Biochemical Pharmacology, Vol. 44, No. 3, pp. 593–596, 1992.
Printed in Great Britain.

0006-2952/92 $5.00 + 0.00
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A novel note on the effect of ara-C on the polyamine content of the male accessory organs of the rat

(*Received* 26 *March* 1992; *accepted* 14 *May* 1992)

Abstract—The effects of 1-β-D-arabinosylcytosine (ara-C) given for 5 days on body weight, on the weights of the prostate, seminal vesicles and testis, and on the polyamine content (putrescine, put; spermidine, spd; spermine, spm) in these organs were examined in rats. The body weights of the ara-C-treated rats showed a decrease of 13% ($P < 0.01$) and the weights of the prostate, seminal vesicles and testis were reduced by 20% ($P < 0.05$), 13% and 5.0% ($P < 0.1$), respectively. However, put, spd and spm contents in the ara-C-treated prostate were increased 1.2, 1.8 and 2.9 times, respectively. The contents of these polyamines in the ara-C-treated seminal vesicles increased about 40% for all polyamines. Both spd and spm in the treated testis showed a significant increase of about 20% ($P < 0.05$) as compared with the control. In addition, the total polyamines in the whole organs increased significantly in both the prostate and testis.

1-β-D-Arabinosylcytosine (ara-C*) [1–3] is a cell cycle specific nucleoside analog which potently inhibits DNA synthesis through incorporation of its triphosphate into cells. On the other hand, polyamines [4–7], which usually increase in cells undergoing active growth, not only play an important role in cell proliferation and DNA synthesis, but also are used in the monitoring of the therapeutic effects of cancer chemotherapy [8, 9]. Inhibition of polyamine biosynthesis by α-difluoromethylornithine causes rapid depletion of intracellular polyamines and has resulted in the inhibition of tumor growth in a number of transplanted [10] and chemically induced animal tumors [11, 12], and in the inhibition of metastases [13–15]. Because there is stimulation of cell proliferation by polyamines and inhibition of it by ara-C, the effect of ara-C on the content of polyamine rich accessory organs was examined. The present experiments dealt with ara-C-induced alteration of the polyamine system.

Materials and Methods

Chemicals. All the polyamines and diamine used for the preparation of standard solutions were purchased from the Sigma Chemical Co. (St Louis, MO, U.S.A.). 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, Inc. (Kyoto, Japan).

HPLC. Chromatographic analysis was carried out using the JASCO analytical chromatographic system (Japan Spectroscopic Co., Ltd, Tokyo Japan), which includes 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 analytical procedures, we used a polyamine-pak column (35 mm × 6 mm) protected by a guard-pak column, both made by JASCO. The flow-rates were 0.7 mL/min for both the mobile phase solution and the OPA reagent. The temperature of the column oven was kept at 70° throughout the experiment. After post-column derivatization with OPA, the fluorescence intensity was measured with the intelligent spectrofluorometer (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, from 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 [16] 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 to the mixture, the solution was filtrated 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°.

Animal treatment. Male Sprague–Dawley rats (50 days old, 170–180 g) were kept on 24-hr 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. Twelve rats were divided into two equal groups. One group was successfully injected subcutaneously with ara-C in saline solution at 63 mg/kg body weight daily for 5 days. The other group used as the control was injected with an equal volume of saline. All rats in the two groups were anesthetized with diethylether on the sixth day and all accessory organs were immediately removed, weighed and then kept in 2.0 mL of 10% trichloroacetic acid 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 rpm for 15 min. The supernatants were washed twice with 5 mL of diethylether to eliminate the trichloroacetic acid in the water layer. The water layer was kept in a refrigerator below −20° until measurement. Just before measurement, 250 μL of the water layer was diluted with the mobile buffer to 500 μL. The solution was filtrated by a millipore (45 μm, Cosmonice from Nakarai, Tokyo, Japan) and 10 μL of the filtrate was charged with an autosampler.

Results

The body weight of the rats treated with ara-C increased 13% ($P < 0.01$) in 6 days. Therefore, this dose of ara-C

* Abbreviations: ara-C, 1-β-D-arabinosylcytosine; put, putrescine; spd, spermidine; spm, spermine; OPA, *o*-phthalaldehyde.

Table 1. The wet weights (g), total polyamine content (TP, μmol) and ratio of spd/spm of the prostate, seminal vesicles and testis of rats treated with ara-C

Tissue	Drug	Weight (g)	TP/organ	spd/spm
Prostate	Control	0.240 ± 0.037	2535.53 ± 1159.12	1.89 ± 0.25
	Ara-C	0.193 ± 0.029†	4350.59 ± 1134.39†	1.18 ± 0.17*
Seminal	Control	0.701 ± 0.069	587.37 ± 62.50	1.26 ± 0.13
vesicles	Ara-C	0.607 ± 0.130	792.37 ± 205.97	1.27 ± 0.08
Testis	Control	1.463 ± 0.094	823.19 ± 109.95	0.42 ± 0.02
	Ara-C	1.401 ± 0.084‡	975.40 ± 105.48†	0.42 ± 0.02

Data represent the mean ±SD for each column.
* $P < 0.01$, †$P < 0.05$, ‡$P < 0.1$.

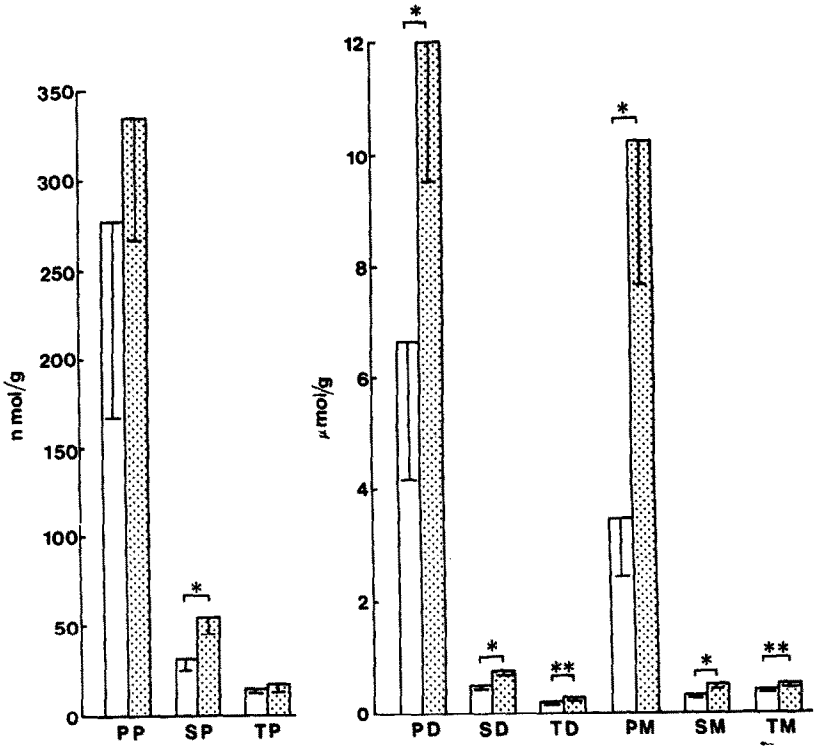

Fig. 1. Put, spd and spm contents in the prostate, seminal vesicles and testis in rats treated with ara-C. PP, SP and TP, PD, SD and TD, and PM, SM and TM correspond respectively to put, spd and spm contents in the prostate, seminal vesicles and testis per gram of wet weight. Unshaded: control column; shaded: treated column. Data represent the mean ±SD for each column. *$P < 0.01$, **$P < 0.05$.

seemed to be the optimum for examination of its effect. The wet weights of the prostate, seminal vesicles and testis were reduced by 20% ($P < 0.05$), 13% and 5% ($P < 0.1$), respectively, as shown in Table 1. These findings indicate that ara-C had the strongest cytotoxic action on the prostate among these organs of the male rat. However, the contents of Putrescine (put), Spermidine (spd) and Spermine (spm) in the ara-C-treated prostate as compared with those in the control rats showed increases of 1.2, 1.8 ($P < 0.01$) and 2.9 ($P < 0.01$) times per gram of wet weight as shown in Fig. 1. These results indicate that ara-C significantly affected spd content and greatly affected spm content in the prostate. When weight loss of the prostate is considered, the statistically significant difference observed in spd per gram of the organ disappears, but a significant increase ($P < 0.05$) in the total polyamine content still remains (Table 1). The ara-C-treated seminal vesicles did not show any significant loss of weight, but the content of each polyamine increased significantly by about 40% ($P < 0.01$) per gram of wet weight as shown in Fig. 1. The spd and spm in the treated testis showed an increase of about 20% ($P < 0.05$) as compared with the control. No significant

increase of put in the testis similar to that in the prostate was observed. The ratio of spd/spm, which is regarded as an index of growth rate, was lower in the ara-C-treated prostate than that in the control, but no change in this ratio was observed in either seminal vesicles or testis, as shown in Table 1.

Discussion

Extensive research has been done regarding the relationship between polyamines and cancer [4, 6]. Many researchers have shown that cell proliferation of the tumor is strictly dependent on polyamines and that it clearly requires a certain level of polyamines. In addition, the polyamine content in the tissues and serum is considered to be strongly related to the regulation of the cell cycle [11, 17–19]. The depletion of polyamines by α-difluoromethylornithine has been shown to result in an accumulation of cells in S-phase. The S-phase specific drug like ara-C results in a preferential synergistic killing of the tumor cells in the S-phase. The observation of a polyamine increase suggested that ara-C should be used with α-difluoromethylornithine but not separately. Thus, to achieve the best therapeutic effect of this drug, it is necessary to clarify the effect of ara-C on the polyamine content in polyamine rich organs.

Judging from the increases in put, spd and spm in these accessory organs of the ara-C-treated rats (Table 1), the tumor regression brought about by ara-C must compete with the tumor growth mediated by the increased polyamine content caused by the action of the drug. It can be postulated that the increased polyamine deposits with successive treatment for 5 days allowed regrowth of the tumor with invasion into the tissue after termination of the drug treatment and promotion of the invasion of metastatic tumor cells. Furthermore, great attention must be paid to the spd level in sera when monitoring the chemotherapeutic effect of ara-C because its increase seems to be followed by elevation of the spd level in the sera. Also an increase in polyamines might cause serious complications, such as hypotension, bradycardia and hypertrophy [20].

The proliferative activity of a tissue is reflected by its spd/spm ratio [21]. The ratio of the treated prostate decreased significantly, but was still slightly higher than 1.0 (Fig. 1). Furthermore, the values in the seminal vesicles and testis were quite similar to those in the control organs. The present results, as reflected by the spd/spm ratio, indicate that there was ordinary cell growth in these tissues under ara-C treatment in spite of the weight losses. This growth must be due to the increased polyamine levels caused by ara-C. Therefore, the additional increase in polyamines due to ara-C could lead to a worsening of a patient's condition after termination of the drug treatment, even if temporary regression of the tumor is observed.

Acknowledgements—The authors are grateful to Misses K. Yoshida, Y. Yamanaka, Y. Yoneda, S. Fukuda and A. Kuroki for technical assistance. This study was supported in part by Research Project Grants No. 3-403 and 3-408 from Kawasaki Medical School.

Departments of Pharmacology
Kawasaki Medical School
577 Matsushima Kurashiki City
Okayama 701-01, Japan
†*Department of Medical Technology*
Kawasaki College of Allied Health Professions
316 Matsushima Kurashiki City
Okayama 701-01, Japan

SATORU WATANABE*
SHOICHI SATO†
SUMIKA NAGASE†
TAIICHI SAITO

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*Corresponding author. FAX (81) 0864 (62) 1199.

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Biochemical Pharmacology, Vol. 44, No. 3, pp. 596–600, 1992.
Printed in Great Britain.

0006-2952/92 $5.00 + 0.00
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Induction of rat hepatic and intestinal glutathione *S*-transferases by dietary butylated hydroxyanisole

(*Received* 2 *February* 1992; *accepted* 4 *May* 1992)

Abstract—To obtain insight into the protection mechanism of butylated hydroxyanisole (BHA), a widely used food preservative with anticarcinogenic properties, we investigated the effects of dietary BHA on rat hepatic and intestinal glutathione *S*-transferase (GST) enzyme activity, and GST isozyme levels. In the proximal small intestine and liver, BHA supplementation significantly increased GST enzyme activity as compared with controls (2.3- and 1.7-fold, respectively, $P < 0.05$). GST class α and μ contents were significantly higher only in the small intestine (1.6–2.1-fold and 1.3–1.5-fold, respectively, $P < 0.05$), whereas GST class π was significantly induced in liver (4.6-fold, $P < 0.05$).

Humans are exposed daily to complex mixtures of chemical compounds in their food [1]. An important portal of entry for these compounds is the gastrointestinal tract [1, 2]. In this case the intestinal mucosa is the first-line barrier. The capability of mucosal cells to detoxify these substances is essential for good protection against these xenobiotics. The detoxification or biotransformation is the total of biochemical reactions which results in the modification and excretion of the exogenous molecules. Important biotransformation enzymes are glutathione *S*-transferases (GSTs*), consisting of a family of isoenzymes with partly overlapping substrate specificities [3, 4]. Therefore, not only the total enzyme activity but also the levels of the different isoenzymes may determine the risk of damaging effects or even the development of cancer [5, 6].

Colon cancer is a major health problem in Western society. It is the second most frequent malignancy and the incidence is still increasing. Epidemiological studies showed that environmental factors, such as dietary habits, may play a role in colon carcinogenesis [7]. However, food also contains compounds which are considered to be anticarcinogenic [7–9]. Butylated hydroxyanisole (BHA) is a widely used food preservative with anticarcinogenic properties [10–12]. A possible mechanism of the chemopreventive action of BHA may be the induction of detoxification enzymes resulting in lower levels of reactive electrophilic metabolites of many xenobiotics [13–17]. Knowledge of the exact protection mechanisms of anticarcinogenic compounds present in food may be of importance for the reduction or prevention of colon cancer. This paper reveals the induction of GSTs in the liver and intestine by BHA as a possible anticarcinogenic mechanism.

* Abbreviations: BHA, 2(3)-*tert*-butyl-4-hydroxyanisole; GST, glutathione *S*-transferase; CDNB, 1-chloro-2,4-dinitrobenzene.

Materials and Methods

Treatment of animals. Male Wistar rats (200 ± 10 g), were obtained from the Central Laboratory Animal Center (University of Nijmegen, The Netherlands). The animals were housed individually on wooden shavings in macrolon cages maintained at 20–25° and 30–60% relative humidity. A ventilation rate of seven air changes/hr and a 12 hr light/dark cycle were used.

The rats were randomly assigned into two groups (nine animals each). Both groups were fed powdered RMH-TM lab chow (Hope Farms, Woerden, The Netherlands). After acclimatization for 3 days, the animals were fed either the basal diet (RHM-TM) or the experimental diet, which was prepared by supplementation with 2(3)-*tert*-butyl-4-hydroxyanisole (BHA) at a concentration of 1% (w/w). A food processor was used to obtain a homogeneous mixture of BHA and the powered lab chow. Body weight was recorded daily and freshly prepared food was given every 2–3 days. During the experiment, the rats had free access to diet and tap water. After 2 weeks the animals were killed by decapitation.

Tissue preparation. The intestine and liver were immediately excised and the intestine was dissected into four segments: proximal, middle and distal small intestine, and large intestine. Each segment was slit longitudinally and the contents were removed by washing with chilled buffer A (0.25 M saccharose, 20 mM Tris, 1 mM dithiothreitol, pH 7.4). The organs were frozen in liquid nitrogen and stored at −80°. For preparation of the cytosolic fraction, the tissue was thawed quickly using cold running water. The mucosal surface of the intestine segments was collected by scraping with a scalpel and the mucosal scrapings were homogenized in buffer A (4 mL/g tissue) in a glass/glass Potter–Elvehjem tube. The liver was also homogenized in buffer A (4 mL/g tissue) with 10 strokes at 1000 rpm of a motor-driven Potter–Elvehjem