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Therapeutic significance of the polyamine level in tissues of rats treated with adriamycin and cisplatin

Satoru Watanabe, Shoichi Sato,[1] Sumika Nagase[1] and Taiichi Saito

Department of Pharmacology, Kawasaki Medical School, 577 Matsushima, Kurashiki City, Okayama 701-01, Japan. Tel: (+81) 86 462-1111; Fax: (+81) 86 462-1199. [1] Department of Medical Technology, Kawasaki College of Allied Health Professions, 316 Matsushima, Kurashiki City, Okayama 701-01, Japan.

The effects of adriamycin (Adr) and cisplatin on body weight, organ weight, and the contents of putrescine, spermidine and spermine in 15 different tissues were examined in rats that had been given these drugs for 5 days. The weight of the spleen in cisplatin-treated rats, and of the thymus, spleen, kidney and heart in Adr-treated rats showed statistically significant decreases. Neither Adr nor cisplatin should be used to treat cancers in the seminal vesicle, and Adr should not be used to treat those in the large intestine due to significant increases in spermidine and spermine, which are associated with a risk of re-growth. However, Adr is recommended in the treatment of cancers in the thymus, kidney, skeletal muscle (femoral), heart, prostate, testis, liver, small intestine and lung, based on decreases in some of the polyamines, while cisplatin is not recommended in the thymus, kidney, skeletal muscle or heart for the same reason that Adr is not recommended to treat cancers of the large intestine (rectum). The ratio of spermidine to spermine was significantly higher in the skeletal muscle of cisplatin-treated rats and in the small intestine and brain of Adr-treated rats, and was lower in the prostate, seminal vesicle and spleen of Adr-treated rats than in control rats.

Key words: Adriamycin, cisplatin, polyamine, putrescine, spermidine, spermine.

Introduction

Adriamycin (Adr)[1,2] and cisplatin,[3,4] which have been widely used to treat several types of cancer, act via intercalation into the DNA of tumor cells, and are potent inhibitors of DNA synthesis. In contrast, polyamines, which are usually increased in cells undergoing active growth,[5,6] not only play an important role in normal cell and tumor cell proliferation,[7] DNA synthesis[8] and repair of damaged tissues,[9] but are also used to monitor the therapeutic effects of drugs.[10,11]

Previous data have shown a contrast between the stimulation of cell proliferation by polyamines and inhibition by Adr and cisplatin. Analysis of the effects of Adr and cisplatin on the polyamine content in tissues with various cell-cycle kinetics is necessary to identify drugs which do not induce polyamine elevation in tumor-bearing tissues to avoid the re-growth which results from trace cells in surviving and/or tumor cells that are tolerant to these drugs after the cessation of Adr and cisplatin therapy. Our previous experiments suggested that the antimetabolite anticancer drugs ara-C and fluorouracil (5-FU),[12,13] which increase the polyamine content in many tissues in rats, may lead to re-growth.

In addition to these effects of polyamines, the amount of polyamines in tissues seems to control the chemotherapeutic effects of Adr and cisplatin. Their toxicity is closely related to polyamines, which modify the formation of the lipid peroxide[14–21] and free radicals[21] produced from Adr, which in turn compete with Adr in binding on the inner membrane of cardiac mitochondria.[22,23] Polyamines also modify the intercalation of cisplatin in DNA.[24] Since it depends on the drug whether polyamine in the tissues bearing the cancer is beneficial or detrimental in cancer treatment, an examination of the polyamine content in the tissues of rats given these drugs could be very useful in identifying drugs which induce a decrease in polyamine and thereby bring about the greatest chemotherapeutic effect. In the present study, we used HPLC to determine the content of individual polyamines and total polyamine content in 15 tissues in rats given Adr and cisplatin for five successive days.

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Correspondence to S Watanabe

Materials and methods

Chemicals

Adr, cisplatin, and all of the polyamines and diamines used to prepare the standard solutions were purchased from Sigma (St Louis, MO). Potassium hydroxide, 2-mercaptoethanol, boric acid, *o*-phthalaldehyde, perchloric acid (60%), Brij-35, methanol and tri-sodium citrate dihydrate were obtained from Nacarai Tesque (Kyoto, Japan), and were used without further purification.

HPLC

Chromatographic analysis[25] was carried out using the JASCO analytical chromatographic system (Japan Spectroscopic, Tokyo, Japan) equipped with a JASCO 802-SC system controller, two JASCO 880-PU intelligent HPLC pumps, a JASCO 851-AS intelligent autosampler, a JASCO 860-CO column oven, a JASCO 821-FP intelligent spectrofluorometer, a JASCO 880-51 degasser and a JASCO 805-GI graphic integrator. For analytical procedures, we used a polyamine-pak column (35 × 6 mm), which was protected by a guard-pak column, both of which were made by JASCO. The flow rates were 0.75 ml/min for both the mobile phase solution and the OPA reagent. The temperature of the column oven with the OPA reaction coil was kept at 70°C throughout the experiment. After post-column derivatization with OPA, the fluorescence intensity was measured with the intelligent spectral fluorometer (excitation at 340 nm, emission at 450 nm), and the amount of each polyamine was calculated from the peak area relative to the internal standard (1,6-diaminohexane).

Buffer and OPA reagent

The buffer solution for the elution system was prepared by dissolving 1.0 mol tri-sodium citrate dihydrate in water in a final volume of 1.0 l and the pH was adjusted to 5.3 with the addition of perchloric acid. This solution was filtered with a membrane filter (45 μm; Advantec, Tokyo, Japan) and degassed under a water aspirator at room temperature for 20 min.

The OPA-2-mercaptoethanol for the post-column derivatization procedure was prepared according to the method of Seilar and Knodgen[26] with minor modifications. Boric acid (24.7 g) and potassium hydroxide (23.0 g) were dissolved in water in a final volume of 1.0 l. After the addition of 2.0 ml 2-mercaptoethanol to the mixture, the solution was filtered in the same manner as the buffer solution. This degassed solution was mixed with 2.0 ml Brij-35 solution and 1.6 g OPA dissolved in 10 ml methanol. The OPA reagent, which was mixed with the solution for the HPLC system behind the polyamine-pak column, was allowed to react with each separated polyamine within the reaction coil with the column oven at 70°C.

Animals

Male Sprague-Dawley rats (42–45 days old, 170–180 g) were maintained on a 24 h light/dark cycle with light from 6.20 a.m. to 6.30 p.m. The conditions of animal housing were strictly controlled, and food and water were available *ad libitum*. Eighteen rats were divided into three equal groups: two experimental groups and a control group. The experimental groups received i.p. injections of Adr or cisplatin in saline solution at 2.0 or 5.0 mg/kg body weight, respectively, daily for 5 days. The control group received an injection of saline solution of the same volume. All of the rats were anesthetized with diethylether on the sixth day and the tissues were immediately removed, weighed and kept in 2.0 ml of an aqueous 10% trichloroacetic acid (TCA) solution containing 0.1 mmol/l 1,4-diaminohexane as an internal standard in an ice bath. Each organ in the cold solution was homogenized with a homogenizer (Kinematica, TCU-2-110, Littau/Luzern, Switzerland) and then centrifuged at 2500 r.p.m. for 15 min. The supernatants were washed twice with 5 ml of diethyl ether to eliminate the TCA in the water layer. The water layer was kept in a refrigerator at below −20°C until measurement. The solution was passed through a millipore filter [45 μm, Cosmonice, Nacarai Tesque (Kyoto, Japan)] and 10 μl of the filtrate was applied to HPLC by an autosampler.

Results

The effects of Adr and cisplatin on body weight, the weights of the prostate, thymus, spleen, kidney, heart, seminal vesicles and testis, and the polyamine contents in these organs as well as in the liver, skeletal muscle (femoral), tongue, small intestine (jejunum), large intestine (rectum), stomach, lung and brain (cortex) were examined in rats that had been

given the drugs for 5 days. The mean body weight decreased by 13% ($p < 0.01$) in Adr-treated rats and by 11% ($p < 0.01$) in cisplatin-treated rats compared with control rats. The mean wet weights of the thymus, spleen, kidney and heart of the rats treated with Adr and that of the spleen of cisplatin-treated rats decreased significantly (Table 1). Therefore, the doses we used, 2.0 (Adr) and 0.5 (cisplatin) mg/kg body weight, seemed to be optimal for examining the effects of Adr and cisplatin. The ranges of the concentration of the polyamines were about 0.02–0.18 nmol/mg for putrescine, 0.1–6 nmol/mg for spermidine, 1.0–14.0 nmol/mg for spermine and 2–5 nmol/organ for total polyamines in each organ, except the prostate. The total amount of polyamines in each whole organ was relatively constant between organs, as compared with the amount per mg of wet weight of tissue. The concentration of putrescine (Figure 1) was about 1/10–40th that of spermidine (Figure 2) and about 1/50–300th that of spermine (Figure 3). Spermidine and spermine in the seminal vesicles of rats treated with either Adr or cisplatin ($p < 0.01$) and in the large intestine of rats treated with Adr ($p < 0.05$) showed statistically significant increases per mg wet weight in comparison with the values in control rats (Figures 2 and 3). No significant increase in polyamines was observed in any of the tissues of rats treated with cisplatin, except for the seminal vesicle. On the other hand, the putrescine (Figure 1) content in the thymus, kidney and skeletal muscle, spermidine (Figure 2) in the heart, and spermine (Figure 3) in the heart and skeletal muscle of Adr- and cisplatin-treated rats showed significant decreases. Furthermore, putrescine in the prostate, testis, liver, heart, small intestine and lung, spermidine in the prostate, thymus, spleen and skeletal muscle, and spermine in the spleen and small intestine of Adr-treated rats, and spermine in the lung of cisplatin-treated rats showed statistically significant decreases.

The spermidine/spermine ratio (Figure 4), which is considered an index of a high growth rate, was significantly lowered in the prostate, seminal vesicle and spleen in Adr-treated rats. However, it was higher than those in control rats in the small intestine and brain (cortex) of Adr-treated rats and in the skeletal muscle of cisplatin-treated rats (Figure 4). The ratio ranged from 0.2 to 1.4

Discussion

We previously observed increases in polyamine content in many accessory organs and other tissues of male rats treated with the antimetabolite anticancer drugs ara-C and 5-FU.[12,13] We noted that tumor re-growth in these organs may be caused by the increased polyamine contents induced by these

Table 1. The wet weights of the prostate, seminal vesicles, testis, thymus, spleen, kidney and heart, and total polyamine contents (TP = nmol/mg × wet weight) in these organs in rats given Adr or cisplatin for 5 days

Tissue	Drug	Weight (g)	TP/organ
Prostate	control	0.227 ± 0.070	3829.02 ± 1135.62
	cisplatin	0.214 ± 0.043	4101.45 ± 833.38
	Adr	0.207 ± 0.027	3277.63 ± 262.20
Seminal vesicles	control	0.270 ± 0.064	627.63 ± 201.74
	cisplatin	0.264 ± 0.065	1086.93 ± 330.83*
	Adr	0.263 ± 0.033	1033.62 ± 222.11*
Testis	control	1.080 ± 0.108	4046.00 ± 1013.49
	cisplatin	1.140 ± 0.038	4669.39 ± 635.76
	Adr	1.131 ± 0.096	4029.72 ± 370.50
Thymus	control	0.540 ± 0.081	2540.13 ± 528.68
	cisplatin	0.439 ± 0.090	2179.74 ± 445.08
	Adr	0.360 ± 0.074**	1450.98 ± 399.35**
Spleen	control	0.548 ± 0.038	1900.91 ± 154.71
	cisplatin	0.472 ± 0.059	1571.86 ± 184.10**
	Adr	0.296 ± 0.023**	814.34 ± 61.30**
Kidney	control	0.817 ± 0.059	2184.59 ± 282.18
	cisplatin	0.807 ± 0.059	1980.38 ± 148.28
	Adr	0.750 ± 0.026*	1859.04 ± 143.24*
Heart	control	0.736 ± 0.069	1459.99 ± 161.61
	cisplatin	0.682 ± 0.071	1156.71 ± 91.16**
	Adr	0.602 ± 0.023**	932.24 ± 53.96**

Data represent the mean ± SE for each group. The significance of differences was exmained by Student's *t*-test. * $p < 0.05$, ** $p < 0.01$.

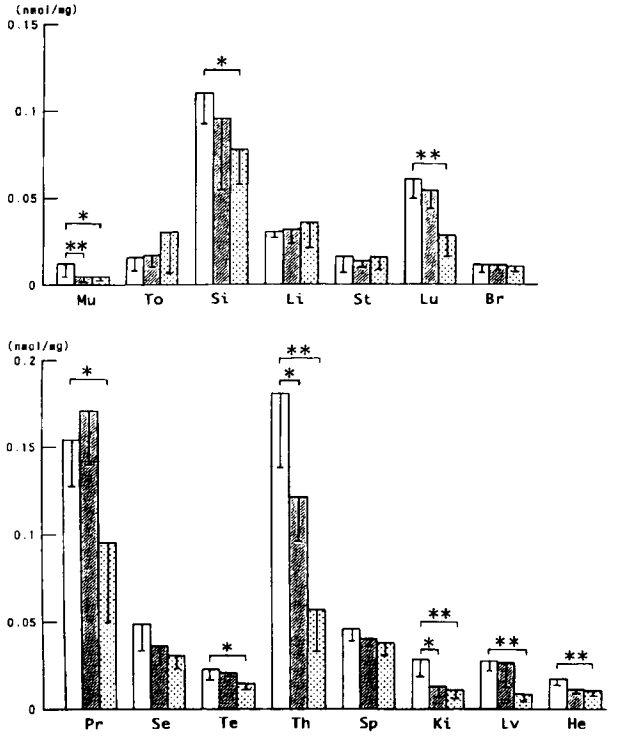

Figure 1. The content of putrescine per milligram of the wet weight of the prostate (Pr), seminal vesicles (Se), testis (Te), thymus (Th), spleen (Sp), kidney (Ki), liver (Lv), heart (He), skeletal muscle (Mu), tongue (To), small intestine (Si), large intestine (Li), lung (Lu), stomach (St) and brain (Br) of rats given Adr or cisplatin for 5 days. Unshaded, control column; shaded, treated columns (right, Adr; left, cisplatin). Columns represent the mean ± SE for each group. Statistical differences were examined by Student's *t*-test. * $p < 0.05$, ** $p < 0.01$.

drugs, based on the stimulatory effects of polyamine on cell proliferation. To avoid re-growth due to the use of antitumor drugs, it is necessary to avoid using drugs which induce polyamine elevation in tumor-bearing tissues. In the present study, we examined the effects of DNA cross-linking anticancer drugs, Adr and cisplatin, on the polyamine levels in 15 tissues.

It is well known that cell proliferation is strictly dependent on polyamines and that a certain basal level of polyamines is required.[5] Therefore, the tumor regression brought about by Adr and cisplatin must compete with the tumor growth mediated by the increased polyamine content caused by these drugs. The polyamines deposited in the tissues by these drugs might cause re-growth of surviving and/or tolerant tumor cells after the termination of drug treatment. In addition to this action, polyamines also play a role in the repair of damaged tissues.[9] Polyamines thus regulate the therapeutic

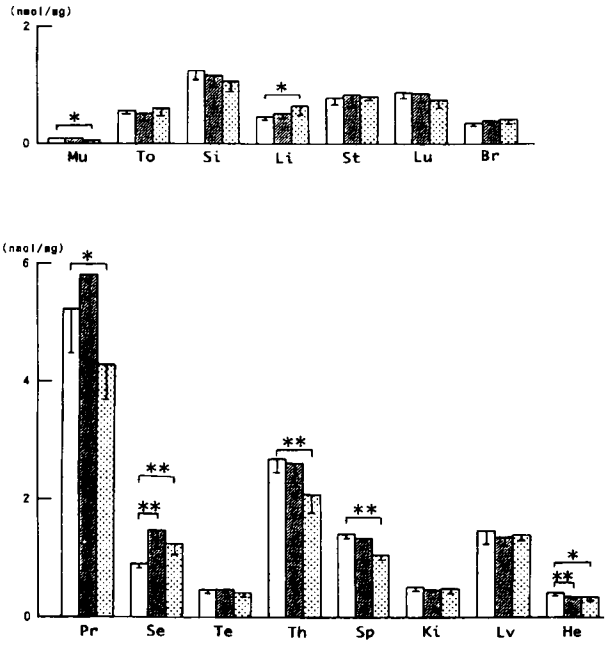

Figure 2. The content of spermidine per mg wet weight of various tissues. Abbreviations and explanations are the same as in Figure 1.

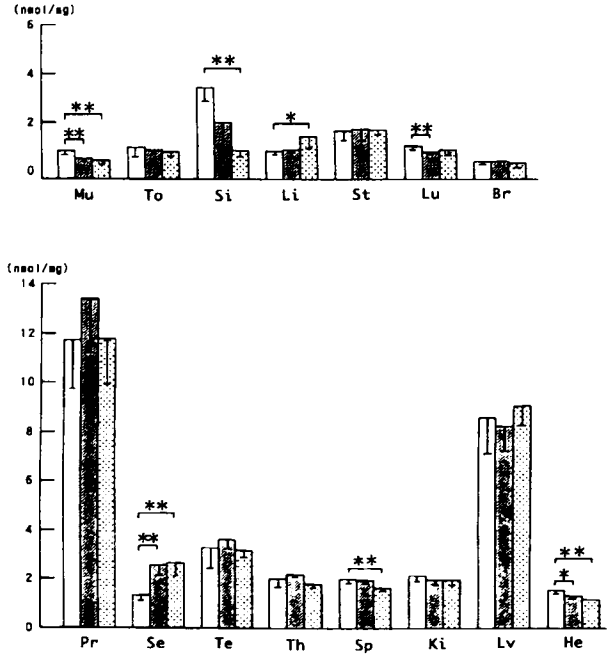

Figure 3. The content of spermine per mg wet weight of various tissues. Abbreviations and explanations are the same as in Figure 1.

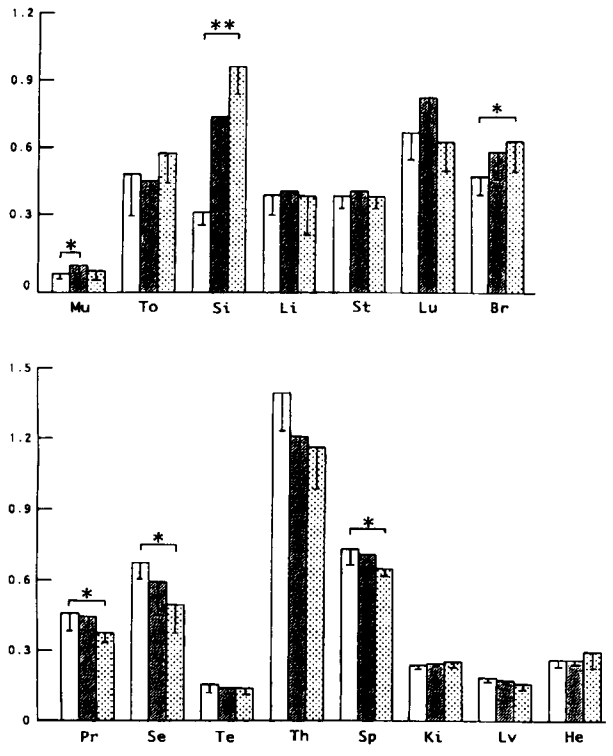

Figure 4. The ratio of spermidine/spermine in various tissues. Abbreviations and explanations are the same as in Figure 1.

effects of these drugs through the stimulation of cell growth and/or repair of tissues damaged by these drugs. The remarkable increases in spermidine and spermine in the seminal vesicles of rats treated with Adr and cisplatin, and in the large intestine of Adr-treated rats, clearly shows that these drugs should not be used to treat tumors in these tissues. However, an increase in polyamines may be partially beneficial in treating cancers in these tissues considering the phenomena described below.

In contrast to the effects of the antitumor drugs ara-C and 5-FU on the polyamine content in many tissues of rats,[12,13] Adr decreased putrescine levels in the prostate, testis, liver, heart, small intestine and lung, decreased spermidine in the prostate, thymus, heart and skeletal muscle, and decreased spermine in the thymus, heart, skeletal muscle and small intestine. Adr is limited in its clinical application because it can induce a specific cardiotoxicity which is cumulative and total dose dependent.[27,28] The molecular mechanism of this toxicity is thought to involve several phenomena, including the binding of the drug to the inner membrane of heart mitochondria,[22,23] stimulation of free radical formation[21] and membrane lipid peroxidation,[14–21] with consequent impairment of their functions. The free-radical nature of Adr toxicity is further supported by the finding that α-tocopherol, a known free radical scavenger, greatly reduces Adr-induced oxidative challenge and toxicity without diminishing its antitumor potential.[29,30] Polyamines are thought to be closely related to these phenomena. It has been reported that polyamines seem to lower the cardiotoxicity of Adr (in the following order of effectiveness: spermine, spermidine and putrescine), since polyamines antagonize the binding of this drug to the inner membrane of heart mitochondria.[22] Furthermore, these polyamines in general seem to be effective in reducing free radical formation, which potentiates its cytotoxicity, based on findings that polyamines were able to significantly counter paraquat-induced augmentation of lipid peroxidation and superoxide dismutase activity in the lung[20] and liver[19] of rats. Therefore, the cardiotoxicity of Adr may increase due to the decrease in polyamines in the heart in Adr-treated rats, as shown in Figures 1–3. Combination therapy with Adr and α-difluoromethylornithine, a specific and non-toxic irreversible inhibitor of ornithine decarboxylase, which inhibits polyamine production, has been suggested for the treatment of animal cancers,[31] since a decrease in the intracellular polyamine level could destabilize DNA, which is presumed to be normally stabilized by non-covalent cross-bridges of spermidine or spermine.

In contrast to Adr, DFMO-mediated polyamine depletion has been thought to lower the toxicity of cisplatin based on the decrease in the formation of cross-linked DNA,[24] since an alteration in the DNA structure caused by polyamine depletion would make the cross-linking reaction with cisplatin mechanistically unfavorable. This result indicates that cisplatin would not be expected to have its best therapeutic effect in the treatment of tumors in the tissues such as the spleen, kidney, skeletal muscle, heart and lung, which showed decreases in some polyamines.

The ratio of spermidine/spermine, which is considered an index[32] of growth suggesting hypertrophy, was high in the small intestine of Adr-treated rats, but the possibility of re-growth is extremely low because of the abnormal decrease in spermine. Adr is a suitable choice for the treatment of tumors in the prostate and spleen based on the spermidine content and the spermidine/spermine ratio. Cisplatin appears to be unsuitable for the treatment of tumors of the skeletal muscle based on the decrease in spermine and the increase in the spermidine/spermine ratio in this tissue. To be able to choose the optimal antitumor drug to obtain the

best therapeutic effect, it is necessary to study in greater detail the role of each polyamine in cell growth. The present results, as reflected by the spermidine/spermine ratio, indicate that ordinary cell growth took place in all of the tissues except for the prostate, seminal vesicle, spleen, small intestine and brain under Adr treatment and except for the skeletal muscle under cisplatin treatment.

Conclusions

Our findings regarding the effects of Adr and cisplatin on the polyamine content in various tissues should be useful for deciding whether to use these antitumor drugs. Increases in polyamine content induced by some antitumor drugs stimulate the re-growth of trace cells in surviving cells and/or tumor cells that are tolerant to these drugs. Accordingly, this results in a worsening of the patient's condition after the termination of drug treatment even if temporary regression of the tumor is observed. In addition, Adr may be used to treat cancer-bearing tissues in which it induced a depletion of these polyamines, while cisplatin cannot. The present results will hopefully encourage physicians to not only note the cell-killing action of the antitumor drug in use, but also its effect on polyamine levels in tumor-bearing tissues.

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