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Hyperthermic intraperitoneal doxorubicin: pharmacokinetics, metabolism, and tissue distribution in a rat model

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Abstract *Background*: The cytotoxic effect of several anticancer agents, including doxorubicin, can be enhanced by hyperthermia. The purpose of this study was to evaluate the effect of hyperthermia on the pharmacokinetics, metabolism, and tissue distribution of intraperitoneal (i.p.) doxorubicin in a rodent model. *Methods*: Doxorubicin was given i.p. to 20 Sprague-Dawley rats at a dose of 2 mg/kg over 60 min. Rats were randomized into two groups according to the temperature of the peritoneal perfusate: group NT received normothermic (37 °C) i.p. doxorubicin; group HT received hyperthermic (43 °C) i.p. doxorubicin. During the course of i.p. chemotherapy, peritoneal fluid and blood were sampled every 10 min. At the end of the procedure, rats were sacrificed and tissue samples (liver, spleen, small bowel, omentum, bladder, diaphragm, abdominal wall, heart) were collected. Concentrations of doxorubicin and its aglycone metabolites were determined in peritoneal fluid, plasma, and tissues by HPLC. *Results*: No significant differences in areas under the curve (AUC) of peritoneal fluid doxorubicin and plasma doxorubicin were found between group NT and group HT. AUC ratios (AUC peritoneal fluid/AUC blood) were 87.9 for group NT and 82.9 for group HT. Group HT exhibited increased doxorubicin concentrations for all intraabdominal tissues. These differences were significant for spleen ($P = 0.03$), small bowel ($P = 0.03$), and omentum ($P = 0.03$). Doxorubicin aglycone was detected in plasma of both groups within the first 10 min of the procedure. There was a significant ($P < 0.001$) increase in plasma aglycone AUC for group HT when compared with group NT. Group HT exhibited increased aglycone concentration for all tissues. This difference was significant for liver ($P < 0.001$) and bladder ($P < 0.001$). *Conclusion*: Hyperthermia did not affect significantly the pharmacokinetics of i.p. doxorubicin. Tissue concentrations of doxorubicin in small bowel, omentum, and spleen were significantly increased when the drug was administered by hyperthermic i.p. perfusion. Hyperthermia increased significantly the doxorubicin aglycone concentrations in plasma, liver, and bladder.

Key words Doxorubicin · Intraperitoneal chemotherapy · Hyperthermia · Pharmacokinetics

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Introduction

Doxorubicin is one of the most frequently used antineoplastic agents in the therapy of human cancer. Definite activity has been reported in treating intraabdominal malignancies such as ovarian carcinomas, stomach carcinomas, pancreas carcinomas, sarcomas, and mesotheliomas [1]. However, systemic doxorubicin does not penetrate into the peritoneal cavity at concentrations high enough to eliminate such tumors effectively [2]. For cancer dissemination restricted to peritoneal surfaces, the therapeutic index of doxorubicin may be improved by changing the route of administration. Intraperitoneal doxorubicin may be considered a treatment option for minute sites of peritoneal seeding. With intraperitoneal administration, a high drug level can be achieved in the peritoneal cavity with low systemic exposure. Pharmacokinetic studies have demonstrated a peritoneal/plasma peak ratio of 200 to 400 [3–6].

The major factor determining the theoretical and practical limitations of intraperitoneal therapy is a limited accumulation of cytotoxic agents in normal or tumor tissues [7]. In experimental studies, the depth of direct penetration of doxorubicin has been shown to be limited to a few cell layers from the surface [8, 9]. Further improvement in drug exposure and cytotoxicity may be obtained by combining intraperitoneal

doxorubicin treatment with regional hyperthermia. The basic principle of this combination is that heat may increase the cytotoxic effects of an anticancer agent [10]. Several studies have shown that in in vitro cell culture systems, doxorubicin is a compound that consistently shows synergistic interaction with heat [11, 12]. Thermal enhancement of doxorubicin cytotoxicity has also been demonstrated in vivo using local hyperthermia or whole-body hyperthermia [13–15].

With increasing clinical interest in intraperitoneal hyperthermia, a better understanding of interactions between heat and cancer chemotherapy agents is required. The physiological stress induced by hyperthermia may cause redistribution of blood flow to various intraabdominal organs and alter drug metabolism. The present experimental study was therefore conducted in a rodent model to determine the effects of regional hyperthermia on pharmacokinetics, tissue distribution, and metabolism of intraperitoneal doxorubicin administered at different temperatures.

Materials and methods

Animals

A total of 20 Sprague Dawley male rats (4–6 months old) weighing between 400 g and 470 g were obtained from a single breeding colony (Harlan Sprague Dawley, Indianapolis, Ind.). Animals were individually housed and they were allowed free access to food and water.

Surgical procedures

Rats were anesthetized with an intramuscular injection of sodium phenobarbital (60 mg/kg) and underwent laparotomy for drain placement. Two outflow multiperforated drains (Silicone tubing, 3.2 mm ID, 6.4 mm OD; Fisher Scientific, Norcross, Ga.) were placed in both subphrenic spaces. Both drains were coated with gauze to prevent occlusion by omentum or small bowel loops [16]. One inflow tube (Silicone tubing, 1.6 mm ID, 3.2 mm OD; Fisher Scientific) was placed in the pelvis. Rat temperature was monitored by two thermistor probes connected to a thermometer (Digital dual channel thermometer, Fisher Scientific). One probe was placed at the small bowel mesentery root in the abdominal cavity (intraperitoneal temperature), the other into the esophagus (body core temperature). After the closure of the abdominal incision with a running suture, one catheter (polyethylene tubing, 0.58 mm OD; Becton Dickinson, Cockeysville, Md.) was inserted into the left femoral vein for blood sampling.

Experimental design

At the start of each experiment, doxorubicin (Ben Venue Laboratories, Bedford, Ohio) at a dose of 2 mg/kg was diluted in 300 ml 5% dextrose solution (Peritoneal Dialysis Solution; Abbot Laboratories, North Chicago, Ill.). This total dose and concentration of doxorubicin was selected to approximate the drug tolerance in humans determined in prior human studies [3, 4]. A closed perfusion system adapted from the experiments of Shiu and Fortner [17] was utilized. The perfusate was heated in a tube coil in a thermostatically regulated water bath and infused into the peritoneal cavity with a roller pump (Varistaltic pump; Cutin Matheson Scientific, Kennesaw, Ga.) at a rate of 100 ml/min for 60 min. Rhythmic massage of the abdomen was necessary to ensure even heat distribution inside the peritoneal cavity.

Animals were randomized into two groups according to the intraabdominal temperature: group NT received normothermic intraperitoneal chemotherapy with intraperitoneal temperatures maintained between 35.5 °C and 36.5 °C, and group HT received hyperthermic intraperitoneal chemotherapy with intraperitoneal temperatures maintained between 41.5 °C and 42.5 °C. For each animal, 1 ml of peritoneal fluid and 0.6 ml of blood were collected at 10-min intervals over 1 h after the initiation of intraperitoneal chemotherapy. The venous catheter was flushed with heparinized saline after blood sampling. At the end of the procedure (60 min), rats were sacrificed and tissues (liver, spleen, omentum, small bowel, abdominal wall, diaphragm, bladder and heart) were sampled. The doxorubicin concentration in peritoneal fluid, plasma, and tissue samples was analyzed by high-performance liquid chromatography (HPLC).

Adriamycin assays by HPLC

Adriamycin levels were determined using a modification of the method of Israel et al. [18]. The HPLC system consisted of a Shimadzu LC7A instrument equipped with a SPD-6AV detector (set at 495 nm UV) along with a C-R6A Chromopac data processor. A reversed phase column (250 × 4.6 nm) of Dynamax 300A 5 μm silica was used, coupled to a guard column of the same chemical consistency. The mobile phase consisted of a mixture of acetonitrile (35% v/v) in 0.1% ammonium formate buffer (pH 4) run isocratically at 0.9 ml/min. Sample injections were 50 μl. All solvents used were HPLC grade (Fisher Scientific, Norcross, Ga.).

Plasma extraction

Blood samples were centrifuged, and 300 μl of the separated plasma was treated with 5 ml methanol/chloroform (3:2) and mixed thoroughly. After centrifugation, the organic phase was transferred to another polypropylene tube and blown down under nitrogen. The residue was resuspended in 300 μl of the mobile phase and filtered for HPLC injection.

Peritoneal fluid extraction

Peritoneal fluid samples were diluted with mobile phase and filtered before HPLC injection.

Tissue extraction

All tissue measurements were performed on dried tissues, and thus reflect the actual intracellular drug concentrations. Each tissue sample (300 to 500 mg) was dried of surface moisture then accurately weighed and homogenized in approximately ten times its volume of methanol/chloroform (3:2). The homogenate was then transferred to a 15-ml polypropylene centrifuge tube and centrifuged at 3000 rpm for 10 min. The organic solution was transferred to another polypropylene tube and vacuum extracted at 45 °C under a stream of nitrogen. The residue was resuspended in 1 ml of the mobile phase and filtered through a 0.45 μm nylon filter for HPLC injection.

Statistical procedures

To obtain the area under the curve (AUC) of peritoneal fluid versus time, and plasma versus time, a computer program LAGRAN-S [19] was used. All pharmacokinetic data and tissue concentrations were compared between the two groups using the Wilcoxon Rank Test using SAS for Windows, version 6.8 (SAS Institute, Cary, N.C.). For all statistical procedures, P-values < 0.05 were taken as significant.

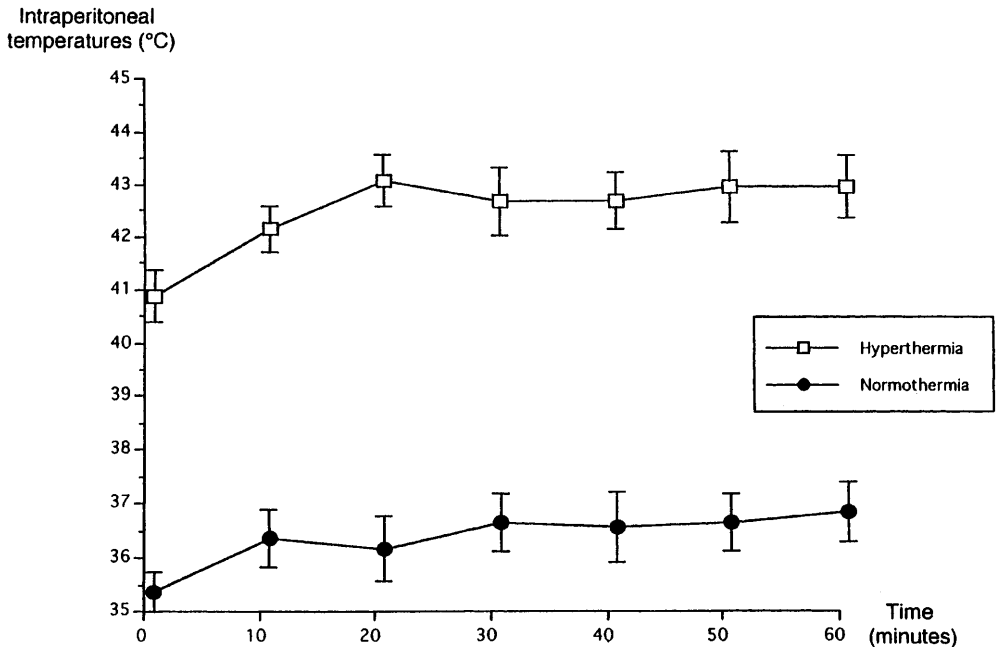

Fig. 1 Intraperitoneal temperature of rats treated with normothermic or hyperthermic intraperitoneal infusion of doxorubicin. Data points represent the means ± SD (bars) of ten measurements

Results

Temperature measurements

The mean (±SD) core temperature over the 1-h procedure was 35.8 °C (±0.7) in group NT and 36.2 (±0.6) in group HT. The mean intraperitoneal temperature over the 1-h procedures was 36.4 °C (±0.6) in group NT and 42.5 °C (±0.8) in group HT (Fig. 1). Intraperitoneal temperatures were maintained at ±0.8 °C of the selected treatment temperature and the temperature steady-state was achieved within the first 10 min of hyperthermic intraperitoneal infusion.

Effects of hyperthermia on doxorubicin pharmacokinetics

The peritoneal fluid and plasma pharmacokinetics of doxorubicin are shown in Fig. 2. The mean peak peritoneal fluid levels were 2.98 (±0.62) μg/ml for group NT and 2.95 (±0.88) μg/ml for group HT (Table 1). In the plasma, the mean peak levels were 0.04 (±0.01) μg/ml for group NT and 0.05 (±0.02) μg/ml for group HT. The mean peak (peritoneal fluid/plasma) ratios for intraperitoneal doxorubicin were 70.1 (±24.4) for group NT and 64.6 (±28.2) for group HT. There was no significant difference between the peak ratios of group NT and group HT.

The mean AUCs of doxorubicin in peritoneal fluid and plasma, and peritoneal fluid/plasma ratios are shown in Table 1. The AUCs for peritoneal fluid doxorubicin were 149.3 (±42.2) μg/ml/min for group NT and 144.9 (±24.4) μg/ml/min for group HT. The AUCs for plasma doxorubicin were 1.84 (±0.53) μg/ml/min for group NT and 1.97 (±0.61) μg/ml/min for group HT. The mean AUC ratios were 87.9 (±37.8) μg/ml/min for group NT and 82.8 (±37.1) μg/ml/min for group HT. There were no significant differences in the peritoneal fluid and plasma AUC levels or in the AUC ratios between the treatment groups.

Effects of hyperthermia on doxorubicin tissue concentrations

Doxorubicin concentrations in the different tissue samples after 60 min of intraperitoneal chemotherapy are shown in Table 2. For both groups, the highest doxorubicin tissue concentrations were found in bladder and abdominal wall. Except for heart and diaphragm, rats of group HT exhibited higher tissue concentrations of doxorubicin (Fig. 3). These increases in tissue concentrations were significant for small bowel ($P = 0.03$), omentum ($P = 0.03$), and spleen ($P = 0.03$).

Effects of hyperthermia on doxorubicin metabolism

In peritoneal fluid, no doxorubicin metabolites were detected in either group. In plasma, a single doxorubicin metabolite was detected in both groups after 10 min of treatment. This metabolite was identified as doxorubicin aglycone. As shown in Fig. 4, group HT exhibited the highest doxorubicin aglycone concentrations in plasma. The mean aglycone peak levels were 0.01 (±0.01) μg/ml

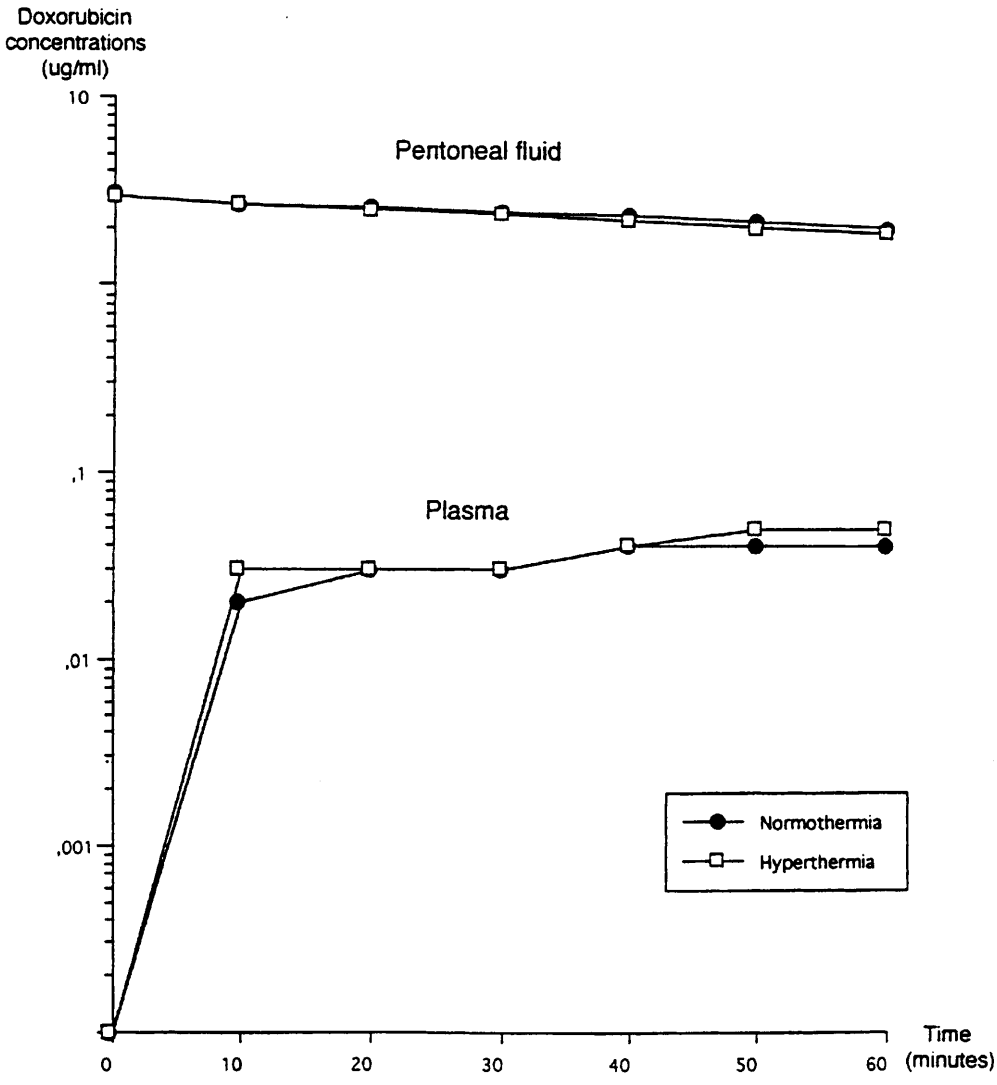

Fig. 2 Semilogarithmic doxorubicin concentration versus time in peritoneal fluid and plasma of rats treated with normothermic or hyperthermic intraperitoneal infusion of doxorubicin. Data points represent the means of ten measurements

Table 1 Effects of hyperthermia on doxorubicin pharmacokinetics. Values are means ± SD. (*NS* not significant ($P > 0.05$), *ND* nondetectable concentrations, *NA* not applicable)

Parameters	Compound	Normothermia	Hyperthermia	*P*-value
Peritoneal fluid peak (μg/ml)	Doxorubicin	2.98 ± 0.62	2.95 ± 0.88	NS
	Aglycones	ND	ND	
Plasma peak (μg/ml)	Doxorubicin	0.04 ± 0.01	0.05 ± 0.02	NS
	Aglycones	0.01 ± 0.01	0.12 ± 0.06	<0.001
Peritoneal fluid/plasma ratio	Doxorubicin	70.1 ± 24.4	64.6 ± 28.2	NS
	Aglycones	NA	NA	
Peritoneal fluid AUC	Doxorubicin	149.3 ± 42.4	144.9 ± 24.4	NS
	Aglycones	ND	ND	
Plasma AUC	Doxorubicin	1.84 ± 0.53	1.97 ± 0.61	NS
	Aglycones	0.40 ± 0.15	3.10 ± 1.31	<0.001
Peritoneal fluid/plasma AUC ratio	Doxorubicin	87.9 ± 37.8	82.8 ± 37.1	NS
	Aglycones	NA	NA	

for group NT and 0.12 (±0.06) μg/ml for group HT, and these levels were significantly different ($P < 0.001$). The mean plasma AUC was 0.40 (±0.15) for group NT and 3.10 (±1.31) for group HT, and these values were also significantly different ($P < 0.001$).

As shown in Table 2, doxorubicin aglycone was detected in all tissues of group NT, except the bladder. For group HT, doxorubicin aglycone was detected in all tissues and at higher levels than for group NT (Fig. 5). These increases in doxorubicin aglycone concentrations

Table 2 Effects of hyperthermia on doxorubicin distribution and metabolism in tissues. Values are means ± SD. (*NS* not significant ($P > 0.05$), *ND* nondetectable concentrations)

Organ	Compound	Concentrations (μg/g)		*P*-value
		Normothermia	Hyperthermia	
Liver	Doxorubicin	0.44 ± 0.19	0.47 ± 0.29	NS
	Aglycones	0.21 ± 0.18	0.88 ± 0.38	< 0.001
Spleen	Doxorubicin	0.16 ± 0.17	0.37 ± 0.17	0.03
	Aglycones	0.06 ± 0.03	0.10 ± 0.11	NS
Small bowel	Doxorubicin	0.75 ± 0.37	1.67 ± 0.64	0.03
	Aglycones	0.29 ± 0.29	0.80 ± 0.27	NS
Omentum	Doxorubicin	0.92 ± 0.36	2.36 ± 0.78	0.03
	Aglycones	0.01 ± 0.01	0.04 ± 0.04	NS
Diaphragm	Doxorubicin	0.74 ± 0.35	0.73 ± 0.39	NS
	Aglycones	0.01 ± 0.04	0.04 ± 0.04	NS
Bladder	Doxorubicin	2.75 ± 0.69	3.73 ± 1.15	NS
	Aglycones	0 (ND)	0.23 ± 0.21	< 0.001
Abdominal wall	Doxorubicin	1.43 ± 0.57	1.77 ± 0.96	NS
	Aglycones	0.02 ± 0.04	0.04 ± 0.06	NS
Heart	Doxorubicin	0.08 ± 0.04	0.10 ± 0.06	NS
	Aglycones	0.01 ± 0.02	0.03 ± 0.02	NS

after hyperthermic intraperitoneal treatment were significant for liver ($P < 0.001$) and bladder ($P < 0.001$).

Discussion

The primary goal of regional chemotherapy is to augment the efficacy of chemotherapy agents by achieving high levels of drugs in the target tissue, while reducing the level of systemic toxicity. Recent experimental studies have demonstrated that regional cytotoxicity of intraperitoneal chemotherapy may be improved by delivery of the drug by hyperthermic perfusion [11, 20]. Heated intraperitoneal chemotherapy is now used in different clinical settings as an adjuvant and palliative treatment of gastric, colon, and appendix cancers [21–24]. Although mitomycin C has been the drug most frequently tested in these clinical trials, doxorubicin may be the drug of choice for peritoneal sarcomatosis, peritoneal mesothelioma, or ovarian adenocarcinoma with peritoneal carcinomatosis [4]. Because of its cell cycle-nonspecific action, doxorubicin may be administered intraperitoneally over a short period of time, preferentially at the end of the surgical resection of an intraperitoneal cancer [25]. Clues to a potential clinical advantage of this chemotherapy technique can be obtained from pharmacokinetic investigations such as these in the present study.

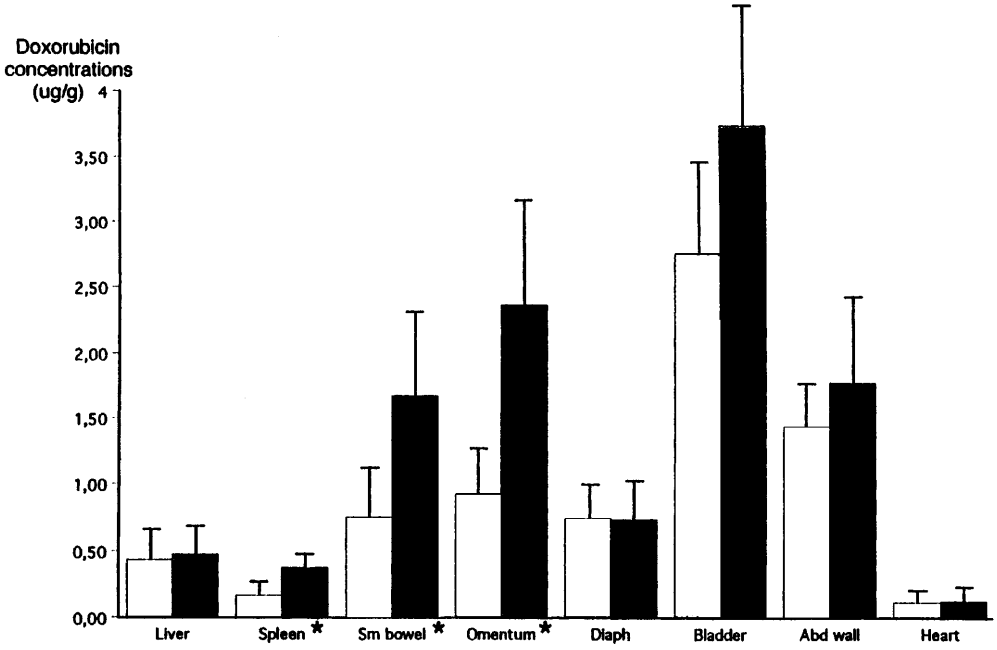

Fig. 3 Doxorubicin concentrations in tissues after 1 h of normothermic (□) or hyperthermic (■) intraperitoneal infusion

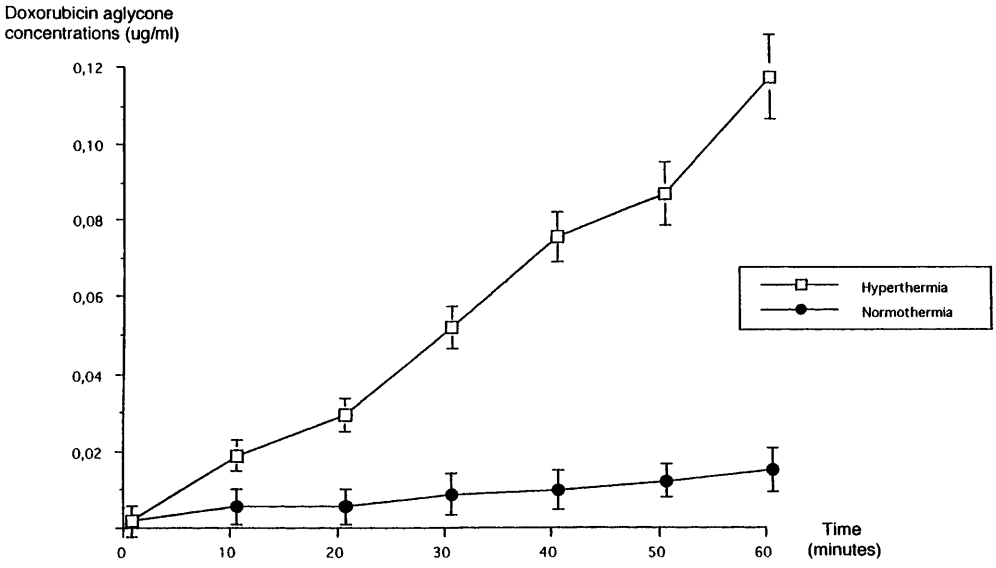

Fig. 4 Doxorubicin aglycone concentration versus time in plasma of rats treated with normothermic or hyperthermic intraperitoneal infusion of doxorubicin. Data points represent the means ± SD (bars) of ten measurements

The main purpose of this study was to assess the effect of the hyperthermia on the pharmacokinetics of intraperitoneal doxorubicin. Our data suggest that hyperthermia did not change significantly the pharmacokinetics of intraperitoneal doxorubicin. For both groups, a high peritoneal fluid/plasma peak ratio and a high AUC ratio were found after the 1-h perfusion. These results confirm the results of previous studies which have demonstrated that doxorubicin is a pharmacokinetically advantageous drug for intraperitoneal administration [9, 25]. Because of its high molecular weight and hydrophilic properties, doxorubicin has a slow peritoneal clearance and may act at higher concentration on tumor cells implanted in the peritoneal cavity.

Although Minaugh et al. [26] have shown that hyperthermia does not alter the pharmacokinetics of intravenous doxorubicin, a recent study by Los et al. has shown that abdominal hyperthermia increases the peritoneal clearance of cytotoxic agents administered into

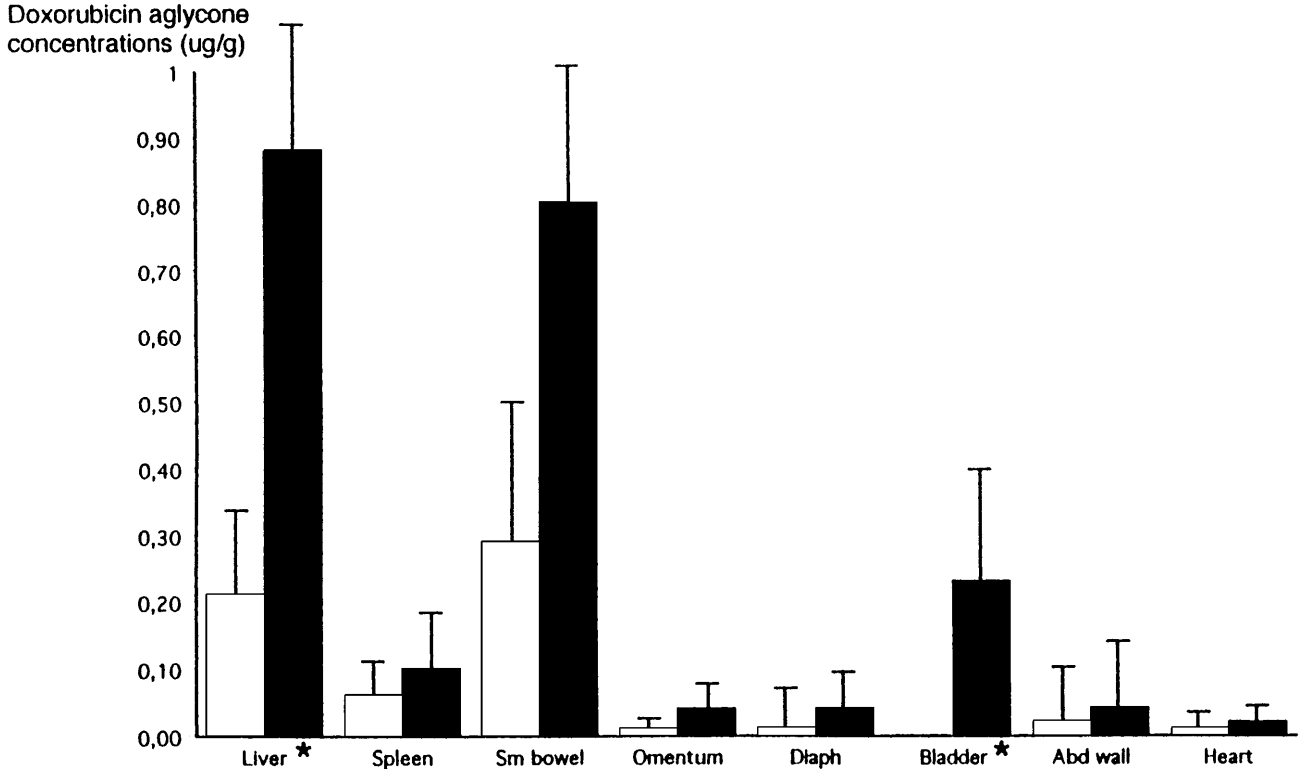

Fig. 5 Doxorubicin aglycone concentrations in tissues after 1 h of normothermic (□) or hyperthermic (■) intraperitoneal infusion

the peritoneal cavity [21]. In their model, Los et al. analyzed the effect of 1 h of abdominal hyperthermia on the pharmacokinetics of intraperitoneal carboplatin over a 24-h period. They showed that the drug exchange between the peritoneal cavity and the circulation was not changed during the heating period. The peritoneal clearance of carboplatin began to increase only 1.5 h after hyperthermia treatment.

Rats treated at 42.5 °C exhibited increased concentrations of doxorubicin in all tissues. Because doxorubicin measurements were performed on dried tissues, these results suggest that the increment in tissue concentrations was a result of a direct increase in drug uptake by tissues rather than of a simple osmotic drug diffusion in heat-related tissue edema. This increased uptake of doxorubicin was statistically significant only for small bowel, omentum, and spleen. No significant increase in doxorubicin concentration was found in heart tissue. This may suggest that the effect of regional hyperthermia was limited to the peritoneal cavity without affecting the systemic toxicity of doxorubicin. Dewhirst et al. [27] have demonstrated that normal tissues consistently respond to hyperthermia with a marked increase in blood flow as long as tissue temperature levels do not exceed 45 °C. The physiological stress induced by hyperthermia may have caused redistribution of blood flow to various intraabdominal organs. Because of its relatively high vascularization, small bowel may have been exposed to more doxorubicin delivered via both direct diffusion and the systemic circulation. This phenomenon of increased drug delivery to the gastrointestinal tract is supported by a report of bloody diarrhea in dogs treated by hyperthermic adriamycin [28]. Although no rational explanation may be provided for the increased concentration of doxorubicin in spleen, this phenomenon has been previously described [29].

The metabolism of doxorubicin has been studied in both experimental animals and humans [30]. Two major metabolic pathways are responsible for the biotransformation of doxorubicin. One route leads to the formation of the alcohol derivative, doxorubicinol, and the other to the nonpolar products, aglycones. In our experiments, no doxorubicinol metabolites were detected in rats treated at either 35.5 °C or 42.5 °C. This absence of increased alcohol conversion of doxorubicin by hyperthermia has been previously demonstrated in a rabbit model [26].

On the other hand, nonpolar products of doxorubicin were detected in our experiments. Aglycone metabolites were found in plasma of both groups within the first 10 min of intraperitoneal doxorubicin perfusion, and this aglycone conversion of doxorubicin was significantly increased by hyperthermia. Such a phenomenon may be explained by an increased passage of doxorubicin into the liver when intraabdominal temperatures are maintained above 40 °C. Experimental studies have shown that hyperthermia increases the portal blood flow [31]. Because aglycone metabolites are produced in the liver and excreted in the urine, higher concentrations of aglycones were found in plasma, liver and bladder of rats treated with hyperthermic intraperitoneal doxorubicin. However, this pharmacokinetic event has no impact on toxicity or antitumor effect of intraperitoneal doxorubicin because of the absence of antiproliferative and antineoplastic actions of such metabolites [32].

In conclusion, this study demonstrated that abdominal hyperthermia did not affect the pharmacokinetic advantages of intraperitoneal doxorubicin. High intraperitoneal concentrations of doxorubicin were achieved over the 1-h procedure with low plasma levels and heart tissue concentrations. Hyperthermia increased significantly doxorubicin concentrations in small bowel, omentum, and spleen. Because of the high incidence of tumor deposits in such intraabdominal organs, hyperthermia may be beneficial in improving drug distribution and penetration at these sites.

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