PROTECTIVE EFFECTS OF JUZENTAIHOTO, DRIED DECOCTUM OF 10 CHINESE HERBS MIXTURE, UPON THE ADVERSE EFFECTS OF MITOMYCIN C IN MICE

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(Received July 12, 1983)

In view of the reduction of side effects as well as the enhancement of anti-tumor activities of anticancer drugs, we have been interested in the combined use of Chinese medicines with them.

In the present study, we attempted to examine the effects of Chinese prescription, Juzentaihoto (JTX), combined with mitomycin C (MMC). JTX is consisted of Astragali radix, Chinnamomi cortex, Rehmanniae radix, Paeonia radix, Cnidii rhizoma, Atractylodis lanceae rhizoma, Angelicae radix, Ginseng radix, Hoelen and Glycyrrhizae radix.

In BDF₁-mice which were implanted with P-388 leukemic cells, JTX prolonged significantly the average survival days of MMC-treated group. In tumor-free BDF₁-mice, JTX improved the leukopenia and the body weight loss which were caused by MMC. Additionally, JTX delayed the appearance of deaths by lethal dose of MMC.

These results elucidate that JTX enhances the anti-tumor activity of MMC and lessens the adverse effects of it. JTX may be useful for patients undertaking MMC treatment.

Keywords — Juzentaihoto; mitomycin C; Chinese medicine; mitomycin C side effects reduction; anti-tumor activity enhancement; P-388 leukemic cell

INTRODUCTION

Many anticancer drugs are known to have various side effects (leukopenia, thrombocytopenia, depilation, liver injury, anorexia and so on), which are big problems in cancer treatment, regarding the safety of the patients to whom powerful drugs are given.

Recently, studies on the combination of anticancer drugs with some detoxifying agents¹⁻⁴ or on the derivatives with weaker side effects have been carried out.

We have been interested in the combined use of Chinese medicines with anticancer drugs.

As is already known, mitomycin C (MMC) has a strong anti-tumor activity, but the clinical use is limited because of its side effects such as leukopenia, appetite loss etc.⁵⁻¹⁰

Up to now, we have attempted to examine influences of 116 Chinese medicines upon MMC, whether or not their combined use with MMC can promote anti-tumor activity and at the same time can lessen toxicities, and have found that about 15 prescriptions reduced the adverse effects of MMC and/or enhanced the anti-tumor activity of it. Among the prescriptions which showed beneficial effects, the effect of Juzentaihoto was most remarkable.

In the present paper, we report the protective effects of Juzentaihoto, dried decoctum of 10 Chinese herbs mixture, upon the adverse effects of MMC in mice.

MATERIALS AND METHODS

Animals — Male BDF₁ (C57BL/6 × DBA/2) strain mice aged 4 to 4.5 weeks (weighed 16 to 20 g) were used. Each group contained 10 mice.

Materials — Juzentaihoto (JTX) was prepared as follows: A mixture of crude drugs consisting of Astragali radix (3.0 g), Chinnamomi
cortex (3.0 g), Rehmanniae radix (3.0 g), Paenoniae radix (3.0 g), Cnidii rhizoma (3.0 g), Atractylodes lanceae rhizoma (3.0 g), Angelicae radix (3.0 g), Ginseng radix (3.0 g), Hoelen (3.0 g) and Glycyrrhizae radix (1.5 g) was added to 285 ml of water and extracted at 100°C for 1 h. The extracted solution was filtered and spray-dried to obtain dry extract powder (2.3 g), LD_{50} of which is over 15.0 g/kg, p.o. in mice and rats.

Mitomycin C (MMC) was purchased from Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan. JTX extract powder and MMC were dissolved in distilled water and physiological saline solution respectively just before the administration.

Tumor Cells — P-388 leukemic cells were used. The cells were subcultured and implanted according to the primary screening protocol of National Cancer Institute (NCI), U.S.A.

Methods — I. Effect of Juzentaihoto on Antitumor Activity of MMC: On day 0, p-388 leukemic cells (1 × 10^6 cells/mouse) were implanted into the abdominal cavity, to examine effects of MMC, JTX and their combination on the survival days. MMC (3.0 mg/kg) was administered intraperitoneally on day 1 and/or 7 after the implantation. JTX (2.0 g/kg) was orally administered from day 1 daily during the experimental period.

II. Effect of Juzentaihoto on Leukopenia and Body Weight Loss Caused by MMC: This experiment was carried out using tumor-free mice whose leukocyte numbers were in the normal region before the administration (on day 1). MMC (3.0 mg/kg) was given intraperitoneally on day 1 and 7. JTX was orally given at a daily dose of 2.0 g/kg from day 1 continuously for 15 d. Every day during the experiment, blood was collected from the tail vein to count the leukocytes using the automatic leukocyte counter (Toa Micro Cell Counter CC-108, Toa Medical Electronics Co., Ltd., Japan).

III. Effect of Juzentaihoto on Lethal Toxicity of MMC: MMC was given intraperitoneally to tumor-free mice by 3 shots of 3.0 mg/kg repeatedly on day 1, 4 and 7, or by one shot of 9.0

![FIG. 1. Effect of Mitomycin C (MMC) on Survival Ratio in BDF1-Mice Implanted P-388](image)

![FIG. 2. Effect of Juzentaihoto (JTX) on The Antitumor Activity of Mitomycin C (MMC) in BDF1-Mice Implanted P-388](image)

* T/C (%) = midian survival time of treated group / midian survival time of control group × 100
mg/kg on day 1, to examine the combination effect of JTX on the appearance of deaths caused by toxic dosage of MMC. JTX was given orally at 2.0 g/kg/d, continuously through the experimental period (from day 1 to 23–29).

RESULTS

I. Effect of Juzentaihoto on Anti-tumor Activity of MMC

Fig. 1 represents the effect of MMC on survival ratio in mice implanted leukemic cell. MMC showed an anti-tumor activity at 1.5–5.0 mg/kg, i.p. dose-dependently. Particularly at 3.0 mg/kg of MMC, the survival days were significantly prolonged and deaths appeared at about the same time. Therefore, we investigated the effect of JTX on the anti-tumor activity at 3.0 mg/kg, i.p. of MMC.

As seen in Fig. 2, JTX markedly enhanced the anti-tumor activity of MMC given on day 1 and 7, whereas it was ineffective after single administration on day 1. And JTX itself did not show any anti-tumor effect.

II. Effects of Juzentaihoto on Leukopenia and Body Weight Loss Caused by MMC

MMC dose-dependently caused leukopenia and body weight loss at 1.0–10.0 mg/kg, i.p. in tumor-free mice. At 3.0 mg/kg, i.p. of MMC, leukocyte counts were significantly decreased and then recovered to control level, and body weight loss was not so severe as that at 5.0 or 10.0 mg/kg, i.p.

These results are shown in Fig. 3. Thus 3.0 mg/kg, i.p. of MMC was used, in order to examine effect of JTX on side effects (leukopenia and body weight loss) of MMC.

As seen in Fig. 4, the combination of JTX and MMC improved the leukopenia and body weight loss caused by MMC. However, these effects of JTX were rather weak.

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**FIG. 3.** Effect of Mitomycin C (MMC) on Leukocyte Counts and Body Weight in Tumor-free BDF1-Mice

- × — × control (vehicle 0.1 ml/10g, b.w., i.p.; n=10), ○ — ○ MMC 1.0 mg/kg, i.p. (n=10), ● — ● MMC 3.0 mg/kg, i.p. (n=10), □ — □ MMC 5.0 mg/kg, i.p. (n=10), Δ — Δ MMC 10.0 mg/kg, i.p. (n=10), ▲ — ▲ normal (intact animal; n=10).

Each point represents the mean ± S.E.

Significant difference from control values; a) \( p < 0.01 \), b) \( p < 0.001 \).
III. Effect of Juzentaihoto on Lethal Toxicity of MMC

JTX delayed the appearance of deaths induced by toxic doses (at 3 shots of 3.0 mg/kg, i.p. and at one shot of 9.0 mg/kg, i.p.) of MMC. These results are shown in Fig. 5.

DISCUSSION

In the present study, we used 2.0 g/kg as a daily dose of JTX, in consideration of the following facts: It is difficult to find pharmacological actions of extracts of Chinese medicines at small doses. Toxicity of JTX is very weak; LD₅₀ of JTX extract powder was over 15.0 g/kg, p.o. in mice and rats.

In the experiment with P-388 leukemic cells implanted mice, JTX markedly enhanced the anti-tumor activity (life prolonging effect) of MMC given on day 1 and 7, but it did not have any effects on anti-tumor activity of MMC given at a single dose. JTX itself, on the other hand, had no anti-tumor effect. We cannot understand the reason why the enhancing effect of JTX on the anti-tumor activity induced by the single administration of MMC was not detected, but this phenomenon may be partially due to the interaction of administration periods of JTX and MMC.

In the experiments with tumor-free mice, JTX improved the leukopenia and the body weight loss caused by 3.0 mg/kg, i.p. of MMC. It also prolonged the survival days of mice which had been given fatal dosis of MMC. These results suggest that JTX attenuates the side effects of

![Graph showing effect of Juzentaihoto (JTX) on Leukopenia and Weight Loss Caused by Mitomycin C (MMC) in Tumor-free BDF₂ Mice]

Each point represents the mean ± S.E.

Statistical significances were evaluated by making a comparison between JTX + MMC treated group and MMC treated group.

a) p < 0.05, b) p < 0.01.
FIG. 5. Effect of Juzentaihoto (JTX) on Toxicity of Mitomycin C (MMC) in Tumor-free BDF1-Mice

A) × — × control (n=10), • — • MMC 3 mg/kg, i.p. × 3 (n=10), ■ — ■ MMC 3 mg/kg, i.p. × 3 + JTX 2 g/kg/d, p.o. (n=10).

B) × — × control (n=10), • — • MMC 9 mg/kg, i.p. (n=10), ■ — ■ MMC 9 mg/kg, i.p. + JTX 2 g/kg/d, p.o. (n=10).

MMC.

In conclusion, JTX is useful to assist anticancer effect of MMC, enhancing the anti-tumor activity and lessening the adverse effects. We consider that the combination of JTX will play an important role to extend the clinical use of MMC and that JTX is useful for patients with neoplasms undertaking MMC treatment.

Acknowledgement We are deeply grateful to Dr. Ichiro Yoshioka and Dr. Tazuko Tashiro for valuable advices.

REFERENCES