Increase of Urinary Putrescine in 3,4-Benzopyrene Carcinogenesis and Its Inhibition by Putrescine

Keisuke Fujita,1 Toshiharu Nagatsu, Kan Shinpo, Kazuhiro Maruta, Hisahide Takahashi, and Atsushi Sekiya

Institute for Comprehensive Medical Science [K. F., K. S., K. M.], Research Center for Laboratory Animals [H. T.], and Department of Pharmacology [A. S.], Fujita-Gakuen University School of Medicine, Toyoake, Aichi 470-11, Japan, and Laboratory of Cell Physiology, Department of Life Chemistry, Graduate School at Nagatsuta, Tokyo Institute of Technology, Yokohama 227, Japan [T. N.]

ABSTRACT

A significant increase in putrescine was noted in the urine of mice with experimental s.c. tumors induced by a single injection of 3,4-benzopyrene solution (2.52 mg of 3,4-benzopyrene in 0.5 ml of tricaprylin). When 10 mg of putrescine were added to the 3,4-benzopyrene solution, the development of tumors was completely inhibited and the increase of urinary putrescine in mice was suppressed simultaneously. Animal weight data of a control group receiving only putrescine indicated that the inhibitory effect of putrescine is not due to its toxicity.

INTRODUCTION

We reported that total polyamines (putrescine, spermidine, and spermine) in urine of patients with blood and solid cancers are significantly high and that putrescine, which increased most significantly, could be of diagnostic aid (1). Our results on the increase of urinary total polyamines in blood and solid cancers agree with early reports by Russell et al. (5, 7), except for the pronounced increase in urinary putrescine (1).

We also reported a significant increase in urine not only of total polyamines but also of putrescine in rats with experimental stomach tumors induced by N-methyl-N-nitro-N'-nitrosoguanidine; this increase in urinary putrescine was observed earlier and was more pronounced (1).

On the other hand, Kalistratos et al. (2-4) recently reported that 3,4-benzopyrene carcinogenesis can be delayed or even completely inhibited by putrescine. Their results prompted us to examine the effects of the injection of 3,4-benzopyrene together with putrescine to mice, and it was found that urinary putrescine increased during tumor development and that injected putrescine inhibited both 3,4-benzopyrene carcinogenesis and the increase in urinary putrescine.

MATERIALS AND METHODS

3,4-Benzopyrene and putrescine were purchased from Sigma Chemical Co., St. Louis, Mo., and tricaprylin was from Tokyo Kasei Kogyo Co., Tokyo, Japan. The design of the experiments on 3,4-benzopyrene carcinogenesis and on the effect of putrescine was essentially the same as that reported by Kalistratos (2) except that putrescine controls were included. Thirty female BALB/c mice, 19 to 20 weeks old and 25 to 30 g body weight, were given s.c. injections of 2.52 mg of 3,4-benzopyrene in 0.5 ml of tricaprylin (Group B) or of 2.52 mg of 3,4-benzopyrene plus 10 mg of putrescine dissolved in 0.5 ml of tricaprylin (Group B + P). As controls, 30 mice were given injections of 0.5 ml of tricaprylin (Group C), and 15 mice received 10 mg of putrescine in 0.5 ml of tricaprylin (Group P).

The diameter of the tumor on the skin was measured by vernier calipers, for each mouse during carcinogenesis, and the surface area was calculated on the assumption that tumors are round. When no tumor was detected the surface area was expressed as zero, and the mean surface area of the tumors of all mice was calculated. For example, at 8 weeks, 2 mice had 7-sq mm tumors, and the remaining 28 mice had no tumor. Therefore, the mean surface area of the tumor of the 30 mice was calculated as follows: (2x7 + 28 x 0) / 30 = 0.5 (sq mm).

Blood pressure was measured in the right carotid artery with an electronic manometer (MPU-0.5 290; San-ei, Japan).

Twenty-four-hr urine specimens were collected in a metabolism cage with a urine collector. Putrescine, spermidine, and spermine in 24-hr urine samples were determined by our assay method (1). In principle polyamines were isolated with Dowex 50 column chromatography, separated by high-voltage paper electrophoresis, and finally measured on a chromatogram with a Shimadzu CS-900 dual-wavelength thin-layer chromatography scanner at 505 and 700 nm by the zigzag scanning method. Urinary polyamines were expressed as μmol/mg creatinine.

RESULTS

The experimental results of putrescine as inhibitor of 3,4-benzopyrene carcinogenesis are graphically summarized according to the method of Kalistratos (2) (Chart 1). Animals died within 23 weeks after a single s.c. injection of 3,4-benzopyrene due to the tumor development which was 100% (Group B). In contrast tumor development was completely inhibited in Group B + P (group treated with 3,4-benzopyrene plus putrescine). In this group 7 mice died 75 days after the injection, but none had developed a tumor locally. In the control Group C, only 1 mouse died of pneumonia 90 days after the injection. In the control Group P, 3 mice died within 1 day, and a total of 7 mice died within 50 days, but none of the 7 had developed a tumor. In both the B + P and P groups, while death occurring within 3 days seemed to be due to acute toxicity of putrescine,
especially respiratory inhibition, death later in the experiment was due to pneumonia.

While the B group showed no early mortality, the 17 and 20% mortality rates of the B + P and P groups occurred relatively early in the experiment, and putrescine administration might have been toxic. Therefore, the toxicity of putrescine was examined in detail and its 50% lethal dose by s.c. injection for BALB/c mice was found to be 1770 mg/kg (range, 1650 to 1894 mg/kg; \( p = 0.95 \)); this toxicity was acute and caused death by respiratory inhibition and a fall in blood pressure. From the weight data of the surviving animals (Chart 2), the inhibition of tumorigenesis does not seem to be due to poor general health of the animals, which might have occurred from the chronic toxic effects of putrescine.

Changes in urinary polyamines (putrescine, spermidine, and spermine) were studied during the 3,4-benzopyrene carcinogenesis experiments; s.c. tumor was noticed to develop from 8 weeks after the 3,4-benzopyrene injection (Group B). The mean size of tumors increased markedly between 14 and 18 weeks. A significant increase in urinary putrescine (Chart 3) of mice of Group B which were given injections of 3,4-benzopyrene alone was observed as early as 9 weeks (0.305 ± 0.193 \( \mu \)mol/mg creatinine; \( p < 0.05 \)), became marked from 12 weeks (0.373 ± 0.188 \( \mu \)mol/mg creatinine; \( p < 0.01 \)), and after 16 weeks reached a maximum (0.661 ± 0.490 \( \mu \)mol/mg creatinine; \( p < 0.01 \)). The decrease in urinary putrescine at Weeks 17 and 18 was due to death of mice that had been excreting higher levels of urinary putrescine. On the other hand, the level of urinary spermidine (Chart 4) in the 4 groups showed no significant difference at 18 weeks. Urinary spermine also did not increase or decrease significantly with the passage of weeks.

**DISCUSSION**

In agreement with the results of Kallistratos et al. (2–4), we found that putrescine completely inhibited 3,4-benzo-
Pyrene carcinogenesis. In this study we first noticed a significant and specific increase of urinary putrescine in mice with experimental tumors induced by a single s.c. injection of 3,4-benzopyrene. This increase of urinary putrescine in mice agrees with our previous findings that putrescine increased significantly in urine of patients with blood and solid cancers and in rats with experimental stomach tumors induced by N-methyl-N-nitro-N′-nitrosoguanidine (1). When compared to control rats (0.29 ± 0.065 μmol/mg creatinine), a significant increase in urinary putrescine in experimental rats with stomach tumors after administration of N-methyl-N-nitro-N′-nitrosoguanidine was observed as early as 20 weeks (0.32 ± 0.082 μmol/mg creatinine; p < 0.01) and became marked after 36 weeks (0.52 ± 0.343 μmol/mg creatinine; p < 0.01) (1). Therefore, the present results further support the concept that putrescine concentrations in urine can be of diagnostic aid in cancers, as proposed in our previous study (1).

Interestingly, this increase of urinary putrescine in 3,4-benzopyrene carcinogenesis was completely inhibited by the injection of putrescine with 3,4-benzopyrene.

From the animal weight data of the control Group P receiving only putrescine (Chart 2), changes in tumor yield and urinary polyamines may not be due to poor caloric intake caused by illness of the animals. Hence, it seems likely that the inhibition of tumorigenesis seen here is due to the interaction of benzopyrene and putrescine at the injection site and/or an effect of benzopyrene metabolism. The effects of the injection of putrescine at a different site and at different times relative to the carcinogen remain to be investigated.

Russell has recently proposed that polyamines could be biochemical markers of tumor kinetics (6). Our previous report (1) and the present results suggest that of the polyamines putrescine may be the biochemical marker of tumor kinetics.

REFERENCES

Increase of Urinary Putrescine in 3,4-Benzopyrene Carcinogenesis and Its Inhibition by Putrescine

Keisuke Fujita, Toshiharu Nagatsu, Kan Shinpo, et al.