Continuous Microinstillation of Phenol Red on Liver Surface for Liver Site-Selective Delivery

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Received December 4, 1998; accepted April 21, 1999

We report a very promising approach for liver site-selective drug delivery through drug instillation on liver surface. Phenol red, which was selected as a model drug, was accumulated in the instillation site after instillation on the rat liver surface. The site-selective localization was enhanced by gradually and continuously instilling a small amount of drug solution on the liver surface.

Key words drug delivery system; liver; continuous microinstillation; liver surface; phenol red

The liver plays an important role in maintaining homeostasis, thus many liver diseases such as hepatitis, cirrhosis and hepaticoma are lethal. When drugs are administered via common routes (orally, intravenously, and so on), they are distributed to other organs and to non-pathological regions in the liver, increasing the possibility of side effects. One method of liver targeting involves changing the route of administration. Although hepatic arterial, portal venous and peritoneal administration have been attempted as targeted delivery methods, the administered drugs are distributed to the entire liver. We have previously shown that direct injection is not suitable for site-selective drug delivery to the liver with a high blood flow, since directly injected drugs are rapidly cleared from the injection site and enter systemic circulation. In the present study, we have attempted to perform drug instillation on the liver surface for liver site-selective drug delivery.

MATERIALS AND METHODS

Animal Study All animal experiments in the present study conformed to the Nagasaki University Guideline for Animal Experimentation.

Male Wistar rats (230—270 g), which were not starved, were anesthetized with sodium pentobarbital (50 mg/kg body weight, intramuscular injection), and an incision was made in the middle of the abdomen. Additional sodium pentobarbital was administered as necessary during the experiment to maintain anesthesia. Phenol red (Nacalai Tesque, Inc., Kyoto, Japan) solution was prepared in isotonic phosphate buffer (pH 7.4) to yield a concentration of 10 mg/ml, and was administered as follows.

Continuous Microinstillation on the Liver Surface: The drug solution (235 μl) was instilled using a polyethylene tube fixed by a clamp on the surface of the left lateral lobe at a flow rate of 0.047 ml/min with an infusion pump (Natsume, Tokyo, Japan).

Bolus Instillation on the Liver or Small Intestine Surface: The drug solution (235 μl) was instilled on the surface of the left lateral lobe or distal small intestine with a syringe.

Intravenous Injection: The drug solution (235 μl) was injected into the jugular vein in rats in which the abdomens were opened.

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After administration of the drug solution, the liver was perfused in situ via the portal vein with saline (above 50 ml). The liver was excised and homogenized in three-fold of its weight of isotonic phosphate buffer (pH 6.5). After 1.5 ml of acetone was added to 2.5 ml of the liver homogenate, the mixture was shaken for 15 min, followed by centrifugation at 2000×g for 20 min at 4°C. The phenol red concentration in the resulting supernatant was determined spectrophotometrically at 560 nm after dilution with 1 N NaOH.

Statistical Analysis Statistical analysis was performed by applying a paired t-test or unpaired t-test. p<0.05 was considered to be statistically significant. All results were expressed as the mean value±standard error of at least four experiments.

RESULTS AND DISCUSSION

Phenol red was selected as a model drug because its disposition characteristics in liver are known. We instilled 235 μl of phenol red solution (10 mg/ml) on the rat liver surface at the area of the left lateral lobe. Figure 1 shows the liver concentration of phenol red at 5 min after bolus instillation of a drug solution. The concentration of phenol red in the instillation site (left lateral lobe) was significantly higher than that in the other lobes of the liver. On the other hand, when the same volume of drug solution was instilled around the distal small intestine or injected intravenously, no significant difference in drug concentration between the left lateral and other lobes was found (Fig. 1), suggesting uniform distribution of phenol red in the liver. These results indicate that direct drug instillation on the liver surface should result in local drug distribution.

After instillation of drug solutions on the liver surface, drugs are believed to spread throughout the peritoneal cavity and to be diluted by serous fluid and ascites. The spread and dilution of the instilled drug leads to a reduction of drug accumulation at the instillation site. In order to keep a high concentration of the drug on the liver surface, we gradually and continuously instilled the drug solution on the left lateral lobe. Figure 2A shows the liver concentration of phenol red after the instillation of phenol red solution (10 mg/ml) at a rate of 0.047 ml/min for 5 min. The drug concentration in the liver after bolus instillation of the same dose is also shown in...
Phenol Red Concentration in the Left Lateral Lobe (Solid Column) and the Other Lobes (Open Column) at 5 min after Administration in Rats

Each value is the mean±S.E. Numbers in parentheses represent the number of experiments. Statistical significance represents the difference from drug concentration in other lobes with the use of a paired *t*-test (** p<0.01).

Fig. 2. Phenol Red Concentration in the Left Lateral Lobe (●) and the Other Lobes (○) after Continuous Microinstillation (A) or Bolus Instillation (B) on the Rat Liver Surface

Each value is the mean±S.E. Numbers in parentheses represent the number of experiments. Statistical significance represents the difference from drug concentration in other lobes with the use of a paired *t*-test (** p<0.05, *** p<0.01). Statistical significance represents the difference from drug concentration after bolus instillation with the use of an unpaired *t*-test (** p<0.05, *** p<0.01).

Fig. 2B. In either case, the drug concentration in the left lateral lobe was significantly higher than that in the other lobes. The ratio of drug concentration in the left lateral lobe to that in the other lobes at 5 and 10 min after continuous microinstillation was larger than that after bolus instillation (Fig. 3). Furthermore, continuous microinstillation resulted in a significantly higher accumulation of the drug in the instillation site when compared to bolus instillation (Fig. 2). These results indicate that the accumulation efficacy of phenol red to the local site in the liver is enhanced with a decrease in the rate of drug instillation.

Previously, we examined the absorbability of various model drugs from the liver surface using an experimental system consisting of a cylindrical glass cell attached to the rat liver surface.2-9 Our results demonstrated that the drugs tested were effectively absorbed from the liver surface. For clinical application of drug administration on the liver surface, we investigated in this study the accumulation efficacy of phenol red to a local site in the liver after instillation on the liver surface. In spite of its hydrophilicity and low tissue affinity,10 phenol red demonstrated site-selective localization after instillation on rat liver surface. Gradual and continuous instillation of the drug solution resulted in an improvement of drug accumulation in the instillation site (Figs. 2 and 3). The accumulation efficacy should be increased by elevating the drug concentration and vehicle viscosity of the dosing solution, and by administering drugs with selective affinity for the liver. Additional studies are needed using other drugs with different characteristics. Recently, implantable infusion pumps have been developed for the treatment of several diseases,11 and endoscopic and laparoscopic operation techniques have made remarkable progress.12 These advanced medical technologies should make possible the clinical application of continuous microinstillation of drugs on the liver surface.

Acknowledgements We thank Miyuki Ando for skilled technical assistance. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture, Japan.

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