Enhancement of anti-tumor effects of mitomycin C by Rokumi-gan

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(Received November 28, 1990. Accepted February 16, 1991.)

Abstract

Effects of Rokumi-gan on the survival effects of mitomycin C (MMC) were studied using mice transplanted with Sarcoma 180 (S-180). Rokumi-gan was found to enhance survival effects of MMC significantly. It was thought that Poria, one of the constituents of Rokumi-gan is important in producing the effect. The strongest effect was found in a water extracted fraction (MH) from the methanol-extracted residues of Poria. The extract MH exhibited the enhancement effect even in cases of administration prior to S-180 transplantation. These findings suggest that MH might be related to biophylaxis system.

Key Words Rokumi-gan, mitomycin C, Sarcoma 180, anti-tumor activity, BRM, tonic, Poria.

Abbreviations H, hot water extract of Poria ; M, methanol extract of Poria ; MH, water extracted fraction from the methanol-extracted residues of Poria ; MMC, mitomycin C ; S-180, Sarcoma 180

Introduction

Since most current anti-cancer agents have strong side effects, restriction on each clinical dose of the agents produces a large barrier for medical treatment of cancer patients. To make up for those deficits, Kampo-hozai has been recently utilized for cancer treatment. Adachi found that administration of Juzen-taiho-to with anti-cancer agents has favorable therapeutic effects on progressive breast cancer.[1] Haranaka *et al.* demonstrated by animal experiments that the side effects of various anti-cancer agents were protected without loss of anti-tumor effects by a combination of anti-cancer agents with Juzen-taiho-to or Sho-saiko-to.[2] Aburada *et al.* also reported that administration of Juzen-taiho-to with mitomycin C (MMC) was found to enhance the anti-tumor effect of MMC and reduce its side effect.[3] Further, we found that many Kampo-hozai, such as Juzen-taiho-to, Gorei-san and Chorei-to have some effects of reducing renal toxicity by cis-diaminedichloroplatinum (cis-platin).[4]

From these findings, it has been gradually clarified that Kampo-hozai compensates for the deficits of dose-restriction by anti-cancer agents. To demonstrate these effects more clearly, it is necessary to identify what the effective crude drug contained in the Kampo-hozai is, to identify the effective component in such crude drugs, and to further elucidate the mechanism of the effective component.

Rokumi-gan is a typical Kampo-hozai applied to clinical uses for the purpose of increasing yin deficiency (zi-yin). Jiang *et al.* found that Rokumi-gan has a repressive effect on the formation of the tumor by chemical carcinogens, spontaneous carcinogenesis in mice and mutagenisity of cyclophosphamide. Then, it was further suggested that the zi-yin effect of Rokumi-gan might have some effect on bio-phylaxis system.[5] These results suggest a possibility that Rokumi-gan brings a compensatory effect on the deficits of anti-cancer agents.

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Journal of Medical and Pharmaceutical Society for WAKAN-YAKU **8**, 29－33, 1991

In the present experiment, we investigated effects of the combined administration of Rokumi-gan and MMC. Further, we tried to clarify the role of crude drugs constituting Rokumi-gan and to identify the effective components in the crude drug.

Materials and Methods

Preparation for water extract of Rokumi-gan: The crude drugs used in this experiment were a Chinese product supplied by Tsumura Co. The clinical dose per day of the crude drugs combined in Rokumi-gan was Rehmanniae Radix (5 g), Moutan Cortex (3 g), Poria (3 g), Dioscoreae Rhizoma (3 g), Alismatis Rhizoma (3 g) and Corni Fructus (3 g). The mixed crude drugs combining these components in such weight ratio were extracted with hot water and then lyophilized to give water extract in a yield of 23.3%.

Preparation of water extract from prescriptions excluding one constituent from Rokumi-gan: Preparation of the water extract was employed as described above. The yield of each water extract was 14.7% (Rokumi-gan minus Rehmanniae Radix), 23.5% (Rokumi-gan minus Moutan Cortex), 23.3% (Rokumi-gan minus Poria), 24.7% (Rokumi-gan minus Dioscoreae Rhizoma), 20.2% (Rokumi-gan minus Alismatis Rhizoma) and 18.8% (Rokumi-gan minus Corni Fructus).

Preparation of water extract from each constituent drug: Preparation of the extract was employed as described above. The yield of water extract from each crude drug was 54.2% (Rehmanniae Radix), 2.0% (Poria) and 20.0% (Discoreae Rhizoma).

Preparation of Poria extract: Poria was extracted with hot water and the extract was lyophilized to give the water extract (H) in a yield of 2.0%. On the other hand, Poria was extracted with hot methanol and the extract was dried up under a reduced pressure to obtain the methanol extract (M) in a yield of 2.5%. The residues of the methanol extraction were extracted by overnight stirring in water at room temperature and then lyophilized to give water extract of methanol residues (MH) in a yield of 0.6%.

Bioassay: Sarcoma 180 cells (S-180) were intraperitoneally transplanted at 5×10^6 cells/mouse into male ddY mice weighing 20 g (Japan SLC, INC., Japan) at day 0. MMC (Wako pure chemicals Co.) was intraperitoneally given once at day 1, or twice at day 1 and day 7. The test samples were perorally administered daily from day 1 to day 21 in cases of injection after S-180 transplantation (post-injection); whereas in cases of pre-injection these were perorally administered once a day from day-14 (day minus 14) to day-1 (day minus 1). The effect of the test samples in mice was evaluated by a comparison of the mean number of survival days and the number of animals surviving for more than 60 days. The mean survival days were calculated by regarding "more than 60 days" as "just 60 days".

Statistics: The experimental data were expressed as mean±standard deviation of the mean and significance was tested by student's t-test.

Results

Combination effect of MMC and Rokumi-gan

Results of combined administration of MMC (2.5 mg) at day 1 and Rokumi-gan at 3, 4 or 5-fold dosage for clinical use after S-180 transplantation are shown in Table I. These results show that Rokumi-gan administered at a 5-fold dosage significantly enhanced the survival effect by MMC (Table I).

Table I Combination effects of MMC and Rokumi-gan on survival days of S-180 transplanted mice.

Treatment	Dose (mg/kg)	Survival days Mean±S.D.[c]	Number of survivors[d]
None	–	13.2±3.5	0/5
MMC[a] alone	–	18.6±5.2	0/5
MMC[a] + Rokumi-gan[b]	280.0	32.2±16.4	0/5
	373.0	28.0±9.0	0/5
	466.0	34.0±13.4*	1/5

a) MMC (2.5 mg/kg) was intraperitoneally given once at day 1.
b) Rokumi-gan was perorally given daily from day 1 to day 21.
c) Significant difference from survival days of the MMC alone group at *; $p<0.05$.
d) The number of animals surviving for more than 60 days.

Combination effect of MMC and the prescription excluding one constituent from Rokumi-gan

When the prescription excluding one constituent from Rokumi-gan was administered to the mice transplanted with S-180 at a 5-fold dosage for clinical use in addition to MMC injection (2.5 mg / kg) at day 1, the enhancement effect by Rokumi-gan disappeared or was reduced. The reducing effect was distinct, especially in cases of the following three prescriptions excluding Rehmaniae Radix, Poria or Dioscoreae Rhizoma from Rokumi-gan (Table II).

Table II Combination effects of MMC and the prescription of one constituent excluded Rokumi-gan on survival days of S-180 transplanted mice.

Treatment	Dose (mg/kg)[b]	Survival days Mean±S.D.	Number of survivors[c]
None	–	13.4±2.9	0/5
MMC[a] alone	–	16.6±1.7	0/5
MMC[a]+Rokumi-gan- Rehmanniae Radix	220	16.8±1.8	0/5
MMC[a]+Rokumi-gan- Corni Fructus	320	25.8±17.4	1/5
MMC[a]+Rokumi-gan- Dioscoreae Rhizoma	420	17.0±2.8	0/5
MMC[a]+Rokumi-gan- Moutan Cortex	400	20.4±5.7	0/5
MMC[a]+Rokumi-gan- Poria	400	16.6±2.2	0/5
MMC[a]+Rokumi-gan-Alismatis Rhizoma	343	18.4±3.4	0/5

a) MMC (2.5 mg/kg) was intraperitoneally given once at day 1.
b) Test samples were perorally given daily from day 1 to day 21.
c) The number of animals surviving for more than 60 days.

Combination effect of MMC and the crude drugs constituting Rokumi-gan

Table III shows the results of administration of water extract from Rehmaniae Radix (271, 542 mg/kg/day), Poria (6, 12 mg/kg/day) or Dioscreae Rhizoma (60, 120 mg/kg/day) to mice transplanted with S-180 and injected with MMC (2.5 mg/kg) at day 1 and day 7. As shown in these results, Poria enhanced the survival effect by MMC even at a low dose (12 mg/kg/day) and also, three of the five mice tested survived for more than 60 days (Table III).

Table III Combination effects of MMC and Rehmanniae Radix, Poria or Dioscoreae Rhizoma on survival days of S-180 transplanted mice.

Treatment	Dose (mg/kg)[b]	Survival days Mean±S.D.[c]	Number of survivors[d]
None	–	11.8±2.8	0/5
MMC[a] alone	–	22.8±6.6	0/5
MMC[a]+ Rehmanniae Radix	271	39.2±17.8	2/5
	542	30.4±16.8	1/5
MMC[a]+Poria	6	34.0±13.8	1/5
	12	48.8±13.8*	3/5
MMC[a]+ Dioscoreae Rhizoma	60	30.6±15.2	1/5
	120	29.4±15.9	1/5

a) MMC (2.5 mg/kg) was intraperitoneally given twice at day 1 and day 7.
b) The crude drugs were perorally given daily from day 1 to day 21.
c) Significant difference from surival days of the MMC alone group at *; $p<0.01$.
d) The number of animals surviving for more than 60 days.

Combination effect of MMC and Poria extract in post-administration

The effect of administration of Poria extracts H (12, 24 mg/kg/day), M (15, 30 mg/kg/day) and MH (3.6, 7.2 mg/kg/day) to mice transplanted with S-180 and injected with MMC (2.5 mg/kg) at day 1 is shown in Table IV. This shows that extract H, M or MH produced a prolongation effect by simultaneous administration of MMC, although extract M or extract MH did not prolongate the survival days by itself. The order in enhancement activity was MH>M>H. The extract MH significantly enhanced the effect by MMC and of the 15 mice tested, 4 survived for more than 60 days (Table IV).

Combination effect of MMC and Poria extract MH in pre-administration

Table V shows data of pre-administration effect of extract MH (3.6, 7.2 mg/kg/day) on S-180 transplanted mice injected with MMC (2.5 mg / kg) at day 1. These data demonstrated that extract MH produced a prolongation effect by simultaneous administration of MMC, even when

administered prior to S-180 transplantation, although the extract MH alone did not enhance the survival effect (Table V).

Table IV Combination effects of MMC and Poria extract on survival days of S-180 transplanted mice in post-administration.

Treatment	Dose (mg/kg)[b]	Survival days Mean±S.E.[c]	Number of survivors[d]
None	–	16.7±1.2	0/14
M alone	15.0	16.6±1.2	0/5
	30.0	15.6±2.0	0/5
MH alone	3.6	15.4±1.0	0/5
	7.2	15.2±1.0	0/5
MMC[a] alone	–	26.3±2.0	0/15
MMC[a]+H	12.0	29.0±3.0	1/15
	24.0	33.2±4.3	3/15
MMC[a]+M	15.0	34.0±4.5	3/15
	30.0	34.1±3.5	2/15
MMC[a]+MH	3.6	38.1±3.9**	4/15
	7.2	38.0±4.1*	4/15

a) MMC (2.5 mg/kg) was intraperitoneally given once at day 1.
b) The test samples were perorally given daily from day 1 to day 21.
c) Significant difference from survival days of the MMC alone group at *; $p<0.05$ and **; $p<0.02$.
d) The number of animals surviving for more than 60 days.

Table V Combination effects of MMC and Poria extract MH on survival days of S-180 transplanted mice in pre-administration.

Treatment	Dose (mg/kg)[b]	Survival days Mean±S.E[c]	Number of survivors[d]
None	–	17.6±2.4	0/5
MH alone	3.6	19.8±4.3	0/5
	7.2	8.4±1.9	0/5
MMC[a] alone	–	28.6±6.2	0/5
MMC[a]+MH	3.6	34.2±9.4	0/5
	7.2	45.8±12.1*	2/5

a) MMC (2.5 mg/kg) was intraperitoneally given once at day 1.
b) The test samples were perorally given daily from day-14 to day-1.
c) Significant difference from survival days of the MMC alone group at *; $p<0.05$.
d) The number of animals surviving for more than 60 days.

Discussion and Conclusion

The prolongation effects of Rokumi-gan on survival effects of MMC were investigated using mice transplanted with S-180. Though peroral administration of Rokumi-gan alone at 5-fold clinical dosage had no survival effects, Rokumi-gan in a combination with MMC produced significant enhancement with the same dosage as in the above case.

In treatment of diseases, traditional Chinese medicine lays stress on fuzheng quxie. Quxie means elimination of the cause of a disease and fuzheng means supplement of the resistance against diseases. The effect by combination of Rokumi-gan and MMC as described above is thought to be caused by combination of fuzheng and quxie.

Next, the effect of various prescriptions excluding one of the constituents from Rokumi-gan was investigated to clarify the role of each crude drug contained in Rokumi-gan. The combination effect of MMC and Rokumi-gan on survival days disappeared or was reduced in the case of those prescriptions. The tendency was distinctive, especially with the exclusion of Rehmanniae Radix, Poria or Dioscoreae Rhizoma. From these results, it was thought that all the crude drugs constituting Rokumi-gan could be concerned in producing the effect and especially, the three crude drugs may play an important role to elicit the effect.

Accordingly, the combination effects of Rehmanniae Radix, Poria or Dioscoreae Rhizoma and MMC were examined. It was found that the combination of Poria and MMC produced the strongest effect. This fact suggests that Poria contributes largely in producing this effect. Although Poria is a crude drug that is frequently combined in Kampo-hozai, there have been few studies as to the bioactive substances of the drug. Therefore, the extraction method of Poria was examined to identify the effective component of the effect. The order of its activity was MH>M>H. The extract MH significantly enhanced the survival effect by MMC. This result demon-

strates that the effective component in Poria is not sufficiently extracted with hot water. Stepwise extraction with methanol or water is more effective. The prescription combined with Poria was mainly utilized as pills or powders such as Rokumi-gan, Hachimi-jio-gan, Keishi-bukuryo-gan, Gorei-san, Toki-shakuyaku-san, Choto-san, *etc.* The reason why the pill or powder form was chosen for these prescriptions remains unknown. It may have relation to the fact that the effective component of Poria can not be sufficiently extracted with water.

Next, the combination effect of MMC and Poria extract MH, which is administered before S-180 transplantation, was examined and it was demonstrated that MH significantly enhanced the survival effect by MMC even in the case of administration before S-180 transplantation. In addition, administration of MH alone was observed to produce no survival effects in cases of pre-administration or post-administration. These findings suggest that MH might not directly affect tumor cells, but that it would act on the homeostatic system. Accordingly, it is suggested that MH has such action that corresponds to the concept of fuzheng expressed in traditional Chinese medicine. To elucidate a mechanism of the effect produced by Poria, it is urgent to isolate the effective component and determine its structure. Isolation of the effective component is now in progress in our laboratory.

和文抄録

Sarcoma 180 担癌マウスを用い，マイトマイシンCの延命作用に及ぼす六味丸の影響を検討したところ，六味丸はマイトマイシンCの延命作用を有意に増強した。六味丸構成生薬のうち，特に茯苓が本作用発現に重要な役割をはたしていると考えられた。茯苓の抽出物中，メタノール抽出残渣を水で抽出した部分 (MH) に最も強い作用が認められた。また，抽出物MHには，Sarcoma 180 移植前の投与でも，本作用が認められ，MHは生体防御系に作用していることが示唆された。

References

1) Adachi, I.: Studies on combined administration of Juzen-taiho-to with anti-cancer agents to patients of progressive breast cancer by a randomized study (in Japanese). *J. Med. Pharma. Soc. WAKAN-YAKU* **4**, 338-339, 1987.

2) Haranaka, R., Kosodo, H., Hirama, N., Hanawa, T., Hasegawa, R., Nakagawa, Y., Haranaka, K., Satomi, N., Sakurai, A., Yasukawa, K., and Takido, M.: Studies on anti-tumor effects of Juzen-taiho-to and Cinnamomi Ramulus (in Japanese). *J. Med. Pharm. Soc. WAKAN-YAKU* **3**, 312-313, 1986.
Haranaka, R., Hasegawa, R., Gen, M., Liu Ai Min, Hirama, N., Hanawa, T., Nakagawa, Y., Haranaka, K., Satomi, N., Sakurai, A., and Iimura, H.: Studies on anti-tumor effects of Juzen-taiho-to and Cinnamomi Ramulus II (in Japanese). *J. Med. Pharm. Soc. WAKAN-YAKU* **4**, 336-337, 1987.
Haranaka, R., Hasegawa, R., Okado, H., Nakagawa, Y., Satomi, N., Sakurai, A., Iimura, H. and Haranaka, K.: Studies on combination effects of anti-tumor agents and Juzen-taiho-to or Sho-saiko-to (in Japanese). *J. Med. Pharm. Soc. WAKAN-YAKU* 5, 368-369, 1988.

3) Aburada M., Takeda, S., Morita, T., Itoh, H., Matsusita, M., Hosoya, H., Kamatani, N., Matuta, K.and Miyamoto, A.: Studies on Kampo-hozai as tonic for tumor treatment ; Reducing effects of Juzen-taiho-to on side effects of MMC (in Japanese). *J. Med. Pharm. Soc. WAKAN-YAKU* **1**, 68-69, 1984.

4) Sugiyama, K., Yokota, M., Ueda, H. and Ichio, Y.: Reducing effect of traditional Chinese medicines on cisplatin-induced toxicity (in Japanese). *J. Med. Pharm. Soc. WAKAN-YAKU* **5**, 290-291, 1988.

5) Liang, T., Yan, S., and Zhao, L.: Preventing effect of Liuwei-dihuang decoction on esophageal carcinoma. International Symposium of Traditional Chinese Medicine on Oncology, Symposium Paper, Beijing, China, p. 71, 1987.