Pharmacokinetics of Iopromide Liposomes in Rabbits

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Purpose. The dose-proportionality of pharmacokinetics of an iodinated contrast medium, iopromide, encapsulated into liposomes was investigated. ***Methods.*** Following single intravenous administration of 150 mg iodine/kg (potential diagnostic dose) and a five-fold higher dose in rabbits the pattern of elimination was studied until 7 d and the blood concentrations were monitored up to 72 h after administration. The iodine concentration in the liver was calculated on the basis of the blood concentration and related to the concentration measured in the rabbit liver. ***Results.*** The dose-normalized blood concentration-time profiles of the encapsulated iodine were not superimposable. Contrary to the low dose a steady-state concentration of 2.8 mg iodine/mL was observed in blood for 60 min after the high dose administration indicating a saturation of the liposomal liver uptake. For both doses the elimination of iodine occurred predominantly via the kidneys and was complete 7 d after administration. The dose-normalized amounts of iodine excreted with the urine were similar for both dose groups. From the blood data it was calculated that doses up to about 300 mg iodine/kg should result in a dose-proportional increase of liposomal liver uptake before saturation occurs. This was confirmed by the measured iodine liver concentrations after increasing the doses stepwise from 150 to 750 mg iodine/kg. ***Conclusions.*** In rabbits for the dose range 150 to 750 mg iodine/kg iopromide liposomes reveal dose-dependent pharmacokinetics due to a saturation in liver uptake which occurrs for doses of 300 mg iodine/kg corresponding to 300 mg lipid/kg onwards.

KEY WORDS: iopromide liposomes; dose-dependent pharmacokinetics; rabbit; liver-specific contrast agent.

INTRODUCTION

Particulate carriers such as liposomes loaded with iodinated x-ray contrast media have attracted increasing attention aiming at the diagnosis of liver lesions. Following intravenous administration, the particles are rapidly taken up by the Kupffer cells of the liver and the macrophages of the spleen. Based on the observation that the phagocytic activity, however, is predominantly found in normal but not in tumourous tissue, liposomes containing x-ray contrast agents might be helpful tools in the diagnosis of liver tumours and lesions [1-5]. Iopromide (Ultravist®) is a water soluble, hydrophilic and nonionic iodinated contrast agent for x-ray imaging. After intravenous administration iopromide is rapidly distributed into the extracellular fluid space and predominantly eliminated via the kidneys through glomerular filtration [6]. The main diagnostic potential of iopromide lies in the area of uro- and angiography and computed tomography [7].

The encapsulation of iopromide into liposomes yielded iopromide liposomes [8]. After encapsulation into liposomes, the hydrophilic character of iopromide was masked changing the pattern of biodistribution in a way that the liposomally encapsulated iopromide is specifically taken up by the Kupffer cells of the liver. Thus the diagnostic potential of iopromide liposomes mainly lies in the detection of liver lesions.

It was the aim of this study to investigate the pattern of elimination and the concentration-time profile in blood after single intravenous administration of a low -potentially diagnostic- dose and a five-fold higher dose of iopromide liposomes in rabbits. Opsonization of liposomes by blood constituents (mainly complement) is considered as the main factor for the recognition of the liposomes by the Kupffer cells of the liver. The depletion of blood from the opsonins after administration of increasing doses is discussed as a one reason for the dose-dependency observed in the pharmacokinetics of liposomal preparations [14, 15]. Therefore, if dose-dependent pharmacokinetics of iopromide liposomes were observed in the rabbit it would be of interest to calculate which volume of blood was needed for the opsonization of the iopromide liposomes and to calculate the maximum dose of iopromide liposomes that can be opsonized in rabbits.

MATERIALS AND METHODS

Characterization of Liposomes

Iopromide liposomes were prepared as described elsewhere [8]. Briefly, iopromide liposomes consisted of soy phosphatidylcholine, cholesterol and stearic acid at a molar ratio of 4:5:1 and were prepared by adding iopromide (Ultravist-370®) and using the ethanol-evaporation technique with subsequent lyophilization. The iodine:lipid ratio was 1. The freeze-dried powder had to be reconstituted by the addition of four volumes of 135 mM mannitol solution.

In the rehydrated suspension about 30 % of the iopromide was encapsulated into liposomes. The remainder represented free, unencapsulated iopromide. In the rehydrated suspension the mean diameter of the liposomes was 0.5± 0.1 μm and the iodine content was about 100 mg iodine/mL. The freshly rehydrated suspension was used for intravenous administration.

Note. In order to clearly determine the pharmacokinetic properties of the liposomal iopromide it was important to separate the free iopromide from the encapsulated (liposomal) iopromide in each blood sample. This was performed by equilibrium dialysis as described below. In urine no separation between free and liposomal iopromide was possible and necessary. Following release of iopromide from the liposomes in the Kupffer cells of the liver, the renally excreted iopromide contained free iopromide only.

Contrast Medium Administration and Dose

The suspension of iopromide liposomes was infused into the marginal ear vein of the animals using an infusion pump

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called "Precidor" (Informs, Basel). Two doses were investigated: 150 mg of total iodine/kg BW corresponding to 150 mg lipid/kg BW and 750 mg of total iodine/kg BW corresponding to 750 mg lipid/kg BW. The iopromide liposomes were infused using the same speed of administration (0.75 mL/kg/min) for both dose groups resulting in an infusion period of about 2 min for the low and 10 min for the high dose.

Animals and Experimental Procedure

The performance of the animal experiments adhered to the "Principles of Laboratory Animal Care". The experiments were performed in female hare-rabbits (breeder: Wulf, Germany) weighing 2.0-3.1 kg. Groups of five animals per dose were investigated. Twelve hours before the start of the experiment and during the first 3 h after administration of the contrast medium the animals had no access to food. Then the animals had free access to food and water. For the collection of urine a balloon catheter was introduced into the bladder before contrast medium administration after the animals had been anaesthetized by intramuscular administration of 0.55 mL/kg of a mixture of Rompun® /Ketavet® (1.5/1; v/v). The administration of the contrast medium started after the animals had fully recovered from the anaesthesia. This was approximately 2 hours after the awakening of the animals. During the application and during the collection of blood and urine samples the animals were kept in a restraining cage for 3 hours. Then the animals were transferred to metabolism cages. Seven days after contrast medium administration the animals were sacrificed by exsanguination under Rompun®/ Ketavet® anaesthesia.

Sampling Procedure

For both dose groups investigated blood samples of 2 mL each were taken from the central artery of the ear before and 5, 10, 20, 30 and 45 min, 1, 1.5, 2 and 3 h after the end of the infusion. For the high dose group (750 mg iodine/kg) blood sampling was extended and the sampling points were as such: during the 10-min infusion period (t = -10, -7.5, -5, -2.5 and 0 min) and after the end of infusion (5, 10, 20, 30, and 45 min, 1, 1.5, 2, 4, 6, 8, 24, 48 and 72 h). In this latter group a saline infusion of 30 mL was given over a 120-min period after the end of the contrast medium infusion in order to substitute for the loss of blood due to the frequent blood sampling. Urine was collected quantitatively before and 0.5, 1, 2 and 3 h after the end of infusion via a catheter and then daily until 7 d p.a. in metabolism cages. Feces was collected daily until 7 d p.a.

Separation Between Liposomal and Free Iopromide

Immediately after sampling 1 mL of each blood sample underwent equilibrium dialysis for 1 h at 37 °C against 0.125 M phosphate buffer (Sörensen buffer), pH = 7.4, in order to separate between the free iopromide at the buffer side (Cbuffer) and the total (encapsulated + free) iopromide at the blood side (Cblood). The percentage of the encapsulated iopromide in blood was calculated according to:

$$\%\ \text{iodine}\ \textit{encapculated} = (\text{Cblood} - \text{Cbuffer}) \cdot 100 / (\text{Cblood} + \text{Cbuffer})$$

In an earlier experiment it was shown that 1 h dialysis time is sufficient for achieving the equilibrium concentration of free iopromide on both sides of the membrane. The dialysis membranes used had a 5000 D cut off (Dianorm, Munich, Germany).

Quantitative Analysis

The iodine concentration was measured both in the solution injected into the animals and in all biological samples by means of x-ray fluorescence [10]. The blood and urine samples were measured directly without further sample preparation whereas the feces samples were homogenized in an aqueous solution of 5 %-KOH before measurement. The lower limit of determination of this method is 0.01 mg iodine/ mL. The linear range of measurement was 0.01 to 2 mg iodine /mL.

Pharmacokinetic Analysis

Model-independent (noncompartmental) analysis was applied using the computer program Topfit (2.0) [9]. The concentration-time data obtained from the *free* and *encapsulated* iodine concentration in blood after intravenous administration of the low and the high dose underwent pharmacokinetic analysis.

Total area under the blood concentration-time curve (AUCdata) was calculated for both dose levels according to the time period that was determinable in blood: from time 0 to 3 h for the low dose study (free and encapsulated iodine); from time 0 to 8 h (encapsulated iodine) for the high dose study; from time 0 to 4 h (free iodine) for the high dose study. For calculation of AUC the beginning of the contrast medium infusion time was set to t = 0. Total area under the blood concentration time curve, AUC, was obtained from $AUC = AUC(0\text{-tlast}) + AUC(\text{tlast}\text{-}\infty)$, where AUC(0-tlast) and AUC(tlast-∞) correspond to the truncated area up to the time of the last measurable blood concentration, tlast, and the truncated area from tlast to infinite time. The value for AUC(0-tlast) was computed by applying the linear trapezoidal rule. The estimate for AUC(tlast-∞) was obtained from $AUC(\text{tlast}\text{-}\infty) = \text{Clast}/\lambda z,b$. Total clearance was computed from CL = Dose/AUC. The steady-state volume of distribution was calculated from $VSS = \text{Dose} \cdot AUMC/AUMC^2 - T \cdot \text{Dose}/2AUC$, where AUMC and T correspond to the area under the first moment curve and the infusion time, respectively. Estimates for AUMC were obtained from $AUMC = AUMC(0\text{-tlast}) + AUMC(\text{tlast}\text{-}\infty)$. The value for AUMC(0-tlast) was estimated on application of the linear trapezoidal rule and AUMC(tlast-∞) was calulated from $AUMC(\text{tlast}) = \text{Clast}/\lambda z,b^2 + \text{tlast}*\text{Clast}/\lambda z,b$. The terminal half-life in blood, $t_{1/2},z,b$, of the *free* iodine was calculated from t = 0 to 3 h for the low and from 0 to 4 h for the high dose group. The terminal half-life of the *encapsulated* iodine was calculated from 0-3 h for the low and from 1.5 to 8 h for the high dose group.

The plot of the urinary excretion rates versus time revealed a biphasic profile. Therefore, two half-lives were calculated from the urinary excretion data, e.g. $t_{1/2}\lambda z,u1$ (excretion rates 0-3 h p.a.) and $t_{1/2}\lambda z,u2$ (excretion rates 1-7 d p.a.).

Prediction of Liver Concentration from Blood Data

It was assumed that due to the particulate character the biodistribution of the liposomal iopromide is mainly confined to the blood volume, to the liver and the spleen. This pattern of biodistribution was shown for iopromide liposomes in rats [8]. Since in rabbits the volume of the spleen is a hundred-fold lower than the volume of the liver [11] the amounts of the liposomal iopromide distributed to the spleen was considered to be negligible (based on the spleen concentration measured in some animals the error by neglecting the spleen was assessed to be in the order of 2-3 %).

The concentration C of iopromide in the liver L at time t ($C_{L,t}$) was calculated according to

$$C_{L,t} = A_{L,t} / V_L$$

where $A_{L,t}$ represents the amount of iodine in the liver tissue at time t, and V_L represents the volume of the liver tissue. $A_{L,t}$ was calculated according to:

$$A_{L,t} = (D \cdot F / V_b - C_{b,t}) \cdot V_b$$

where D represents the dose (total dose of iodine/kg), F represents the fraction of the iodine dose encapsulated in liposomes, V_b represents the blood volume, and $C_{b,t}$ represents the concentration of encapsulated iodine in blood at time t. V_b was 60 mL/kg and V_L was 43 g/kg [11].

Measurements of the Liver Concentrations After Increasing Doses

In order to follow the concentration-time profile of the iodine concentrations in the liver after increasing doses of iopromide liposomes a computed tomography study (125 kV; Somatom, Siemens) was performed in hare rabbits (2.2-4.2 kg) using the following doses: 150 / 250 / 300 / 400 and 750 mg of total iodine/kg BW. For the highest and lowest dose three animals were investigated, each, whereas for the other doses one animal per dose was used. The animals were anaesthetized with a mixture of xylazine (Rompun®) and ketamine (Ketavet®) before injection of the contrast material. The absorption of the x-rays in the liver tissue was measured at various time points up to 60 min p.i. and expressed in Hounsfield Units (HU). The increase in HU compared to baseline was calculated. Before the animal experiment a calibration curve was established at the computed tomography instrument using aqueous solutions of iopromide with increasing concentrations. This calibration curve indicated a direct proportional linear increase in HU (1 mg iodine corresponding to 27 HU) with increasing iopromide concentrations in the range of 0.1 to 3 mg iodine/mL. Based on this calibration curve the liver iodine concentration at 60 min was calculated.

Biostatistics

The difference of pharmacokinetic parameters between the 150 and 750 mg of total iodine/kg dose groups was evaluated by the analysis of variance (α =5 %) after normal distribution had been confirmed by the Bartlett Test. In case the groups were inhomogenous the Kruskal Wallis Test was applied with α = 5 %.

RESULTS

In iopromide liposomes about 30 % of the iodine concentration represented liposomal iopromide whereas the remainder represented free, unencapsulated iopromide. Therefore, the pharmacokinetic parameters were calculated based on the *free* and the *encapsulated (liposomal)* iodine concentration in blood whereas the urinarily excreted amounts of iopromide represented total iopromide (free iopromide administered and released from the Kupffer cells).

Free Iopromide Concentration

In blood the *free* iodine concentration was determinable up to 3 h p.i. and declined monophasically with a mean (sd) half-life ($t_{½\lambda z,b}$) of 0.65 (0.11) h and 0.54 (0.04) h for the low

Table I. Pharmacokinetic Parameters as Evaluated in Ten Female Hare Rabbits After Single Intravenous Injection of a Low (150 mg Total Iodine/kg) and a High Dose (750 mg Total Iodine/kg) of Iopromide Liposomes by Means of Noncompartmental Analysis[a]

From blood concentration data		150 mg total iodine/kg 0–3 h p.i.		750 mg total iodine/kg 0–72 h p.i.	
		Free iodine Mean (SD)	Encapsulated iodine Mean (SD)	Free iodine Mean (SD)	Encapsulated iodine Mean (SD)
AUC0–∞[b]	[mg * h/mL]	0.32 (0.04)	1.09 (0.17)	1.61 (0.09)	30.7 (2.62)
AUCdata[c]	[mg * h/mL]	0.29 (0.04)	0.66 (0.15)	1.58 (0.09)	16.2 (1.85)
Vss[d]	[L/kg]	0.48 (0.07)	0.33 (0.03)[g]	0.66 (0.05)	0.25 (0.03)[g]
$t_{1/2}\lambda z$, b[e]	[h]	0.65 (0.11)	1.70 (0.35)	0.54 (0.04)	7.09 (1.04)
CL[f]	[mL/min/kg]	7.88 (0.89)	2.35 (0.38)[g]	7.79 (0.46)	0.41 (0.04)[g]

[a] The pharmacokinetic parameters are described separately based on the free and on the encapsulated (liposomal) iodine concentration in blood since the liposomal preparation administered contained about 30% liposomal iopromide and 70% of free iopromide.

[b] AUC0–∞: area under the blood concentration-time curve from time 0 extrapolated to infinity.

[c] AUCdata: area under the blood concentration-time curve of the measured samples.

[d] Vss: volume of distribution at steady-state.

[e] $t_{1/2}\lambda$ z,b: elimination half-life in blood.

[f] CL: total blood clearance.

[g] The values are calculated based on the dose expressed as total iodine/kg BW; due to the fact that the liposomal preparation contained only about 30% of encapsulated iopromide the real values of the liposomal CL and the liposomal Vss are about three-fold lower.

and the high dose group, respectively (Table I, Figure 1). Statistically no significant difference was found for this parameter between the dose groups ($p>0.05$). The dose normalized values of AUCdata were also not statistically different ($p>0.05$) between the low and the high dose group for the free iopromide concentration indicating dose-proportional pharmacokinetics. The steady-state volumes of distribution reflected the extracellular fluid space. 3 h after administration the free iodine concentration measured in blood corresponded to 1.16 and 0.65% of the administered dose for the low and the high dose group, respectively, and more than 90 % of the administered dose of free iodine was renally excreted (see below).

Encapsulated (Liposomal) Iopromide Concentration

The corresponding blood concentration-time profile of the *encapsulated* iopromide did not decline in parallel for both dose groups and was not superimposable as can be seen from Figure 2 indicating dose dependence in the pharmacokinetics of iopromide liposomes in rabbits.

For the high dose group in blood the observation period of 3 h was not sufficient for adequate interpretation of pharmacokinetics because 3 h p.i. the liposomal iodine concentration in blood corresponded to about 19 % of the total iodine dose administered whereas for the low dose group the corresponding value had declined to 5 %. Therefore, an observation period in blood from 0 to 72 h was used for adequate interpretation of the pharmacokinetics of the high dose.

Immediately after administration of the high dose the *encapsulated* iodine concentrations showed a mean constant level of 2.84 mg iodine/mL for the time period 0 to 60 min indicating a saturation of the liposomal uptake by the liver.

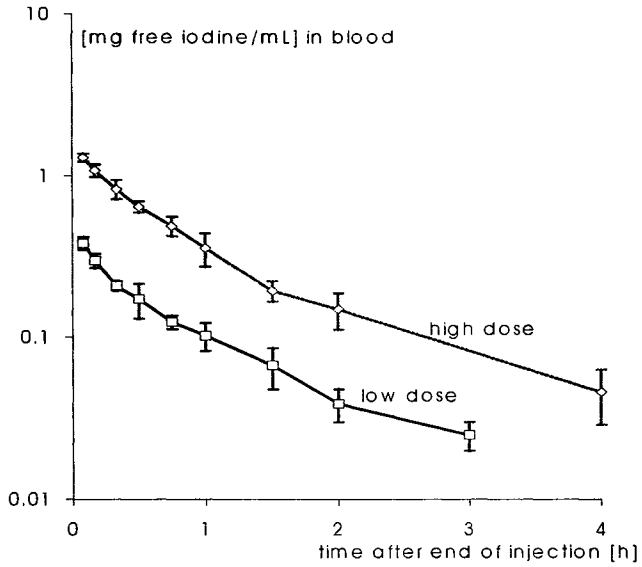

Fig. 1. Concentration time-profile of the free iodine in blood after single intravenous administration of 150 mg of total iodine/kg (low dose) and 750 mg total iodine/kg of iopromide liposomes in rabbits. The two curves decline in parallel and confirm the dose-proportional pharmacokinetics of free (not encapsulated) iopromide (Ultravist®). Each data point represents the mean and standard deviation of five animals.

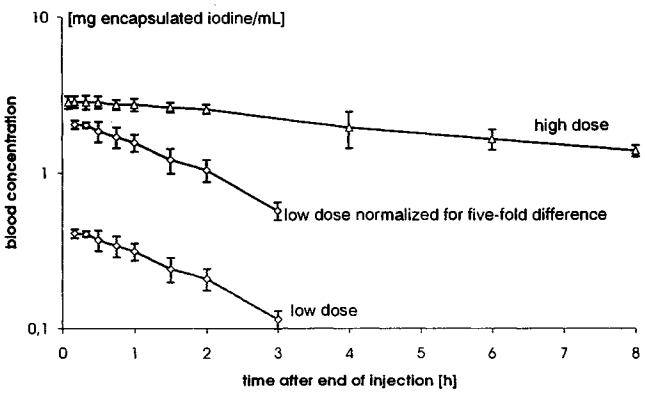

Fig. 2. Concentration time-profile of encapsulated (liposomal) iodine in blood after single intravenous administration of 150 mg of total iodine/kg (low dose) and 750 mg total iodine/kg of iopromide liposomes in rabbits. The measured values of the low dose were normalized for the five-fold difference and included in the figure. The figure clearly demonstrates that the two curves do not decline in parallel and are not superimposable after dose normalization indicating dose-dependent pharmacokinetics of the liposomal iodine in iopromide liposomes. Each data point represents the mean and standard deviation of five animals.

Thereafter, in the time period up to 8 h the encapsulated iopromide declined with a mean (sd) half-life ($t½\lambda z,b$) of 7.1 (1.0) h.

Following the high dose administration the *encapsulated* iodine concentrations in blood were determinable up to 8 h p.i. At 8 h p.i. no free iodine was determinable in blood. Consequently, the total iodine concentration in blood at 8 h p.i. was identical with the liposomal iodine concentration. This was confirmed by measuring the degree of encapsulation in the 8 h blood sample which was > 98 %. For the determination of the liposomal iodine in blood the blood samples underwent equilibrium dialysis. After dialysis the iodine content had to be determined at both sides of the membrane. Therefore, the buffer and blood samples had to be diluted three-fold. Consequently, the limit of determination of the liposomal iodine is three-fold lower than the determination of total iodine in blood which was 0.01 mg iodine/mL because the blood sample was measured undiluted. Knowing that from 8 h onwards the total iodine concentration in blood is identical with the liposomal iodine concentration the blood concentration of the total iodine was followed further on. Measurable iodine concentrations were obtained for 24- and 48-h-blood samples (not for the 72 h sample) corresponding to 4.5 and 0.7 % of the totally administered iodine dose, respectively. Once more the elimination half-life of the liposomal iopromide in blood was calculated from 1.5 to 48 h (including the 24 and 48 h data points) resulting in a value of 8.75 ± 1.66 h. This calculation is based on an observation period which coveres five half-lives, and therefore is slightly different from the value of 7.1 h reported above which covered one half-life only (0-8 h p.i.).

Elimination

The urinarily excreted amounts of iopromide represent iopromide which has to be considered as a mixture of: 1. the excreted amounts of the free iopromide injected by the lipo-

somal preparation, 2. the excreted amounts of the iopromide that is released from the liposomal preparation (encapsulated iopromide) that had been phagocytosed by the Kupffer cells and macrophages of the spleen.

In urine and feces for the low dose group the iodine concentrations were determinable up to 6 d after administration whereas for the high dose group the iodine concentrations were determinable up to 7 d p.a. For both dose groups investigated renal elimination was the major route of elimination. Seven days after administration the mean (sd) values of the recovery [%] of iodine in urine were 79.2 (12.8) for the low and 92.7 (5.73) for the high dose group whereas the mean (sd) recovery values [%] of iodine in feces were 11.4 (5.91) for the low and 7.16 (3.25) for the high dose group (Table 2). A statistically significant difference was found between the low and the high dose group for the dose normalized amounts of iodine recovered from urine whereas for the extrarenal elimination no difference was found between the two dose groups. The difference in renal recovery is probably not dose related but might be explained by the incompleteness in the urine collection of the low dose group because the total recovery of iodine was also significantly lower for the low dose group when compared to the high dose group (89.6 ± 4.77 % versus 99.9 ± 5.03 %).

From 3 h after application onwards the free iopromide that was injected with the liposomal preparation had most likely left the body because at 3 h p.i. the renal excretion of iopromide amounted to 68.9 % of the total iodine dose which would correspond to 98 % of the free iopromide dose that was injected with the liposomal preparation. The renal excretion rates of total iodine versus the midpoint of the collection interval showed a parallel decrease for the first 3 h after application of the contrast medium for the two dose levels with mean (sd) urinary half-lives [h] of 1.00 (0.36) for the low and 0.80 (0.18) for the high dose group ($p>0.05$). However, when calculated from the late renal excretion rates (1 d to 7 d p.a.) the mean (sd) urinary half-lives [h] were slightly different ($p<0.05$) with values of 25.1 (2.36) for the low and 30.0 (2.38) for the high dose group (Table II and Figure 3).

Predicted and Measured Liver Concentration

The values of the iodine concentration in liver at 60 min after injection as calculated from the blood data were 0.73 mg iodine/g for the low and 1.9 mg iodine/g for the high dose (n = 5 per dose). The values of the iodine concentration in the liver tissue as derived from the density of the liver using computed tomography were 0.77 (n = 3), 1.23 (n = 1), 1.90 (n = 1), 2.0 (n = 1) and 2.1 mg iodine/g (n = 3) at 60 min following 150, 250, 300, 400 and 750 mg iodine/kg administration. The data are summarized in Figure 4.

Table II. Elimination of Iodine in Urine After Single Intravenous Injection of a Low (150 mg Total Iodine/kg) and a High Dose (750 mg Total Iodine/kg) of Iopromide Liposomes in Ten Female Hare Rabbits

		High dose	Low dose
$t_{1/2}\lambda z,u1$[a]	[h]	0.80 (0.18)	1.00 (0.36)
$t_{1/2}\lambda z,u2$	[h]	30.0 (2.38)	25.1 (2.36)
Ae (0–7d)[b]	[% of dose]	92.7 (5.73)	79.2 (12.8)
Aef (0–7d)[c]	[% of dose]	7.16 (3.25)	11.4 (5.91)
Recovery	[% of dose]	99.9 (5.03)	89.6 (4.77)

[a] $t_{1/2}\lambda z,u1$ + 2: elimination half-life calculated from the renal excretion rates from 0–3 h (u1) and from 1–7d (u2).
[b] Ae: dose normalized amounts recovered from urine.
[c] Aef: dose normalized amounts recovered from feces.

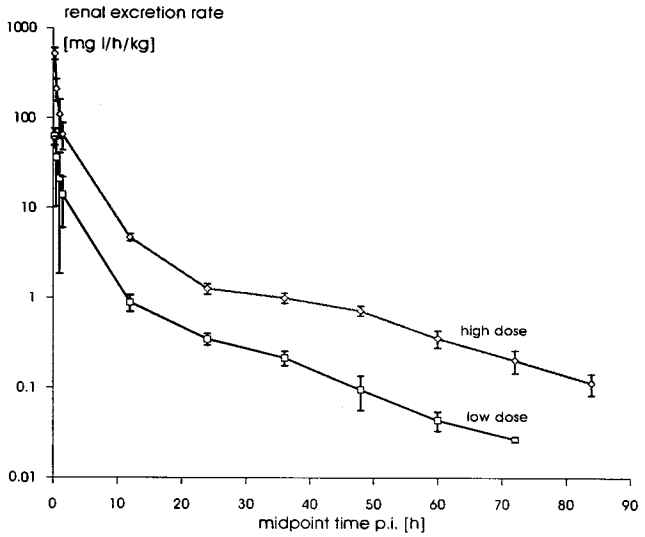

Fig. 3. Renal excretion rates of iopromide in [mg iodine/h/kg] versus the midpoint of the collection interval after single intravenous administration of 150 mg of total iodine/kg (low dose) and 750 mg total iodine/kg of iopromide liposomes in rabbits. Each data point represents the mean and standard deviation of five animals.

DISCUSSION

In the rehydrated and filtered suspension of iopromide

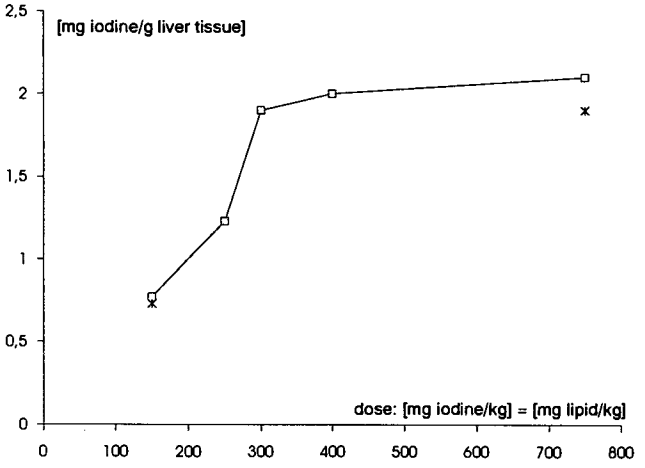

Fig. 4. Accumulation of iopromide liposomes in the liver tissue of rabbits after single intravenous administration of increasing doses of iopromide liposomes (150, 250, 300, 400 and 750 mg total iodine/kg corresponding to 50, 85, 100, 130 and 250 mg liposomal iodine/kg). The squares represent the data obtained by measuring the density in the liver by means of computed tomography. The stars represent the data calculated (predicted) on basis of the liposomal iodine concentration in blood. The predicted data points (stars) represent the mean of five animals whereas the squares represent the data of one animal except for the lowest and for the highest dose (n = 3).

liposomes about 30 % of the total iopromide content was encapsulated into liposomes whereas the remainder represented free, unencapsulated iopromide [8]. As a consequence the pharmacokinetics of iopromide liposomes were determined both by the kinetics of the free and the liposomally entrapped iopromide. Separating between the free and the encapsulated fraction of iodine in blood allowed for the specific determination of the pharmacokinetics of the liposomally entrapped iopromide. In addition, the pharmacokinetics of the free iopromide were determined in blood. In blood the half-life of elimination of the *free* iopromide was 0.65 h (39 min) and 0.54 h (32 min) for both dose groups and dose-proportional pharmacokinetics was observed. However, the plots of the *encapsulated* iopromide concentration versus time of the two dose groups were not superimposable after dose normalization and did not decline in parallel indicating dose-dependent pharmacokinetics of iopromide liposomes in rabbits. This was most likely due to a saturation in the uptake of the liposomes by the liver and spleen since the distribution and elimination of the free, non-encapsulated iopromide was shown -and is well known- to be of linear pharmacokinetics in the dose range studied.

In the rabbit 7 d after single intravenous administration of 150 and 750 mg iodine/kg the excretion of iopromide liposomes was complete and occurred predominantly via the kidneys. 3 h after application the urinarily excreted iopromide amounted to 68.9 % of the total iodine dose corresponding to 98 % of the free iopromide dose that was injected with the liposomal preparation. Consequently, in the time period from 3 h onwards the renally excreted iopromide most likely represented the iopromide released from the liposomes that had been phagocytosed by the Kupffer cells of the liver and spleen.

For both dose levels investigated a fast elimination phase was observed in urine with a half-life of about 35 minutes representing mostly the renal elimination of the free, unencapsulated iopromide. This half-life correlates well with the half-lives of the free iopromide measured in the blood of 39 and 32 min (see above). The late elimination phase calculated from urinary excretion data was different for the two dose levels (25 and 30 h, respectively) and most likely represents the elimination of the liposomally entrapped iopromide that is released from the Kupffer cells of the liver or from the macrophages of the spleen.

In general the uptake of liposomes by the Kupffer cells of the liver is dependent on the liposome composition, on the liposome size and on the effect of the opsonization of the liposomes [12-16]. The opsonization of liposomes by the interaction with the complement system might cause destabilization and leakage of liposomes in blood or plasma depending on the size and composition of the liposomes [17, 18]. The liposomes studied here have shown to be sufficiently stable in vitro after incubation with plasma [19]. On the other hand the opsonization of liposomes by the complement system is believed to be a determinant factor for the recognition of the liposomes by the Kupffer cells of the liver. The depletion of the blood from opsonines is considered as one reason for the observed saturation in liver uptake of liposomes [14, 15, 16]. Another possible reason for saturable liver uptake is a saturation in the phagocytic activity of the Kupffer cells [14]. Therefore, it was of interest to investigate at which dose a saturation in the accumulation of iodine by the rabbit liver is observed after application of iopromide liposomes.

Following administration of 750 mg iodine/kg of iopromide liposomes a constant blood concentration of 2.8 mg iodine/mL was observed for 60 min indicating saturation of the liposomal liver uptake. Based on the measured iodine concentration in the liver of 2.1 mg iodine/g and based on the volume of the liver of 43 g/kg it was calculated that about 90 mg liposomal iodine/kg were taken up by the liver at saturation (2.1 mg iodine/g multiplied by 43 g/kg). Consequently, doses of 300 mg of *total* iodine/kg and above should result in the maximum liver iodine concentration in rabbits. If opsonization of liposomes is a prerequisite for recognition and uptake by the liver it was calculated that about 0.2 mL of rabbit blood were able to opsonize 1 mg of *total* iodine corresponding to 1 mg of lipid (60 mL/kg of blood volume in rabbits divided by 300 mg total iodine/kg). If the depletion of blood from opsonins is the determinant factor for the dose-dependence in the liver uptake the "threshold" dose for saturation should be about 300 mg total iodine/kg BW. Indeed, this value is in good agreement with the value found from the imaging study using computed tomography where the saturation occurred at a dose of about 300 mg of total iodine/kg BW (300 mg lipid/kg) as is demonstrated in Figure 4.

Another interesting conclusion can be drawn from Figure 4: at the low dose e.g. 150 mg of total iodine/kg corresponding to 50 mg of liposomal iodine/kg the accumulation of iodine in the liver reaches 0.77 mg iodine/g liver corresponding to a total amount 66 mg of iodine for a rabbit of 2 kg of BW based on the liver volume of 43 g/kg. Assuming that only the liposomal iodine is taken up by the liver this value corresponds to 66 % of the liposomal iodine dose administered. A similar observation is made for the 250 mg total iodine/kg dose where the corresponding value is 62 %. In other words, eventhough the saturation of the liver uptake of iopromide liposomes is not reached yet only 60-70 % of the liposomal dose is recognized and taken up by the Kupffer cells of the liver. One reason for this selection in liver uptake might be the size of the liposomes. It was shown by Harashima et al. [15] that the size of the liposomes affected the recognition by the complement (=opsonization), and the liposomes were taken up by the liver depending on the extent of opsonization; liposomes smaller than 200 nm in diameter were not opsonized whereas best liver uptake and opsonization was found for liposomes of 800 nm in diameter. The mean diameter of the iopromide liposomes studied here was about 500 nm.

Therefore we conclude from our study that the size of the iopromide liposomes could be further optimized for recognition and uptake by the liver. Furthermore, we conclude that the dose-dependency in liver uptake that we observed for iopromide liposomes in the rabbit might be due to the depletion of the blood from opsonins. However, we do not know at which dose level saturation of hepatic uptake would occur provided enough complement is available for opsonization. The latter question can be answered by studying the hepatic uptake after increasing doses using an isolated perfused liver model.

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