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Effect of tolmetin sodium dihydrate on adhesion formation by intraperitoneal administration of antineoplastic agents

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Summary. Antineoplastic agents are currently being administered through catheters placed intraperitoneally to treat cancer localized to the peritoneum. This route allows for high local concentrations of antineoplastic drug at the tumor site with low levels of the drug systemically, thereby reducing the systemic toxicity. However, there are complications with this mode of delivery, including a decrease in catheter patency and induction of adhesion formation, which leads to decreased drug dispersion and limits continuing drug administration. A model was developed in rats to mimic this method of antineoplastic drug administration that produced fibrin deposition around the catheter and adhesion formation involving bowel, intestines and liver. All antineoplastic agents tested, including Adriamycin, methothexate, bleomycin, mitoxantrone and cisplatin, induced moderate to severe adhesion formation with varying effects on catheter patency. When an intraperitoneal bolus of tometin encapsulated in liposomes was tested with Adriamycin delivered via an osmotic minipump, a reduction in adhesion formation was observed. However, highly significant adhesion reduction was found when tolmetin was coadministered with the antitumor agents.

Introduction

In recent years, the administration of antineoplastic drugs directly into the abdomen through indwelling catheters was found to treat many intraperitoneal cancers effectively [1, 3, 4, 8]. This route of administration allows treatment of intraperitoneal tumors with a high concentration of antineoplastic drug at the tumor site thereby allowing for low systemic levels of drug [7]]. The reduction in the systemic levels of the drug thereby minimizes the systemic toxicity while maximizing the concentration of the drug delivered to the tumor. However, several complications occur from this route of administration owing to the presence of the catheter in the peritoneal cavity and irritation of the parietal and visceral peritoneum by the antineoplastic drug. The formation of severe and extensive intraperitoneal adhesions may limit the utility of this route of administration because of pain and localization of the drug to areas immediately around the catheter [2,4]. These complications result in decreased drug dispersion and limits continuing drug administration. In addition, the resultant pain can be so severe that the patient will often discontinue treatment via this route. A model was developed in rats to mimic this method of antineoplastic drug administration which produced fibrin deposition around the catheter and adhesion formation.

Several studies are ongoing in an attempt to reduce the formation of intraperitoneal adhesions after intracavitary administration of chemotherapeutics. These methods include (a) alterations in catheter placement techniques, (b) alterations of the composition of the catheter to provide a more biocompatible catheter, (c) alterations in the volume, time course and frequency of drug administration and (d) administration of additional drugs that reduce intraperitoneal adhesion formation such as dextran and ibuprofen [6]. In this study, the effect of a nonsteroidal anti-inflammatory drug (NSAID), tolmetin, on the formation of adhesions resulting from continuous intraperitoneal administration of antineoplastic drugs was examined.

Materials and methods

Medicaments. Tolmetin sodium dihydrate, produced by McNeil Laboratories (West Point, Pa.), was provided as a gift from Ethicon Inc. (Somerville, N. J.). The following antineoplastic agents were purchased: bleomycin (Bristol Myers Oncology Division, Syracuse, N. Y.) methotrexate (Bristol-Myers Oncology Division), cisplatin (Bristol-Myers Oncology Division) and Adriamycin (Adria Laboratories, Columbus, Ohio). All drugs were diluted in phosphate-buffered saline (PBS: 0.02 M Na_2PO_4/0.15 M NaCl, pH 7.0), which was also used as a vehicle control.

Liposome preparation and NSAID encapsulation. All liposome/NSAID preparations, a gift from Vestar Inc. (San Dimas, Calif.), were performed under aseptic conditions. Sterile PBS, pH 7.4, was added to water-

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jacketed beakers and preheated to 65° C. Tolmetin sodium (McNeil Laboratories, Ft. Washington, Pa.) was then dissolved in the 65° C PBS. L-α-Distearoylglycerophosphocholine and cholesterol were dissolved in chloroform. α-Tocopherol was added to a concentration of 4% (w/v) and the mixture dried to a fine powder. The resulting powder was stored until needed. The powdered lipid mixture was then added and the suspension allowed to hydrate with stirring for 24 h. The hydrated suspension was apportioned into vials of appropriate size and autoclaved for 40 min at 121° C. Samples vials were removed for HPLC analyses for each liposome component as well as total and encapsulated drug, and the remaining vials stored at 5° C. The liposome/NSAID preparations are stable for several months under these conditions.

The liposome/NSAID preparations used in these studies were made in 1- to 3-l batches. The total lipid concentration was in the range of 35–40 g/l and the total NSAID concentration was in the range of 15–18 g/l [10].

Model. Sprague Dawley female rats (175–225 g), obtained from Simonson (Gilroy, Calif.), were housed in wire rack cages on a 16- to 8-h light/dark cycle in the University of Southern California School of Medicine Vivarium. Food (Purina Rat Chow, Purina Ralston, St. Louis, Mo.) and water were available ad libitum. Prior to surgery, the rats were anesthetized with ketamine (750 μg/kg, Park-Davis, Marris Plan, N. J.) and rompun (250 μg/kg, Bryet Division, Miles Laboratory Inc., Schawnee, Kan.). The rats then received a standardized shave of the abdomen with electrical animal clippers and a betadine preparation. The anesthetized rats then underwent a midline laparotomy to place an Alzet mini-osmotic pump (Alza Laboratories, Palo Alto, Calif.). The pump (model 2M1) was placed in the subcutaneous space and a catheter, previously sterilized by soaking overnight in ethanol, was connected between the pump and the peritoneal cavity. The catheter was attached to the peritoneal side-wall with two 5–0 Ethilon sutures (Ethicon Inc.). Various concentrations of the antineoplastic drugs, with or without tolmetin sodium, were placed in the Alzet pumps (2 ml, 10 μl/h) for continuous administration of the drug. The abdominal incision was then closed with two layers of continuous sutures (5–0 Ethilon) and the rats were observed daily during the postoperative interval for signs of toxicity or pain. No signs of gross toxicity (including lethargy or unkempt coat) were observed, possibly because of the relatively low concentrations of antineoplastic drugs used.

Seven days after the initiation of treatment, the rats were sacrificed by pentobarbital overdosage and the extent of adhesion formation in the peritoneal cavity was noted. Adhesions were evaluated in the areas formed around the catheter, between the catheter and intestines or liver, and between the lobes of the liver. The animals were evaluated by two independent observers and if there was a disagreement as to the score, the higher one was given. In addition, the treatment group was blinded to the observers during scoring. The scoring system used was as follows:

0, no adhesions found in the peritoneal cavity.

0.5+, only a few, very filmy adhesions between the bowel and catheter. Essentially no fibrin-like substance covering the catheter.

1.0+, adhesions present between the lobes of the liver; no adhesions between the intestines. The adhesions between the bowel and the catheter are very filmy but more extensive than in 0.5+. Fibrin-like substance is found covering the catheter.

1.5+, adhesions involve the liver and the bowel as in 2.0+ but the adhesions between the bowel and the catheter are less thick than 2.0+. The covering on the catheter is more evident than 1.0+.

2.0+, there are one or two points of adherence between the lobes of the liver and a few points of attachment between the loops of bowel. The adhesions between the bowel and the catheter are thick.

2.5+, the points of adherence between the lobes of the liver and the bowel are more sparse than in 3+ and there are thick adhesions between the bowel and the catheter. The catheter is completely covered with adhesions/fibrin.

3+, lobes of the liver are joined together; the bowel is adherent to itself and partially adherent to the bladder.

4+, lobes of the liver are joined together; the bowel is adherent to the liver as well as to itself. The catheter is stuck to the bowel so that it is difficult to remove.

Table 1. Intraperitoneal tolmetin encapsulated in liposomes for prevention of adhesion formation by Adramycin

Via pump	Intaperitoneal bolus[d]	Response	
Adriamycin (23.2 μg/ml)[a,b]	–	2+ 2+ 3+	
Adriamycin[a,b]	1 ml liposome (35.6 mg/ml) 9.06 mg/ml tolmetin	1+ 0.5+ 2+	$P = 0.10$
Adriamycin[a,b]	1 ml liposome (33.04 mg/ml) 5.79 mg/ml tolmetin	2+ 2+ 1.5+	$P = 0.5$
Adriamycin[a,c]	6 ml liposome (32.33 mg/ml)	2.5+ 2.5+ 3+	
Adriamycin[a,c]	3 ml liposome (32.59 mg/ml) 4.44 mg/ml tolmetin*	2+ 1+ 2+	$P < 0.05$*
Adriamycin[a,c]	6 ml liposome (32.59 mg/ml) 4.44 mg/ml tolmetin	3+ 2+ 4+	$P = .65$

[a] Adriamycin was administered via an Alzet mini-osmotic pump at a concentration of 23.2 μl/ml for 7 days (until the animals were sacrificed for necropsy)

[b,c] Experimental groups were conducted simultaneously and the results were compared within these groups for statistical analysis

[d] A single injection of the indicated medicament at the indicated volume was given at the end of surgery

* These values were significantly reduced compared to control ($P < 0.05$)

Test for statistical significance were performed by the Mann Whitney *U*-test. A value of $P < 0.05$ was considered to be significant.

Results

Table 1 shows that Adriamycin, an antineoplastic drug commonly given for the treatment of ovarian cancer, delivered intraperitoneally over a 7-day period via an Alzet minipump caused extensive adhesion formation in all test animals and that different doses of tolmetin encapsulated in liposomes, which were previously shown to reduce intraperitoneal adhesion formation following surgical trauma [10], and placed intraperitoneally as a bolus at the time of closure after catheter placement, were only partially effective in reducing the extent of adhesion formation. However, in this model, Adriamycin was administered to rats continuously for 7 days and therefore the irritation that results from the administration of the drug would continue for 7 days. Therefore, further studies were conducted with tolmetin placed in the pump with the Adriamycin. Coadministration of Adriamycin and tolmetin was then tested as shown in Table 2, and a significant reduction in adhesion formation was observed. When vehicle (phosphate-buffered saline, PBS) containing antineoplastic agent was delivered through the catheter, fibrin-like substances formed, which covered the catheter in the peritoneum, and adhesions were consistently formed between the catheter, the intestine, and occasionally the liver. Such fibrin-like

Table 2. Reduction of adhesion formation by Adriamycin with continuous administration of tolmetin sodium (* $P < 0.05$)

Via pump[a]	Response
Adriamycin (23.2 μg/ml)	1.5+
	4+
	3+
Adriamycin (23.2 μg/ml)+	0.5+
23 mg/ml tolmetin Na*	1+
	0
Adriamycin (23.2 μg/ml)+	0.5+
46 mg/ml tolmetin Na*	1+
	0.5+

[a] The indicated drugs (Adriamycin and tolmetin) were given at the stated concentrations for a period of 7 days (10 μl/h) at which time the rats were sacrificed and necropsy performed

* These adhesion scores were determined to be significantly reduced compared to control levels ($P < 0.05$; Mann Whitney U-test)

substances and adhesions did not form when the PBS alone was administered through the Alzet pump.

Several other antineoplastic drugs were also tested in this model during continuous administration of tolmetin by the pump (Table 3). The antineoplastic agents studied include methotrexate, bleomycin, mitoxantrone and cisplatin. In all cases, continuous administration of tolmetin reduced the adhesion formation caused by the antineoplastic agent. Many of these antineoplastic agents also induced white fibrotic covering over the liver capsule. Administration of tolmetin with the antineoplastic drugs did not alter the white covering over the liver induced by the antineoplastic agent (data not shown).

Discussion

Antineoplatic drugs, such as Adriamycin and cisplatin, are currently being administered through catheters placed intraperitoneally to treat cancer localized to the peritoneum [1, 3–5, 8]. This route of administration allows for treatment of intraperitoneal tumors with a high concentration of antineoplastic drug at the tumor site thereby allowing for reduced systemic levels of drug. The rationale for this route of administration involves allowing the tumor to be exposed to maximal concentrations while reducing systemic concentrations and systemic toxicities. However, intraperitoneal administration of antineoplastic agents leads to a decrease in catheter patency and induces adhesion formation [2, 4]. These complications result in decreased drug dispersion and limit the patient's tolerance of the drug. In this report, an animal model of adhesion formation after administration of antineoplastic drugs was used to test the efficacy of a NSAID, tolmetin sodium dihydrate, in the reduction of adhesion formation. Several antineoplastic drugs, Adriamycin, bleomycin, methotrexate, mitoxantrone and cisplatin, induced adhesion formation in this model. However, saline alone in the pump did not induce adhesion formation. While the placement of the catheter alone did not induce adhesion formation (over a period of 7 days), the presence of the catheter was necessary to pro-

Table 3. Effect of tolmetin on adhesions formed after administration of methotrexate, bleomycin, mitoxantrone, and cisplatin

Antineoplastic drug[a]	Conc.	Tolmetin conc. (mg/ml)	Response
Methotrexate	0.77 μg/ml	0	2+
			3+
			4+
			2.5+
			3.5+
	0.77 μg/ml	23	1+
			4+
	0.77 μg/ml	46**	0.5+
			1.5+
			1.5+
			0.5+
			0
	0.77 μg/ml	92**	0
			1+
			0
			1+
Bleomycin	0.0077 U/ml	0	2.5+
			4+
	0.0077 U/ml	23*	0.5+
			1+
			1.5+
	0.0077 U/ml	46*	0.5+
			1+
	0.77 U/ml	0	1.5+
			2+
			3.5+
	0.77 U/ml	23*	0
			0.5+
			0
	0.77 U/ml	46*	0
			1.5+
			0.5+
Mitoxantrone	1.16 mg/ml	0	3+
			3.5+
	1.16 mg/ml	23*	1+
			1+
	1.16 mg/ml	46*	0
			1+
Cisplatin	387 μg/ml	0	2+
			4+
			2.5+
	387 μg/ml	23*	0
			0.5+
			1+
	387 μg/ml	46*	0.5+
			1+
			1.5+

[a] The antineoplastic drug indicated was administered by Alzet miniosmotic pump at the concentration indicated for 7 days. The scores of the animals treated with tolmetin were compared to those of the control group, given the same concentration of antineoplastic drug, for statistical analysis

* These values were significantly reduced compared to control ($P < 0.05$)

** These values were significantly reduced compared to control ($P < 0.01$)

duce adhesions. In preliminary studies it was found that administration of drug continuously through the pump alone or as a single bolus (i.e., not employing the catheter) did not induce adhesion formation (data not shown). This is not consistent with what is observed in patients. This may occur for two reasons: (a) these rats did not contain tumors, which would contribute an inflammatory and immune stimulus in the peritoneal cavity, or (b) the rat forms adhesions less easily than humans.

Administration of tolmetin (either free or in liposomes) at the time of catheter placement only partially reduced adhesion formation. In this model the drug is continuously administered for 7 days and the anti-inflammatory effects of tolmetin administered only a single time would not continue for this period. However, continuous administration of tolmetin for the entire experimental period significantly reduced adhesion formation. Although drug administration to patients is intermittent, the catheter is continuously in place. Consequently, the administration of tolmetin may need to occur more frequently than drug administration especially if catheter placement in the abdominal cavity contributes to adhesion formation.

Administration of antineoplastic drugs at concentrations much lower than recommended human doses (literature values) was able to induce adhesion formation in rats. In addition, as the administered drug concentration increased, the severity of adhesion formation decreased (Table 3, bleomycin). This may be due to a direct effect of these drugs on the tissue repair mechanisms that leads to adhesion formation, since most antineoplastic drugs are either antimetabolites or antiproliferative.

Additional benefits to the patient, besides reduction of adhesion formation, may be gained by administration of tolmetin. Tolmetin administration will reduce inflammation and hence may reduce pain. In addition, previous studies showed that intraperitoneal administration of tolmetin to rats at the time of surgery increased the tumoricidal, respiratory burst and phagocytic activities of postsurgical peritoneal macrophages [9]. Since administration of tolmetin to rats at the time of surgery elevated the tumoricidal activity of peritoneal macrophages, administration of tolmetin to patients in conjunction with antineoplastic agents may enhance the efficacy of the treatment through enhancing the immune response of the patient.

In summary, tolmetin was highly effective at reducing the formation of adhesions caused by intraperitoneal administration of antineoplastic drugs. Further studies should be conducted to determine if tolmetin alters the antineoplastic efficacy of these drugs.

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