Studies in Pharmacokinetics and Tolerance of Substances Temporarily Retained in the Liver by Microsphere Embolization

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Earlier investigation has shown that a temporary arrest of arterial blood flow can be achieved by embolization with degradable microspheres. This study was made to investigate the change in pharmacokinetics and drug tolerance which takes place when a substance is retained in the liver by a microsphere embolization. ^{14}C-labelled inulin and 5-fluoro-uracil were studied. The administration of these substances with microspheres led to a delay in their systemic distribution. Furthermore there was an increased tolerance to 5-fluoro-uracil, probably due to a prolonged first pass effect when the substance was temporarily retained in the liver by a microsphere embolization.

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THE THERAPEUTIC EFFECT of drugs is firmly tied to their pharmacokinetics. According to Notari,[9] an ideal drug should: a) reach the site of action, b) arrive rapidly and in sufficient quantity, c) remain for a sufficient length of time, d) be excluded from other sites and e) be removed from the target site at the right time. These properties can be assured by chemical modification of the drugs. Another way may be to deliver the drug directly to the target organ and hold it there for as long as necessary. By means of arterial regional infusion, drugs can be delivered to the target organ and can reach a high local concentration. This type of administration is used most commonly in the treatment of malignancy with cytotoxic drugs where the toxic side effects prevent a high systemic dosage.

With the introduction of a new type of degradable microsphere, it has become possible to achieve a temporary arterial flow reduction in a localized area or organ by intra-arterial embolization of microspheres. This means that a substance could be retained in the organ for a longer time, which might change both its therapeutic effects and its toxicity.

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The aim of this study was to investigate the retention of inulin in the liver when injected simultaneously with degradable microspheres. Furthermore, the tolerance to a cytotoxic substance (5-fluoro-uracil) was studied under the same conditions.

Materials and Methods

The experiments were made on male Wistar–Fur rats (S.P.F. Möllegaard, Denmark) weighing 250–350 g. All animals were anesthetized by intraperitoneal injection of chloral hydrate 35 mg/100 g rat, 15 minutes before the experiment.

Forty-two rats were used to study the pharmacokinetics of inulin when injected into the liver artery, alone (21 animals) or in combination with microspheres (21 animals). In five rats from each group, blood and urine concentration of inulin were followed for 35 minutes after administration. The other 16 rats in each group were used for analysis of the inulin level in the liver parenchyma. These rats were killed at different intervals (2, 15, 30, and 60 minutes) after the administration of inulin, and the liver taken for examination.

Inulin (NEC–164 P Inulin carboxyl-^{14}C, New England Nuclear Corp., Boston) was purchased in aqueous solution of 1.96 mg/ml with a radioactivity of 5 μCi/ml. In every experiment 0.5 ml of this solution was injected.

To study drug tolerance, 5-fluoro-uracil (Roche) 300 mg/kg rat was injected into the liver artery without and in combination with microspheres. Eighteen rats were given 5-FU alone, and 16 rats were injected with the 5-FU-microsphere combination. White blood

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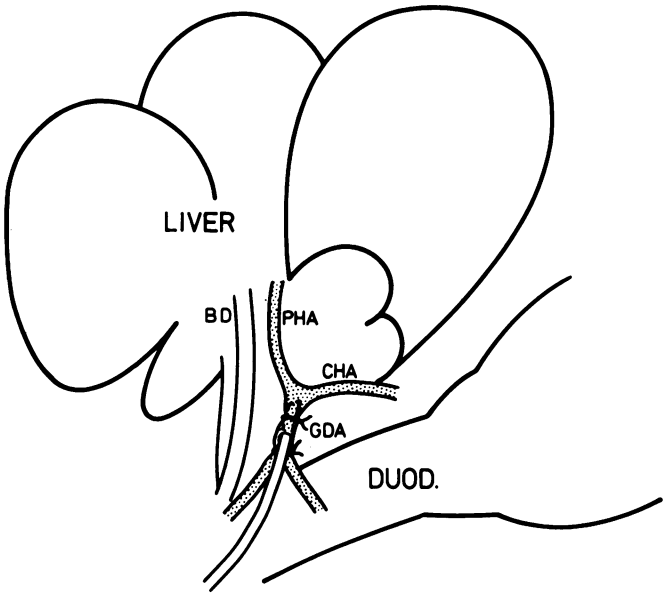

FIG. 1. Position of catheter for injection of test substances into the liver artery. PHA = Proper Hepatic Artery. CHA = Common Hepatic Artery. GDA = GastroDuodenal Artery. BD = Bile Duct

cell count, body weight and survival were registered every day for two weeks.

Administration of Test Substances

The injection of test substances into the liver artery was made through a retrograde catheter in the gastroduodenal artery (Fig. 1) according to a model described previously.[7] To prevent reflux to the common hepatic artery, this artery was occluded by a string during the injection. The catheter was then flushed with 0.2 ml heparinized saline (500 U Heparin Vitrum/100 ml saline).

Degradable Microspheres

The particles used for embolization are spheres made by crosslinked polysaccharide derivatives (starch). The microspheres were produced and supplied by Pharmacia AB, Uppsala, Sweden, and investigation at their laboratories has shown the following properties of the particles: The microspheres are completely digested by endohydrolases (amylase) in body fluids. Degradation time is dependent on the degree of crosslinking in the microsphere and the enzyme concentration. Furthermore, the size and number of microspheres injected will influence the time for flow retardation. The particles are built like a three-dimensional network, and because of the configuration with a denser structure towards the surface of the sphere its size will remain intact until the final dissolution. During the disintegration the microspheres become plastic and may permit a plasma flow in the capillary bed.

The microspheres were delivered in batches of defined sizes and cross-linking levels. In this investigation a microsphere 19 ± 3.1 μm in size and with 420 $\times$ 10^6 microspheres per gram was used. The half time *in vitro* of this microsphere is nine minutes at amylase 25 μkat./l (Batch Ph BR 42 B 19B 92789 b).

The microspheres were kept dry and added to the solution to be injected just before use. The suspension was then vigorously vibrated. A concentration of 2 g% (w/v) microspheres was used. Inulin was injected in a volume of 0.5 ml which gave 42 $\times$ 10^4 spheres per injection when the microsphere combination was tested. To reach the toxic 5-FU dose (75 mg/250 g rat), 1.5 ml 5-FU solution (Roche) was needed, resulting in 126 $\times$ 10^4 spheres per injection.

Urine Sampling

Urine was collected during five minute periods via a plastic catheter (PE 10 Intramedic) introduced into the left ureter and fixed in place with a ligature. To facilitate the urine collection and to control all filtrated inulin, a right nephrectomy was performed. Diuresis was initiated 15 minutes before injection of the test substance by an intravenous injection of 2 ml 25% mannitol.

Blood Sampling

The rats undergoing the pharmacokinetic study with inulin had their tail artery catheterized (PE 50 Intramedic, Clay-Adam) to facilitate blood sampling. The blood was collected in 50 μl disposable pipettes and immediately transferred to the counting vials. Blood was sampled every 15 seconds during the first minutes. When examining the tolerance to 5-FU, 50 μl blood was collected once a day from a cut in the tail and used for counting white blood cells.

Sample Preparation and Measurement of Radioactivity

The different samples for measurement of inulin-carboxyl-^{14}C activity were prepared as follows: to 50 μl blood was added 0.4 ml 35% hydrogen peroxide and 1 ml of a 1/1 mixture of Soluene 100 (Packard Instrument Co., Inc., solubilizer) and isopropanol. Fifteen minutes later 10 ml Insta-Gel (Packard Instrument Co., Inc.) with 0.5 ml hydrochloric acid (0.5 normal) was added and the sample was then ready for counting. The urine which was collected during five minute periods was prepared by adding 10 ml Insta-Gel. The liver samples were weighed and cut into

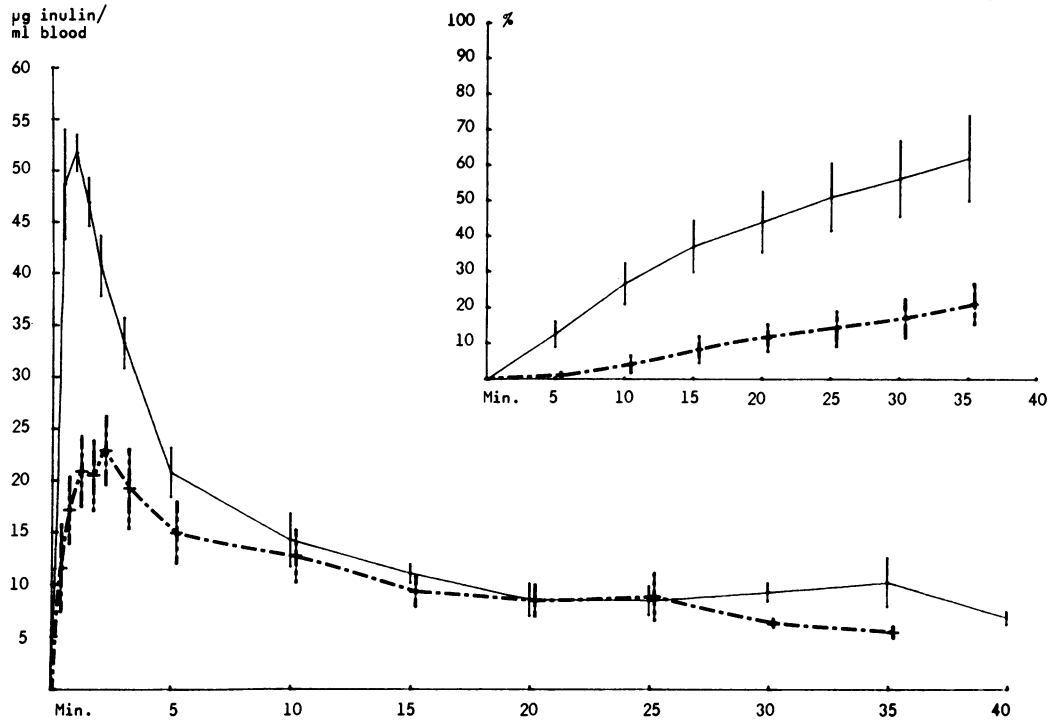

FIG. 2. Mean ^{14}C-inulin concentration (±SE) in blood. Solid line = after injection of 980µg inulin into liver artery. Broken line = after injection of 980 µg inulin and microspheres into liver artery. Chart top right shows renal inulin excretion in percentage of administered dose.

small pieces and then dissolved at 50° in Soluene 350 (Packard Instrument Co., Inc.) using 10 ml Soluene per gram liver tissue. After two days, 1 ml of this homogenized solution was diluted with 15 ml Insta-Gel and measured for activity.

The samples were measured in a liquid scintillation counter (LKB Wallac LSC 81000). The counting efficiency was determined with internal standard, and each sample count was corrected for quenching. The sample was measured for activity during a period of six minutes and a minimum net count of 1000 cpm was registered in every sample.

Results

The injection of inulin into the liver artery gave a rapid elevation of the inulin blood concentration to a maximum of 50–55 µg/ml blood in one minute (Fig. 2). The concentration then rapidly fell to a level of about 10 µg/ml blood after 20 minutes. The inulin was excreted with the urine, and after 35 minutes more than 60% of the administered dose was collected in the urine (Fig. 2).

When inulin was injected in combination with microspheres, the initial high peak concentration failed to appear, and the maximum blood concentration reached was 20–25 µg/ml blood. This difference was significant at 1% level. Twenty minutes after administration, the concentration curve in the blood was almost identical to the concentration found when inulin was injected alone (Fig. 2). The injection of inulin with microspheres also led to a significantly (at 1% level) delayed excretion of inulin with the urine, and after 35 minutes only 20% of the administered dose was found in the urine (Fig. 2).

Injection of inulin with microspheres resulted in an increased inulin concentration in the liver. During the first 30 minutes after injection of inulin and microspheres, the mean inulin concentration in the liver was raised by 42% (significant at 5% level, t = 1.86; one tail test) as compared to when inulin was injected alone (Fig. 3).

Injection of 5-FU alone caused a leukocytopenia in the rats. When 5-FU was administered with microspheres, leukocytopenia was less marked and was, furthermore, reversed at days four and nine when a significantly (at 1% level) higher number of circulating leukocytes was found than when 5-FU was injected alone (Fig. 4).

The 5-FU injection caused a decrease in the body weight with the most marked weight loss during the first four days. No significant difference was found. No difference was found when microspheres were added at the injection (Fig. 5).

The survival rate after this toxic dose of 5-FU showed a striking difference in the two groups. The survival rate in the group given 5-FU alone was 22% as compared to 75% when the drug was injected with microspheres (Fig. 6) and the difference was

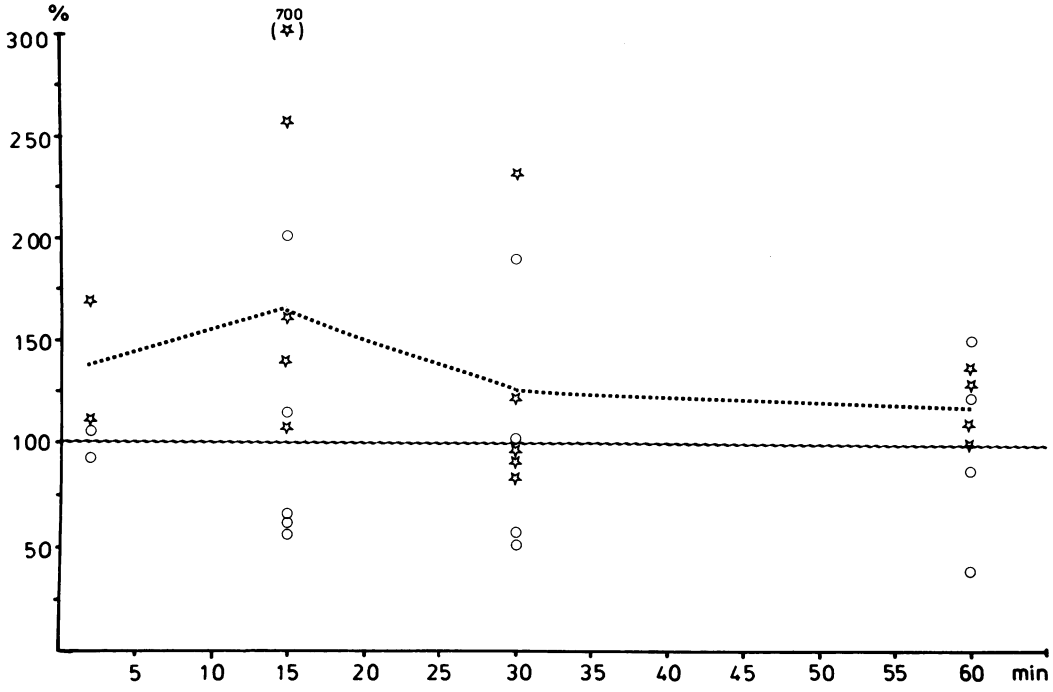

FIG. 3. Comparison of inulin amount in liver parenchyma when injected alone (Individual score = ○ Mean = wavy line) and in combination with microspheres (Individual score = star Mean = broken line). The points are calculated as percentage of the mean inulin value (set at 100%) reached each time after inulin is injected alone.

significant at 1% level (Chi square with Yate correction 7.47).

Discussion

Injection of a substance into the liver artery together with microspheres causes a delay in the distribution of the substance in the body. Because of slow release, the initial blood peak concentration will be reduced. The delay in the systemic distribution will then be influenced by the diffusion properties of the retained substance, affecting the portal washout during the arterial occlusion. This will mean that the microsphere

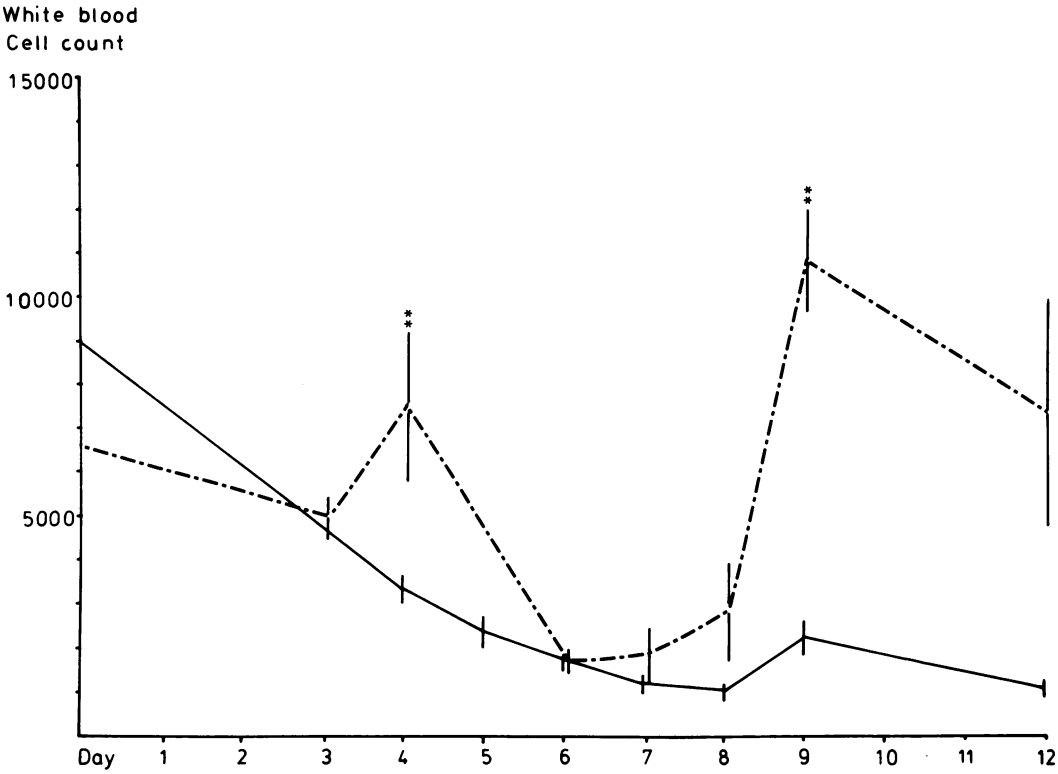

FIG. 4. Mean white blood cell count (±SE) after injection of 5-FU (300 mg/kg) into liver artery. Solid line = 5-FU. Broken line = 5-Fu + microspheres.

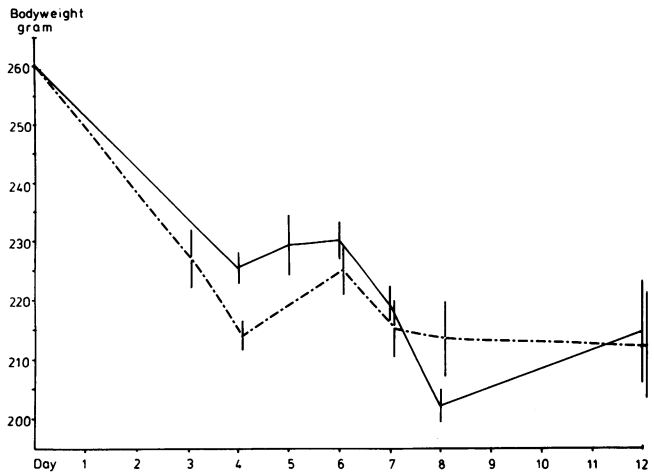

FIG. 5. Mean body weight (±SE) after injection of 5-FU (300 mg/kg) into liver artery. Solid line = 5-FU. Broken line = 5-FU + microspheres.

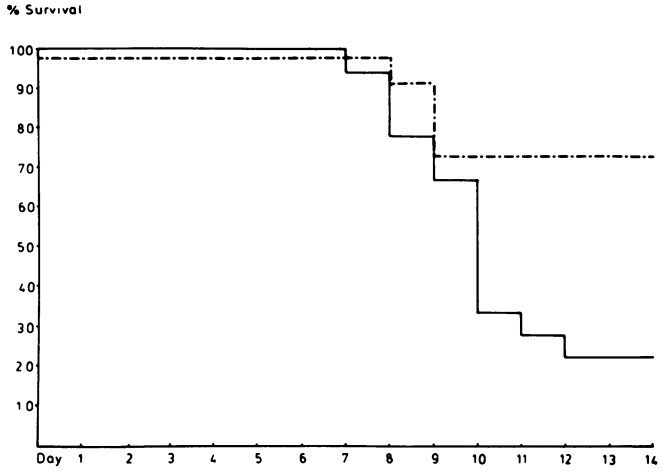

FIG. 6. Survival after injection of 5-FU (300 mg/kg) into liver artery without (Solid line) and in combination with microspheres (Broken line).

embolization will cause only a minor delay in the washout of a highly diffusible substance like xenon.[8]

The regional drug metabolism will also be decisive for the systemic distribution of a drug delivered directly to the liver. Substances metabolized in the liver and excreted by the bile will be reduced in systemic availability after passage through the liver. The first pass effect may then be increased when the liver contact is prolonged, as will be the case when drugs are administered with microspheres. Inulin is, however, not metabolized in the liver or excreted by the bile, and the reduced activity found in blood and urine after administration together with microspheres is, therefore, thought to depend on a slow release effect in the liver.[4]

Since 5-FU is a substance which is mainly metabolized in the liver, the first pass effect will be valid for this drug.[2,5] The depression of the bone marrow activity after a single dose of this drug appears to be related to the early plasma concentration.[3] Correspondingly, retention of the drug in the liver by microspheres gives a reduced depression of the bone marrow as revealed by the white blood cell count. The reduced leukopenia when 5-FU was administered with microspheres is probably also the reason why the survival increased after this treatment as compared to when 5-FU was injected alone. The gastrointestinal toxic effect sometimes observed after 5-FU treatment, with diarrhoea and loss of appetite, was not prominent in either group, and the body weight developed identically after the 5-FU injection whether or not the microspheres were added.

The change in drug tolerance which can be affected by microsphere administration depends on the metabolic pathway of the drug. The ideal drug for this administration into the liver would then be a cytotoxic substance with a high affinity to tissues in the liver, where it is degraded to atoxic metabolites. There is evidence that chemotherapy infusion with simultaneous tumor blood flow restraint is highly injurious to tumor cells,[1] and that microsphere embolization can be repeated several times. Liver retention of the appropriate drug would through concomitant microsphere injection then be an effective new approach to the treatment of liver malignancy.

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