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Antagonistic Effects of Tetrodotoxin on Aconitine-induced Cardiac Toxicity

Takiyoshi Ono[1], Makiko Hayashida[1], Akito Tezuka[2], Hideyuki Hayakawa[3] and Youkichi Ohno[1]

[1]Department of Legal Medicine, Nippon Medical School
[2]Division of Regenerative Medicine and Cardiology, Department of Internal Medicine, Nippon Medical School
[3]Tsukuba Medical Examiner's Office

Abstract

Aconitine, well-known for its high cardiotoxicity, causes severe arrhythmias, such as ventricular tachycardia and ventricular fibrillation, by opening membrane sodium channels. Tetrodotoxin, a membrane sodium-channel blocker, is thought to antagonize aconitine activity. Tetrodotoxin is a potent blocker of the skeletal muscle sodium-channel isoform $Na_v1.4$ (IC_{50} 10 nM), but micromolar concentrations of tetrodotoxin are required to inhibit the primary cardiac isoform $Na_v1.5$. This suggests that substantial concentrations of tetrodotoxin are required to alleviate the cardiac toxicity caused by aconitine. To elucidate the interaction between aconitine and tetrodotoxin in the cardiovascular and respiratory systems, mixtures of aconitine and tetrodotoxin were simultaneously administered to mice, and the effects on electrocardiograms, breathing rates, and arterial oxygen saturation were examined. Compared with mice treated with aconitine alone, some mice treated with aconitine-tetrodotoxin mixtures showed lower mortality rates and delayed appearance of arrhythmia. The decreased breathing rates and arterial oxygen saturation observed in mice receiving aconitine alone were alleviated in mice that survived after receiving the aconitine-tetrodotoxin mixture; this result suggests that tetrodotoxin is antagonistic to aconitine. When the tetrodotoxin dose is greater than the dose that can block tetrodotoxin-sensitive sodium channels, which are excessively activated by aconitine, tetrodotoxin toxicity becomes prominent, and the mortality rate increases because of the respiratory effects of tetrodotoxin. In terms of cardiotoxicity, mice receiving the aconitine-tetrodotoxin mixture showed minor and shorter periods of change on electrocardiography. This finding can be explained by the recent discovery of tetrodotoxin-sensitive sodium-channel cardiac isoforms ($Na_v1.1$, 1.2, 1.3, 1.4 and 1.6).
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Key words: aconitine, tetrodotoxin, arrhythmia, tetrodotoxin-sensitive channel, tetrodotoxin-resistant channel, breathing rate, arterial oxygen saturation

Correspondence to Takiyoshi Ono, Department of Legal Medicine, Nippon Medical School, 1–1–5 Sendagi, Bunkyo-ku, Tokyo 113–8602, Japan
E-mail: takiono@nms.ac.jp
Journal Website (http://www.nms.ac.jp/jnms/)

Introduction

Aconitum plants (Ranunculaceae) are distributed worldwide in mountainous regions or cold areas[1]. The raw root of these plants is highly toxic; however, it has been used in Asian medicine for centuries. The major toxic compounds of *Aconitum* plants are alkaloids, such as aconitine, mesaconitine, hypaconitine, and jesaconitine. The alkaloid contents in *Aconitum* plants change quantitatively and qualitatively depending on the species, place of origin, and time of harvest[2]. *Aconitum* alkaloids are neurotoxins that bind to the neurotoxin-binding receptor site II on the α subunit of voltage-sensitive sodium channel[3,4]. This binding results in a sodium channel that stays open longer. Aconitine suppresses the conformational change of the sodium channel from the active state to the inactive state. The membrane stays depolarized owing to the constant sodium influx; therefore, the membrane cannot be repolarized[5]. Thus, *Aconitum* alkaloids cause nerve excitation, followed by paralysis. Concerning the pharmacological or toxic effects of *Aconitum* alkaloids, various types of sensory and motor manifestations can be included or elicited. Among these manifestations, cardiotoxicity is the most important, causing severe arrhythmia and possible death. Because of its high cardiotoxicity, the median lethal dose (LD_{50}) of aconitine in mice is 1.8 mg/kg when administered orally and 0.308 mg/kg when administered intraperitoneally. The LD_{50} of aconitine for humans is considered to be 1 to 2 mg. Fatal accidents and murder due to ingestion of *Aconitum* plants have been reported. In 1986, a Japanese man was accused of attempting to murder his wife to receive a large insurance benefit. However, his wife developed severe vomiting, abdominal pain, and numbness of the limbs and died 90 minutes after his departure, which was inconsistent with the known latency of aconitine intoxication. The puffer fish toxin, tetrodotoxin, was later found to have been co-administered with aconite alkaloids in this case[6].

Tetrodotoxin is a selective sodium-channel blocker that obstructs depolarization of the excitable membrane. Voltage-gated sodium channels are responsible for initiating action potentials in excitable cells. These channels are composed of a pore-forming α subnuits and auxiliary β subunits[7]. Ten genes that encode α subnuits have been identified and 9 have been functionally expressed[8]. These different isoforms have distinct patterns of development and localization in the nervous system, skeletal muscle, and cardiac muscle and possess different pharmacological properties. Isoforms that are preferentially expressed in the central nervous system ($Na_v1.1$, 1.2, 1.3, and 1.6) are inhibited by tetrodotoxin at nanomolar concentrations and are similar to the isoforms present in adult skeletal muscles, including those responsible for respiration ($Na_v1.4$; half maximal inhibitory concentration [IC_{50}], 10 nM)[9,10]. In contrast, the primary cardiac isoform ($Na_v1.5$; IC_{50} > 1 μM) requires micromolar concentrations of tetrodotoxin for inhibition because of the presence of a cysteine instead of an aromatic residue in the pore region of domain I[9–13]. Thus, the cardiac isoform $Na_v1.5$ is classically called tetrodotoxin-resistant I_{Na}, and the $Na_v1.1$, 1.2, 1.3, 1.4, and 1.6 isoforms are called tetrodotoxin-sensitive I_{Na}.

Tetrodotoxin blocks sodium conductance and neuronal transmission in skeletal muscles[14]. It acts on the central and peripheral nervous systems (i.e., autonomic, motor, and sensory nerves). Patients with severe poisoning may lapse into a coma, and death may occur within 4 to 6 hours of ingestion, typically due to respiratory failure caused by respiratory muscle paralysis[15]. At the membrane sodium channel, aconitine and tetrodotoxin counteract each other. Several studies have found that tetrodotoxin shows anti-arrhythmic activity as a selective sodium-channel blocker[16–18]. However, in contrast to the skeletal muscle isoform ($Na_v1.4$; IC_{50}, 10 nM), the primary cardiac isoform ($Na_v1.5$) requires micromolar concentrations of tetrodotoxin for inhibition, suggesting that it is difficult for tetrodotoxin to alleviate the cardiac toxicity caused by aconitine. In 1992, Ohno reported that when aconitine and tetrodotoxin were administered simultaneously, the time of death due to aconitine was significantly delayed in proportion to the dose of tetrodotoxin administered, compared with that when only aconitine was administered, and the mortality rate of

aconitine was decreased by tetrodotoxin when the dose ratio of the 2 toxins was in a particular range[19]. This discovery was important, although the reason for this interaction has not yet been clarified.

The present study was designed to investigate the effects of simultaneous aconitine and tetrodotoxin administration on the electrocardiogram (ECG), breathing rates, and arterial oxygen saturation (SpO_2) of experimental animals and to elucidate the interaction between aconitine and tetrodotoxin in the cardiovascular and respiratory systems. The results are discussed in light of the recent discovery of tetrodotoxin-sensitive sodium channel cardiac isoforms ($Na_v1.1$, 1.2, 1.3, 1.4, and 1.6).

Materials and Methods

Reagents

Aconitine and tetrodotoxin were purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA). Other reagents used were of analytical grade.

Animals

Five-week-old male inbred ICR mice (weight, 30–35 g) (Charles River Laboratories, Yokohama, Japan) were used in all experiments. The experiments were performed according to the guidelines of the Ethical Committee on Animal Experimentation of Nippon Medical School (Tokyo, Japan).

Administration of Drugs

The mice were divided into the following 4 groups: the aconitine group (Group A), the tetrodotoxin group (Group B), the aconitine-tetrodotoxin mixed-toxicity group (Group C), and the control group (Group D). Each group consisted of 50 to 60 mice. Aconitine and tetrodotoxin were dissolved in 0.1 M acetate buffer (pH 5) and administered intraperitoneally to the mice. In Group A, 0.10, 0.15, 0.3, or 0.4 mg/kg of aconitine was administered. In Group B, 5, 10, 15, 20, or 40 μg/kg of tetrodotoxin was administered. In Group C, 0.15 mg/kg of aconitine and 5 or 10 μg/kg of tetrodotoxin, or 0.4 mg/kg of aconitine and 15 μg/kg of tetrodotoxin were administered. In Group D, acetate buffer was administered.

ECG

ECG was performed with a preamplifier (RMP-6004, Nihon Kohden, Tokyo, Japan) and a 2-channel power lab system (model 2/25, Nihon Kohden, Tokyo, Japan). Mice were fastened belly up onto a small animal restraint,. ECG was performed for 3 hours, starting at the time of the administration. The ECG was analyzed with Chart version 5.3, ECG Analysis Module (AD Instruments, Tokyo, Japan). The appearance and duration of arrhythmias were recorded. Severe arrhythmias, such as ventricular tachycardia (VT) and ventricular fibrillation (VF), were analyzed.

Breathing Rate and SpO_2

Breathing rate was analyzed with a force transducer (AD Instruments). The SpO_2 was determined with a portable animal pulse oximeter (Nonin Medical Inc. Minneapolis, MN, USA), and analyzed with a signal conditioner (Bridge Pod, Nihon Kohden, Tokyo, Japan) and a data acquisition unit (PowerLab, Nihon Kohden). An animal anesthesia apparatus (TK-4m, model 8600V, Nonin Medical Inc.) was used to analyze the breathing rate and SpO_2 of mice under fluothane anesthesia. Fluothane was administered at 2% to 2.5% for anesthetic induction and at 1% for maintenance of anesthesia.

The breathing rates and SpO_2 of only the following groups were analyzed: Group A (0.4 mg/kg of aconitine), Group B (15 μg/kg of tetrodotoxin), Group C (0.4 mg/kg of aconitine and 15 μg/kg of tetrodotoxin), and Group D. Each group consisted of 50 to 60 mice.

Statistical Analysis

Welch's *t*-test was used to determine statistically significant differences between the control group and the other groups and between Group A and Group C. Differences with p values < 0.05 were considered to be statistically significant. The chi-square test was used to certify mortality.

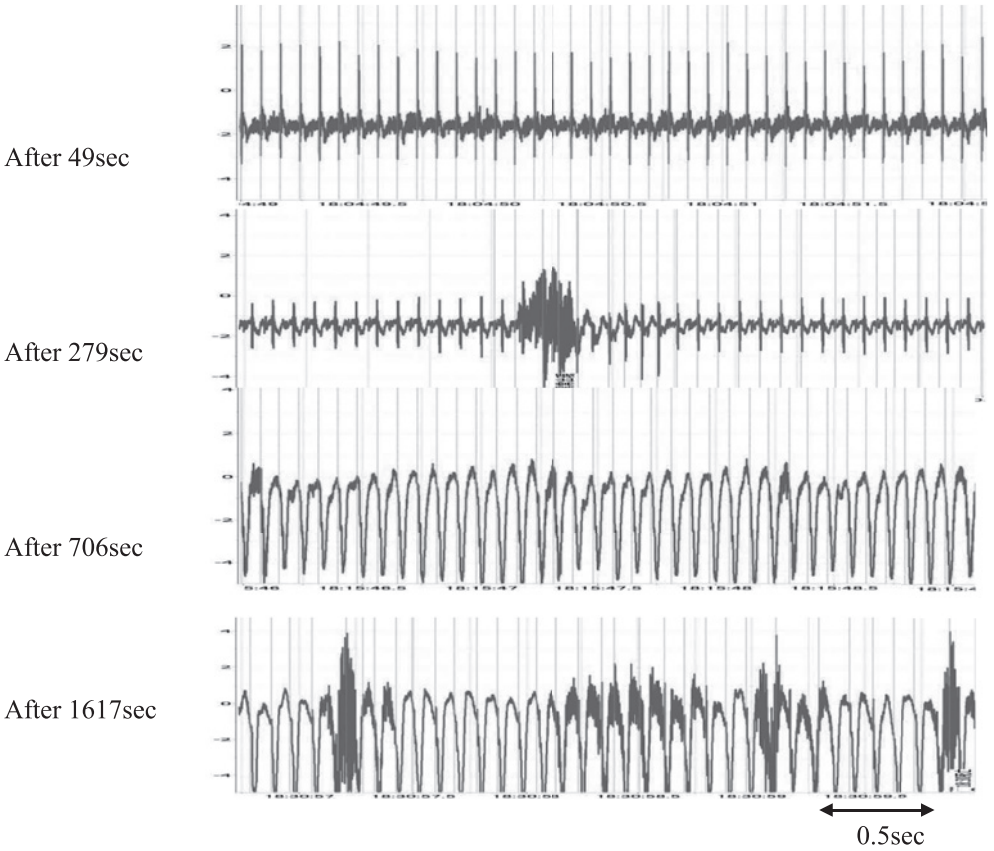

Fig. 1 Electrocardiogram of a mouse given 0.4 mg/kg of aconitine.

Results

Because we examined 50 to 60 mice in each group, it is impossible to describe the outcome for each mouse in detail. Hence, ECG waveforms, ECG time courses, breathing rates, and SpO_2 shown in **Figure 1～8** are those of typical mice from each group.

ECG Changes

There were no ECG changes in the mice in Group B, which received tetrodotoxin doses of 5 to 40 μg/kg. The ECGs confirmed the absence of VT and VF after the administration of 0.1 mg/kg of aconitine, and there were no deaths. Both VT and VF were detected in most of the mice to which 0.15 mg/kg of aconitine had been administered, but regular sinus rhythm returned within 1,800 seconds (30 minutes). The mortality rate was approximately 17%. Both VT and VF were seen in all mice within 600 seconds (10 minutes) after the administration of 0.3 mg/kg of aconitine, and the mortality rate increased to approximately 40%. Both VT and VF were detected since the early-stage ECG and tended to be sustained after administration of 0.4 mg/kg of aconitine, compared with the mice in Group A to which 0.3 mg/kg of aconitine was administered. All mice died within 1,800 seconds (30 minutes) after the administration of aconitine (mortality rate, 100%). **Figure 1** shows the ECG of a mouse given 0.4 mg/kg of aconitine. Various types of arrhythmia were detected, such as VF, VT, atrioventricular block, bundle branch block, and torsade de pointes[20]. In particular, VT and VF were detected on early-stage ECGs.

The ECG of a mouse that was simultaneously given 0.4 mg/kg of aconitine and 15 μg/kg of tetrodotoxin is shown in **Figure 2**. Compared with the mice in Group A that were given 0.4 mg/kg of aconitine, mice in Group C that were given 0.4 mg/kg of aconitine and 15 μg/kg of tetrodotoxin showed the controlled emergence of VT and VF on ECG and the delayed appearance of arrhythmias. Moreover, approximately half the mice in Group C did not show any lethal arrhythmias, such as VT and VF, on

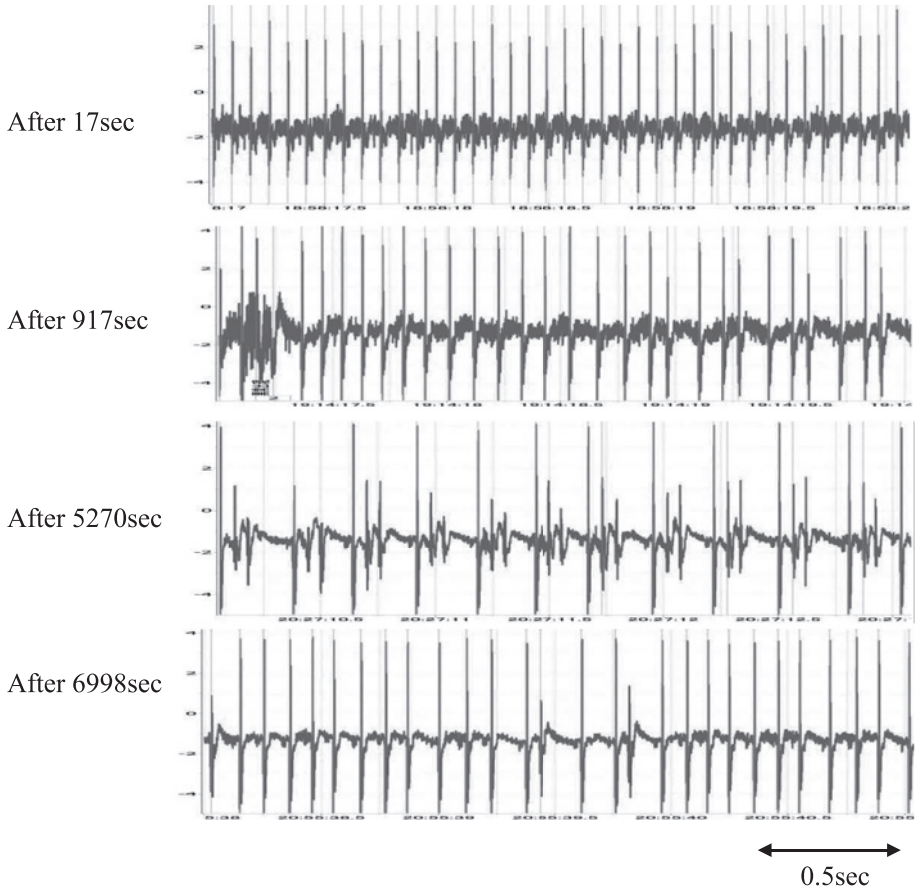

Fig. 2 Electrocardiogram of a mouse given 0.4 mg/kg of aconitine and 15 μg/kg of tetrodotoxin.

ECG.

In **Figure 3**, the time courses of ECG changes in the mice in Group A that were given 0.15 mg/kg of aconitine and those in mice in Group C are shown. Row 1-1 shows the time courses of ECG changes in mice that were given 0.15 mg/kg of aconitine alone. Both VT and VF were detected, and regular sinus rhythm returned within 1,800 seconds (30 minutes); some of the mice did not show any VT or VF. The mortality rate was approximately 17%.

Row 2-1 shows the time courses of ECG changes in mice in Group C that were simultaneously given 0.15 mg/kg of aconitine and 5 μg/kg of tetrodotoxin. Compared with those in the mice in Group A given 0.15 mg/kg of aconitine (Row 1-1), the time span of VT and VF tended to decrease in the mice in Group C (0.15 mg/kg of aconitine and 5 μg/kg of tetrodotoxin, simultaneously). The mortality rate was 0%, which was lower than that in Group A (0.15 mg/kg of aconitine). Row 2-2 shows the time courses of ECG changes in mice that were simultaneously given 0.15 mg/kg of aconitine and 10 μg/kg of tetrodotoxin. In the mice in Group C, the rates of VT and VF were lower and the duration of VT and VF tended to be shorter than in Group A (0.15 mg/kg of aconitine). However, the number of deaths was much higher; 87% mice died during the experiment, and most of the deaths, approximately 77%, occurred in the absence of lethal arrhythmias.

In **Figure 4**, the time course of ECG changes in mice in Group A that were given 0.4 mg/kg of aconitine is compared with that in mice in Group C. Row 1-1 shows the time course of ECG changes in mice that were given 0.4 mg/kg of aconitine alone. In all mice, VT and VF were confirmed within approximately 420 seconds (7 minutes) after aconitine administration, and all mice died within 1,800 seconds (30 minutes); the mortality rate was 100%. Row 2-1 shows the time course of ECG changes in mice in Group C that were simultaneously given 0.4 mg/kg of aconitine and 15 μg/kg of tetrodotoxin. A few mice died within 1,800

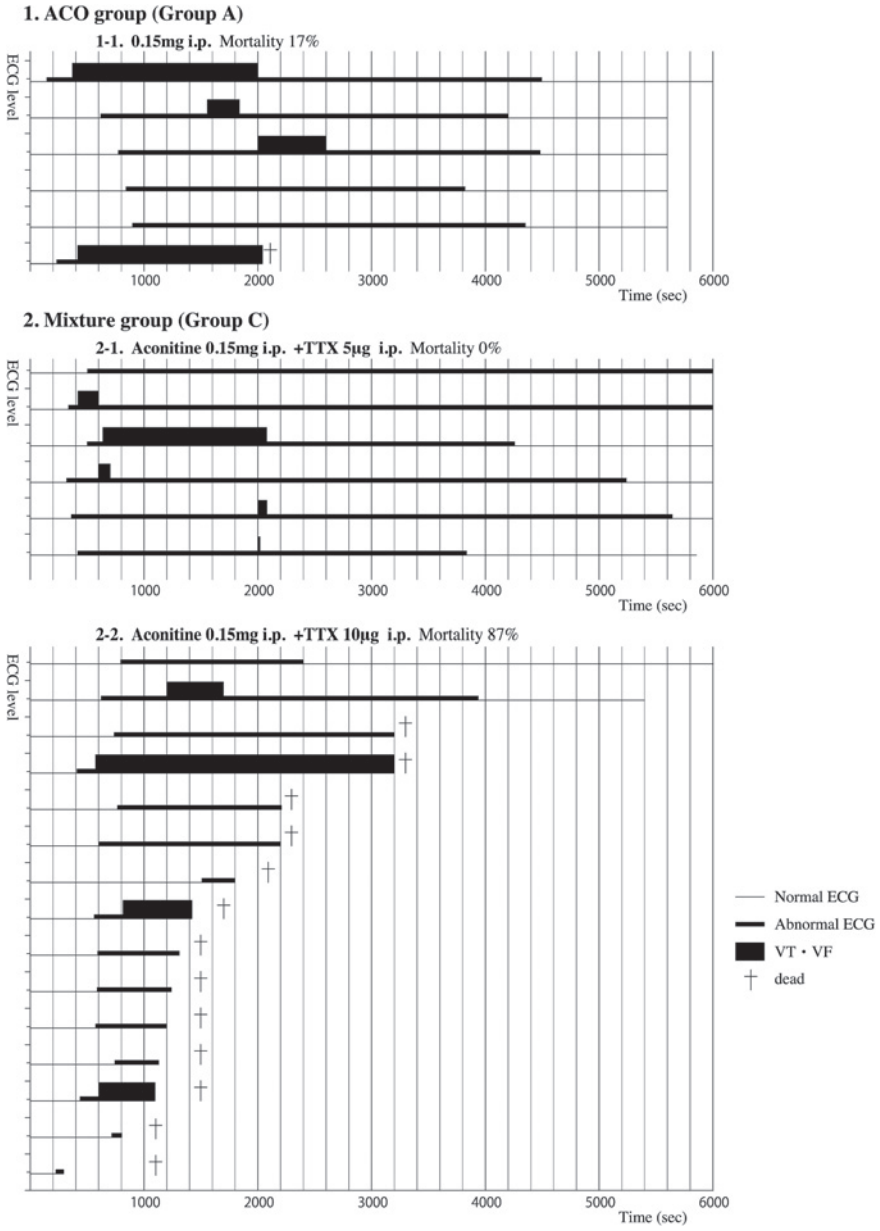

Fig. 3 Time courses of electrocardiogram changes in mice given 0.15 mg/kg of aconitine (Group A) compared with those in mice given aconitine and tetrodotoxin (Group C). Row 1-1 (0.15 mg/kg aconitine), Row 2-1 (0.15 mg/kg of aconitine and 5 μg/kg of tetrodotoxin), and Row 2-2 (0.15 mg/kg of aconitine and 10 μg/kg of tetrodotoxin).

seconds (30 minutes) after simultaneous administration of aconitine and tetrodotoxin, without showing any lethal arrhythmias before death. In the mice that survived, VT and VF developed but then stopped in some mice, which survived despite regular sinus rhythm not returning, while in the other mice, lethal arrhythmias, such as VT and VF, were not confirmed, and abnormal and normal sinus rhythms were mixed together. The mortality rate was approximately 34%, which was significantly less than the 100% mortality rate of Group A (0.4 mg/kg of aconitine).

Breathing Rate Changes

The breathing rate of the mice in Group D **(Fig. 5, 6)** was approximately 150 breaths per minute (bpm) at the start of the examination and gradually decreased to approximately 60 bpm because of the effects of anesthesia. The breathing rates experienced an upward trend again from 6,500 seconds (108 minutes) because of the termination of

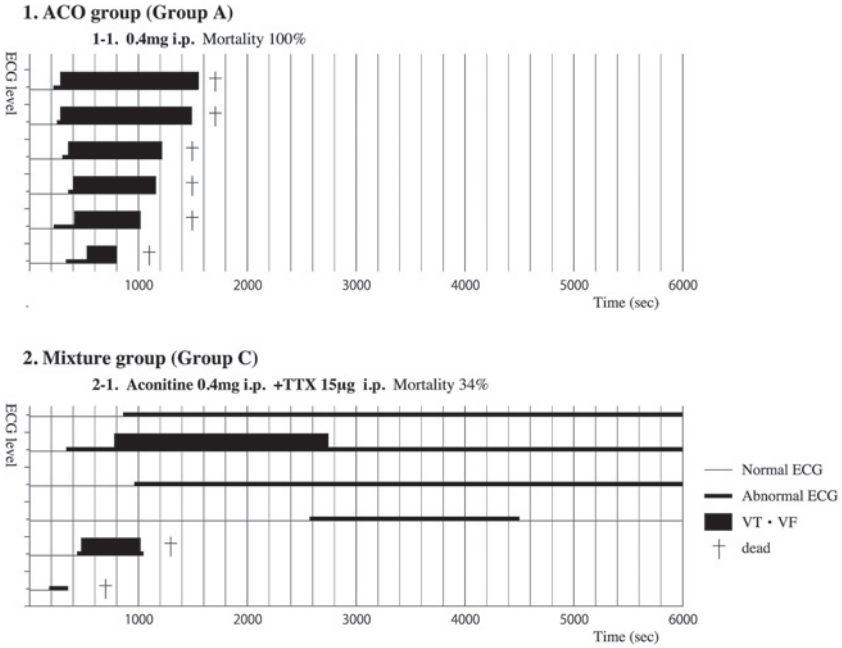

Fig. 4 Time courses of electrocardiogram changes in mice given 0.4 mg/kg of aconitine groups compared with those in mice given aconitine and tetrodotoxin. Row 1-1 (0.4 mg/kg of aconitine), and Row 2-1 (0.4 mg/kg of aconitine and 15 μg/kg of tetrodotoxin).

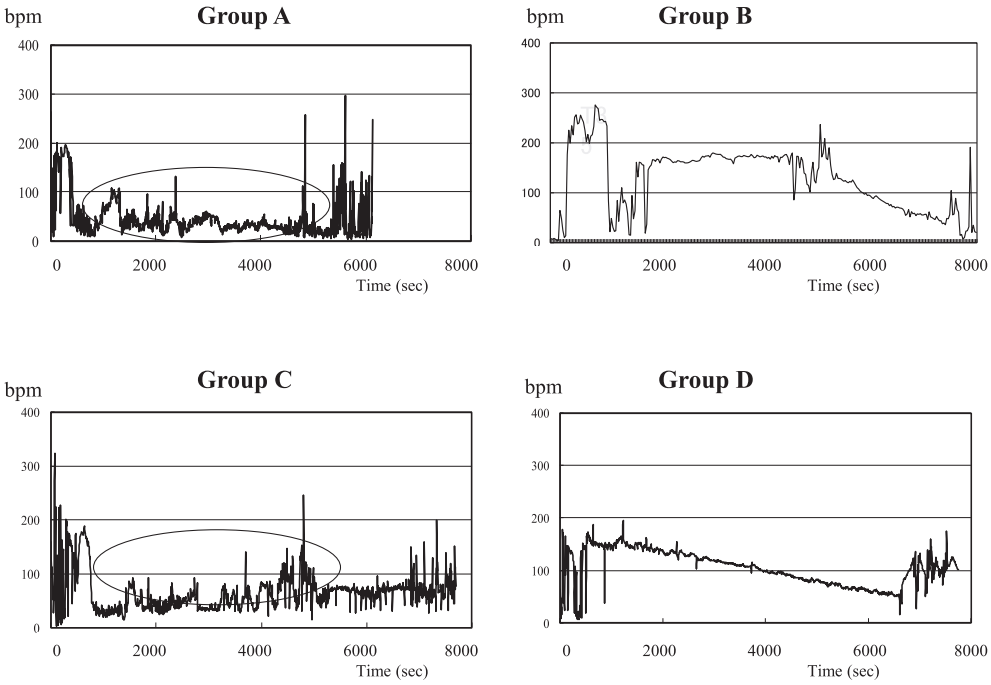

Fig. 5 The breathing rate changes in mice that did not die in early stages; the mice that did not die in early stages of the administration of 0.4 mg/kg of aconitine (Group A); the mice that did not die in early stages of the administration of 15 μg/kg of tetrodotoxin (Group B); the mice that did not die in early stages of the administration of a mixture of aconitine and tetrodotoxin (0.4 mg/kg of aconitine and 15 μg/kg of tetrodotoxin; Group C); and the control group (Group D).

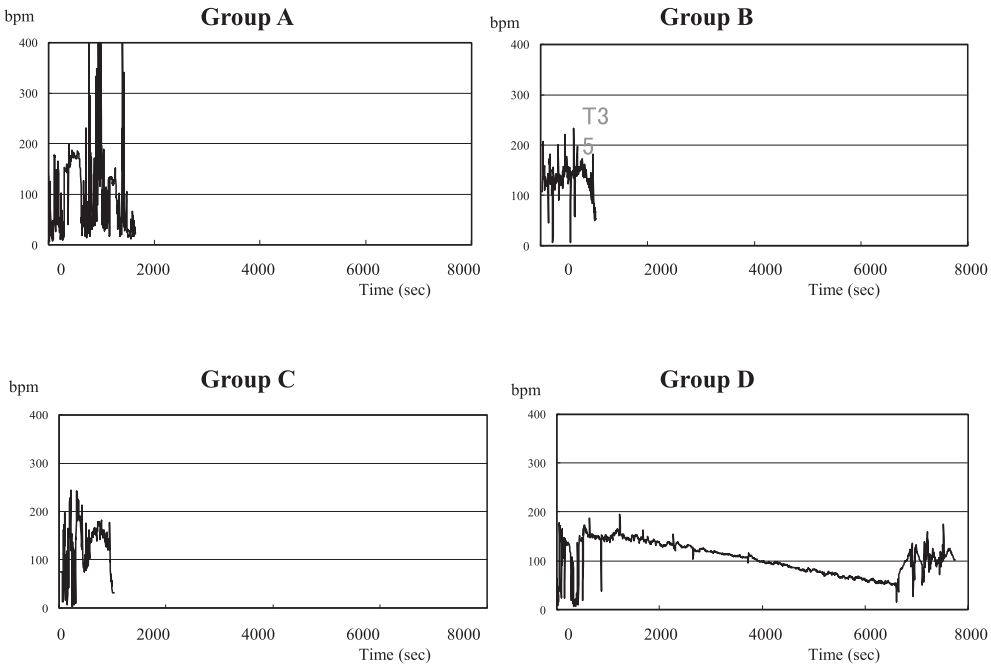

Fig. 6 The breathing rate changes of mice that died in early stages; mice died in early stages of the administration of 0.4 mg/kg of aconitine (Group A); mice died in early stages of the administration of 15 μg/kg of tetrodotoxin (Group B); mice died in early stages of the administration of a mixture of aconitine and tetrodotoxin (0.4 mg/kg of aconitine and 15 μg/kg of tetrodotoxin; Group C); and the control group (Group D).

anesthesia. The breathing rates of the mice that did not die in early stages in Group A (0.4 mg/kg of aconitine) **(Fig. 5)** were approximately 190 bpm at the start of the examination, decreased to 40 bpm at 420 seconds (7 minutes), fluctuated around 40 bpm until 5,500 seconds (91 minutes), and gradually recovered to 80 bpm. The breathing rates of the mice that died in early stages in Group A (0.4 mg/kg of aconitine) **(Fig. 6)** were also approximately 190 bpm at the start of the examination, fluctuated between 20 and 190 bpm at 1,200 seconds (20 minutes), decreased rapidly to 20 bpm, and then decreased to 0 bpm. The breathing rates of mice that did not die in early stages in Group B (15 μg/kg of tetrodotoxin) were approximately 260 bpm at the start of the examination and then decreased rapidly to 20 bpm at 1,200 seconds (20 minutes). Thereafter, the breathing rate steadily increased to approximately 170 bpm, where it remained for 3,000 seconds (50 minutes). After 5,000 seconds (83 minutes) the breathing rate decreased to 20 bpm. The breathing rates of the mice that died in early stages in Group B (15 μg/kg of tetrodotoxin) **(Fig. 6)** fluctuated between 100 and 180 bpm during the first 1,000 seconds (16 minutes), after which breathing stopped.

The breathing rates of mice in Group C that did not die in early stages of the administration of a mixture of aconitine and tetrodotoxin **(Fig. 5)** were approximately 180 bpm at the start of the examination and decreased to 40 bpm at 900 seconds (15 minutes). Subsequently, it fluctuated and slowly increased to 90 bpm. The breathing rates of mice that died in early stages in Group C (aconitine and tetrodotoxin) **(Fig. 5)** fluctuated between 80 and 210 bpm during the first 1,000 seconds (16 minutes), after which breathing suddenly stopped.

SpO_2

The SpO_2 of the mice in Group D **(Fig. 7, 8)** was maintained at 80% to 90%. In contrast, the SpO_2 of the mice that did not die in early stages in Group A (0.4 mg/kg of aconitine) **(Fig. 7)** was 100% at the start of the examination, decreased to 45% at 400 seconds (6 minutes), remained at 45% for 500 seconds (8 minutes), and then increased to 100%; thereafter the SpO_2 fluctuated between 80% and 100%. The SpO_2 of mice that died in early stages in

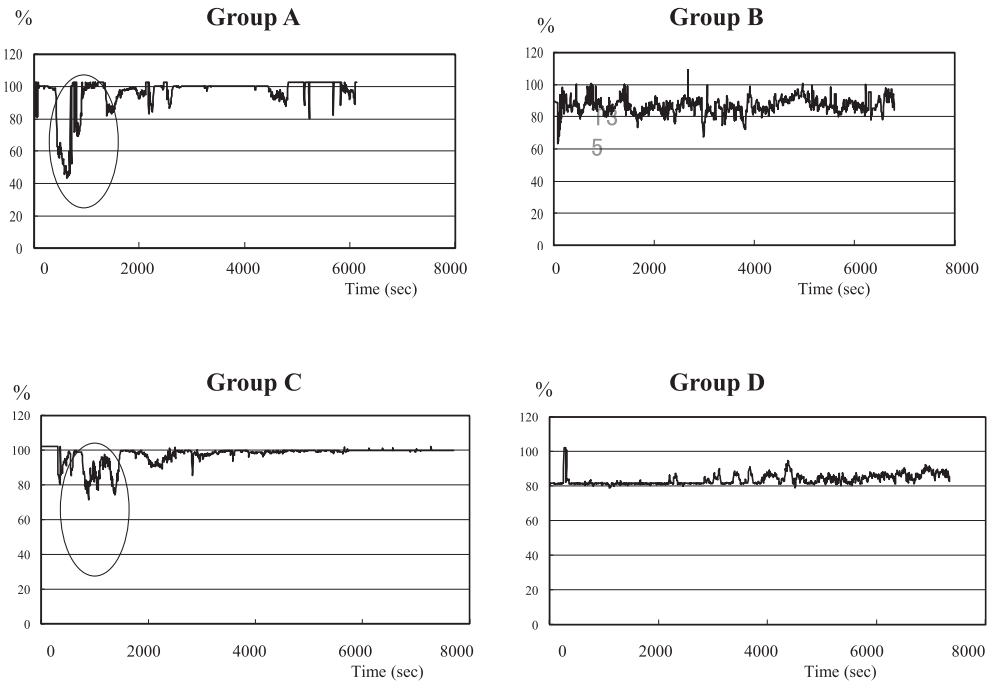

Fig. 7 The SpO2 changes in mice that did not die in early stages; the mice that did not die in early stages of the administration of 0.4 mg/kg of aconitine (Group A); the mice that did not die in early stages of the administration of 15 μg/kg of tetrodotoxin (Group B); the mice that did not die in early stages of the administration of a mixture of aconitine and tetrodotoxin (0.4 mg/kg of aconitine and 15 μg/kg of tetrodotoxin; Group C); and the control group (Group D).

Group A (0.4 mg/kg of aconitine) **(Fig. 8)** was approximately 95% at the start of the examination and rapidly decreased to 55% at 500 seconds (8 minutes). This decrease was followed by an increase to approximately 90% until 1,000 seconds (16 minutes); finally, the SpO_2 decreased to 40% and then to 0%. The SpO_2 of mice that did not die in early stages in Group B (15 μg/kg of tetrodotoxin) **(Fig. 7)** fluctuated between 70% and 100% during the entire period. The SpO_2 of mice that died in early stages in Group B (15 μg/kg of tetrodotoxin) **(Fig. 8)** fluctuated between 75% and 100% and abruptly decreased to 0% at approximately 1,700 seconds (28 minutes).

The SpO_2 of mice that did not die in early stages in Group C (aconitine and tetrodotoxin) **(Fig. 7)** was 90% to 100% at the start of the examination. Thereafter, it decreased to 75% at 800 seconds (13 minutes), where it remained for 800 seconds (13 minutes), and then increased to 100%. Finally, the SpO_2 fluctuated between 90% and 100%. The SpO_2 of mice that died in early stages in Group C (aconitine and tetrodotoxin) **(Fig. 8)** fluctuated between 80% and 100% and then decreased abruptly to 0% at 1,100 seconds (18 minutes).

Discussion

Aconitine causes arrhythmias when it is systemically administered[20,21], and death may result from sustained ventricular arrhythmias, such as VT and VF, caused by the direct action of aconitine on the heart[22,23]. Moreover, lethal arrhythmias, such as VT and VF, have been detected immediately before death.

In the present study, VT and VF were frequently detected on ECG in the mice given aconitine **(Fig. 1)**, and the duration and severity of the arrhythmias depended on the aconitine dose. The mortality rate increased as aconitine doses were increased. These results suggest that lethal arrhythmia is the root cause of death by aconitine.

As mentioned previously, the primary cardiac voltage-gated sodium channel isoform $Na_v1.5$ requires micromolar concentrations of tetrodotoxin for inhibition (IC_{50} > 1 μM)[20], whereas the sodium

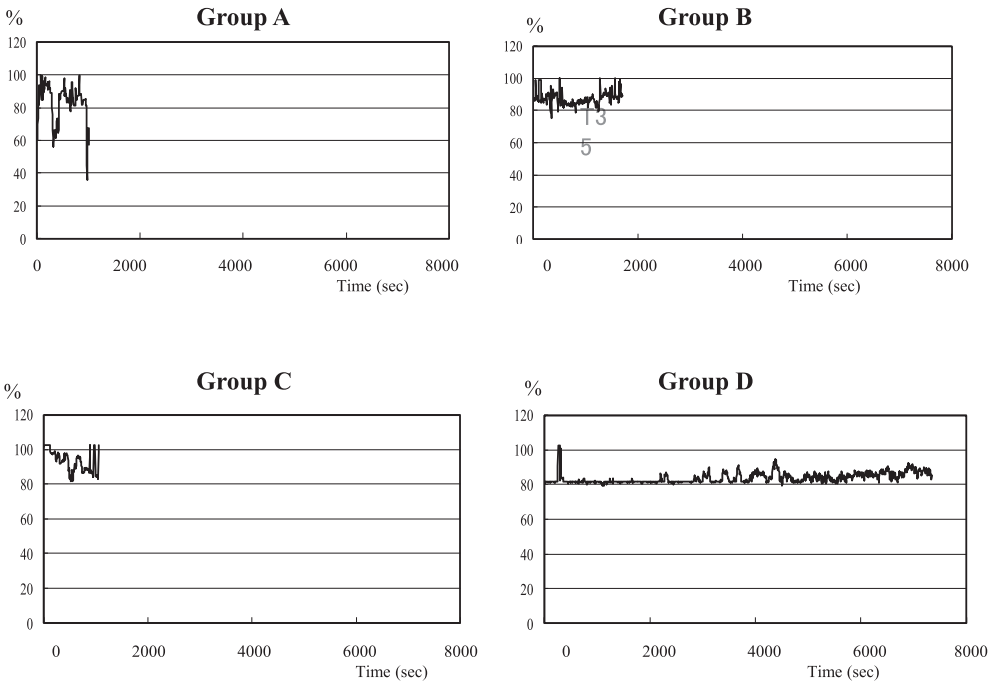

Fig. 8 The SpO2 changes in mice that died in early stages; mice died in early stages of the administration of 0.4 mg/kg of aconitine (Group A); mice died in early stages of the administration of 15 μg/kg of tetrodotoxin (Group B); mice died in early stages of the administration of a mixture of aconitine and tetrodotoxin (0.4 mg/kg of aconitine and 15 μg/kg of tetrodotoxin; Group C); and the control group (Group D).

channels in adult skeletal muscles and the central nervous system ($Na_v1.1$, 1.2, 1.3, 1.4, and 1.6) are considerably more sensitive to tetrodotoxin. During the last 10 years, it has become evident that in addition to the major cardiac tetrodotoxin-resistant I_{Na} ($Na_v1.5$), several tetrodotoxin-sensitive isoforms are expressed in the mammalian heart[24,25]. The isoforms $Na_v1.1$, 1.2, 1.3, 1.4, and 1.6 mediate small sodium currents in ventricular cardiomyocytes compared with $Na_v1.5$, but they contribute significantly to the coupling of cell-surface depolarization to contraction because of their location in the transverse tubules[26,27].

Because the tetrodotoxin IC_{50} of $Na_v1.5$ in ventricular cardiomyocytes is greater than 1 μM, it was impossible to counterbalance the amount of aconitine administered when the amount of tetrodotoxin, which was simultaneously administered, in our experiments was 5 to 15 μg/kg (approximately 17–52 nM). Thus, abnormal ECG patterns were detected in all mice given both aconitine and tetrodotoxin.

In contrast with the survival rate of mice given only aconitine, the survival rates of mice given particular combinations of aconitine and tetrodotoxin increased.

The interaction between aconitine and tetrodotoxin depends primarily on the aconitine dose administered. Compared with the predominant tetrodotoxin-resistant cardiac sodium channel isoform $Na_v1.5$, the tetrodotoxin-sensitive sodium current in the ventricular myocardium represents 20% to 30% of the total sodium current, depending on the membrane potential and experimental conditions[27]. When the tetrodotoxin concentration is 5 to 15 μg/kg, it can affect only the tetrodotoxin-sensitive sodium channels. Hence, the duration of lethal arrhythmias increased as the aconitine dose increased.

In addition, the interaction between aconitine and tetrodotoxin is related to the composition of the aconitine and tetrodotoxin mixture administered. When the tetrodotoxin dose is approximately equal to or less than the dose that can block the tetrodotoxin-sensitive sodium channels, which are excessively activated by aconitine, the toxicity of aconitine is gradually somewhat decreased in accordance with the increase in the tetrodotoxin

dose. Therefore, both the frequency of VT and VF and the mortality rate decrease. This point can be confirmed by the examples of mice (Group C) that were given 0.15 mg/kg of aconitine and 5 μg/kg of tetrodotoxin (row 2-1 of **Fig. 3**) and mice that were given 0.4 mg/kg of aconitine and 15 μg/kg of tetrodotoxin (row 2-1 of **Fig. 4**). Furthermore, in the case reported by Ohno, the victim developed severe vomiting, abdominal pain, and numbness of the limbs before death[6], which were all typical symptoms of aconitine poisoning. However, the latency of her death (> 90 minutes) was due to the effect of tetrodotoxin.

When the dose of tetrodotoxin is greater than the dose that can block the tetrodotoxin-sensitive sodium channels, which are excessively activated by aconitine, the toxicity of tetrodotoxin becomes prominent. The LD_{50} of intraperitoneally administered tetrodotoxin is 10.7 μg/kg in mice[28]. Therefore, some mice died of respiratory failure before recovery from severe arrhythmias, such as VT and VF. This is the reason for most deaths that occurred in the absence of severe arrhythmias in mice that were given 0.15 mg/kg of aconitine and 10 μg/kg of tetrodotoxin (row 2-2 of **Fig. 3**).

The results of our investigations of the breathing rate and SpO_2 of mice are consistent with this hypothesis. We were not able to use implantable electrodes because of the small bodies of the mice. Furthermore, because the probes for measuring the breathing rate and SpO_2 are extremely sensitive, even small movements by the mice may generate noise. Thus, the breathing rate and SpO_2 were measured with the mice under anesthesia.

We divided the mice into 2 groups, those that died in early stages (within 30 minutes of the administration) and those that did not. In mice that died in early stages, the breathing rate and SpO_2 of the mice given both aconitine and tetrodotoxin were more similar to those of mice given tetrodotoxin alone than those of mice given aconitine alone (**Fig. 6, 8**). This result is consistent with the interpretation that the deaths of some mice in Group C were more likely to have been caused by respiratory failure due to tetrodotoxin. This result is also consistent with the result that most of these mice died without showing severe arrhythmias, such as VT and VF, on ECG when the dose of tetrodotoxin was greater than the dose that can block the tetrodotoxin-sensitive cardiac sodium channels, which are excessively activated by aconitine (**Fig. 3, 4**).

The breathing rates of the mice that did not die in early stages of the administration of an aconitine-tetrodotoxin mixture presented waveforms that were similar to those of the mice that received aconitine alone, but the breathing rates recovered more quickly (**Fig. 5**). In addition, the SpO_2 of the mice that received an aconitine-tetrodotoxin mixture showed a waveform that was similar to that of the mice receiving aconitine alone, although the steepness of the decline phase was alleviated from 50% to 80% (**Fig. 7**). When combined with the results of the ECG changes (**Fig. 3, 4**), these experiments strongly suggest that tetrodotoxin antagonized aconitine and ameliorated the toxicity of aconitine in mice, a result that has been previously demonstrated in vitro[29,30].

Conclusions

The results of this study lead us to 4 conclusions.

1) Mortality and toxicity of aconitine are directly proportional to the aconitine dose administered.

2) Abnormal ECGs were observed in all aconitine-tetrodotoxin mixed-toxicity groups when 5 to 15 μg/kg of tetrodotoxin was administered simultaneously with aconitine.

3) Compared with those in mice given aconitine alone, mortality rates were lower and arrhythmia was delayed in mice receiving mixtures of aconitine and tetrodotoxin. Furthermore, the decrease in breathing rates and SpO_2 are ameliorated in the mice that did not die in early stages of the administration of the aconitine-tetrodotoxin mixed-toxicity group. The above conclusions suggest that the action of tetrodotoxin is antagonistic to the action of aconitine.

4) When the dose of tetrodotoxin is greater than the dose that can block the tetrodotoxin-sensitive sodium channels, which are excessively activated by aconitine, the toxicity of tetrodotoxin becomes prominent. Under these circumstances, mortality

increases because of the respiratory effects of tetrodotoxin.

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