

PERCUTANEOUS INJECTING ANTINEOPLASTIC AGENT INTO TRANSPLANTED TUMORS OF MICE BY CT-GUIDED

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Antineoplastic agent and contrast medium were injected into transplanted tumors of mice under guidance with CT, site and range of the intratumoural drug were shown on CT image immediately. It was value of multi-point injections, concentration of 0.1 mg/0.1 ml MMC every point, 1 cm interval of injection. After the injections, the tumor size of mice reduced and at last disappeared (ratio of inhibited tumor 59.32% in 0.05 mg MMC group, 43.86% in 0.1 mg MMC group). The pathologic examination showed coagulative necrosis of the tumor tissues. The higher concentration of antineoplastic agent (0.2 mg MMC) could make the tumors enlarged (ratio of inhibited tumor -15.3%). The tissues and vessels around the tumors were not injured, if MMC overflow out off the tumor.

Key words: CT-guidance, Transplanted tumor, Antineoplastic agent

The form of therapy by antineoplastic agent injection of solid tumors under the image-guidances is a new method treating the tumors locally and having got some curative effects^{1,2} in clinic practice.

The study aimed at antineoplastic agent injection of transplanted tumors of mice under CT-guidance, and offered some references, such as, concentration, quantity, curative effects, complications, side-effects of the antineoplastic agent acting on tumor directly, which was without some systematic theoretical researches for treating malignant solid tumors locally.

MATERIALS AND METHODS

Animals: 96 of normal male and female Kunming mice with weight 20-25 g.

Tumor cell line source: S180 solid tumor cell line from the Pharmacologic Research Room of Beijing Institute for Cancer Research

MMC: Mitomycin C from Kyowa

Contrast medium: Ultravist

Experimental Methods

S180 tumor cell line were transplanted into subcutis of right ainxits of mice with 100% rate of successful transplantation, on day 7 after transplantation, the transplanted tumors of mice were about 1 cm in size (Mean=1.03 cm).

After anesthesia of mice, the transplanted tumors of mice were scanned with 2 mm thickness and interval continuous CT scanning.

MMC and contrast medium (Ultravist) were injected into the centre of the largest slice of the transplanted tumor, under CT-guidance.

All animals were divided into three groups.

Group A: Different quantities of 0.1 mg MMC were injected into the transplanted tumors, in 40 cases.

Group B: Different concentrations of 0.1 ml MMC were injected into the transplanted tumors, in 50 cases.

Control group: 0.1 ml saline and 0.1 ml Ultravist were injected into the transplanted tumors, in 30 cases.

MMC (0.1 mg/0.1 ml) and Ultravist were injected into the retroperitoneums of normal mice, in 6 cases.

The animals were executed periodically, the specimens from the injected tumors were fixed in 10% buffered formalin, embedded in paraffin, and cut into thin sections for HE staining.

RESULTS

Observation of Quantity of Antineoplastic Agent Injection.

Different quantities of MMC were injected into the transplanted tumors of about 1 cm and 4-5 cm in diameter, in order to observe how much quantity of drug liquid (MMC) was held intratumorally.

Table 1 shows that it was value of 0.1 ml of drug liquid every point intratumorally. Overflow means the drug liquid flowed out off a needle tract onto surface of skin. when the quantities of the drug liquid were over 0.2 ml for one-point or multi-point injections and the needle was taken out off the tumor.

Correlations between Distribution of Intratumoural Antineoplastic Agent (and contrast medium) and Intratumoural Pathologic Changes.

MMC and Ultravist were injected into the transplanted tumours, the distribution of Ultravist (and MMC) was shown on CT image immediately (Table 2), and the contrast medium disappeared in about 20 minutes on CT image. After injection, intratumoral necrosis appeared, the range of necrosis related to the distribution of the contrast medium (and

MMC).

Antineoplastic Agent (MMC) Injection of Retroperitoneums of Normal Mice and Pathologic Changes

0.1 mg/0.1 ml MMC and Ultravist were injected into the retroperitoneums of normal mice under CT-guidance with 2 mm thickness continuous scanning, in order to observe whether antineoplastic agent (MMC) injured the adjacent vessels, organs and soft tissues around the tumors, in case the antineoplastic agent overflowed out off the injected tumors. CT image showed the contrast medium (and MMC) remained in the area near the kidney hili of the retroperitoneums of the mice. On day 3, week 2 and week 4 after the injection, the pathologic sections showed none of injury of the vessels, organs and soft tissues, none of the thrombus formed in the lumina of the vessels, none of the differences comparing with the same sites of normal mice without the antineoplastic agent and the contrast medium injection.

Different Concentrations of Antineoplastic Agent Acting on the Transplanted Tumors and Their Pathologic Changes

Different concentrations of 0.1 ml MMC were injected into the transplanted tumours of mice, the concentrations of MMC were given according to "dose conversion among the animals with different weights."⁴ and referred to the dose of chemotherapy by arterial catheter perfusion of human, the concentration of mice = 0.096mgMMC/25g (Table 3).

Table 1. Different quantities of 0.1 mg MMC injection and overflow from needle tract

Quantity (ml)	Diameter of tumor (cm)	Site of injection		Overflow (cases)
		one-point (cases)	multi-point (cases)	
0.05	1.03	10		none
0.1	1.03	10		1
0.2	1.03	10		8
0.1	4 -- 5		5	none
0.2	4 -- 5	5		3

Table 2. Distribution of Ultravist (and MMC) and intratumoural necrosis (with 0.1 mg/0.1 ml MMC and Ultravist)

Site	Cases	Distribution of contrast medium (cm ²)*	Tumor size (cm ²)**	Necrosis of tumor (cm ²)**	Radius of necrosis (cm)	Time (days)
Centre	17	0.38	1.16	0.9	0.48	3 — 7
Decentration	13	0.39	1.19	0.58	0.57	1 — 7

Note: Site: The place, which Ultravist (and MMC) remained on CT image after intratumoural injection.

*: After Ultravist and MMC intratumoural injection, the largest diameter of the distribution of Ultravist was measured on CT image.

** : The diameters were measured from pathologic sections corresponding to the slices of the largest range of the contrast medium (and MMC) on CT image.

Table 3. Inhibition of the transplanted tumors with different concentrations of 0. 1 ml MMC injection

Groups	Cases	Average of tumor size (cm ³)		Ratio of inhibited tumor (%)	P (q test)
		\bar{x}	s		
Control	30	1.352	0.266		
MMC 0.05 mg (T1)	6	0.550	0.210	59.32	< 0.01 (T1 vs. C)*
MMC 0.1 mg (T2)	24	0.759	0.110	43.86	< 0.05 (T2 vs.C)*
MMC 0.2 mg (T3)	20	1.559	0.370	-15.31	> 0.50 (T3 vs.C)*

Note: C: control groups. Ratio of inhibited tumor= (A-B)/A×100%⁽⁵⁾. A: average size of control groups;

B: average size of treated groups

Pathologic Morphologic Changes after Antineoplastic Agent Injection

In 0.1 mg and 0.05 mg MMC injection group, on day 1 after injection, pathologic examination showed that necrotic regions separated and enclosed the tumor tissues as a “insular form”, on day 3 examination, the necrotic zones widened, on days 7 to 10 examination, woven fibrous tissues appeared in the necrotic regions, on day 25, the tumour tissues disappeared and were replaced with fibrous tissues. In 0.05 mg MMC group, on day 25, histologic examination showed the tumor tissues disappeared, and fibrous tissues proliferated. In 0.2 mg MMC group, on day 1 examination, there were some reticular necrotic regions in the injected tumors, but on days 3 to 7, the necrotic regions did not widen, the tumor cells proliferated and the tumorsize enlarged (16/20 cases), in control group, on day 3 to 7 necrosis located only near the points of injections, but on the peripheries of the necrosis, the tumor tissues proliferated and the tumor size enlarged.

Time Interval of Repeated Injection of Antineoplastic Agent

In 0.1 mg MMC group, the 19 cases reduced in the 24 transplanted tumors with MMC injection, 3 cases of them enlarged again, on days 21 to 28 after injection. In 0.05 mg MMC group, one case enlarged on day 23 too, therefore, 0.1 mg/0.1ml MMC were injected into the 4 cases of the transplanted tumors repeatedly. On days 15 to 20 examination, the tumor size of the 4 cases reduced again, however, the repeated injections were necessary after about three weeks, if there were some curative effects in the local treatment of the transplanted tumours.

DISCUSSION

It was great difficult to treat advanced, postoperative, recurrent and metastatic tumours in clinic practice. Because of local hypoconcentration and injury of immunizing function of the body, by oral, intramuscular and intravenous medication of antineoplastic agent, the anti-tumoural effect was restricted. Chemotherapy by arterial catheter perfusion could raise local concentration of antineoplastic agent for a while, but because of blood flow shock and short acting time on the tumour cells. it was limited in clinic

practice.

The form of changing mode and route of medication under image guidances in local treatment of solid tumors set up a new way in resolving the above-mentioned questions.

Quantity of Local Drug Injection, Site of Intratumoral Drug Liquid and Curative Effect of the Tumors

The space of the drug liquid remaining intratumorally was small and tight. If the quantity of the drug liquid for one-point injection in small tumour (about 1 cm^3) or big tumor ($4 - 5\text{ cm}^3$) was over 0.2 ml, the drug liquid was likely to overflow from a needle track and flowed into the soft tissues around the tumors and difficult to act on the tumor cells. Because of intratumoral poor-distributed qualities of materials, the intratumoral drug liquid was not likely to distribute from the centre of the injection point to everywhere as a spherical form, sometimes, directly reached the peripheries of the tumor, (decentration, 13/30 cases). The pathologic sections showed that if the drug liquid was in the centre of the tumor, the necrosis as a reticular form spread from the centre to the periphery of the tumor with a range of about 0.9 cm^2 , if the drug liquid was in the periphery of the tumor, the necrosis spread to the centre of the tumor with a range of about 0.58 cm^2 . The radius of necrosis was 0.48 cm (centre), and 0.58 cm (decentration). Consequently, it was suggested that the 0.1 ml of injected volume, multi-point injections and 1 cm interval were suitable for the antineoplastic agent injection of the intratumors.

Concentration of Drug and Curative Effect of the Transplanted Tumors

The ratio of tumor response to antineoplastic agents depended on contact concentration and constant time, besides configuration, form, dose of antineoplastic agent, among them, the contact concentration was a major factor.³ In the group of 0.2 mg MMC, the ratio of inhibited tumor showed negative (-15.31%). On the earlier (1 - 2 days) pathologic examination, necrotic zones were very thin, on days 3 to 7 examination, the necrotic zones did not widen, on the contrary, the tumor cells proliferated and the tumor size enlarged. In 0.5 mg and 0.1 mg MMC groups, histologically, the necrotic zones

widened, as time passed, the tumor tissues were separated by reticular necrotic zones, and fibrous tissues proliferated, the tumor tissues disappeared. Therefore, when injected with antineoplastic agent locally, the concentration of the drug would be paid attention to, under allowed quantity of the drugs (0.1 ml every point). The reason why the tumor tissues proliferated and the tumor size enlarged with the higher concentration of MMC (0.2 mg/0.1 ml) of local intratumoral injection would be further studied.

Sometimes, the antineoplastic agent liquid might efflux from tumors into the tissues around them. In our test, 0.1 mg/0.1 ml of MMC were injected into the retroperitoneums of normal mice, none of big vessels, organs, connective tissue, and musculus were injured severely.

CT-guided percutaneous intratumoral injection had virtues of correct localization, convenient observation, real time display of lesion site and range on CT image when antineoplastic agent mixed with contrast medium were injected into the tumors, therefore, the interval of the multi-point injections and the quantity of drugs could be decided and the necrotic range of the tumor tissues was assessed in this way. Image-guided antineoplastic agent intratumoral injection was a new, useful way in clinic practice, but some curative mechanism, such as pharmacokinetics of chemotherapeutic agents via local intratumoral injection, diffusion of intratumoral drug, and so on, was not elucidated. The late curative effect was not observed on, therefore, the local anti-tumor treatment should be further studied.

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