Original 和漢医薬学会誌**9**, 126－130, 1992

Increasing effect of Peony root upon anti-acetylcholine action of Magnolia bark in isolated ileum of mouse

Fumihiko YOSHIZAKI,* Takaaki KOMATSU, Miki SAGAE, Yukiko SASAKI, Miki TABEI, Toshio ANDO and Shuji HISAMICHI

Department of Pharmacognosy, Tohoku College of Pharmacy

(Received December 27, 1991. Accepted July 15, 1992.)

Abstract

It was found that Peony root increases anti-acetylcholine action of hot water extract of Magnolia bark in mouse ileum. This action occurs strongly when both crude drugs are mixed and refluxed with water. The mixture of separately refluxed solutions of these crude drugs shows similar action after heating.

The active component, gallic acid, was isolated from the methanolic extract of Peony root by purification of the fractions. This compound alone does not affect the activity of Magnolia bark. However, its aqueous solution also strongly augments the action of hot water extract of Magnolia bark by mixing and consecutive heating of both of them. Relationships between this increasing effect and concentration of gallic acid or heating time were investigated.

Key words Magnolia bark, Peony root, hot water extract, anti-acetylcholine action, mouse, gallic acid.

Abbreviations MBS, hot water extract (solution) of Magnolia bark ; MPS, hot water extract (solution) of mixed Magnolia bark and Peony root ; PRS, hot water extract (solution) of Peony root.

Introduction

The majority of Kampo medicines are usually comprised of plural crude drugs. Therefore, it is possible that blended crude drugs influence each other under the process of decoction.

In previous papers, we investigated the influence of coexisting crude drugs on the extraction of paeonol from Moutan bark into the decoction,[1] then examined the activities of mixed Scallion bulb and Trichosanthes fruit on gastric lesions in rats and compared the effects with them individually.[2]

Muscle relaxation is one of the important efficacies of Magnolia bark which can be applied to medical treatment of Parkinsonism. Therefore, with the object of searching for the possibility of increase of this pharmacological activity by coexisting crude drugs in Kampo formulation, some crude drugs blended with Magnolia bark in Kampo medicines were screened by using anti-acetylcholine action of hot water extract (solution) of Magnolia bark (MBS) in isolated ileum of mouse. Since coexisting Peony root was consequently found to have influence on this action, we report the details and attempt the characterization of the ingredient which has increasing activity in this crude drug.

Subjects and Methods

Materials : Chopped Magnolia bark (from Japan) and Peony roots (from Japan) were purchased from Nakai-kohshindo (Kobe). Acetylcholine chloride and gallic acid were purchased from Nacalai tesque (Kyoto).

Animals : Male ddy mice weighing between

*〒 981 仙台市青葉区小松島 4-4-1
東北薬科大学生薬学教室 吉崎文彦
4-4-1 Komatsushima, Aoba-ku, Sendai 981, Japan

Journal of Medical and Pharmaceutical Society for WAKAN-YAKU **9**, 126－130, 1992

25-30 g were used. They were housed in cages and kept at 23±1 °C, The animals were starved for a day before use but allowed free access to water till the beginning of the experiment.

Action on isolated ileum of mouse: According to the Magnus method, ileum preparation isolated from mouse was suspended in a 50 ml organ bath filled with Tyrode's solution (temperature, 25-26°C). 0.1 ml of acetylcholine dissolved in Tyrode's solution was applied to the bath. The contraction of the ileum was mediated by transducer (Natsume KN - 259) and recorded on a Kimorecorder. The materials for examination were applied to the bath 3 min before acetylcholine administration and contractions were compared.

Preparation and treatment: 6 g of Magnolia bark was refluxed with 60 ml of water at 100°C for 30 min and filtered through absorbent cotton. The extract was concentrated to 10 ml *in vacuo* and 0.5 ml of it was applied to the organ bath before administration of acetylcholine. Independently, mixture of Magnolia bark and Peony root (each 6 g) was refluxed with 120 ml of water for 30 min and similarly concentrated to 20 ml, then 1.0 ml of the solution was applied to the bath. Methanolic extract (1.0 g), chloroform extract (0.5 g), ethyl acetate extract (0.5 g) and *n*-butanol extract (0.5 g) of Peony root were dissolved or suspended in 10 ml of water then mixed with MBS (volume ratio of 1 : 1) followed by heating for 30 min. Each mixture of 1.0 ml was also applied to the bath. Each chromatographed fraction (0.5 g × weight of the fraction / weight of total fractions) was dissolved in 10 ml of water and also investigated in a similar fashion. Mantle heater and volt-slider (3.8A, 60V) were used as heating apparatus.

Extraction and fractionation: Peony roots (1200 g) were extracted three times with methanol (3.6 *l*) at room temperature. Removal of solvent *in vacuo* gave about 212 g of methanolic extract. A portion of the extract (187 g) was dissolved in water and successively distributed between chloroform (0.6 *l*), ethyl acetate (0.6 *l*) and *n*-butanol (0.6 *l*) each three times. After removal of the respective solvents, the individual extracts were obtained. The ethyl acetate extract (8.3 g) was column chromatographed on silica gel (Wakogel C-200) using *n*-hexane-acetone and dichloromethane-methanol as eluents to give a colorless substance (about 260 mg). Recrystallization from acetone-chloroform gave colorless needles, mp 237.0-241.0°C (dec). It was identified as gallic acid by comparison of the IR spectrum and TLC *Rf* value with those of authentic gallic acid.

Results

A typical suppression pattern of hot water extract (solution) of mixed Magnolia bark and Peony root (MPS) is revealed in Fig. 1. As shown in Fig. 2, pre-administered MBS had about 43.5% of relaxing action on the acetylcholine (1×10^{-7} g/ml)-induced contraction of the ileum in mouse. MPS increased this suppression (approximately 64.0 %) by previous application, whereas hot water extract (solution) of Peony root (PRS) itself had no influence on the activity of acetylcholine. Mixture of MBS and PRS also showed similar activity to that of MPS by pre-heating the solution.

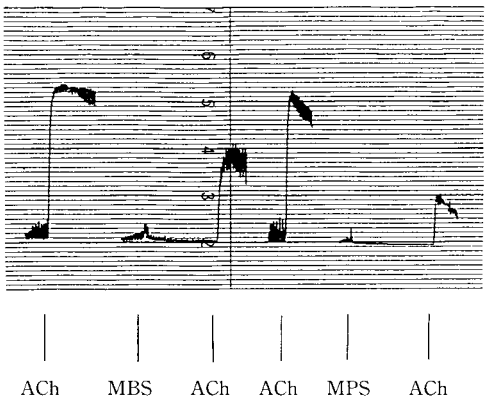

Fig. 1 Comparison of effect of hot water extract (solution) of mixed Magnolia bark and Peony root (extract concentration, 160 mg/ml) (MPS) with that of hot water extract (solution) of Magnolia bark (extract concentration, 98 mg/ml) (MBS) on the contraction of mouse ileum induced by 1×10^{-7} g/ml of acetylcholine.

The mixture of MBS and methanolic extract of Peony root revealed similar action to that of MPS. Accordingly, as shown in Fig. 3, methanol

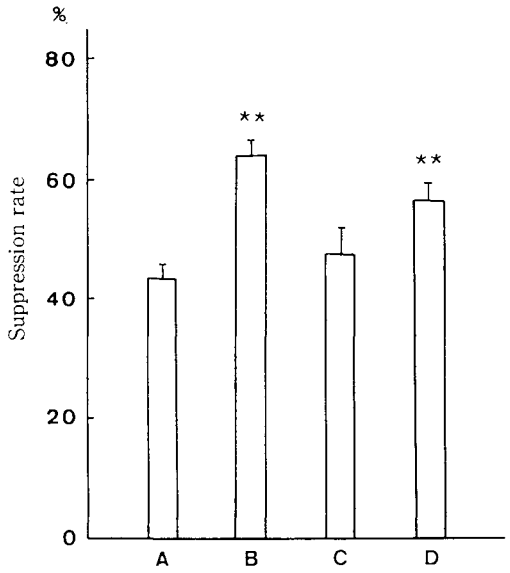

Fig. 2 Comparison of decreasing effects on the contraction of mouse ileum induced by 1×10^{-7}g/ml of acetylcholine.
A, MBS [hot water extract (solution) of Magnolia bark (extract concentration, 98 mg/ml, control)], n=12 ; B, MPS [hot water extract (solution) of mixed Magnolia bark and Peony root (extract concentration, 160 mg/ml)], n=5 ; C, MBS + PRS [hot water extract (solution) of Peony root], without pre-heating, n=6 ; D, MBS +PRS, with 30 min pre-heating, n=6. All columns represent the mean±S.E.. ** exhibit a signficant difference from the control at $p < 0.01$.

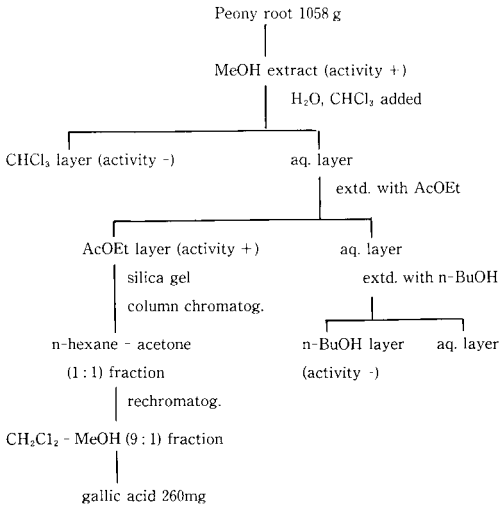

Fig. 3 Fractionation procedure of methanolic extract of Peony root.

extract was fractionated with the guidance of the activity to isolate a colorless crystalline substance, gallic acid.

Relation between concentration of gallic acid (purchased from Nacalai tesque) and increasing effect on action of Magnolia bark (20 min pre-heating) is exhibited in Fig. 4. 8.3×10^{-7} g/ml of gallic acid revealed slight activity, whereas linear augmentation was observed till 1.7×10^{-5} g/ml.

In Fig. 5, correlation of pre-heating time of mixture solution of MBS and gallic acid (purchased from Nacalai tesque, 1.2 mg/ml in water) with change of activity is shown. The activity without heating was 15% and 55% augmentation at 20 min pre-heating was the maximum.

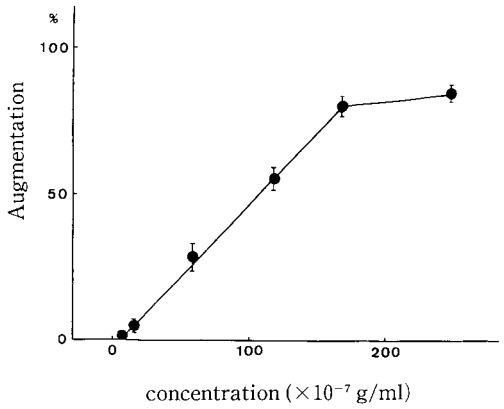

Fig. 4 Relationship between gallic acid concentration and augmentative contribution to Magnolia bark activity.
Applied dose of mixture of hot water extract (solution) of Magnolia bark (extract concentration, 98 mg/ml) and each gallic acid aqueous solution (volume ratio of 1 : 1) was 1 ml and previously heated at 100°C for 20 min. Individual data points represent the mean±S.E. of 4 experiments.

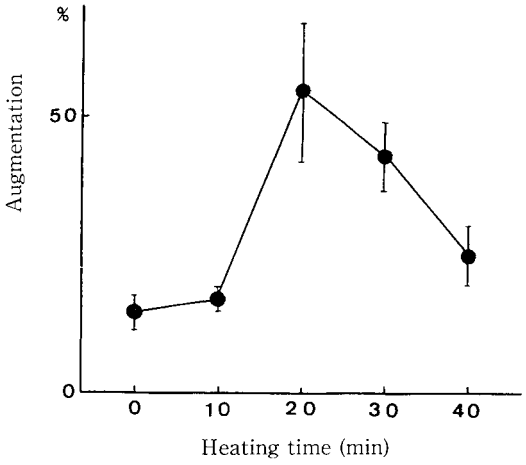

Fig. 5 Relation between change of increasing activity and pre-heating time of mixture of MBS [hot water extract (solution) of Magnolia bark (extract concentration, 98 mg/ml)] and gallic acid.
Applied dose of mixture (volume ratio of 1 : 1) of MBS and gallic acid aqueous solution (1.2×10^{-5} g/ml) was 1 ml. Each datum point represents the mean±S.E. of 4 experiments.

Discussion

Aqueous extract of Magnolia bark exhibits the curare-like action and its active constituent is quaternary alkaloid, magnocurarine. Accordingly it depresses the contraction of frog's rectus abdominis caused by acetylcholine.[3,4] Another representative constituent, magnolol shows the central nervous system suppression and central muscle relaxation.[5,6] Necessarily, MBS is expected to reveal the anti-acetylcholine action in mouse ileum and it actually depresses the contraction of the isolated ileum induced by acetylcholine to 50 %.

On the other hand, anti-cholinergic activity of Peony root in *in vivo* experiments was recently reported.[7] However, no anti-cholinergic action is found in *in vitro* experiments of this crude drug[8] and practically no activity against acetylcholine is observed in mouse ileum.

Nevertheless, it was found that by mixing Magnolia bark and Peony root (1 : 1), the latter augments the anti-acetylcholine action of the former near to 50 %. Furthermore, this activity appears strongly through refluxing with water together. But it does not appreciably occur under merely mixing of individual hot water extracts and augments as well owing to heating.

Gallic acid, already reported on its separation from Peony root,[9] was isolated and identified as an active component in this crude drug. The minimum concentration of gallic acid inducing the increasing effect was 1.7×10^{-6} g/ml and over 80 % augmentation was observed more than 1.7×10^{-5} g/ml. Taking into consideration the amount of gallic acid actually isolated from Peony root, these concentrations seem higher than that in refluxed solution of 6 g of Peony root. Degradation of tannins (such as galloyl glucoses) in Peony root during reflux may have contributed to the activity. However, as the exact content of gallic acid in Peony root is ambiguous, it can not be affirmed.

The mere mixture of MBS and aqueous solution of gallic acid also scarcely shows activity but it is augmented by heating. Some chemical interaction through heating may be correlated with this phenomenon. The decrease of activity in above 30 min pre-heating may be based on the degradable property of gallic acid by heating.

These results are quite suggestive for consideration of the meaning of the decoction procedure of Kampo medicines, namely the heating of plural compound mixtures in water. Further detailed studies on the causes are expected.

和文抄録

厚朴熱水抽出液のマウス腸管における抗アセチルコリン作用を芍薬が増強することを見い出した。この作用は両生薬をいっしょに水で加熱抽出することにより強く発現する。個別に抽出した液の混合物でも加熱すると類似の作用を示す。

芍薬中の活性成分として，gallic acid がそのメタノールエキスを分画精製することにより単離された。本物質単独では厚朴の活性に影響をおよぼさないが，その水溶液は厚朴熱水抽出液と混合加熱する

ことにより，やはり厚朴の作用を強く増大する。この増強効果と，gallic acid の濃度あるいは加熱時間との関係が検討された。

References

1) Fang, Z.Z., Yoshizaki, F. and Hisamichi, S. : Paeonol content in the decoction of Moutan Cortex infused with another crude drug. *Japan. J. Pharmacog.* **44**, 52-54, 1990.

2) Takano, F., Yoshizaki, F., Ahiko, M., Satoh, H., Ando, T. and Hisamichi, S. : Effects of extracts of mixed Allii Bakeri Bulbus and Trichosanthis Fructus on gastric lesions. *J. Med. Pharm. Soc. WAKAN-YAKU* **8**, 23-28, 1991.

3) Ogiu, K., Takagi, H. and Morita, M. : 第4級塩基の蛙腹直筋に対するクラレ様作用並にワゴスチグミンの拮抗に就て. *Folia Pharmacologica Japonica* **51**, 209-217, 1955 (in Japanese).

4) Inoue, K. : 防己科並びにモクレン科植物から分離した第4級アンモニウム塩基, Menisperine, Lourifoline, Magnocurarine, Magnoflorine 及び Salicifoline（各塩化物）の薬理作用に就て. *Folia Pharmacologica Japonica* **53**, 797-818, 1957 (in Japanese).

5) Watanabe, K., Goto, Y. and Yoshitomi, K. : Central depressant effects of the extracts of Magnolia Cortex. *Chem. Pharm. Bull.* **21**, 1700-1708, 1973.

6) Watanabe, K., Watanabe, H.Y., Goto, Y., Yamamoto, N. and Yoshizaki, M. : Studies on the active principles of Magnolia bark. Centrally acting muscle relaxant activity of magnolol and hōnokiol. *Japan. J. Pharmacol.* **25**, 605-607, 1975.

7) Kobayashi, M., Ueda, C., Aoki, S., Tajima, K., Tanaka, N. and Yamahara, J. : Anticholinergic action of Paeony root and its active constituents. *Yakugaku Zasshi* **110**, 964-968, 1990 (in Japanese).

8) Maeda, T., Shinozuka, K., Baba, K., Hayashi, M. and Hayashi, E. : Effect of Shakuyaku-kanzoh-toh, a prescription composed of Shakuyaku (Paeoniae Radix) and Kanzoh (Glycyrrhizae Radix) on guinea pig ileum. *J. Pharm. Dyn.* **6**, 153-160, 1983.

9) Shibutani, S., Nagasawa, T., Oura, H., Nonaka, G. and Nishioka, I. : Effect of extract from Paeoniae Radix on urea-nitrogen concentration in rat serum. *Chem. Pharm. Bull.* **29**, 874-878, 1981.