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 occurs 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.
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 anesthetized by intramuscular administration of 0.55 mL/kg of a mixture of Rompun®/Ketanest® (1:1; 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®/Ketanest® 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 blood loss 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 (Chbuffer) and the total (encapsulated + free) iopromide at the blood side (Cblood). The percentage of the encapsulated iopromide in blood was calculated according to:

\[
\text{% iodine encapsulated} = \frac{\text{Cblood} - \text{Chbuffer}}{\text{Cblood} + \text{Chbuffer}} \times 100
\]

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-tlast) + AUC(tlast-∞), 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(tlast-∞) = Clast/az,b. Total clearance was computed from CL = Dose/AUC. The steady-state volume of distribution was calculated from Vss = Dose · AUMC/AUMC2 · T · 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-tlast) + AUMC(tlast-∞). The value for AUMC(0-tlast) was estimated on application of the linear trapezoidal rule and AUMC(tlast-∞) was calculated from AUMC(tlast) = Clast/az,b2 + tlast/Clast/az,b. The terminal half-life in blood, t1/2,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. t1/2,z,u1 (excretion rates 0-3 h p.a.) and t1/2,z,u2 (excretion rates 1-7 d p.a.).