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Fate of methotrexate albumin microspheres after hepatic intra-arterial injection in dogs[1]

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ABSTRACT Methotrexate (MTX) albumin microspheres (40 μm) were injected into dog hepatic artery. The MTX levels in the hepatic vein maintained at relatively high concentration for over 3 h, whereas in the case of conventional MTX in saline the drug level decreased sharply soon after injection. After ia MTX in microspheres the MTX levels in liver were higher than those after MTX in saline. The microsphere emboli were entrapped in the hepatic precapillary arterioles. Thrombi were found in hepatic arterioles, with microsphere constituting the core. Hence MTX microspheres hepatic intra-arterial injection may be an effective treatment for patients with liver neoplasms.

KEY WORDS methotrexate; albumin microspheres; therapeutic chemoembolization; liver

In the chemotherapy of liver cancer, the hepatic intra-arterial (ia) injection of drug in biodegradable microspheres is superior to conventional drug in saline, for the microspheres prolong the retention time of the drug in the liver[1,2]. Moreover, the microspheres could embolize the arterioles to block the tumor blood supply, resulting in anoxia and ischemic necrosis of the tumor tissue[3]. A new kind of embolizer-albumin microsphere is devised. This paper studied the fate of ia methotrexate (MTX) in human albumin microspheres in dogs.

MATERIALS AND METHODS

MTX-albumin microspheres Microspheres, prepared by emulsion polymerization[4], obtained from Institute of Radiation Medicine, Academy of Military Medical Sciences of the People's Liberation Army, were 40 μm in diameter and the MTX content was about 5% (100 mg microspheres contained 5 mg MTX).

Dog experiment Twelve mongrel dogs of either sex, weighing 13.4±*s* 1.5 kg (Animal Center, First Military Medical University), were anesthetized with iv sodium pentobarbital (30 mg·kg^{-1}). A cannula was inserted into the hepatic artery and another cannula was injected into the inferior vena cava to the inflow junction of the hepatic vein. An amount of 100 mg the microspheres or 5 mg MTX in 5 ml normal saline was injected into the hepatic artery. Inferior vena cava blood and liver tissue were obtained at 0, 5, 15, 30, 60, 120, 150, and 180 min after injection. Dogs were killed on d 1, 3, 5, 10, and 20 after injection, and blood and liver were taken.

Determination of serum MTX concentration Blood samples were kept at 4℃ overnight. Serum MTX was analyzed by fluorescence polarization immunoassay (FPIA)[5]. The TDx System and MTX reagent pack were products of Abbott Laboratories (USA). In this experiment, the range of the MTX calibration curve was 0—1.0 μmol·L^{-1}. Higher concentrations needed dilution. The lowest measurable level was 0.01 μmol·L^{-1}. Reproducibility was measured form 10 runs of five replicates each of human serum with MTX 0.07, 0.40, 0.80, 5.0, 50, and 500 μmol·L^{-1}, yielding CV <10%.

Determination of liver MTX concentration The liver was blotted with filter paper. Liver tissue 0.50 g was hemogenized in KCl (0.15 mol·L^{-1}) 3 ml and stored in ice bath. The supernatant was obtained by centrifugation (3000×*g* for 5 min) and assayed by FPIA.

Data processing The MTX concentrations-time curve was fitted and pharmacokinetic parameters were calculated with a PKBP-N1 program on a SUN 386 computer.

Histology Liver slices were stained with hematoxylin and eosin. The microsphere-embolizing pat-

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terns were examined under light microscope (×400).

RESULTS

Serum MTX levels After the ia MTX in microspheres, the serum MTX concentration remained at a relatively high level for over 3 h, and decreased gradually. By contrast, the serum MTX levels after the ia MTX in saline reached its peak earlier but decreased quickly (Fig 1A). The MTX concentrations-time curves fitted to a 2-compartment model. The pharmacokinetic parameters were shown in Tab 1. Liver MTX levels Within 3 h after ia injection the liver MTX levels after MTX in microspheres were higher than those after MTX in saline (Fig 1B). On d 20 there remained 0.2 nmol·g^{-1} in the liver after MTX in microspheres while almost undetectable after MTX in saline.

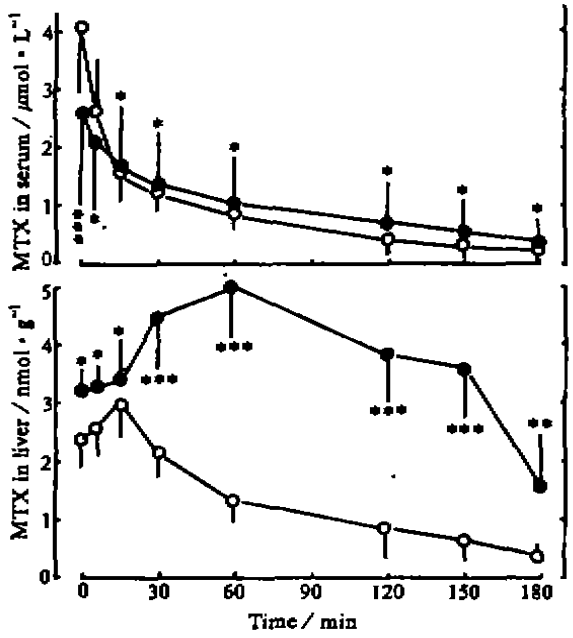

Fig 1. MTX concentrations in serum and liver after ia 100 mg MTX in microspheres (●) or 5 mg MTX in saline (○) in dogs. $n=6$, $\bar{x}\pm s$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ vs in saline.

Tab 1. Pharmacokinetic parameters of ia MTX in microspheres (100 mg) or MTX in saline (5 mg) in dogs. $n=6$, $\bar{x}\pm s$. $^{a}P>0.05$, $^{b}P<0.05$, $^{c}P<0.01$ vs in saline.

	Microspheres	Saline
$T_{\frac{1}{2}\alpha}$/min	9±5^{b}	3.7±2.1
$T_{\frac{1}{2}\beta}$/min	109±64^{a}	77±55
V_c/L	4.3±1.1^{c}	1.3±0.4
AUC/μmol·min·L^{-1}	272±207^{a}	189±69

Liver/serum MTX ratio After ia MTX in microspheres the ratio increased with time. After ia MTX in saline the ratio remained low (Tab 2).

Tab 2. Liver/serum MTX ratios after ia MTX in microspheres (100 mg) or MTX in saline (5 mg) in dogs. $n=6$, $\bar{x}\pm s$. $^{a}P>0.05$. $^{b}P<0.05$. $^{c}P<0.01$ vs in saline.

Time/min	Microspheres	Saline
0	1.8±0.8^{c}	0.48±0.20
15	3.1±1.5^{a}	2.1±1.2
60	5.4±3.4^{a}	2.1±1.1
120	6.2±3.7^{b}	1.8±1.3
180	10.3±3.2^{b}	5.1±1.5

Embolization pattern The microspheres embolized the precapillary arterioles in a single-beaded arrangement. Thrombi were found in arterioles, each with a microsphere constituting the core. The tissue around the thrombi showed coagulation necrosis. The portal areas and hepatocytes remained unaffected in the non-embolized parts.

DISCUSSION

The albumin is a superior ground-plasma for microspheres to starch or fibrinogen. For it was got from the human plasma, it could not be eliminated by the reticuloendothelial

system and phagocyte easily[6].

The fate of MTX albumin microspheres after hepatic ia injection indicated that the MTX microspheres have two strong points: (1) the elimination half-life of MTX in microspheres was prolonged; (2) the microspheres continuously launched a high level in the target tumor. The histology of liver slices showed that embolization of microspheres in arterioles block the tumor blood supply, making it impossible to build up collateral circulation for tumor. Based on the facts above, the conclusion was drawn that the double effects-sustained release and block of tumor blood supply-will be significant in the treatment of hepatic tumor.

In the clinical field of liver cancer chemotherapy, it is desirable that the antitumor drugs are better delivered to the site of liver cancer in a sufficient amount for as long a period of time as possible. The use of MTX microspheres as a sustained-releasing targeting agent meets such a demand. Although some data did not show statistic significance, probably because of the small sample size in our experiment, the trend is evident. Our results were similar to those reported by others[7,8]. The characteristic feature of the MTX microspheres would be capable of improving the antitumor efficacy, and reducing the systemic toxicity. Thus, ia MTX microspheres may be a promising clinical therapeutic means for patients with malignant hepatic tumor.

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甲氨蝶呤白蛋白微球在狗肝动脉灌注的体内过程

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摘要 甲氨蝶呤白蛋白微球(40 μm)经狗肝动脉灌注后, 肝静脉药物浓度维持在一个相对高水平达3 h之久. 而盐水剂型甲氨蝶呤肝动脉灌注后药物浓度骤降. 肝组织药物浓度前者大于后者. 组织形态学观察到微球栓塞在肝毛细管前小动脉, 在肝小动脉以微球为中心形成血栓. 甲氨蝶呤微球肝动脉灌注可能是治疗肝癌的有效手段.

关键词 甲氨蝶呤; 白蛋白微球; 治疗性化学栓塞; 肝