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Pharmacokinetic evaluation of intraperitoneal doxorubicin in rats

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Received June 28, 2013, accepted August 2, 2013

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Pharmazie 69: 125–127 (2014) *doi: 10.1691/ph.2014.3754*

The intraperitoneal (ip) administration of doxorubicin (DOX) is considered to be an important approach for the treatment of peritoneal tumors, because the prognosis of peritoneal cancer is generally poor due to its refractoriness to conventional chemotherapy. In the present study, we examined the disposition behavior of DOX after ip administration in rats to evaluate the adequacy of the ip administration of DOX on the basis of pharmacokinetic aspects. By comparing the area under the serum concentration-time curve (AUC) after ip and intravenous (iv) dosing of 5 mg/kg DOX, the bioavailability of intraperitoneally administered DOX was estimated as 43.8%. This finding suggests that the majority of DOX remained in the abdominal cavity without being incorporated into the systemic circulation. The mean residence time (MRT) of DOX after its ip administration was about 80% longer than that after its iv administration, which indicated the slow absorption process associated with ip application. No significant difference was observed in the elimination rates of systemically absorbed DOX. These results indicate that the ip administration of DOX likely provided an adequate opportunity for it to interact with peritoneal tumors by maintaining sufficient DOX levels while reducing its systemic exposure

1. Introduction

Peritoneal carcinomatosis from gastrointestinal and ovarian carcinomas has been associated with poor survival, and treatments lack standard protocols. However, immediate intraperitoneal (ip) chemotherapy with anti-neoplastic agents offers the greatest survival chances for patients, as well as complete surgical cytoreduction of peritoneal implants (Verwaal et al. 2003). Complete cytoreductive surgery with the administration of immediate ip chemotherapy has become the mainstay for treating locally advanced intra-abdominal cancer (Ceelen et al. 2000). The treatment of peritoneal tumors in humans using ip chemotherapy with doxorubicin (DOX), an anthracycline antineoplastic drug, has been previously reported (Ansaloni et al. 2012; Salti et al. 2012). A clear understanding of ip DOX pharmacokinetics is necessary for its appropriate use in peritoneal cancer therapy. This study was undertaken to examine pharmacokinetic profiles after the ip administration of DOX in a rat model, and these were compared with those after its intravenous (iv) administration.

2. Investigations, results and discussion

The present study showed that serum DOX concentrations after ip administration rapidly rose and attained peak levels, followed by a decline similar to that observed in the iv group (Fig.). This observation was not consistent with a previous report showing that serum DOX concentrations remained at a markedly lower level after ip administration at a dose similar to that employed in the present study (Parker et al. 1982). This discrepancy may be ascribed to a difference in the interval from surgery using pentobarbital until the performance of pharmacokinetic experiments. In contrast to the present study in which pharmacokinetic experiments were carried out using awake rats, DOX was given to anesthetized rats that had undergone surgery only 30 minutes previously in the earlier study. Pentobarbital anesthesia has been reported to affect hemodynamic responses (Pedersen et al. 2007); therefore, the absorptive function after ip administration may be impaired due to the physiological

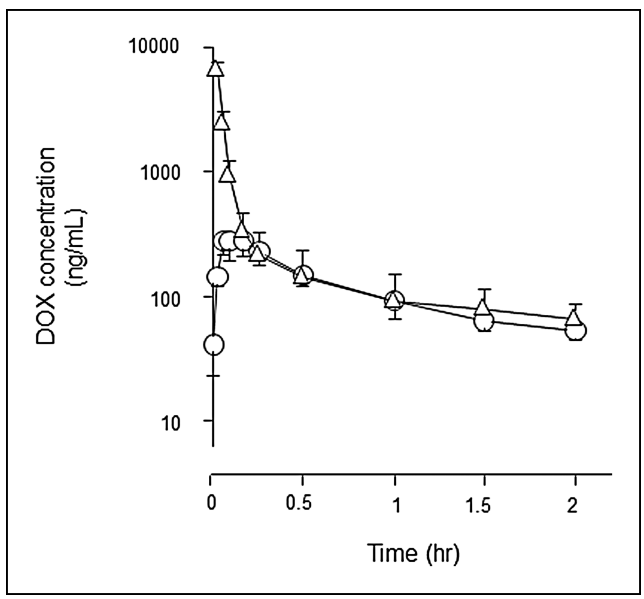

Fig.: Serum concentration-time courses of DOX after its ip and iv administration (5 mg/kg) to rats. Serum concentrations of DOX were measured after its ip (open circle) and iv (open triangle) administration. Results are shown as means ± SD of four rats per group.

Table: Pharmacokinetic parameters of DOX after ip and iv administration

	ip	iv
AUC (mg•h/mL)	0.324 ± 0.053 **	0.736 ± 0.145
Vd_{ss} (mL/kg)	--a)	5.16 ± 2.34
ke (1/h)	0.705 ± 0.096	0.764 ± 0.226
CL_{tot} (mL/h/kg)	--a)	6.89 ± 0.94
MRT (h)	1.353 ± 0.176 **	0.749 ± 0.200

** $p < 0.01$ *vs* iv
a) Not applicable

changes caused by pentobarbital in the previous study. The peaks of DOX metabolites in the serum obtained from rats given the ip and iv applications of DOX were not detected in the HPLC chromatograms (data not shown), indicating that DOX in the serum mostly exists as an unchanged drug. This finding was in agreement with that found in earlier investigations showing the pharmacokinetic profiles when given DOX *via* the iv route (Israel et al. 1978; Tavoloni et al. 1980).

By comparing the area under the serum DOX concentration *versus* time curve (AUC) after administration between the ip and iv routes (Table), bioavailability was estimated as about 40% when administrated intraperitoneally. In view of the lack of a difference in the elimination rate at the terminal phase (ke) values between the ip and iv groups (Table), the elimination capacity was considered not to be altered regardless of the administration route. These results suggested that the majority of DOX remained in the abdominal cavity without affecting elimination characteristics by the ip application, although a small amount of DOX was uptaken into the systemic circulation. Taking into account the about twice prolongation of the mean residence time (MRT) over that after iv injection (Table), the disposition behavior of intraperitoneally administrated DOX provides a fundamental explanation for the rational attempt to treat intra-abdominal cancer.

Ip chemotherapy with DOX after extensive cytoreductive surgery for advanced peritoneal carcinomatosis and sarcomatosis, such as ovarian cancer, is feasible with acceptable morbidity and mortality (Ansaloni et al. 2012; Salti et al. 2012). Since it is likely that therapeutic outcomes are closely associated with DOX concentrations in neoplastic ascites, further studies may be necessary to understand the intra-abdominal pharmacokinetics of DOX. DOX is frequently used in combination with paclitaxel or cisplatin to treat advanced peritoneal tumors (Ansaloni et al. 2012). Therefore, pharmacokinetic examination of DOX after ip administration will also be required under the condition of concurrent dosing with paclitaxel or cisplatin.

Instillation therapy with DOX also plays an essential role in clinical cancer treatment (Formariz et al. 2007). However, these regimens have been associated with several problems such as severe adverse events and the appearance of tumor cells acquiring resistance to anti-cancer agents, which restricts their clinical usefulness (Zhang et al. 2009). The improvement of current cancer therapies is of importance in addition to the discovery of novel chemotherapeutic agents. A number of studies have recently been conducted to explore substances protective against organ damage caused by DOX (Abo-Salem 2012; Hadi et al. 2012). In these experiments, an animal model with tissue injury was constructed by the ip administration of DOX because of its technical simplicity, although DOX is generally administered *via* an iv route in clinical practice. The present results confirmed the validity of the application of ip treatment to the toxicological study because a part of DOX was bioavailable to the whole body. This is also supported by the similarity in the pharmacokinetic profiles of DOX in the systemic circulation when given *via* iv and ip routes, although MRT in the ip group was longer than that in the iv group.

In conclusion, the present study demonstrated that the majority of DOX administered intraperitoneally remained in the abdominal cavity, which explains the reliability of treating advanced peritoneal tumors with the ip application of DOX. Our findings will be helpful in promoting the appropriate use of anthracyclines for cancer chemotherapy.

3. Experimental

3.1. Materials

DOX hydrochloride was obtained from Meiji Seika Kaisha (Tokyo, Japan). Daunorbicin hydrochloride (DNR) was purchased from Sigma Chemical Co. (MO, USA). All other reagents were of commercial or analytical grade requiring no further purification.

3.2. Animal treatment

Male Wister rats aged 9 weeks were obtained from Japan SLC, Inc. (Hamamatsu, Japan). The right femoral artery and vein were cannulated with polyethylene tubing (Natsume Seisakusyo Co., Ltd., Tokyo, Japan) under anesthesia with pentobarbital the day before pharmacokinetic experimentation, and these tubes were then externalized *via* subcutaneous tunneling to the interscapular area. Rats were allowed to recover in regular holding cages with free access to water and food overnight following surgery. DOX at a dosage of 5 mg/kg was administrated into the abdominal cavity or right femoral vein. Serial blood samples were obtained from the right femoral artery 0.25, 1, 3, 5, 10, 15, 30, 60, 90, and 120 min after administration. Blood samples were centrifuged for 10 min to separate serum and stored at $-80\,^{\circ}C$ until assayed. Experimental protocols and animal care methods in the experiment were approved by the Animal Experiment Committee at Osaka Ohtani University.

3.3. Sample preparation and HPLC assay

Serum concentrations of DOX were determined using the HPLC method described below. Fifty μL of 2 μg/mL DNR, an internal standard, 37.5 μL of 4% zinc sulfate, and 37.5 μL of methanol were added to a 50 μL serum sample. The mixture was then vortexed and centrifuged at 5,000 *g* for 10 min. Fifty μL of supernatant were injected into the HPLC. The mobile phase consisted of 50 mM potassium phosphate and acetonitrile (v/v: 1:3) and the flow rate was set at 1.0 mL/min. The eluent was monitored using fluorescence detection with an excitation wavelength set at 480 nm and an emission wavelength of 520 nm.

3.4. Pharmacokinetic analysis

Standard pharmacokinetic parameters of DOX were calculated on the basis of the non-compartmental method. The ke value was determined by linear regression of the log-linear portion of plots of serum concentration against time. The last sampling point ($t_{(last)}$) applicable for data analysis was decided to be time 2 h because the measured concentration fell below the quantitation limit after 2 h in some cases. The AUC was calculated by a linear trapezoidal approximation from time zero to the $t_{(last)}$, with the addition of a correction term by extrapolation to infinity using the ratio of the last measured concentration ($Cp_{(last)}$) to ke. The total clearance (CL_{tot}) was determined by dividing the intravenous dose (5 mg/kg) by the AUC. The area under the first-moment curve (AUMC) was also calculated by a linear trapezoidal approximation from time zero to the $t_{(last)}$ with the addition of a correction term after $t_{(last)}$ to infinity, namely, $t_{(last)} * Cp_{(last)}/ke + Cp_{(last)}/(ke)^2$. The MRT value was determined by dividing AUMC by the AUC. The apparent volume of distribution at steady-state (Vd_{ss}) was estimated by multiplying the CL_{tot} and MRT. Bioavailability (F) was obtained by dividing the AUC of the ip group by that of the iv group.

3.5. Statistical analysis

Data were expressed as means ± S.D. The difference between the mean of two groups was compared using the Student's unpaired *t*-test. Differences with a *p* value of 0.05 or less were considered significant.

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