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# Potential Antidotes for T-2 Toxin Poisoning

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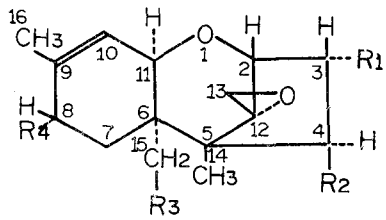
**Abstract**—In order to search for potential antidotes for T-2 toxin poisoning, seven Chinese herbal drug extracts and five natural constituents were tested on mice intoxicated with T-2 toxin. When extracts of *Panax ginseng* and *Atractylodes japonica* (500 mg/kg) were administered p.o. once 3 hrs before and once 1 hr after T-2 toxin treatment, a 30% complete survival rate was noted. In case of *Paeonia albiflora* var. *typica*, a 30% complete survival rate was also produced at a dose of 250 mg/kg. Other extracts, *Glycyrrhiza uralensis*, *Scutellaria baicalensis*, *Rehmannia glutinosa* and *Plantago asiatica* exhibited no significant protection from the T-2 toxin poisoning. A nucleoside, thymidine showed protective activity against T-2 toxin toxicity and it produced a 40% complete survival rate when administered i.p. once 0.5 hr after T-2 toxin treatment. Other natural constituents, aucubin, vitamin C and E, and lipoic acid did not show any significant protective activities.

**Keywords**—T-2 toxin poisoning · antidote · *Paeonia albiflora* var. *typica* · *Panax ginseng* · *Atractylodes japonica* · thymidine

The trichothecenes represent a group of naturally occurring sesquiterpenoids derived from various genera of toxic fungi such as *Trichothecium*, *Trichoderma*, *Myrothecium*, *Cephalosporium*, *Fusarium*, *Stachybotrys*, *Verticimonosporium* and *Cylindocarpon*.[1-4] In the higher plant of *Baccharis megapotamica*, the compounds were also isolated.[5,6] Most trichothecenes are found to contain a double bond and an epoxide ring at C-9, 10 and C-12, 13, respectively, as shown in Fig. 1.

Most trichothecene mycotoxins are extremely toxic to virtually all eukaryotic cells in animals and plants. Moldy cereal contaminated with *Fusarium* and *Stachybotrys* causes alimentary toxicity in man and animal. It is well known that during World War II, the inhabitants of Orenburg, Russia, who had eaten contaminated cereal grain, developed severe ATA (Alimentary Toxic Aleukia) like symptoms with a resultant high mortality rate.[7]

Due to the extremely high toxicities of trichothecene mycotoxins resulting in necrotic angina, leukopenia, hemorrhage, exhaustion of bone marrow and high mortality, these mycotoxins can be used for biological warfare. It has been suggested that Russian may have used




| $R_1$ | $R_2$ | $R_3$ | $R_4$ |
|---|---|---|---|
| OH | OAc | OAc | $OCOCH_2CH(CH_3)$ |

**Fig. 1.** T-2 toxin

T-2 toxin for the purpose of weapon in the Afghanistan and Kampuchean wars.[8,9]

T-2 toxin (3 $\alpha$-hydroxy-4 $\beta$, 15-diacetoxy-8 $\alpha$(3-methylbutyryloxy)-12, 13-epoxy-trichothec-9-ene) belongs to a non-macrocyclic trichothecene mycotoxin. It is frequently found in various species of *Fusarium* fungi such as *F. tricinctum*, *F. solani*, *F. poae*, and *F. acuminatum*. The contamination of cereal grains, especially, wheat, corn, barley and buck wheat by these pathogenic *Fusarium* species is common throughout the world, frequently in the field of high moisture and cold weather. The outbreak of intoxication in men and animals due to consumption of *Fusarium* contaminated agricultural products has been reported in Korea, Japan, Russia, U.S.A. and many European countries.[10]

Because of the high toxicity of T-2 toxin and its contamination of food and agricultural products, a number of attempts have been made to control the contamination by the mycotoxin and to search for a possible antidote, but no significant developments have been reported as yet.

In this regard, the present study aims to search for a potential antidote from natural constituents. To measure the antidotal effects, the survival rates of test groups and a control group were compared. Dose-schedules, and routes of administration were also verified, since the T-2 toxin toxicity is very sensitive to such verification.

## Materials and Methods

### Animals

Young male mice (4 weeks old, ICR) were used for all experiments. All mice were supplied by the Experimental Animal Breeding Laboratory, Seoul National University. All mice were maintained on a commercial diet and water *ad libitum* in a climatized room with an alternating 12 hr light-cycle.

### Preparation of Plant Extracts

All herbal materials were purchased from the local market. Each plant material was extracted in an ethyl alcohol-water solution (EtOH: water, 7: 3 v/v) under reflux conditions for 2 hrs. The extraction was repeated twice. The solvent was then evaporated *in vacuum* at 50°C to dryness. Extracts obtained from each 200 g of dried plant sample are as follows; *Paeonia albiflora* var. *typica* (radix), 61.3 g; *Glycyrrhiza uralensis* (radix), 51.7 g; *Scutellaria baicalensis* (radix), 90.0 g; *Rehmannia glutinosa* (rhizoma), 90.9 g; *Plantago asiatica* (semen), 8.5 g; *Panax ginseng* (radix), 45.4 g; *Atractylodes japonica* (rhizoma), 13.4 g. All plant extracts were dissolved and/or suspended in a 1 % sodium carboxymethyl cellulose solution before administration.

### T-2 Toxin and Other Reagents

T-2 toxin ($C_{24}H_{34}O_9$; M.W. 466.53, 97.6 %) was purchased from Wako Pure Chemical Industries, Ltd. Japan. Other reagents were supplied by Sigma Chemicals Co. from U.S.A. T-2 toxin was dissolved in either 5 % ethyl alcohol or olive oil. Water-soluble drugs were

**Scheme 1.** Dose-Schedules

| Pretreatment (drug) | 0 time (T-2 toxin) | Posttreatment (drug) |
|---|---|---|
| 3 hrs(p.o.) ← | (p.o.) | → 1 hr(p.o.) |
| | (p.o.) → 0.5 hr(i.p.) | → 3 hrs(p.o.) |
| | (s.c.) → 0.5 hr(i.p.) | |

O time; only T-2 toxin was administered to test and control groups

Pretreatment; plant extracts or drugs were administered prior to T-2 toxin administration at 0 time.

Posttreatment; plant extracts or drugs were administered after T-2 toxin administration at 0 time.

Each group consisted of 5-8 mice. Survivors were counted from Day 1.

dissolved in 0.9 % NaCl solution.

**Dose-Schedules**

To obtain the highest antidotal effect of the potential antidote tested, the following dose-schedules were employed (Scheme 1).

## Results

According to various studies on the toxicoses of T-2 toxin, the primary target organ that is mostly damaged by this mycotoxin appears to be the blood-forming organs and severe depression of bone marrow occurs, followed by leukopenia.[11,12] In this regard, the authors have reported that certain herbal extracts exhibited the effect of enhancing DNA synthesis in bone marrow cells or of depressing DNA synthesis.[13] On the basis of such previous observation, seven herbal drugs were selected and their extracts were prepared for testing. The results are shown in Table I. In this experiment, all mice were fasted for 15 hrs prior to the administraion of herbal extracts and T-2 toxin. Initially each mouse in the test groups received p.o. the extracts (500 mg/kg), afterwhich the T-2 toxin (5 mg/kg) was administered p.o. to each mouse, including mice in the control group at 0 time. One hour after 0 time, each mouse was administered p.o. the same herbal extract dose of 500 mg/kg. The number of survivors and deaths were noted from day 1. As the data shown in Table I, all mice in the control group received only T-2 toxin died within two days. In comparison with the survival rate of the control group treated with *P. albiflora* var. *typica*, *G. uralensis*, S. *baicalensis* and *R. glutinosa* was noted. The survival rates in the tests groups receiving *P. ginseng, and A. japonica*, showed slight increases of survival rates(30 %), and survivors of more than two days appeared to overcome the T-2 toxicity, since those were still alive after one week observation period.

Next we conducted a similar experiment, except the dose of herbal drug was reduced to one half of the preceeding experiment, since *P. albiflora* var. *typica* has the property of depressing the bone marrow cells' DNA synthesis which might cause some toxicity rather than protection from T-2 toxicity. As the data in Table II shows, we noted some increased survival rates of the test group treated with *P. albiflora* var. *typica* extract in comparison with that of

**Table I.** Survival study *in vivo* on the antidotal effects on T-2 poisoning

| Treatment | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 |
|---|---|---|---|---|---|---|---|
| T-2 toxin | 1/5 | 0/1 | | | | | |
| *P. albiflora* var. *typica* (radix) | 1/5 | 0/1 | | | | | |
| *G. uralensis* (radix) | 2/4 | 0/2 | | | | | |
| *S. baicalensis* (radix) | 4/2 | 0/2 | | | | | |
| *R. glutinosa* (rhizoma) | 3/3 | 0/3 | | | | | |
| *P. asiatica* (semen) | 3/3 | 1/2 | 1/0 | 1/0 | 1/0 | 1/0 | 1/0 |
| *P. ginseng* (radix) | 3/3 | 2/1 | 2/0 | 2/0 | 2/0 | 2/0 | 2/0 |
| *A. japonica* (rhizoma) | 3/3 | 2/1 | 2/0 | 2/0 | 2/0 | 2/0 | 2/0 |

Numbers of survivors/deaths. 4 week-old ICR mice each weighing 15±2 g were used. Each group consisted of 6 mice. T-2 toxin was dissolved in 5 % EtOH and 5 mg/kg dose was administered p.o. at 0 time on day 0. All mice were fasted prior to administrating drugs. Herbal drug extracts (500 mg/kg) were administered p.o. once, at 3 hr before and 1 hr after T-2 toxin treatment at 0 time.

*Paeonia albiflora* var. *typica*(赤芍藥), *Glycyrrhiza uralensis*(甘草), *Scutellaria baicalensis*(黃芩), *Rehmannia glutinosa*(地黃), *Plantago asiatica*(車前子), *Panax ginseng*(人蔘) and *Atractylodes japonica*(蒼朮).

**Table II.** Survival study *in vivo*

| Treatment | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 |
|---|---|---|---|---|---|---|---|
| T-2 | 4/2 | 0/4 | | | | | |
| *P. albiflora* var. *typica* | 5/1 | 3/2 | 2/1 | 2/0 | 2/0 | 2/0 | 2/0 |
| *G. uralensis* | 2/4 | 0/2 | | | | | |
| *S. baicalensis* | 4/2 | 0/4 | | | | | |
| *R. glutinosa* | 3/3 | 0/3 | | | | | |
| *P. asiatica* | 3/3 | 2/1 | 1/1 | 0/1 | | | |
| *P. ginseng* | 3/3 | 0/3 | | | | | |

Numbers of survivors/deaths.
Each mouse in test groups received p.o. 250 mg/kg dose of extract.
Schedule and route for administration are same as in Table 1.

the preceeding experiment. Two mice appeared to survive longer than two days, whereas there was no significant increase in the survival rates observed in the test groups treated with *S. baicalensis*, *R. glutinosa*, *P. ginseng* and *A. japonica* extracts.

The trichothecene mycotoxins including T-2 toxin exhibit potent cytotoxicity to mammalian cells and such cellular toxicity is closely related to their lethal toxicity, dermal toxicity and severe impairment of immune responses in animals. The possible cellular mechanisms of cytotoxicity by T-2 toxin are results of the inhibition of macromolecule syntheses like protein, RNA and DNA. The translation stage appears to be most sensitive to T-2 toxin toxicity, and consequently cellular protein synthesis is highly inhibited by impairment of ribosomal RNA function.[14,15] Therefore, it has been suggested that the inhibition of cellular RNA and DNA syntheses resulted secondarily from the inhibition of protein synthesis by T-2 toxin. Recently it has been found that T-2 toxin possesses some activity for lipid peroxidation.[16,17]

Related to such cellular toxicities, several natural constituents of biological importance were tested against T-2 toxin toxicity. They were vitamin C and E, which possess antioxidant action, lipoic acid, aucubin and thymidine, antidotes for the depression of RNA synthesis by alpha-amanitin, and inhibitory effect of DNA synthesis, respectively.[18-25] The results are shown in Table III and IV. In the experiment shown in Table III, all mice were fasted for 15 hrs before treatment with drugs. Vitamin E

**Table III.** Survival study *in vivo*

| Treatment | Day 1 | Day 2 |
|---|---|---|
| T-2 toxin | 2/4 | 0/2 |
| Vitamin E | | |
| 400 mg/kg | 4/2 | 0/4 |
| 800 mg/kg | 4/2 | 0/4 |
| Lipoic acid | | |
| 50 mg/kg | 3/3 | 0/3 |
| 100 mg/kg | 3/3 | 0/3 |

Numbers of survivors/deaths
Vitamin E and lipoic acid were dissolved in corn oil.
T-2 toxin dissolved in 5 % EtOH was administered p.o. at doses of 5 mg/kg to each mose.
Schedule for administration was same as in Table I.

**Table IV.** Survival study *in vivo*

| Treatment | Day 1 | Day 2 | Day 3 |
|---|---|---|---|
| T-2 toxin | 5/3 | 1/4 | 0/1 |
| Vitamin C (100 mg/kg) | 2/6 | 0/2 | |
| Thymidine (50 mg/kg) | 7/1 | 2/5 | 0/2 |
| Aucubin (100 mg/kg) | 5/3 | 0/3 | |

Numbers of survivors/deaths Drugs dissolved in 0.9 % saline were administered i.p. once, at 0.5 hr and 3 hr after T-2 toxin treatment at 0 time.
Each group consisted of 8 mice.

**Table V.** Survival study *in vivo*

| Treatment | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 |
|---|---|---|---|---|---|---|---|
| T-2 toxin | 3/2 | 0/3 | | | | | |
| Thymidine | | | | | | | |
| 50 mg/kg | 1/4 | 0/1 | | | | | |
| 100 mg/kg | 4/1 | 2/2 | 2/0 | 2/0 | 2/0 | 2/0 | 2/0 |
| 150 mg/kg | 1/4 | 0/1 | | | | | |
| 200 mg/kg | 0/5 | | | | | | |

Numbers of survivors/deaths
T-2 toxin was dissolved in olive oil and administered s.c. at a dose of 1.8 mg/kg to each mouse.
A single dose of thymidine was administered i.p. 0.5 hr after T-2 toxin treatment at 0 time.
Each group consisted of 5 mice.

and lipoic acid were dissolved in corn oil and administered p.o. 3 hrs prior to T-2 toxin administration at 0 time. At 0 time, all mice each received p.o. a 5 mg/kg dose of T-2 toxin. An hour later, each mouse in the test groups was administered each drug orally. All mice in the control group died within two days and no significant increased life spans of mice in the test groups treated with vitamin E and lipoic acid were observed. In the experiment shown in Table IV, all mice were also fasted 15 hrs prior to administration of drugs or T-2 toxin. Vitamin C, aucubin and thymidine were dissolved in 0.9 % physiological saline and administered i.p. once to each mouse 0.5 hr and 3 hrs after T-2 toxin(5 mg/kg) had been administered at 0 time. As the data shown in Table IV, all mice in the control group died within three days. Treatments with vitamin C and aucubin exhibited no significant increase of survival rates, but there was a slight increase of survival rates in the thymidine-treated group at the dose of 50 mg/kg. Therefore, different doses of thymidine were given and the possibility of life span prolongation was examined as follow; at 0 time all mice received s.c. a 1.8 mg/kg dose of T-2 toxin, then half an hour later, each mouse in the test groups were administered i.p. specified doses of thymidine as shown in Table V. No significant increase of survival rates of mice in the tests groups were noted when administered doses of 50, 150 and 200 mg/kg. But at the dose of 100 mg/kg, two mice (40 % of total experimental mice) survived.

## Discussion

Due to its potent toxicity, contamination with T-2 toxin, one of the Trichothecene mycotoxins, has brought about many problems to food and agricultural science as well as to human and animal hygiene. In addition, efforts have been made to search for a potential antidote, since the possibility of using this mycotoxin as a biological warfare agent has arisen.

Important features of T-2 toxin toxicity can be characterized by the reduction of white blood cells and severe depression of bone marrow cells, followed by ATA-like symptoms and resulting death. On the cellular level, it markedly inhibits protein synthesis in virtually all eukaryotic cells and secondarily, DNA and RNA syntheses are also impaired.[14,15] In addition, it was also found that this mycotoxin enhanced lipid peroxidation and procuded hepatic injury.[16,17]

Previously we observed that certain Chinese herbal drug extracts exhibited some biological activities that either inhibited or enhanced DNA synthesis in bone marrow cells. In this connec-

tion, to investigate their potential protective activities aginst T-2 toxin toxicity, *P. albiflora* var. *typica*, *G. uralensis*, *S. baicalensis*, *R. glutinosa*, *P. asiatica*, *P. ginseng* and *A. japonica* were administered to experimental mice intoxicated with T-2 toxin. Among them, *P. albiflora* var. *typica*, *P. asiatica*, *R. glutinosa*, and *A. japonica* have an inhibitory activity to DNA syntheses in bone marrow cells, whereas the remaining herbal drug extracts have some stimulating activity.[13]

At the dose of 500 mg/kg of each extract (administered once, 3 hrs before and 1 hr after T-2 toxin treatment), *P. ginseng* and *A. japonica* showed protective activites (30 %). To avoid the possibility of an over-dose of the extract, one half the dose was administered. Only *P. albiflora* var. *typica* revealed protective activity at a dosage rate of 250 mg/kg, and the protective activities of *P. ginseng* and *A. japonica* were rather diminished. It is interesting to us that those herbal extracts which have an inhibitory activity in DNA synthesis in bone marrow cells also showed a protective action from T-2 poisoning. In this connection, it is worth noting that some potent inhibitors of protein and RNA synthesis, e.g. cycloheximide and actinomycin D exhibit protective activities against other protein and RNA syntheses inhibitors like $CCl_4$ when they were administered prior to $CCl_4$ treatment.[26] In this regard, it can be implied that when the bone marrow cells' function, which includes DNA synthesis, was reduced by pretreatment with such herbal extracts as *P. albiflora* var. *typica* and *A. japonica*, the cells were damaged less after such treatment when exposed to T-2 toxin.

Vitamin C and E are known to be antioxidants. And lipoic acid, a vitamin, has been used as an antidote for *Amanita* mushroom poisoning which contains alpha-amanitin, a potent inhibitor of RNA synthesis in mammalian cells.[18] Aucubin is an iridoid compound, a natural constituent isolated from *Aucuba japonica* (Cornaceae) and *Plantago asiatica* (Plantaginaceae) and has the biological activity of inhibiting RNA and protein syntheses.[19-22] This compound was reported to exhibit an antidotal activity against alpha-amanitin poisoning.[23,24] Thymidine is a natural precursor for DNA synthesis in all cells. When it is administered in high doses, DNA synthesis is feed-back inhibited, and bone marrow cells are depressed by such feed-back inhibition. Therefore, we tested these natural constituents to find one that might exhibit a potential antidotal action.

As the data in Table III show, at two different doses of 400 and 800 mg/kg of vitamin E (administered as follows; once 3 hrs before and one hr after T-2 toxin treatment), there was no appreciable survival rate increase in comparison with that of the control group. This result appears to be similar to a report published elsewhere.[16] This result indicates that although T-2 toxin possesses some lipid peroxidation activity, it may not be the main cause for lethality produced by the toxin. When vitamin C, an antioxidant, was administered, a similar result was also obtained; no increased survival rates were noted as shown in Table IV. In the case of lipoic acid, even though it has been used clinically for the treatment of *Amanita* mushroom poisoning, it did not show any protective activity against T-2 toxin poisoning. Aucubin was reported to exhibit some inhibitory activity of RNA and protein syntheses. It is also an antidote for alpha-amanitin mycotoxin. But no significant increased survival rate was obtained at a dose of 100 mg/kg, which is an optimal dose of antidotal activity against alpha-amanitin toxicity.

Since we noted some protective activity at a dosage of 50 mg/kg of thymidine, the dose-schedule for thymidine administration and route

of T-2 toxin treatment were verified to obtain a higher protective action. A single dose of thymidine showed a maximum protective activity at 100 mg/kg.

The results so far obtained indicate that a portion of test mice (less than 40 %) fully recovered from the T-2 intoxication when treated with *P. albiflora* var. *typica*, *P. ginseng*, *A. japonica* and thymidine, but we could not produce more that 40 % recovery in all mice in the test groups. It is interesting to note that those materials exhibiting protective activity againts T-2 toxicity also have an inhibitory action on DNA syntheses in bone marrow cells. This observation may warrant further study in searching for potential antidotes for trichothecene mycotoxin poisonings.

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