Influence of coexisting crude drugs on the acute toxicity of Aconite root in mice

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Abstract

For the purpose of studies on crude drugs affecting Aconite root activities, the influence of coexisting crude drugs which are found in the prescriptions containing Aconite root on the acute toxicity was investigated. Each of the crude drugs to be tested was extracted along with Aconite root and the toxicity of the obtained hot water extract was compared with that of Aconite root alone after intraperitoneal administration to mice. Black soybean, Ginger, steamed Ginger and Plantago seed decreased the acute toxicity of Aconite root. However, co-administration of extracts of each of these crude drugs with extract of Aconite root showed no influence on toxicity.

The amount of total aconitine alkaloids eluted into the hot water showed that Plantago seed significantly decreases the quantity of aconitine alkaloid. On the other hand, Cornus fruit promotes the elution of the alkaloids and seemed to increase its acute toxicity under all conditions studied. These influences on alkaloid elution seemed the causes of influences of these crude drugs on toxicity. Implication of the counteracting effects of black soybean, Ginger and steamed Ginger against the virulence of Aconite root is in accord with the tradition from olden times.

Key words Aconite root, hot water extract, acute toxicity, mouse, total alkaloids.

Introduction

Plural crude drugs are normally blended in Kampo medicines. In order to study the mutual influence on their action, we previously investigated the influence of coexisting Peony root on the anticholinergic action of the hot water extract of Magnolia bark in mouse ileum and the effect on the action of Magnolia bark of isolated gallic acid, one of the constituents of Peony root.[1)]

As a result, as one of the studies on mutual influences of crude drugs, we noted the acute toxicity of Aconite root, as easily observable activity, and examined the presence of coexisting crude drugs which exert an influence on the acute toxicity (chosen as an easily recognizable indicator) of this crude drug in terms of lethality, using hot water extracted solutions.

Materials and Methods

Materials: Crude Aconite roots (dried and chopped) were provided by Sanwa Shoyaku Co., Ltd. (from China, used for observation of acute toxicity) and the Experimental Station of Tohoku University for medicinal plants (*Aconitum carmichaeli* DEBEAUX, cultivated plants, utilized for estimation of total alkaloid content). Gingers and steamed Gingers were prepared from fresh gingers (from Kohchi Prefecture, Japan). Black soybeans (from Hokkaido, Japan) were supplied from the market in Sendai. Other curde drugs were purchased from Nakai-kohshindo (Kobe).

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Animals: Male ddy mice weighing between 22-30 g were used. The animals were housed in cages and kept at 23±1°C. They were also starved and denied water for a day before use.

Assay Procedure: Four grams of crude Aconite root was refluxed with 30 ml of water for 30 min and filtered. This filtrate resulted in 100 % mortality (within 10 min) at an intraperitoneal dose of 0.5 ml/20 g. Separately, a mixture of crude Aconite root and a crude drug (each 4 g) was refluxed with 60 ml of water for 30 min. After filtration, the filtrate was concentrated *in vacuo* to the same volume with the extract of Aconite root alone (the invariance of the toxicity during this process was elucidated in advance). The mice were administered each test solution intraperitoneally at a dose of 0.5 ml/20 g (5 mice in a group) and lethal times were measured, then averaged. The operation mentioned above was repeated 3 times and the times compared. The other mice (10 animals in a group) were permitted to stand for 20 h after intraperitoneal administration of the test solution at doses of 0.17-0.22 ml/20 g and the dead individuals were counted. This operation was also repeated 3 times. Independently, 4 g of each crude drug was extracted with 30 ml of hot water in a similar process as above. Each filtrate was intraperitoneally given to 10 mice (0.17 - 0.22 ml / 20 g) separately from the administration of the same volume of Aconite root extract (1 min time lag; saline instead of each crude drug extract was used as a control). The number of dead animals was counted 20 h later and compared. This experimentation was repeated 3 times.

Acute Toxicity Test of Cornus Fruit: 4 g of Cornus fruit was extracted once with boiling water (30 ml) for 30 min, then filtered. The filtrate was intraperitoneally given to mice (15 animals in a group) at a dose of 0.5 ml/20 g and survival after 72 h was noted.

Estimation of Total Alkaloids: According to Hikino *et al.*[2] and Chikazawa *et al.*,[3] mixture of Aconite root (4 g) and a crude drug (each 4 g) was refluxed with 60 ml of water for 30 min and filtered. Two and a half milliliters of ammonia water (**JP** XII) was added to the filtrate, then extracted with chloroform (quadruple volume, three times). After removal of the solvent *in vacuo*, the extract obtained was dried and dissolved in 5 ml of ethanol to which 30 ml of water, 8 drops of methyl red reagent (**JP** XII) and 1 drop of methylene blue reagent (0.1 w/v% in ethanol) were added. The test solution obtained ($n=3$) was titrated with 0.001 N HCl (10 ml corresponds to 6.037 mg of benzoylaconine). As a control, Aconite root alone (4 g) or each crude drug alone (4 g) was also refluxed with 30 ml of water and treated in a manner similar to that described above.

Statistics: Statistical analysis was performed using Student's t-test. Values of $p<0.05$ were regarded as significant.

Results

Twenty - one kinds of crude drugs were examined. Lethal times were compared between hot water extracts of Aconite root alone and each mixture of Aconite root and crude drug with intraperitoneal administration in mice. The crude drugs which significantly extended the times were as follows, black soybean (+ 52.0 %), Ginger (+ 49.0 %), steamed Ginger (+ 47.0 %) and Plantago seed (+38.0 %). Conversely, Cornus fruit showed significant shortening (− 29.1 %). The results with other crude drugs are shown in Table I.

The survival rates of mice (20 h later) at each administration of extract of the mixture when doses of control (Aconite root alone) were adjusted to 50-70 % mortality are shown in Table II; namely, Ginger, 100.0 % (control, 33.0 %); Plantago seed, 100.0 % (control, 30.0 %); black soybean, 96.7 % (control, 30.0 %); steamed Ginger, 73.3 % (control, 33.3 %); Cornus fruit, 16.7 % (control, 50.0 %).

The survival rates of mice administered simultaneous separate injections of the extract of individual crude drugs from the extract of Aconite root alone (adjusted to about 50-80 % lethality) were as follows: black soybean, 33.3 % (control, 23.3 %); Plantago seed, 33.3 % (control, 26.7 %); Ginger, 20.0 % (control, 23.3 %); steamed Ginger, 20.0 % (control, 23.3 %); Cornus fruit,

Table I Changes of lethal times by coextraction of Aconite root with each crude drug.

Crude drug	Lethal time (sec)	Lethal time of control (sec)	Inhibition (%)
Black soybean	666.7±53.7[a)]	438.7±33.3	+52.0
Ginger	775.8±58.9[a)]	519.6± 9.4	+49.3
Steamed Ginger	767.1±61.4[a)]	522.1±33.2	+46.9
Plantago seed	559.6±35.3[a)]	405.5±22.6	+38.0
Glycyrrhiza	577.2±42.8	463.6±16.3	+24.5
Moutan bark	578.7±54.0	471.8±14.1	+22.6
Pueraria root	611.4±61.2	498.7±29.6	+22.6
Ephedra herb	576.8± 7.3	483.2±41.4	+19.4
Cinnamon bark	546.3±28.7	483.2±41.4	+13.1
Peony root	572.4±28.2	513.9±10.2	+11.4
Atractylodes rhizome	564.2±47.2	516.5±48.8	+ 9.2
Rehmannia root	433.2±36.5	404.1±31.4	+ 7.2
Asiasarum root	420.7±20.0	399.2± 8.7	+ 5.4
Saposhnikovia root	414.1± 8.3	405.5±22.6	+ 2.1
Hoelen	399.8±20.3	404.1±31.4	− 1.1
Jujube	393.4±33.4	404.1±31.4	− 2.6
Alisma rhizome	426.5±31.6	442.2±52.0	− 3.6
Atractylodes Lancea rhizome	483.2±12.1	516.5±48.8	− 6.4
Achyranthes root	346.1±11.4	405.5±22.6	−14.6
Dioscorea rhizome	381.6±31.8	498.7±29.6	−23.5
Cornus fruit	287.7±22.4[a)]	405.5±22.6	−29.1

All values represent the maen ±S.E..
a) Significantly different from control, $p<0.05$.

Table II Variation of toxicity of Aconite root in extraction together with other crude drugs.

Crude drug	Dead animals[a)] (/30 mice)	Lethality (%)
Control[b)]	20	66.7
Ginger	0[c)]	0.0
Steamed Ginger	8[c)]	26.7
Control[b)]	21	70.0
Black soybean	1[c)]	3.3
Plantago seed	0[c)]	0.0
Control[b)]	15	50.0
Cornus fruit	25[d)]	83.3

a) After 20 hr.
b) Aconite root only.
Significantly different from control, c) $p<0.01$, d) $p<0.05$.

Table III Alteration of toxicity of Aconite root in simultaneous separate administration from each independent extract of crude drug.

Crude drug	Dead animals[a)] (/30 mice)	Survival rate (%)
Control[b)]	23	23.3
Ginger	24	20.0
Steamed Ginger	24	20.0
Control[b)]	23	23.3
Black soybean	20	33.3
Control[b)]	22	26.7
Plantago seed	20	33.3
Control[b)]	16	46.7
Cornus fruit	27[c)]	10.0

a) After 20 hr.
b) Aconite root and saline (instead of each crude drug extract) were administered.
c) Significantly different from control, $p<0.05$.

10.0 % (control, 46.7 %) (Table III). In addition, the hot water extract of Cornus fruit alone did not show any toxicity in mice within 72 h at a dose of 0.5 ml/20 g.

The influence of these 5 coexisting crude drugs on the elution of aconitine alkaloids from Aconite root into hot water extract was investigated (Table IV). The total alkaloids content in the solution was lower when extracted with Plantago seed ; in contrast, Cornus fruit increased the content significantly. Black soybean, Ginger and steamed Ginger had no influence on the extraction of alkaloids.

Table IV Determination of total alkaloids in hot water extracts of mixed Aconite root and crude drugs.

Crude drug	Content (mg)
Aconite root only	8.965±0.167
Ginger	9.207±0.437
Steamed Ginger	9.131±0.742
Black soybean	8.754±0.332
Plantago seed	6.480±0.568[a)]
Cornus fruit	10.305±0.315[a)]

Content was replaced with an amount of benzoylaconine.
All values represent the mean ±S.E..
a) Significantly different from control, $p<0.01$.

Discussion

Crude Aconite root is not usually clinically used except in treatment to reduce virulence. There have been several reports on the acute toxicity of Aconite root and its active constituents, the aconitine alkaloids,[2,4-8] but none relating to the influence of coexisting crude drugs on its toxicity.

Twenty kinds of crude drugs which are found in the 10 prescriptions containing Aconite root (Kakkon-ka-jutsubu-to, Kanzo-bushi-to, Kei-kyo-so-so-oh-shinbu-to, Keishi-ka-jutsubu-to, Gosha-jinki-gan, Shigyaku-to, Shakuyaku-kanzo-bushi-to, Shinbu-to, Hachimi-jio-gan, Mao-bushi-saishin-to) and black soybean which is not used in Kampo formulations (although tradition says that it frequently detoxifies Aconite root) were tested for changes in toxicity by comparison with the hot water extract of mixed crude Aconite root and each crude drug with that of crude Aconite root alone.

Black soybean, Ginger, steamed Ginger and Plantago seed significantly elongated the lethal times with intraperitoneal administration (more convenient to observe the toxic reaction than oral application) in mice. In contrast, Cornus fruit significantly shortened the lethal time. These results were similar to those in comparison of the number of dead animals with weaker poison (by decrease of dose). Namely, black soybean, Ginger, steamed Ginger and Plantago seed helped raise survival rates over those of the control group, unlike Cornus fruit. But comparison of the number of dead animals after simultaneous separate administration of the hot water extracts of Aconite root and each crude drug showed no difference between balck soybean, Ginger, steamed Ginger, Plantago seed and the control group. This suggests that the toxicity is weakened only with simultaneous extraction. Therefore, there is a possible influence on the acute toxicity of aconitine alkaloids by the components of coexisting crude drugs in the process of extraction, based on such mutual interaction.

Because coextracted Plantago seed decreased the total amount of alkaloids eluted into a hot water solution, it can be presumed that disturbance of alkaloid elution is the main reason for alleviation of toxicity in this case. Promotion of aconitic alkaloid elution seems to be one of the causes of toxic increase with Cornus fruit. Cornus fruit also resulted in more dead individuals than the control when administered separately from Aconite root. As Cornus fruit itself had no acute toxicity under the conditions employed, another still obscure cause of this increasing effect on toxicity is presumed to exist.

It has already been reported that the total amount of aconitine alkaloids in the decoction of Aconite root is different from those of Kampo formulations containing Aconite root.[3] It has also been implied that the elution of these alkaloids varies according to the crude drugs blended.

Tradition says that miso soup or potion of decoction of mixed black soybean and Glycyrrhiza or Kanzo-kankyo-to[9] in Kampo medicine are effective antidotes to Aconite root intoxication. It is said that a similar treatment is used in China[10] as well. Suggestion of the counteracting effects of black soybean, Ginger and steamed Ginger against the virulence of aconitine alkaloids in coextraction (codecoction) with Aconite root is attractive.

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和文抄録

附子の生理活性に影響を与える生薬を検討する目的で，附子といっしょに漢方方剤中に配合されている生薬を選んで共存させ，附子の急性毒性に対する影響の有無について調べた。各被検生薬を附子といっしょに熱水抽出し，その抽出液のマウス腹腔内投与による毒性を，附子単味の抽出液のものと比較検討した。検討した生薬のうち，黒豆，生姜，乾姜および車前子が附子の急性毒性を減少させた。しかしながら，これら生薬単味の抽出液を附子単独のものと同時に投与した場合，毒性に影響はなかった。

熱水中に溶出するアコニチンアルカロイドの総量を定量したところ，車前子が附子と共存すると有意にこれを減少させることがわかった。一方，山茱萸は共存することによりアルカロイドの溶出を促進し，毒性も増強しているように思えた。これら生薬の附子の毒性への影響は，その溶出アルカロイド量への影響で説明できる。また，黒豆や生姜に附子の毒性に対する解毒作用が暗示されることは，昔からの言い伝えとも一致して興味深い。

References

1) Yoshizaki, F., Komatsu, T., Sagae, M., Sasaki, Y., Tabei, M., Ando, T. and Hisamichi, S.: Increasing effect of Peony root upon anti-acetylcholine action of Magnolia bark in isolated ileum of mouse. *J. Med. Pharm. Soc. WAKAN-YAKU* **9**, 126-130, 1992.

2) Hikino, H., Yamada, C., Nakamura, K., Sato, H., Ohizumi, Y. and Endo, K.: Change of alkaloid composition and acute toxicity of Aconitum roots during processing. *Yakugaku Zasshi* **97**, 359-366, 1977 (in Japanese).

3) Chikazawa, K., Araki, S., Tamada, T., Itoh, K., Itoh, H. and Arai, M.: Alkaloid constitution in "Uzu" and "Shujibushi", as well as alkaloid contents in their decoction. *Japanese Journal of Oriental Medicine* **39**, 33-40, 1988 (in Japanese).

4) Kuroda, A.: Aconitin 類の分解及び吸収に関する薬理学的研究 第1 篇 Aconitin, Mesaconitin及び Jesaconitin の分解による毒力減少に就ての薬理学的検索. *Folia Pharmacologica Japonica* **47**, 21-26, 1951 (in Japanise).

5) Gotoh, H.: Aconitine の分解と其の分解物質の薬理作用に関する研究 第2篇 Mesaconitine と其の加熱分解物質の薬理作用の比較検索. *Folia Pharmacologica Japonica* **52**, 511-519, 1956 (in Japanese).

6) Yakazu, S.: 東亞産 Aconite 根（烏頭・附子）の薬理学的研究及び治療応用の検討 第1篇 Aconite 根の生薬利用の諸操作に対する薬理学的検索. *Folia Pharmacologica Japonica* **54**, 880-894, 1958 (in Japanese).

7) Tamura, S. and Tsubouchi, H.: 炮附子末の急性毒性. *Kampo Kenkyu* No.9, 331-341, 1981 (in Japanese).

8) Hikino, H., Takahashi, M., Konno, C., Hashimoto, I. and Namiki, T.: Subacute and subchronic toxicity of Aconitum roots and mesaconitine. *Shoyakugaku Zasshi* **37**, 1-9, 1983 (in Japanese).

9) Shimizu, T. "国医薬物学研究", Hirokawa Publishing Co., Tokyo, Japan, pp. 128-130, 1941.

10) Kobe Chuigaku Kenkyukai trans. and ed., "漢薬の臨床応用", Ishiyaku-syuppan, Tokyo, Japan, pp. 187-190, 1979.