

## The Influence of Tetrodotoxin on the Toxic Effects of Aconitine in vivo

YOUKICHI OHNO, SHOETSU CHIBA\*, SEISAKU UCHIGASAKI\*,  
EIKOH UCHIMA†, HAJIME NAGAMORI†, MICHINAO MIZUGAKI§,  
YOSHIHARU OHYAMA§, KATSUHIKO KIMURA§, and YASUO  
SUZUKI‡

*Department of Legal Medicine, Nippon Medical School, Tokyo 113, \*Department of Legal Medicine, Nihon University School of Medicine, Tokyo 173, †Department of Legal Medicine, Faculty of Medicine, University of the Ryukyus, Okinawa 903-01, and § Department of Pharmaceutical Sciences, Tohoku University Hospital and ‡Pharmaceutical Institute, Tohoku University, Sendai 980*

OHNO, Y., CHIBA, S., UCHIGASAKI, S., UCHIMA, E., NAGAMORI, H., MIZUGAKI, M., OHYAMA, Y., KIMURA, K. and SUZUKI, Y. *The Influence of Tetrodotoxin on the Toxic Effects of Aconitine in vivo*. Tohoku J. Exp. Med., 1992, 167 (2), 155-158 — Both aconite toxins (aconitine, mesaconitine, and hypaconitine) and a pufferfish toxin (tetrodotoxin, TTX) were detected in the blood of a legal autopsy case. In order to elucidate the in vivo influence of TTX on the toxic effects of aconitine, a mixture of aconitine and TTX was administered to male ICR mice orally or intraperitoneally. The animal experiments revealed that the time of death due to aconitine was significantly delayed in proportion to the dose of TTX compared with the case for aconitine alone, and that the mortality of aconitine was lowered by TTX when the dose ratio of the two toxins was in a particular range. Accordingly, it is thought that the toxic effects of aconitine are attenuated by TTX in vivo. ——— legal autopsy; aconitine; tetrodotoxin; poison interaction; animal experiment

A 33-year-old woman suddenly felt nauseous and vomited violently in a hotel, and lost consciousness in an ambulance. She died soon after admission to a hospital due to ventricular fibrillation. The cause of death was initially thought to be heart attack, but macroscopic and microscopic examination of a legal autopsy failed to make the cause of death clear.

After that, aconitine, mesaconitine, and hypaconitine were detected in the victim's blood stored in a freezer ( $-20^{\circ}\text{C}$ ), by means of gas chromatography/selected ion monitoring (Mizugaki et al. 1988). It was thus concluded that the cause of death was aconite poisoning. Later, tetrodotoxin, the poison of a pufferfish, was also detected in the same blood. As tetrodotoxin is known to be an

antagonist of aconitine in the sodium channel in excitable membranes, one would expect tetrodotoxin to delay the appearance of aconitine poisoning and thus the time of death. However, there are no reports on the influence of tetrodotoxin on the toxic effects of aconitine in vivo. The interaction between the two toxins was therefore examined in animal experiments.

## MATERIALS AND METHODS

### *Chemicals*

Aconitine (ACT) and tetrodotoxin (TTX) were obtained from Sigma Chemical Co. (St. Louis, Mo, USA).

### *Animal experiment*

Male mice of the ICR strain weighing 30–35 g were used in all the experiments. ACT and TTX were dissolved in a 0.1 M acetate buffer (pH 5.0) and a total volume of 0.4 ml was administered to the animals orally or intraperitoneally. The doses were adjusted so that mice received orally 2 or 3 mg/kg of ACT together with 0, 0.33, 0.67, or 1.0 mg/kg of TTX, and intraperitoneally 0.4 mg/kg of ACT together with 0, 2.5, 5, 10, 15, 20, 30, or 40  $\mu$ g/kg of TTX. The death of the animals was determined to have been caused by cardiac arrest. Differences in the mortality and the mean time until death of the group receiving only ACT and the group receiving a mixture of ACT and TTX were determined using Fisher's exact probability test and the Welch test, respectively.

## RESULTS

Table 1 shows the mortality data on mice administered 2 or 3 mg/kg of ACT orally together with different doses of TTX.

Regarding the data for 2 mg/kg of ACT, which was close to its LD<sub>50</sub>, about half of the animals died 5.5 min after the administration of ACT alone. The coadministration of TTX significantly delayed the time of death in proportion to the dose ( $p < 0.01$ ), although the mortality increased. An increase in the oral dose

TABLE 1. *Time of death of mice treated orally with aconitine and different amounts of tetrodotoxin*

| Dose of aconitine (mg/kg) | Dose of tetrodotoxin (mg/kg) | Number of mice treated | Time of occurrence of death (min) |      |       |       |       |       | Number of mice that died |                 |
|---------------------------|------------------------------|------------------------|-----------------------------------|------|-------|-------|-------|-------|--------------------------|-----------------|
|                           |                              |                        | 1-5                               | 5-10 | 10-20 | 20-30 | 30-45 | 45-60 |                          | Mean $\pm$ s.d. |
| 2                         | 0                            | 5                      | 1                                 | 1    |       |       |       |       | 5.5 $\pm$ 1.5            | 2               |
| 2                         | 0.33                         | 4                      |                                   |      | 1     | 1     |       |       | 17.5 $\pm$ 3.5**         | 2               |
| 2                         | 0.67                         | 5                      |                                   | 1    | 2     | 1     |       |       | 18.3 $\pm$ 7.3**         | 4               |
| 2                         | 1.0                          | 5                      |                                   | 1    | 1     | 2     | 1     |       | 21.6 $\pm$ 9.8**         | 5               |
| 3                         | 0                            | 5                      |                                   | 3    | 1     | 1     |       |       | 13.0 $\pm$ 7.3           | 5               |
| 3                         | 0.33                         | 5                      |                                   |      |       | 2     | 1     |       | 30.7 $\pm$ 10.3*         | 3               |
| 3                         | 0.67                         | 5                      |                                   | 1    | 2     | 1     | 1     |       | 21.4 $\pm$ 9.3           | 5               |
| 3                         | 1.0                          | 5                      |                                   | 1    | 1     | 2     |       | 1     | 27.4 $\pm$ 16.4          | 5               |

\* $p < 0.05$ ; \*\* $p < 0.01$ .

TABLE 2. *Time of death of mice treated intraperitoneally with 0.4 mg/kg of aconitine and different amounts of tetrodotoxin*

| Dose of tetrodotoxin ( $\mu\text{g}/\text{kg}$ ) | Number of mice treated | Time of occurrence of death (min) |                    |                |       |       |                | Mean $\pm$ S.D.     | Number of mice that died |
|--|------------------------|-----------------------------------|--------------------|----------------|-------|-------|----------------|---------------------|--------------------------|
|  |                        | 1-5                               | 5-10               | 10-20          | 20-30 | 30-60 | 60-            |                     |                          |
| 0  | 9                      | 2                                 | 6                  | 1              |       |       |                | $7.8 \pm 3.2$       | 9                        |
| 2.5  | 5                      |                                   | 2                  |                |       |       |                | $7.5 \pm 1.5$       | 2*                       |
| 5  | 5                      |                                   | 2                  | 1              |       |       |                | $9.7 \pm 2.9$       | 3                        |
| 10   | 9                      |                                   | 2                  | 2              | 1     |       |                | $15.0 \pm 5.2^{**}$ | 5*                       |
| 15   | 5                      | 1 <sup>b</sup>                    | 1                  |                | 1     | 1     | 1 <sup>a</sup> | $43.0 \pm 49.9$     | 5                        |
| 20   | 10                     | 3 <sup>b</sup>                    | 5(3 <sup>b</sup> ) | 2              |       |       |                | $8.5 \pm 4.2$       | 10                       |
| 30   | 4                      | 2 <sup>b</sup>                    | 1 <sup>b</sup>     | 1 <sup>b</sup> |       |       |                | $6.5 \pm 3.4$       | 4                        |
| 40   | 5                      | 4 <sup>b</sup>                    | 1 <sup>b</sup>     |                |       |       |                | $4.6 \pm 0.8^{**}$  | 5                        |

\* $p < 0.05$ ; \*\* $p < 0.01$ .

<sup>a</sup>Died at 140 min. <sup>b</sup>The number determined to have died of TTX poisoning.

of ACT to 3 mg/kg clearly delayed the time of death when 0.33 mg/kg of TTX was coadministered ( $p < 0.05$ ). The mortality was also lower than for a single dose of ACT (60% vs 100%). The external appearance and behavior of animals receiving a mixed dose were visibly different from receiving ACT alone. The toxic signs of ACT (vomiting and spasms) were markedly reduced as the dose of TTX increased. In some mice receiving 1.0 mg/kg of TTX along with 3 mg/kg of ACT, the toxic signs almost completely disappeared within an hour. After some time had elapsed, however, these mice suddenly exhibited vomiting followed by spasms and convulsions, and died.

Table 2 shows the data on mice intraperitoneally administered 0.4 mg/kg of ACT along with various doses of TTX. At TTX dose levels of 2.5-10  $\mu\text{g}/\text{kg}$ , the mortality was lower than ACT alone ( $p < 0.05$  at 2.5  $\mu\text{g}/\text{kg}$  and 10  $\mu\text{g}/\text{kg}$ ). TTX doses of 10 ( $p < 0.01$ ) and 15  $\mu\text{g}/\text{kg}$  clearly delayed the time of death. However, the toxic signs appearing after the intraperitoneal administration of ACT were not so noticeably inhibited by TTX as in the case of oral administration.

## DISCUSSION

Aconites (wolfsbane) are plants in the genus *Aconitum* (the Ranunculaceae family), containing very toxic substances such as aconitine, mesaconitine, hypaconitine and jesaconitine in the root, leaf, and stem. The  $\text{LD}_{50}$  of ACT for mice is 1.8 mg/kg (p.o.) and 0.308 mg/kg (i.p.). The fatal dose for humans per os is thought to be 2 to 3 mg. TTX is also a very toxic substance contained in several tissues of some pufferfish (Fugu), such as, the ovarium, liver, and skin. The  $\text{LD}_{50}$  of TTX for mice is 0.33 mg/kg (p.o.) and 10  $\mu\text{g}/\text{kg}$  (i.p.). The toxic effects of ACT are presumably due to an alteration in the  $\text{Na}^+$  inflow through the sodium channel of excitable membranes. That is, ACT first facilitates excitation

of the membrane by increasing the  $\text{Na}^+$  inflow and then depresses it by prolonging depolarization due to the increased  $\text{Na}^+$  inflow. TTX also affects the functioning of the sodium channel, but its effect is thought to be to inhibit the  $\text{Na}^+$  inflow when the channel is in the open state. Thus, these toxins are antagonistic to each other, and serve as tools to investigate the functioning of excitable membranes (Catterall 1980). As ACT is also known to induce ventricular extrasystole and ventricular fibrillation, it is a useful tool for the pharmacological study of cardiac functions. Besides these physiological effects of ACT and TTX on membranes, there might be some as yet undiscovered interaction when these toxins are administered in vivo at the same time. The only information reported so far is that the arrhythmogenic effects of ACT are inhibited by TTX in the perfused rat hearts (Lagier et al. 1970). The pharmacokinetics of ACT and TTX alone or in combination are not yet known because of the difficulty in measuring the concentration of these toxins and of the very low levels in the blood.

In the present study, animal experiments have shown that the mortality of ACT is lowered by TTX, and that the time of death is delayed by TTX when the ratio of the two toxins is within a particular range, as compared with the administration of ACT alone. Moreover, the toxic signs of ACT on animal behavior were markedly reduced after oral coadministration as the dose of TTX was increased. Accordingly, it is thought that the toxic effects of ACT are attenuated by TTX in vivo, probably due to their mutual antagonistic effect on excitable membranes.

#### References

- 1) Catterall, W.A. (1980) Neurotoxins that act on voltage-sensitive sodium channels in excitable membranes. *Ann. Rev. Pharmacol. Toxicol.*, **20**, 15-43.
  - 2) Lagier, M.G., Auclair, M.-C. & Lechat, M.P. (1970) Suppression de l'effet anti-arythmique de la tétrodotoxine vis-à-vis de l'aconitine, chez le rat, par une perfusion de chlorure de sodium hypertonique. *C.R. Acad. Sci. Paris*, **270**, 3325-3328.
  - 3) Mizugaki, M., Ohyama, Y., Kimura, K., Ishibashi, M., Ohno, Y., Uchima, E., Nagamori, H. & Suzuki, Y. (1988) Analysis of aconitum alkaloids by means of gas chromatography/selected ion monitoring. *Eisei Kagaku*, **34**, 359-365. (in Japanese)
-