PREVENTION OF ABDOMINAL METASTASES AFTER INTRAPERITONEAL INJECTION OF YOSHIDA ASCITES CELLS: USE OF A NEW COLLOIDAL ^{32}P-CHROMIC PHOSPHATE

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Numerous studies describe the use of various chemotherapeutic agents at the time of and after surgery to decrease the incidence of postoperative liver metastases (*1,2*). Because of the high toxicity of these compounds—particularly to bone marrow—their use requires extreme caution. ^{32}P-chromic phosplate has been proposed for radiation prophylaxis following surgical tumor removal (*3–7*). With this radiocompound there is no bone-marrow depression. In these previous studies the type of ^{32}P-chromic phosphate used (particulate) has made intravenous or intralymphatic administration necessary. With the development of a true colloidal ^{32}P-chromic phosphate (*8*) which has a high migration capability, intravenous or intralymphatic injection is not necessary. Intracavitary treatment with this colloid offers the prophylactic properties of a direct injection into the blood or the lymphatic stream.

The different behavior of this radiocolloid compared with the particulate form is based in its unique physicochemical characteristics. When injected intraperitoneally, this colloidal ^{32}P-chromic phosphate migrates almost 50% to the liver during the first 24 hr. Only approximately 8% of the particulate form migrates during the same interval (*9*). Autoradiographic studies of the abdominal organs after intraperitoneal injection of both colloidal and particulate ^{32}P-chromic phosphate reveal that the colloidal form is trapped mainly by the lymph nodes. Particulate chromic phosphate on the other hand, is pocketed in different regions of the peritoneum with a reduced uptake by the intestinal, mesenteric and pancreatic lymph nodes (*10*). Experimentally it has also been found that after intraperitoneal injection, this true colloidal preparation migrates into the thoracic duct, and before entering the blood circulation a significant fraction (approximately 10%) is trapped by the mediastinal lymph nodes (*10*).

Ziedman (*11*) injected tumor cells directly into the thoracic duct of rabbits which resulted in the development of metastatic tumors in mediastinal lymph nodes, indicating a pathway for mediastinal node involvement. The similarity between the radiocolloid transport by the lymph stream and the lymphatic spread of tumor cells suggests that this radiocompound can be used for prophylactic irradiation.

The basic process for preparing this true colloidal chromic phosphate is to reduce Cr(VI) to Cr(III). The aquo-complex $Cr(H_2O)_6^{3+}$ that is formed replaces some of its water molecules by phosphate ions in a process called anation or anion penetration. When it is heated, this coordination compound then polymerizes into polynucleated complexes (olation) which finally—by forming larger aggregates—precipitates as a basic chromic phosphate (particulate form; particle size 0.6–2 microns). If this polymerization is done in a medium containing gelatin, the complexes will combine with the protein, and one avoids the formation of very large aggregates which could lead to an insoluble hydrate. The result is a true colloidal solution (particle size 0.1–0.3 micron) with the phosphate incorporated into the protein complex (*12*).

We have studied the ability of this ^{32}P-labeled true colloidal chromic phosphate to prevent metastases in the abdominal cavity in rats following intraperitoneal injection of Yoshida ascites tumor cells.

METHODS

The ^{32}P-labeled colloidal chromic phosphate was prepared according to one of the authors' technique (*8*) as follows: 2.5 ml of CrO_3 solution (10 mg/ml) was added to 1 ml of H_3PO_4 solution (10 mg/ml)

Received Dec. 20, 1967; revision accepted April 3, 1968.

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containing the ^{32}P activity as PO_4^{3-}. The mixture was heated for 5 min in a boiling-water bath. Then 150 mg of Na_2SO_3 dissolved in 1 ml of 6% gelatin solution was added. After boiling for 10 more min, the clear blue-greenish colloidal solution was dialyzed against distilled water; the water was changed at least 3 times during 4–5 hr. The final dialysis was performed against normal saline.

Adult albino rats weighing 250–300 gm were injected intraperitoneally with 0.2 ml of Yoshida ascites fluid followed in 20 min by intraperitoneally administered colloidal ^{32}P-chromic phosphate. In all the experiments, a control group of animals was injected with exactly the same amount of "cold" colloidal chromic phosphate 20 min after the injection of the same number of Yoshida ascites cells. Groups of animals were sacrificed at different times (7 days, 15 days and 30 days), and the presence or absence of metastases in the different organs and tissues was recorded.

In Experiment I the number of cells injected was 5,600,000, and the radioactivity was administered in a single dose of 75 μCi (specific activity: 100 μCi/mg chromic phosphate and 300 μCi/ml colloidal solution). The radioactivity in various tissues was measured after hydrolysis of the samples with 2*N* HCl. Radioactivity was recorded by counting an aliquot using a thin-window counter; corrections for self-absorption were made. Tables 1 and 2 list the results obtained in this experiment.

In Experiment II the number of cells injected was 9,100,000, and the radiocolloid was administered in a single dose of 50 μCi (specific activity: 90 μCi/mg chromic phosphate and 300 μCi/ml colloidal solution) to a group of female rats.

In Experiment III the number of cells injected was

TABLE 1. EFFECT OF ^{32}P-CHROMIC PHOSPHATE (COLLOIDAL) ON PREVENTION OF YOSHIDA ASCITES METASTASES

	Animal Number									
	1	2	3	4	5	6	7	8	9	10
Control group after 7 days										
Sex	F	F	F	M	M	F	M	M	M	M
Metastases:										
Pancreas	−	+	+	+	+	−	+	+	+	−
Mesentery	−	+	+	+	+	−	+	+	+	−
Small intestine	+	+	+	+	+	−	+	+	+	+
Injection site	−	−	−	+	−	−	−	−	−	−
Fluid:										
Volume (ml)	−	−	5	−	6	1.5	2.5	12	7	−
Cells/ml ($\times 10^6$)	−	−	133	−	7	26	125	52	77	−
Treated group after 7 days										
Sex	M	F	M	M	F	F	F	F	F	F
Metastases:										
Mesentery	−	−	−	−	+	−	−	−	−	−
Injection site	−	−	−	−	−	−	−	−	+	−
Fluid:										
Volume (ml)	1.5	1	1.8	−	−	1.8	−	−	−	−
Cells/ml ($\times 10^6$)	19	3.7	8.2	−	−	37	−	−	−	−
Control group after 15 days										
Sex	F	F	M	M	M	F	F	F	M	M
Metastases:										
Mesentery	−	−	+	−	−	−	−	−	−	−
Pancreas	−	−	+	−	−	−	−	−	−	−
Inguinal lymph node	+	−	−	+	−	−	−	−	−	−
Injection site	+	−	+	+	+	+	+	−	+	+
Extensive	+	+	−	+	+	+	+	+	+	−
Fluid:										
Volume (ml)	25	38	−	40	4	30	5	48	16	−
Treated group after 15 days										
Sex	M	F	M	M	M	F	F	F	F	F
Metastases:										
Pancreas	−	−	+	−	−	−	−	−	−	−
Mesentery	−	−	+	−	−	−	−	−	−	−
Parametrium	−	−	−	−	−	+	+	+	+	+
Injection site	−	−	−	−	−	−	−	−	−	+
Extensive	+	+	−	+	+	−	−	−	−	−
Fluid:										
Volume (ml)	28	31	−	28	61	−	−	−	−	−

TABLE 2. RADIOACTIVITY DISTRIBUTION (μCi/gm TISSUE) IN ANIMALS TREATED WITH 75 μCi OF ^{32}P-COLLOIDAL CHROMIC PHOSPHATE (EXPERIMENT I)

Animal	Liver	Spleen	Mesentery	Pancreas*	Fluid	Parametrium
			After 7 days			
1	1.67	0.54	0.51	0.45	0.15	0.42
2	1.13	0.41	0.21	1.00	0.13	0.32
3	1.25	0.61	0.32	0.56	0.08	0.20
4	1.45	0.53	0.06	9.60	—	—
5	1.18	0.41	0.12	4.93	—	—
6	1.04	0.35	0.15	5.21	0.12	0.18
7	1.22	0.43	0.27	4.94	—	—
8	1.58	0.69	0.09	5.53	—	—
9	1.38	0.41	0.25	3.34	—	—
10	1.39	0.64	0.08	4.68	—	—
			After 15 days			
1	0.80	0.92	0.17	2.78	0.01	—
2	1.10	0.34	0.08	0.25	0.01	0.12
3	0.73	0.12	0.03	2.12	—	—
4	1.25	0.29	0.03	1.26	0.01	—
5	1.06	0.35	0.02	0.98	0.01	0.07
6	1.18	0.42	0.11	1.96	—	0.09
7	1.01	0.33	0.24	1.46	—	0.17
8	0.84	0.23	0.02	0.76	—	0.17
9	0.76	0.28	0.09	2.45	—	0.09
10	1.02	0.42	0.10	1.26	—	0.12

* Lymph node.

8,500,000, and the radiocolloid was administered in a three-dose schedule: 50 μCi administered 20 min after the cells, 25 μCi 3 days after the cells and 25 μCi 6 days after the cells (specific activity: 95 μCi/mg chromic phosphate and 300 μCi/ml colloidal solution). The development of metastases was checked after a longer incubation time: 15 days for the control group and 30 days for the treated one.

In Experiment IV a group of female rats was injected with 7,500,000 cells. The radioactivity was administered in a three-dose schedule: 46 μCi 20 min after the cells, 25 μCi 3 days after the cells and 12 μCi after 6 days (specific activity: 95 μCi/mg chromic phosphate and 300 μCi/ml). The animals were sacrificed 30 days after injection of the cells.

The results of these three experiments are shown in Table 3.

RESULTS

A single injection of 50–75 μCi of the ^{32}P-chromic phosphate considerably reduces the metastases after a 7-day incubation period (control group: 90–100% "take"; treated group 20–30%). After 15 days the animals showed an increase in metastases, but this increase was considerably less than in the survivals of the control group (Tables 1 and 3). The values of the radioactivity concentration in Experiment I indicate that the metastases have a tendency to develop in locations where the radioactivity is lower such as the parametrium. Also, a considerable decrease of radioactivity is observed after 15 days, a phenomenon which can be related to the colloid migration (Table 2). This decrease in the concentration of radioactivity is more significant in tissues like mesentery, pancreas and parametrium which also has a higher metastases "take." To avoid this variation, the incubation period was prolonged, a three-dose administration of radiocolloid was used and the study was extended to 1 month after injection of the tumor cells (Experiments III and IV). With multiple injection of colloid a considerable increase in the survival (control group 30% and treated group 68–87%) has been observed as well as a decrease in the metastases "take" (Table 3).

A considerable difference in the "take" by metastases of the control and treated groups should be pointed out. In the control animals the abdominal contents appear as a hard mass conglomerated in a dense stromal fibrous tissue formation (Fig. 1). On the other hand, even in the cases of total "take" ("extensive" in the tables), the treated animals present a significantly different appearance; there is a clean and normal distribution of the organs except for organ enlargement due to the metastases development (Figs. 2 and 3).

In no case were macroscopic metastases observed in liver, lung or spleen. Another interesting observa-

TABLE 3. EFFECT OF COLLOIDAL ^{32}P-CHROMIC PHOSPHATE ON PREVENTION OF YOSHIDA ASCITES METASTASES: EXPERIMENTS II, III AND IV

	Experiment II				Exp. III		Exp. IV	
	7 days		15 days		15 d.	30 d.	30 days	
	T	C	T	C	C	T	T	C
Number of animals	10	10	10	10	30	30	50	20
Survival (%)	100	100	60	10	30	87	68	30
Metastases								
Pancreas (%)	10	10	10	—	80	—	—	—
Mesentery (%)	10	20	20	—	80	10	—	—
Small intestine (%)	—	—	—	—	70	—	—	—
Parametrium (%)	20	10	—	—	—	—	20	—
Inguinal lymph node (%)	—	—	30	10	—	—	—	—
Injection site (%)	—	10	30	10	10	30	24	—
Extensive (%)	10	70	20	—	20	40	18	30
None (%)	70	—	—	—	—	—	38	—
Fluid (%)	20	70	20	—	60	30	35	—

T = Treated group.
C = Control group.

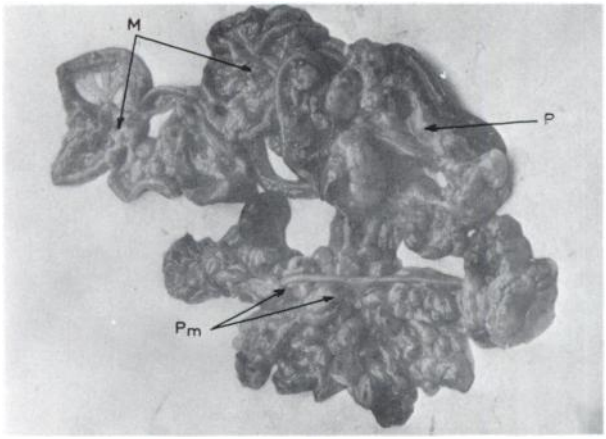

FIG. 1. Abdominal contents of animal from control group after 15 days (Experiment I). M is mesentery; P is pancreas; Pm is parametrium.

tion was the faster multiplication of the tumor cells in female rats than in male, suggesting a possible hormonal influence. Generally the metastases in females begin on the parametrium proximal to the injection site.

DISCUSSION

According to the experimental results, a considerable decrease in the metastatic incidence is obtained with a relatively low therapeutic dose (50–75 μCi). The three-dose treatment schedule (after 20 min, 3 days and 6 days) resulted in a significantly prolonged survival as well as fewer metastases. The radioactivity measurements in a variety of local tissues indicates that there is a direct relationship between the dose of radiation delivered and the prophylactic effect. This suggests that the use of a higher radiation dose or control of the colloid migration (*10*) may result in improvement.

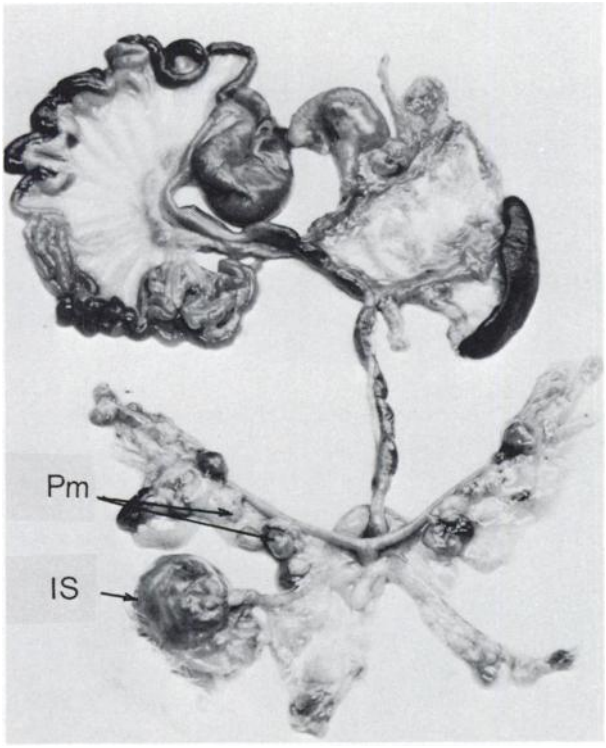

FIG. 2. Abdominal contents of animal from treated group after 15 days (Experiment I). IS is injection site; Pm is parametrium.

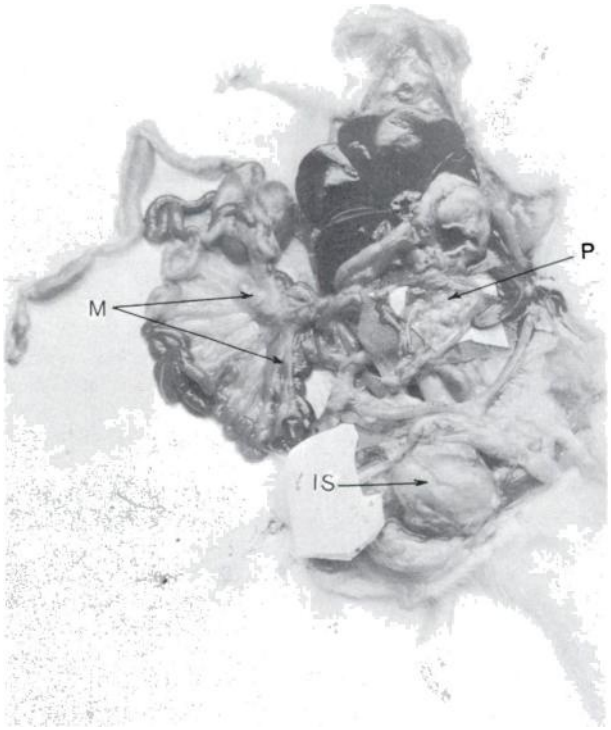

FIG. 3. Abdominal contents of animal from treated group after 1 month (Experiment IV). M is mesentery; P is pancreas; IS is injection site.

With both the particulate and the colloidal form of ^{32}P-chromic phosphate a part of the radioactivity is deposited in bone. With colloidal form, approximately 5% of the injected dose is deposited in bone, but 60–70% of this activity is localized in the mineral tissue (*9*). Because no bone-marrow depression has been observed with the particulate form (*7*), no bone-marrow depression is expected following injection of the radiocolloid.

From these experimental results, it can be stated that this new colloidal form of ^{32}P-chromic phosphate has distinct advantages over the classical particulate form. First, the colloid is much more easily prepared (*10,12*). Second, because this is a colloidal solution rather than a particle suspension, the administered dose can be measured much more accurately. Finally, intravenous or intralymphatic injections are avoided for a prophylactic treatment because when it is in-

stilled intracavitarily this radiocolloid reaches both the lymphatic and blood streams.

ACKNOWLEDGMENT

The authors wish to thank Ulli Mohr of the Experimentale Pathologie Abteilung (Deutsches Krebsforschungszentrum) for his collaboration in this study.

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