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Comparison of the antinociceptive action of crude Fuzei, the root of Aconitum, and its processed products

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Abstract

The antinociceptive effects of crude Fuzei, the root of *Aconitum carmichaeli* and of Fuzei processed by three different methods were determined in mice and rats using the light tail-flick assay. A dose-dependent and significant increase in pain threshold was found at 60 min post treatment, with doses of 20–60 mg/kg crude Fuzei. The analgesic effects of processed Fuzei (20–60 mg/kg) exhibited a dose-dependent inhibition of tail-flick, but the effects were lower than those produced by crude Fuzei in the same tests. The analgesic effect of Yan-Fuzei, the salt baking product, was the most potent of the processed products and was nearly that provided by crude Fuzei. Although the concentrations of aconitine were significantly lower in the processed Fuzei than in the crude Fuzei, a higher oral LD_{50} was found for all of the processed Fuzei formulations. Moreover, antinociception of crude Fuzei and its processed products was attenuated but not totally blocked by naloxone at doses sufficient to block opioid μ-receptors. Furthermore, the analgesic effect of crude Fuzei and its processed products was decreased in opioid μ-receptor knockout mice, but the effect remained unaltered in mice with opioid μ-receptors, indicating that the analgesic effect of Fuzei is centrally mediated. These results demonstrate that Fuzei processed by salt baking possesses analgesic effects within a large therapeutic range, probably via a mechanism involving central opioid receptors that mediate the antinociception.

Keywords: Antinociceptive effect; Fuzei; Tail-flick assay; Opioid receptors

1. Introduction

Medicinal plants are a major component of traditional medical systems in Asia. Preparations of Aconitum roots are employed in Chinese and Japanese medicine for analgesic, antirheumatic and neurological indications (Sato et al., 1979; Hikino et al., 1980). Fuzei, the root of Aconitum (*Aconitum carmichaeli*), contains the highly toxic C19 diterpenoid alkaloids of aconitine, mesaconitine and hypaconitine (Ding et al., 1993). After ingestion, patients may present with signs and symptoms typical of aconitine poisoning. Death may occur from ventricular arrhythmias, which are most likely to develop within the first 24 h (Sawanobori et al., 1996; Ameri, 1998). Aconitine poisoning is far more common in Asia, particularly, China and Hong Kong, than in Western countries, perhaps because of the widespread use of herbal medicines by the Asian population. Thus, Aconitum processed by methods that reduce potential toxicity is essential. This study was conducted to determine the analgesic effects and toxic properties of crude Fuzei and Fuzei processed by salt baking, fire processing, or sulfuration in a model of acute pain in mice and rats.

2. Materials and methods

2.1. Plant preparations

Fuzei (*Aconitum carmichaeli* Dex.), family of Ranunculaceae, was collected in Chengdu, the capital of Sichuan Province in China, and identified by Prof. Y.C. Wu (Graduate

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Institute of Natural Products, School of Pharmacy, Kaoshiung Medical University, Taiwan). The fresh Fuzei was separated into three groups by size (diameter of plant): large, medium and small. The fresh Fuzei was chopped into small pieces and processed by three different methods. The large size Fuzei was incubated with salt to produce Yan-Fuzei. Briefly, 500 g of fresh Fuzei was incubated for 72 h with 1.5 L distilled water containing 100 g NaCl and 100 g $MgCl_2$. Then, the material was dried at room temperature to obtain Yan-Fuzei. The medium Fuzei was processed with fire to produce Hei-Shug-Pian. The fresh Fuzei (500 g) was incubated for 72 h with 1.5 L distilled water containing 100 g $MgCl_2$. After boiling in distilled water contained 100 g $MgCl_2$ for 45 min, the product was stir-fried with sugar and soy to brown the product. Then, the product was dried at room temperature to obtain Hei-Shug-Pian. The small Fuzei was processed to produce Bai-Shug-Pian by incubating 500 g with 1.5 L distilled water containing 100 g $MgCl_2$ for 72 h, followed by sulfuration to whiten the product after drying at room temperature.

2.2. Aconitine extraction

Aconitine concentrations were measured by high-performance liquid chromatography (HPLC) (Chen et al., 2002). Before HPLC analysis, the sample (200 mg) was first acidified with 1 mol/L HCl and the acidic solution extracted using $CHCl_3$. The extract was made basic with ammonia, and 0.1 mol/L $NaHCO_3$ and 0.1 mol/L Na_2CO_3 added to bring the pH to 10. The basic solution was then extracted with $CHCl_3$. The $CHCl_3$ extract was evaporated and a measured amount of methanol was added to the residue. After addition of the internal standard, the sample was injected onto a column of chemically bonded octadecylsilane using a liquid phase of MeOH–H_2O–$CHCl_3$–triethylamine (68:32:2:0.1, volume ratio). The alkaloids were quantified by the ratio of the aconitine peak to the internal standard. This method demonstrated high recovery (> 92%) and good reproducibility (R.S.D. < 3.2%).

2.3. Animals

Male Wistar rats weighing 180–220 g and male BALB/c mice weighing 18–22 g were obtained from the Animal Center of National Cheng Kung University Medical College. Male wild-type (BDF1 mice) and opioid μ-receptor knockout mice (Loh et al., 1998), 8–10 weeks of age, were obtained from Professor H.H. Loh (Department of Pharmacology, University of Minnesota Medical School, Minneapolis, USA). The animals were fed standard chow (Purina Mills Inc.), given water ad libitum, and maintained under well-ventilated conditions with a 12-h light cycle. All animal procedures were performed according to the *Guide for the Care and Use of Laboratory Animals* of the U.S. National Institutes of Health, as well as the guidelines of the Animal Welfare Act.

2.4. Acute toxicity studies: LD_{50}

The peroral (p.o.) acute toxicity (LD_{50}) profile of the crude Fuzei and its processed products was evaluated in BALB/c mice according to the method of Lorke, 1983. Male mice were separated into four groups of animals, each consisting of 10 mice. Food was withheld overnight and the crude Fuzei or a processed product was administered orally at the desired doses the next day. Animals were observed for 24 h after treatment and the final LD_{50} value was calculated as the square root of the product of the lowest lethal dose and highest non-lethal dose, i.e., the geometric mean of the consecutive doses for which 0 and 100% survival rates were recorded.

2.5. Light tail-flick assay

Acute antinociception was assessed using the Tail-Flick Analgesia Meter (1430, Columbus Instruments, 950 N. Hague Ave., Columbus, OH 43204, USA) following the method described previously (D'Amour and Smith, 1941). Briefly, Wistar rats or BDF1 mice with or without opioid μ-receptors were placed in a clear plastic cage with their tails extending through a slot located at the rear of the cage. Thermal stimulation was provided by a beam of high-intensity light focused on the tail 2–3 cm proximal to the end. The time between the start of the stimulation and tail withdrawal was considered the tail-flick latency. The cutoff time in the absence of a response was 14 s to prevent injury to the tail tissue. Threshold measurement was made 60 min after oral administration of the crude or processed Fuzei with saline (0.9% NaCl in distilled water) at the desired concentrations. Further experiments were performed with naloxone, the antagonists of opioid μ-receptors (Research Biochemical Inc., Natick, MA). The inhibitor was injected intravenously into the tail veins of rats 30 min before oral administration of the crude or processed Fuzei.

2.6. Statistical analysis

Data are expressed as mean ± S.E.M. and significance of the difference between control and treated groups was determined using one-way ANOVA following by Dunnett range post hoc comparisons. A P-value < 0.05 was considered statistically significant.

3. Results

The concentration of aconitine in the crude Aconitum was 50.3 μg/mL. However, the content of aconitine in Yan-Fuzei was significantly reduced to 6.7 μg/mL. Similarly, concentrations of aconitine were reduced to 2.8 μg/mL in Hei-Shug-Pian and to 2.1 μg/mL in Bai-Shug-Pian, respectively (Table 1).

The LD_{50} of the crude Fuzei and its three processed products were then evaluated. Treated animals exhibited