually

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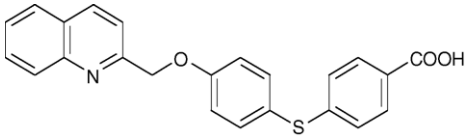

Fig. 1. Chemical structure of quinlukast (M_w = 387).

the principle metabolite in the first stage of biotransformation of such drugs. The other biotransformation reactions probably involve the formation of polar conjugates of QL and its S-oxide and/or formation of further metabolites, e.g., the formation of two dihydrodiol derivates, identified by means of mass spectrometry [10].

The present paper is aimed to evaluate the pharmacokinetics, in vivo metabolism and elimination pathways and rate of QL in rats. For these purposes, the ^{3}H-radiolabelled compound was administered i.v. to rats.

2. Materials and methods

2.1. Chemicals

QL isotopically radiolabelled with ^{3}H (when one hydrogen atom in the drug structure is replaced by its radioactive isotope tritium) was used in the present study. The radiolabelling was carried out in the Nuclear Research Centre (Rez, The Czech Republic). Its specific activity was 2.8 MBq/mg and radiochemical purity was >95%. Therefore this substance was pre-purified and concentrated immediately before its administration. Purifications were performed repeatedly on the HPLC system under the same conditions as described below. Only the peak of QL was collected. QL in the mobile phase from several purifications was neutralized (pH 7.0) with 0.5 M sodium hydroxide and evaporated under a stream of nitrogen. The purity was checked on the HPLC system under the same conditions as described below. The traces of impurities appear in the retention time period of 17 and 27 min (both together <1% of total radioactivity).

Standards of non-radiolabelled QL and its S-oxide were provided by VUFB (Leciva, Prague, The Czech Republic). Methanol and acetonitrile (Sigma, St. Louis, USA) were of HLPC grade; other chemicals were of analytical reagent. The water was purified by reverse osmosis (Millipore system, Molsheim, France).

2.2. Biological samples

For the purpose of this pharmacokinetic study [11], male Wistar rats weighing 240–330 g were used. In short, animals were fastened 18–22 h before the experiment. The estimative quantity of QL was purified immediately before the experiments and then it was re-dissolved in 50 μl of dimethylsulfoxide and solubilized in 0.7 ml of blank plasma for 3 h. The doses (mg/kg) were determined from QL specific activity, measured radioactivity in the administered volume and the mass of an animal additionally. The bile duct of the anaesthetized rat (Pentobarbital, i.p., 45 mg/kg) was cannulated [12]. QL in a dose of 0.20–0.36 mg/(kg of animal mass) was administered into the Saphena vein in a volume of 0.6 ml. A cannula was inserted into the carotid artery and blood samples (200 μl) were repeatedly withdrawn to heparinized PE vials and were centrifuged at 17 000 × *g* for 60 s. Bile samples were collected during 30 min periods. The experiments were performed for 120 min after drug administration. At the end of the experiment, the sample of the urine was taken as well, directly from the urinary bladder.

As another example of biological fluids, also the samples of the perfusion medium and the bile from in situ perfused rat liver preparation were employed [12]. In short, the preparation of QL was the same as in the case of the bile duct cannulated rat study. The estimative quantity of QL was purified immediately before the experiments and then it was re-dissolved in 60 μl of dimethylsulfoxide and solubilized in 10 ml of blank perfusion medium for 3 h. The doses (μg/g of liver mass) were determined from QL specific activity, measured radioactivity in the administered volume and from liver mass additionally. After pentobarbital anaesthesia, the bile duct and the portal vein of the rat were cannulated on a temperature-controlled plate. After the vena cava cannulation, a recirculating perfusion medium was infused into the hepatic portal vein. After the equilibration period, the flow of the perfusate was kept at 25 ml/min and the agent under study was added to a reservoir (total volume of perfusion medium was 150 ml) in a volume of 10 ml and in a dose of 12–20 μg/(g of liver mass). Volumes of 1 ml of input and outflow perfusate samples were repeatedly removed at 10 min intervals in the middle of 10 min periods of bile collection. Samples of perfusate were centrifuged at 17 000 × *g* for 10 s and the supernatant was decanted. Then, 45 and 90 min after the drug loading, 5 ml volumes of the outflow perfusate were removed. The experiments were performed for 95 min after the drug loading.

At the end of both experiments, the liver was detached, weighted, cut to small pieces and dissolved in five aliquots of 4% (w/w) sodium hydroxide in 50% (v/v) ethanol. Then the solubilized tissue was adjusted with 10 N trihydrogenphosphoric acid to pH 7, shaken for 2 min and centrifuged at 170 00 × *g* for 4 min. The supernatant was further analyzed on the HPLC system (after extraction and concentration).

All animal experiments were approved by the Ethics commission of the Faculty of Pharmacy, Charles University.

2.3. Analytical method

The extraction columns Discovery DSC-18LT (55 μm, 100 mg, 1 ml cartridge; Supelco, Bellefonte, USA) equipped with needle (0.9 mm × 40 mm) were used for the extraction [13]. In short, 90 μl of plasma was adjusted with 9 μl of 100 mM phosphate buffer (pH 4.9) and aliquot was loaded onto the conditioned cartridges (methanol, 9 mM phosphate

buffer pH 4.9) and allowed to flow by gravity. The column was washed with 9 mM phosphate buffer (pH 4.9) and the analyte of interest was eluted from the cartridge with methanol and evaporated to dryness under a gentle stream of nitrogen. The evaporation residue was re-dissolved in a mixture of dimethylsulfoxide and water (3:7, v/v). The recovery of QL extraction in rat plasma was over 99.5% in a concentration range of 0.65–4.00 μg/ml [13]. The same procedure was used for QL extraction in the samples of the centrifuged perfusion medium with the same recovery. The solubilized liver preparation was made mainly for a qualitative liver analysis, therefore the recovery of QL extraction was not determined. Samples of bile and urine were injected directly without any treatment or pre-separation to the HPLC system. In the preliminary experiments, an addition of radiolabelled QL to blank samples (bile, urine) had no effect on its HPLC – radiometric determination.

An aliquot of the reconstituted sample was analyzed at 30 °C with an Agilent Technologies 1100 HPLC system using a Zorbax Eclipse XDB-C_8 column (5 μm, 80 Å, 150 mm × 4.6 mm ID; Agilent Technologies, Waldbronn, Germany), equipped with a Wakosil II 5C18-RS guard column (5 μm, 10 mm × 4 mm, SGE, Austin, TX, USA) [13]. The flow rate was 0.8 and 1.1 ml/min in the time period of 0–45 and 45.01–55 min, respectively. The mobile phase A was composed of acetonitrile, methanol and 15 mM trihydrogenphosphoric acid in water pH 2.3 (25:5:70, v/v/v), the mobile phase B was acetonitrile, methanol and 15 mM trihydrogenphosphoric acid in water pH 2.3 (45:5:50, v/v/v) and the mobile phase C was methanol and water (80:20, v/v). The time programme of gradient was: 0–22 min 100% of phase A, 22.01–45 min 100% of phase B and 45.01–55 min 100% of phase C. The fractions were collected by a fraction collector FC 204 (Gilson, Middelleton, USA). Detection was effected by a multiple-wave length detector with UV absorbance at 240 nm and by a liquid scintillation counter Rackbeta 1219 (LKB, Turku, Finland) after taking fractions and by addition of Sigma liquid scintillation cocktail to them.

2.4. Radioactivity assay

Aliquots of plasma, bile, urine, perfusion medium and solubilized liver were mixed with Sigma liquid scintillation cocktail and analyzed in the LKB LS 1219 counter. The values of DPM were calculated from measured CPM values by using of Quench standard curve fitting with the external standard [SQP (E)] by the method of Smoothed Spline according to the recommendation of the Beta Counter Producer.

2.5. Quantification of QL

Quantification of QL and its metabolites was performed on the basis of the known specific activity with regard to the linear dependence of radiolabelled compound concentration on measured radioactivity. Concentrations of QL and its metabolites, present in directly injected samples (bile and urine), were calculated from measured radioactivity after an addition of LS cocktail to the collected fractions. QL concentration in plasma and perfusate samples, respectively, was calculated from percentage of radioactivity extracted with organic phase during SPE procedure and from percentage of radioactivity associated with QL peak in the radiochromatogram.

2.6. Pharmacokinetics methods and calculations

The plasma concentration–time data were analyzed using non-linear least-square regression analysis by means of the Gauss–Newton method [14] weighting of concentration data according to 1/C. Plasma concentration curves could be described adequately by a biexponential equation

$$C = C_1 \, e^{-\lambda_1 t} + C_2 \, e^{-\lambda_2 t}$$

where C is the plasma concentration, t the time after administration, λ_1 and λ_2 are the rate constants characterizing the distribution and elimination phases, respectively, and C_1 and C_2 the hypothetical intercepts with ordinate. The following pharmacokinetic parameters related to the whole animal were calculated from QL plasma concentration–time course [15]: the pharmacokinetic volume of the central compartment, the volume of distribution at steady state, the total body clearance of QL from plasma, the distribution half-life and elimination half-life.

2.7. Protein binding determination

Binding of QL to rat plasma proteins was determined in vitro by equilibrium dialysis, at 37 °C, in a temperature-controlled rotating Plexiglass block with eight dialysis chambers [16,17]. A volume of 0.45 ml of plasma containing QL in a concentration of 250 ng/ml was dialyzed against an equal volume of isotonic phosphate buffer, pH 7.4; equilibrium was reached after 3 h. The free fraction of the drug was calculated from the ratio of the radioactivity detected in the dialysate and in plasma.

3. Results and discussion

HPLC analyses of non-radiolabelled QL (UV detection) and the ^{3}H-radiolabelled compound (radiometric detection) confirmed the identity both in the methanol solution and bile samples, respectively.

Under the chromatographic conditions described in the experimental part, QL and its S-oxide gave peaks with t_R 35.7 and 16.5 min, respectively.

S-oxide of QL was identified by comparison with synthetic non-radiolabelled standard.

Radiometric detection was used for all determinations; UV detection was employed only for the purpose of comparison of the synthetic standards retention times with the radiometric-determined retention times of QL and its

S-oxide, present in biological fluids. Quantification of QL and its metabolites was performed on the basis of the known specific activity. QL concentration (ng/ml) was calculated from determined radioactivity (DPM) in the analyzed volume and from a known specific activity (Bq/mg, DPM/mg). Concentration of QL metabolites (nmol/ml) were calculated from its specific activity (Bq/mol). If radiolabel is still present in the metabolite structure, molar activity of the parent drug and that of the metabolite was the same. The exception is a hypothetical cleavage of a drug molecule, where some non-radiolabelled fragments may arise and thus only the radiolabelled part of the drug structure is determined.

The fractions were collected in 1 min intervals within the analyses of the samples of plasma and perfusion medium (Figs. 3 and 8). Radiochromatograms were made from the radioactivity determined in collected fractions. On the other hand, during the analyses of samples of bile, urine and solubilized liver (Figs. 5, 6, 9 and 10), the fractions were collected in 25 s and 1 min intervals in a time period of 0–18 and 18–45 min, respectively. Radioactivity determined in 1 min fractions was re-counted for four 25 s intervals and a radiochromatogram was made from the values of radioactivity corresponding to 25 s intervals (the area under this peak is the same as in the case of the 1 min fraction). The peak shape was sharp in the radiochromatograms of Figs. 3 and 8, and the peaks became flat in the top of Figs. 5, 6, 9 and 10 for reasons given.

3.1. Pharmacokinetics and metabolism in rats

For pharmacokinetic experiments bile duct cannulated animals were used. Total radioactivity and unchanged QL–time curves in plasma are presented in Fig. 2. Plasma analyses showed that total radioactivity was slightly higher than that for unchanged QL. Nevertheless, QL represented the main form of radioactivity determined in plasma and in comparison with the parent drug metabolites were present in lower

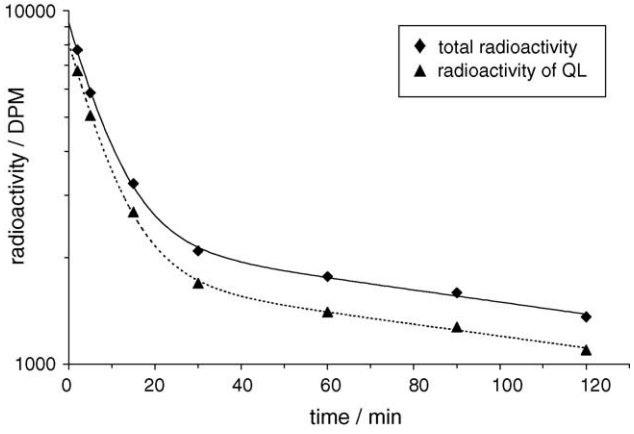

Fig. 2. Rat plasma time courses for total radioactivity and for unchanged QL in semi-logarithmic scale. The values represent experimental points and calculated curves of a randomly selected animal.

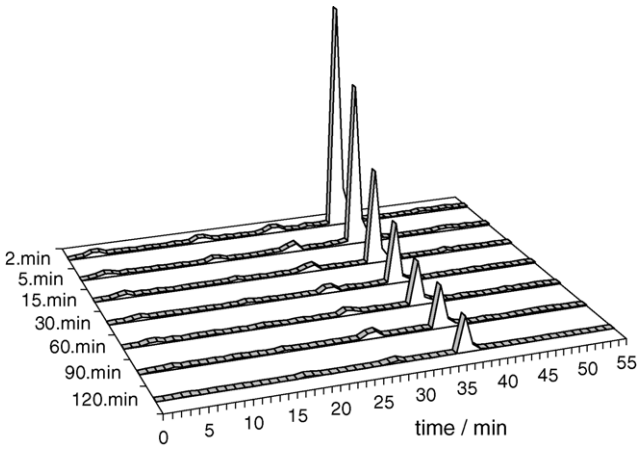

Fig. 3. Representative radiochromatograms of plasma withdrawn in selected time intervals after the drug i.v. administration.

levels in the systemic circulation (Fig. 3). Portions of these extractable components including S-oxide of QL ranged from 7 to 16% of total radioactivity, measured in the radiochromatogram of plasma. A certain part of plasma radioactivity (values increased with time, 4.6–12.5%) stayed in the aqueous phase probably in the form of more polar metabolites. This portion of radioactivity, not retained on the extraction column, was not further analyzed.

Binding of QL to rat plasma proteins was determined to be 99.7 ± 0.1% (mean ± S.D., $n = 4$).

The model selection was taken on the basis of Akaike's information criterion (AIC) [18,19]. A two-compartment model adequately describes the pharmacokinetics of QL during the course of the experiment. The pharmacokinetic parameters of QL in rat plasma are listed in Table 1. Even if lipophilicity of the agent is very high, its distribution volumes (both the volume of the central compartment and the volume at steady state) are relatively low (about 115 and 430 ml, respectively). High protein binding (free fraction in rat plasma being about 0.3%) is probably the cause of that finding. Nevertheless, both distribution volumes are several times higher than the blood volume (15–20 ml) that indicates also intensive binding of the drug in extravascular tissues. Distribution of QL in the body is relatively fast and its distribution half-life is approximately 6 min. On the other hand, the elimination half-life is more than 2 h. This value is in a good agreement with the amount of radioactivity (QL and its metabolites) eliminated by bile and urine during 2 h after administration (approx. 43%). However, the calculated value of

Table 1
Summary of QL pharmacokinetic parameters related to the whole animal (mean ± S.D., $n = 3$), calculated from QL rat plasma concentration–time courses

Distribution volume of the central compartment (ml)	115.9 ± 28.9
Distribution volume at steady state (ml)	430.0 ± 130.9
Total plasma clearance (ml/min)	3.47 ± 2.85
Half-life of distribution (min)	6.02 ± 3.65
Elimination half-life (min)	135 ± 63

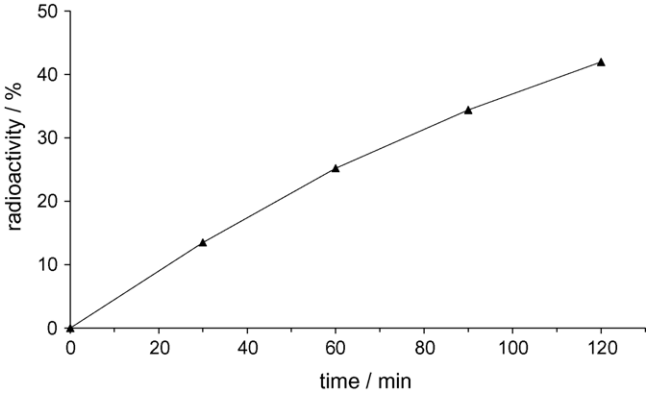

Fig. 4. Cumulative radioactivity elimination in % of administered dose measured in bile of randomly selected rat, collected at 30 min intervals during pharmacokinetic experiments.

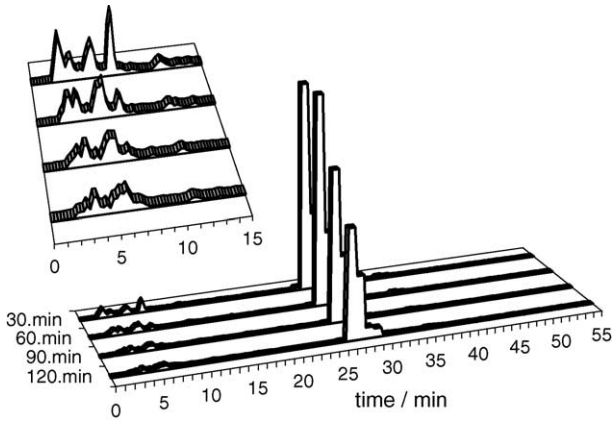

Fig. 5. Representative radiochromatograms of the bile from bile duct cannulated rat collected at 30 min time intervals.

the elimination half-life can be considered only as a preliminary one because of duration of the experiment (restricted by both methodological reasons and frequency of blood sample withdrawing). For this reason, further prolonged studies are required. Relatively low elimination rate is evidently connected with high binding of the agent in the blood. Its high protein binding lowers the unbound concentration in blood and thereby markedly decreases its rate of elimination by glomerular filtration. Moreover, QL is a high lipid-soluble drug that can diffuse rapidly from the glomerular filtrate back into blood. In addition, high protein binding of QL would also decrease the rate of its metabolism by liver enzymes. Thus, QL bound to plasma proteins should be considered as its storage depot in the body.

For an analysis of elimination pathways of QL, the bile duct of rats was cannulated and bile samples were repeatedly collected at 30 min intervals. The quantitative analysis of the parent drug and its metabolites in faeces is very difficult and in addition to it, drug-related metabolites present in faeces may not represent true biliary metabolites due to the possible involvement of colo-rectal microbes, which are known to catalyse several reactions, e.g., hydrolyses and reductions of the present compounds [20]. Analyses of bile confirmed an intensive biliary excretion of radioactivity. Fig. 4 shows a plot of radioactivity excreted to the bile during the course of experiment. Total radioactivity eliminated by the bile was approx. 42% of the dose over a period of 120 min, indicating that a reasonable portion of the drug and metabolites was excreted into the bile. Metabolic profiles of bile samples were mutually similar in intersubject comparison both for bile duct cannulated rats and in situ perfused rat liver, respectively. Relative proportions of metabolites were also quantitatively similar in different time points of bile collection. Radiochromatografic analyses of the bile of bile cannulated rats (Fig. 5) showed the presence of one major and several minor metabolites including S-oxide of QL and only small amounts of the unchanged parent drug.

Only a negligible portion (<0.5%) of radioactivity was eliminated via urine during 2 h after dosing. Mostly polar metabolites – but no parent drug – were detected in the urine (Fig. 6).

3.2. Perfused rat liver studies

As the liver is the major organ responsible for drug metabolism, the perfused rat liver preparation was used for the study of QL metabolism. The perfused rat liver employment makes it possible to study the liver metabolism without an influence of extra-hepatic factors. Analyses of perfusion medium (Fig. 7) showed moderate clearance of radioactivity. The input-to-outflow radioactivity ratio decreased with time and in the last intervals this value approached to one. QL extraction by the liver and a partial turnover of its metabolites back to the perfusion medium were the main processes involved in radioactivity–time profile in the perfusion medium. Binding of QL to bovine serum albumin was also determined in the perfusion medium. It was calculated to be $99.5 \pm 0.1\%$ (mean $\pm$ S.D., $n = 4$). The HPLC picture (Fig. 8) was practically identical with that found in plasma; QL represented the main form of radioactivity determined in the perfusion medium.

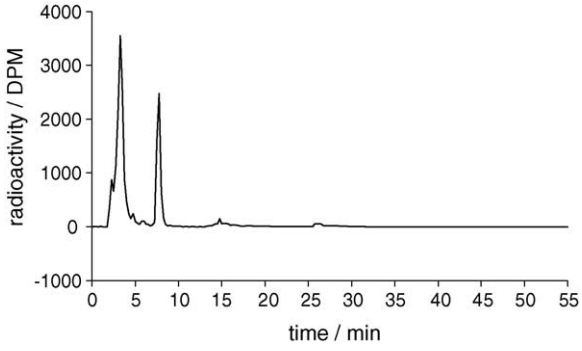

Fig. 6. Representative radiochromatogram of urine withdrawn after the end of the experiment.

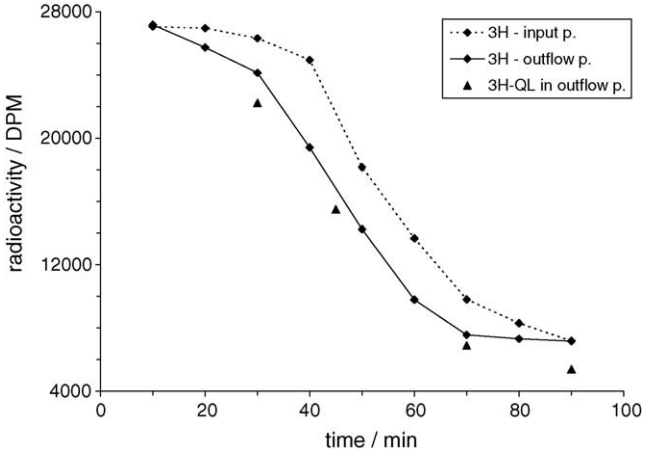

Fig. 7. Representative time courses of radioactivity measured in input and outflow perfusion medium. Triangles correspond to QL concentration in outflow perfusate determined in four time points.

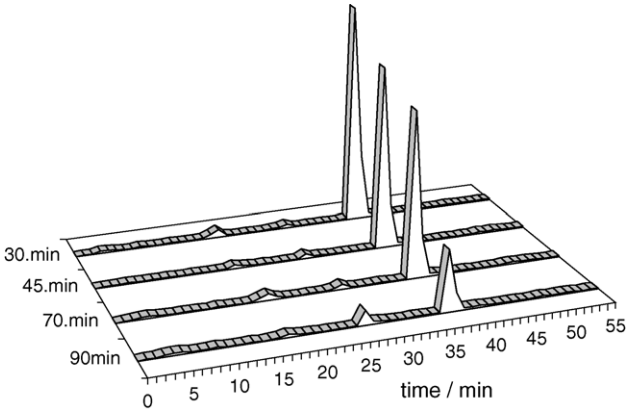

Fig. 8. Representative radiochromatograms of outflow perfusate withdrawn in selected time intervals after the drug loading.

Relative proportions of polar metabolites (eluted in shorter retention times) in the bile of the perfused rat liver (Fig. 9) were higher than that of the bile of bile duct cannulated rats.

3.2.1. Liver

HPLC analyses of the extract from both perfused rat liver and liver of bile duct cannulated rats (Fig. 10) showed the presence of three components: QL, its S-oxide and the major metabolite determined in the bile (t_R 26.5 min). Percentage of radioactivity in the solubilized tissue, not retained on the extraction columns (probably unextractable metabolites), ranged from 5.4 to 11.2% of total radioactivity measured in solubilized liver. These portions were not further analyzed.

Contrary to amount of S-oxide found in in vitro experiments [9,10], only traces of this metabolite were detected in plasma and bile in in vivo testing. The reason of this fact is probably a rapid conjugation of this compound in intact rats. In addition to S-oxide, QL was metabolized to sulphone

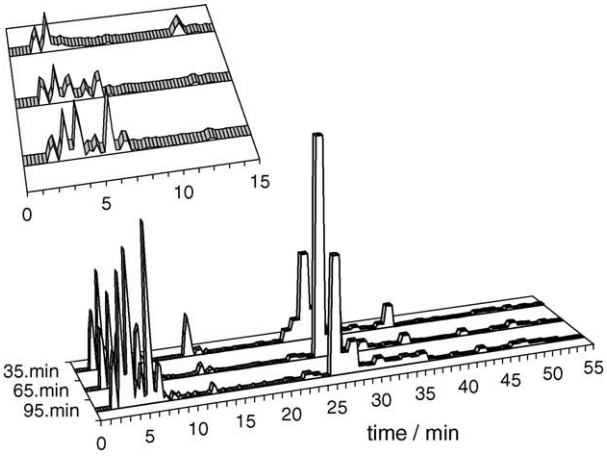

Fig. 9. Representative radiochromatograms of bile of perfused rat liver collected at 10 min time intervals.

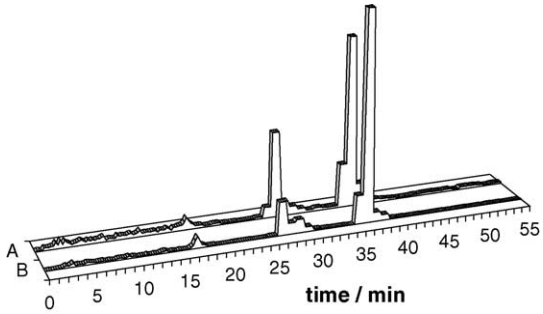

Fig. 10. Representative radiochromatograms of the extract from the liver of bile duct cannulated rat (A) and perfused rat liver (B).

and two dihydrodiols derivates in rat microsomes. Unfortunately, these potential metabolites were not available as standards. Nevertheless with regard to the polarity and the profile of metabolites detected in the HPLC analyses and results achieved in in vitro experiments, one can speculate that they are products of conjugation and/or that they could originate as the products of other metabolic pathways, e.g., hydroxylation. In metabolism of structurally similar drug montelukast [21,22], six metabolites and their diastereomers, including its S-oxide, were determined as products of oxidation, conjugation and hydroxylation. We presume that QL could be metabolized by similar metabolic pathways.

In conclusion, this study is the first view of the metabolism, elimination pathways and preliminary pharmacokinetic parameters' estimation of QL in rats. Further studies specializing in identification of unknown metabolites, as products of in vivo metabolism, should be performed to complete presented information.

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