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Polyamine increase in rat tissues treated with 1-β-D-arabinosylcytosine and 5-fluorouracil

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The effects of 1-β-D-arabinosylcytosine (ara-C) and 5-fluorouracil (5-FU) on body weight, and the contents of putrescine, spermidine and spermine in 14 different tissues were examined in rats given these drugs for 5 days. There were statistically significant increases in all three polyamines in the small intestine, in spermidine in the lung, and in spermine in the thymus, spleen and liver of rats treated with ara-C and 5-FU. Putrescine content in the spleen, heart, muscle and liver, and spermidine and spermine contents in the stomach of the 5-FU-treated rats also showed significant increases.

Key words: 1-β-D-Arabinosylcytosine, 5-fluorouracil, polyamine, putrescine, spermidine, spermine.

Introduction

1-β-D-Arabinosylcytosine (ara-C) and 5-fluorouracil (5-FU), which have been widely used for the treatment of various cancers, are nucleoside analogs and act as potent inhibitors of DNA synthesis. In contrast, polyamines, which usually increase in cells undergoing active growth,[1,2] not only play an important role in normal cell and tumor cell proliferation,[3] DNA synthesis[4] and repair of damaged tissues,[5] but are also used to monitor the therapeutic effect of drugs.[6,7] Previous data have shown a contradictive relation between the stimulation of cell proliferation by polyamines and inhibition by ara-C and 5-FU. Analysis of the effects of ara-C and 5-FU on the polyamine content in tissues with various cell cycle kinetics is necessary to choose the drug which does not induce polyamine elevation in tumor-bearing tissues because it can avoid the regrowth which results from trace cells in surviving and/or tumor cells tolerant against these drugs after the cessation of ara-C and 5-FU therapy. Examination of the polyamine contents of tumor-bearing tissues could be useful in choosing drugs which also induce a decrease in the polyamine contents of these tissues and thereby bring about the greatest chemotherapeutic effect. In the present study, we used high performance liquid chromatography (HPLC) to determine the content of individual polyamines and total polyamine content in various tissues in rats treated with ara-C and 5-FU. The importance of polyamine content in tumor-bearing tissues in choosing anti-tumor drugs that can prevent the tumor regrowth observed after drug cessation of some drugs is discussed.

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Materials and methods

Chemicals

Ara-C, 5-FU and all polyamines and diamines used for the preparation of standard solutions were purchased from Sigma (St Louis, MO, USA). Potassium hydroxide, 2-mercaptoethanol, boric acid, *o*-phthalaldehyde (OPA), perchloric acid (60%), Brij-35, methanol and tri-sodium citrate dihydrate were obtained from Nakarai Tesque (Kyoto, Japan), and were used without further purification.

HPLC

Chromatographic analysis[8] was carried out using the JASCO analytical chromatographic system (Japan Spectroscopic Co., Ltd, Tokyo, Japan) equipped with a JASCO 802-SC system controller, two JASCO 880-PU intelligent HPLC pumps, a JASCO 851-AS intelligent sampler, 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 the

analytical procedure, we used a polyamine-pak column (35 mm × 6 mm), which was protected by a guard-pak column, both made by JASCO. The flow rates were 0.7 ml/min for both mobile phase solution and the OPA reagent. The temperature of the column oven 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).

Buffer and OPA reagent

The buffer solution for the eluation system was prepared by dissolution of 1.0 mol of tri-sodium citrate dihydrate into 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 filtrated by 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 Knödgen[9] 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 of 2-mercaptoethanol into the mixture, the solution was filtered in the same manner as the buffer solution. This degassed solution was mixed with 2.0 ml of Brij-35 solution and 1.6 g of OPA dissolved in 10 ml of methanol. The OPA reagent which was mixed with the solution of the HPLC system behind the polyamine-pak column was allowed to react with each separated polyamine within the reaction coil in the column oven at 70°C.

Animals

Male Sprague-Dawley rats (50 days old, 170–180 g) were kept on 24 h cycles of light/darkness with light from 6.30 a.m. to 6.30 p.m. The conditions of animal housing were strictly controlled, and food and water were continuously available. Eighteen rats were divided into three equal groups: two experimental groups and a control group. The experimental groups received subcutaneous injections of ara-C or 5-FU in saline solution at 63.0 or 33.4 mg/body weight, respectively, daily for 5 days; the control group received an injection of saline solution of the same volume. All the rats were anesthetized with diethylether on the sixth day and the tissues were immediately removed, weighed and then kept in 2.0 ml of 10% trichloroacetic acid (TCA) aqueous solution in an ice bath. Each organ in the cold solution was homogenized with a homogenizer (Kinematica, TCU-2-110, Switzerland) and then centrifuged at 2500 r.p.m. for 15 min. The supernatants were washed twice with 5 ml of diethylether to eliminate the TCA in the water layer. The water layer was kept in a refrigerator at below −20°C until measurement. Immediately before measurement, 250 μl of the water layer was diluted with mobile buffer to 500 μl. The solution was filtrated by a millipore (45 μm; Cosmonice; Nakarai, Kyoto, Japan) and 10 μl of the filtrate was charged by an autosampler.

Results

The effects of ara-C and 5-FU on the body weight, weights of the prostate, seminal vesicles, testis, thymus, spleen and kidney, and on the polyamine contents in these organs as well as the lung, liver, heart, muscle (femoral), tongue, small intestine (jejunum), large intestine (rectum) and stomach were examined in rats given the drugs for 5 days.

Table 1. The wet weights of the prostate, seminal vesicles, testis, thymus, spleen and kidney, and total polyamine contents (TP) in these organs in rats given ara-C and 5-FU for 5 days.

Tissue	Drug	Weight (g)	TP/organ
Prostate	control	0.240 ± 0.037	2535.53 ± 1159.12
	Ara-C	0.193 ± 0.029*	4350.59 ± 1134.39**
	5-FU	0.207 ± 0.047**	2468.99 ± 447.91
Seminal vesicles	control	0.701 ± 0.069	587.37 ± 62.50
	Ara-C	0.607 ± 0.130	792.37 ± 205.97
	5-FU	0.598 ± 0.149	687.35 ± 173.36
Testis	control	1.463 ± 0.094	823.19 ± 109.95
	Ara-C	1.401 ± 0.084***	975.40 ± 105.48**
	5-FU	1.352 ± 0.108	789.49 ± 154.88
Thymus	control	0.414 ± 0.041	1079.46 ± 489.88
	Ara-C	0.112 ± 0.017*	441.02 ± 179.93**
	5-FU	0.098 ± 0.020*	291.02 ± 161.64**
Spleen	control	0.597 ± 0.049	1091.42 ± 383.27
	Ara-C	0.457 ± 0.046*	1156.48 ± 278.13
	5-FU	0.446 ± 0.054*	1316.73 ± 253.50
Kidney	control	1.083 ± 0.060	1194.01 ± 146.73
	Ara-C	0.917 ± 0.071*	1169.55 ± 284.79
	5-FU	0.913 ± 0.88*	1163.89 ± 238.94

Data represent the mean ±SE for each column. Statistical differences were examined by Student's *t*-test. *$p < 0.01$, **$p < 0.05$, ***$p < 0.1$.

The mean body weight decreased by 13% ($p < 0.01$) in the ara-C-treated rats and 11% ($p < 0.01$) in the 5-FU-treated rats when compared with control rats. The mean wet weights of the prostate, thymus, spleen and kidney of the rats treated with these drugs and that of the testis in ara-C-treated rats decreased significantly (Table 1). Therefore, the doses we used, 63.0 (ara-C) and 33.4 (5-FU) mg/body weight, seemed to be the optimal doses for examining the effects of ara-C and 5-FU. The concentration of putrescine (Figure 1) was about 1/10th that of spermidine (Figure 2) and spermine (Figure 3). All three polyamines in the small intestine, spermidine in the lung, and spermine in the thymus, spleen and liver of the rats treated with ara-C and 5-FU showed statistically significant increases per milligram of their wet weights in comparison with those in the control rats (Figures 1–3). In addition, the putrescine (Figure 1) content in the spleen, heart, muscle and liver, and spermidine (Figure 2) and spermine (Figure 3) in the stomach of the 5-FU-treated rats also showed

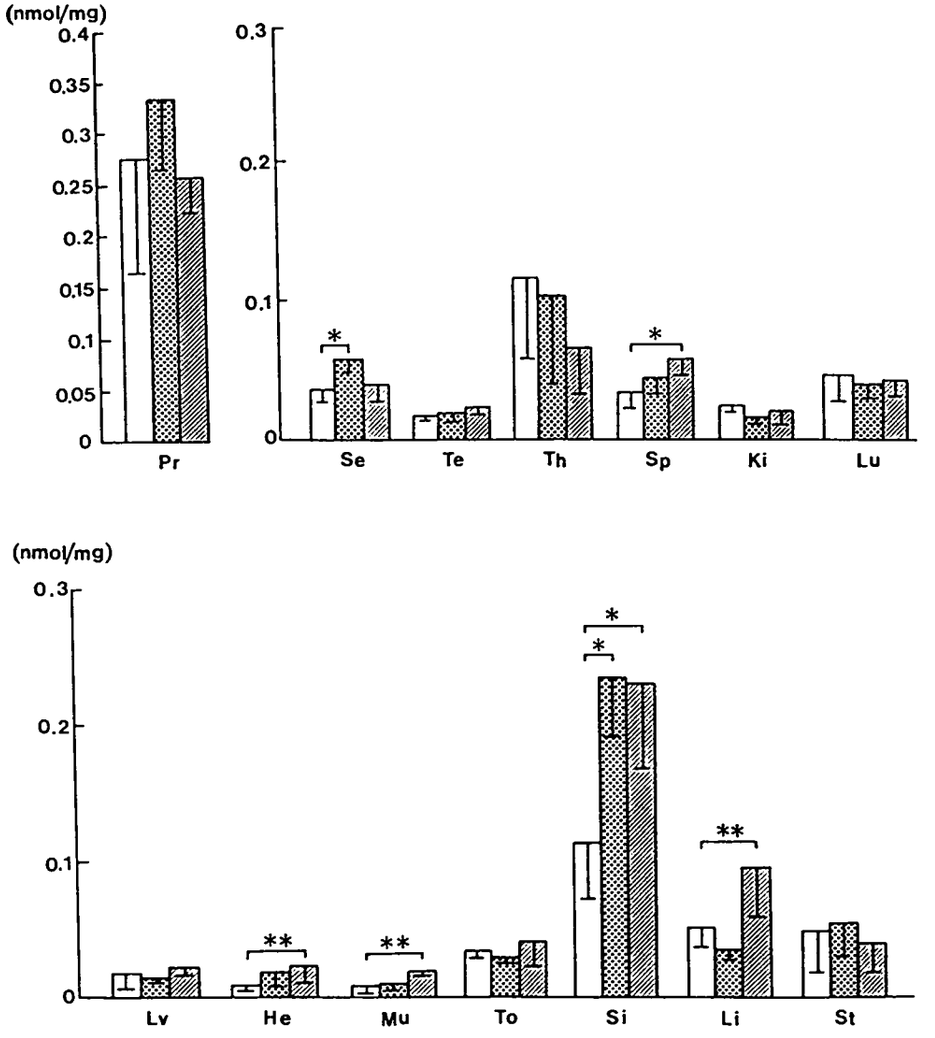

Figure 1. The content of putrescine per milligram of the wet weight of the prostate (Pr), seminal vesicles (Se), testis (Te), thymus (Th), kidney (Ki), lung (Lu), liver (Lv), heart (He), skeletal muscle (Mu), tongue (To), small intestine (Si), large intestine (Li) and stomach (St) of rats given ara-C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

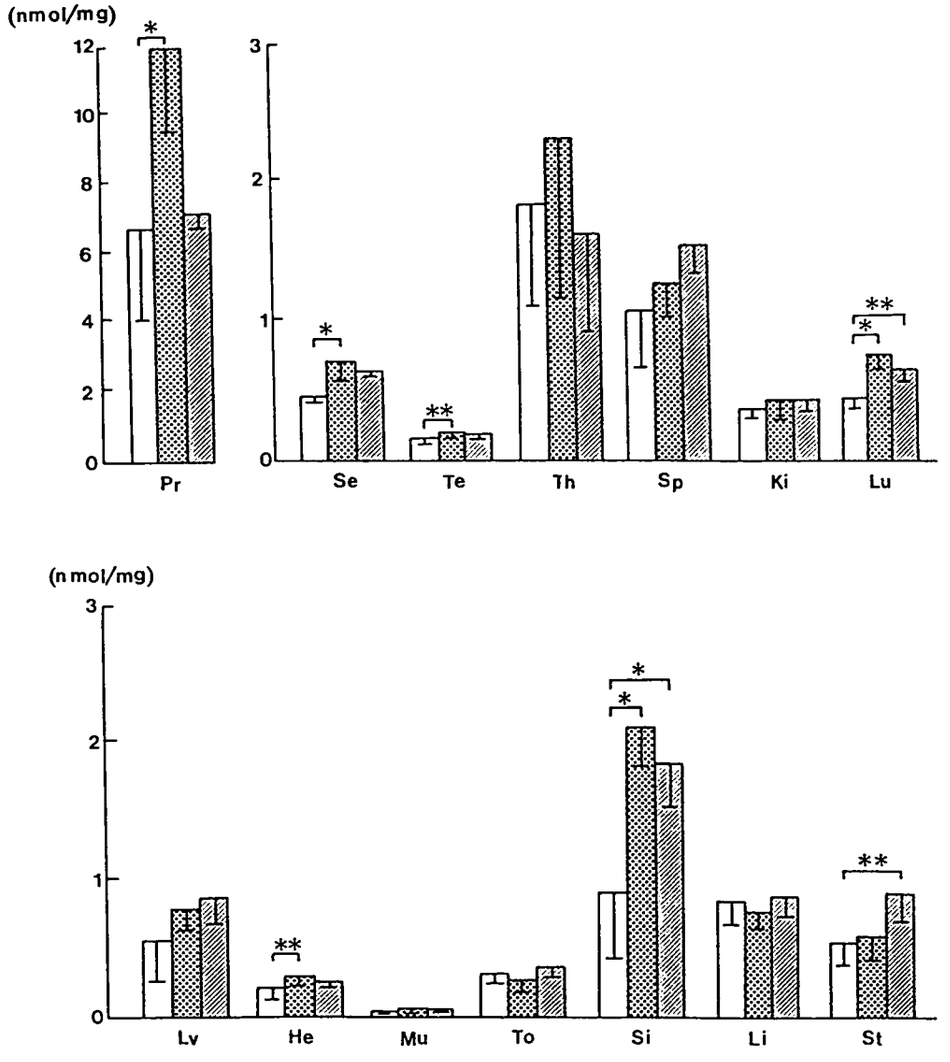

Figure 2. The content of spermidine per milligram of the wet weight of various tissues. Abbreviations and explanations are the same as in Figure 1.

significant increases. No significant decrease was observed in any tissues of rats treated with the drugs.

The spermidine: spermine ratio (Figure 4), which is regarded as the index of a high growth rate, was lowered significantly in the thymus, spleen and liver in the ara-C- and 5-FU-treated rats, in the large intestine of the 5-FU-treated rats, and in the prostate, tongue and stomach of the ara-C-treated rats. However, it was higher than that in the control rats in the testis, lung and muscle of the 5-FU-treated rats (Figure 4). All data in the accessory organs of the ara-C-treated rats are cited from our previous report.[10]

Discussion

We observed an increase in polyamine content in many accessory organs of male rats treated with ara-C.[10] In that paper, we commented that tumor regrowth in these organs may be caused by the increased polyamine content induced by ara-C, judging from the stimulation effects of polyamine on cell proliferation. To avoid regrowth due to the anti-tumor drug employed, it is necessary to avoid using drugs which induce polyamine elevation in the tumor-bearing tissues. In the present study, we examined the effects of ara-C and 5-FU on the polyamine levels in many tissues.

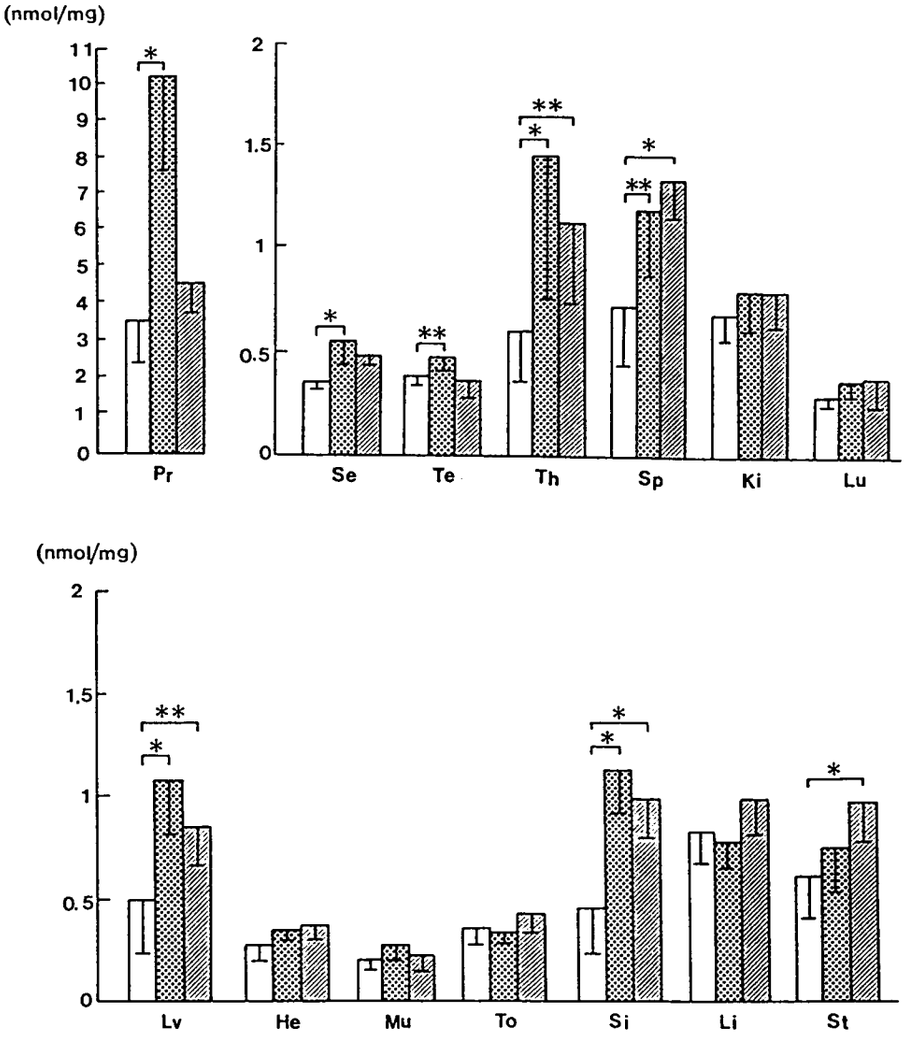

Figure 3. The content of spermine per milligram of the wet weight of various tissues. Abbreviations and explanations are the same as in Figure 1.

It is well known that cell proliferation is strictly dependent on polyamines and that a certain basal level of polyamines is required.[1] Therefore, tumor regression brought about by ara-C and 5-FU must compete with tumor growth mediated by the increased polyamine content caused by the action of these drugs. The polyamines deposited in the tissues by these drugs might cause regrowth of surviving and/or tolerant tumor cells after termination of the drug treatment. In addition to this action, polyamines also play a role in the repair of damaged tissues.[5] Polyamines thus regulate the therapeutic effects of these drugs through the stimulation of cell growth and/or repair of tissues damaged by these drugs. The remarkable increases in all polyamines in the small intestine of the rats treated with ara-C and 5-FU clearly show that these drugs should not be used in the treatment of tumors of the intestine. Furthermore, this seems to be strongly supported by the ratio of spermidine: spermine, which is regarded as the index[11] of growth. Not only were the values high (equal to about 2), suggesting hypertrophy, but there was also no significant difference in the small intestine between the treated rats and untreated rats. The choice of either drug for the treatment of tumors of the lung may be unsuitable on the basis of the spermidine content and the spermidine:spermine ratio. The choice of 5-FU also seemed unsuitable for the treatment of tumors of the heart, muscle or

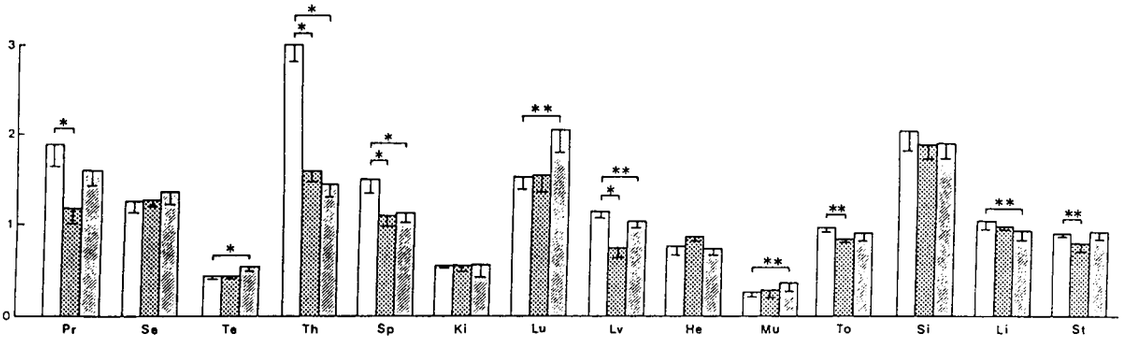

Figure 4. The ratio of spermidine:spermine in various tissues. Abbreviations and explanations are the same as in Figure 1.

stomach, judging from the increase in any one polyamine and the spermidine:spermine ratios in these tissues. However, use of these drugs cannot be excluded only the basis of the present results, i.e. an increase in spermine and a decrease in the spermidine:spermine ratio in the thymus, spleen and liver of the ara-C- and 5-FU-treated rats as compared with the levels of the control. In order to be able to choose the optimal anti-tumor drug to obtain the best therapeutic effect, it is necessary to study in more 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 tissues except the thymus, spleen and liver under ara-C and 5-FU treatment, the prostate, tongue and stomach under ara-C treatment, and the liver under 5-FU treatment.

Conclusions

Our findings regarding the effects of ara-C and 5-FU on the polyamine content in various tissues should be useful in making a decision whether to use these anti-tumor drugs. Increases in polyamine content induced by some anti-tumor drugs stimulate the regrowth of trace cells in surviving cells and/or tumor cells tolerant against these drugs. Accordingly, this then results in the worsening of a patient's condition after the termination of drug treatment even if temporary regression of the tumor is observed. In addition, the increase in spermidine due to the drug action must be carefully watched when monitoring the chemotherapeutic effect on the spermidine level in the sera.[6,7] The present results, hopefully, will encourage physicians not only to note the cell-killing action of the anti-tumor drug in use, but also its effect on the polyamine levels in tumor-bearing tissues.

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