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The role of the chemical burns caused by hydroxide ion in the toxicity of dermal exposure to tetramethylammonium ion in a rat model

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ABSTRACT

Objective: To evaluate the role of the chemical burns caused by hydroxide ion in the fatal effects of tetramethylammonium ion (TMA) in dermal exposure to tetramethylammonium hydroxide (TMAH), we conducted a rat study consisting of two-step treatments with dermal exposure to NaOH and tetramethylammonium chloride (TMACl).
Methods: In the first step, NaOH or saline was administered in the gauze on the shaved skin for 5 min, and in the second step, TMAH, TMACl, or saline was administered in the same way. The mean blood pressure (MBP), heart rate (HR), and survival in rats were compared among seven groups.
Results: Dermal exposure to saline and then 2.75 M TMACl introduced limited and temporary non-fatal effects. Exposure to 2.75 M NaOH and then saline had almost no effects and caused no deaths. Treatments with more concentrated NaOH or TMACl resulted in suppressions of MBP and HR, and deaths were observed after the dosing of TMACl.
Conclusion: The toxicity of dermal exposure to TMA alone is limited, but fatal effects can be introduced by pre-treatment with hydroxide ion. Therefore, the chemical burn caused by hydroxide ion plays an essential role in the toxicity, implicating that effective neutralizing may help decreasing the fatality rate.

1. Introduction

Tetramethylammonium hydroxide (TMAH; $(CH_3)_4NOH$) is a colorless to light yellowish liquid with a slight amine odor [1]. It is widely used as a key agent in the manufacture of integrated circuits, liquid crystal displays, printed circuit boards, and many other electronic products as an anisotropic etchant or a cleaning agent. A single company can produce more than 40,000 tonnes of TMAH annually [2]. In Taiwan alone, hundreds of thousands of workers involving manufacture, transport, storage, or waste reduction are at risk of exposure to TMAH, and numerous intoxication cases have

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been reported, including three fatalities through dermal exposures in the recent years [3]. Although TMAH is regarded as having a relatively low level of toxicity [4], the three fatality cases were exposed to 25% TMAH which resulted in chemical burns on only 7–29% of the total body surface area (TBSA) [5,6]. Their conditions deteriorated so quickly that all three cases lost vital signs on the way to the hospital in less than 30 min, even though they all went through decontamination in the safety shower immediately. These cases have drawn the public's attention [7], but only a few studies have been conducted on health effects of dermal exposures to TMAH.

With a high dissolubility, TMAH dissociates to tetramethylammonium ion (TMA; $(CH_3)_4N^-$) and hydroxide ion in water. TMA is an autonomic ganglion blocker with the characteristic of cardiac suppression [8] and is considered as the source of TMAH systemic toxicity [3,5,6]. It is classified as a cholinergic agonist that binds to the nicotinic and muscarinic receptors in skeletal muscles [9], smooth muscles [10] and cardiac muscles [11]. Although the hydroxide ion in TMAH solution produce strong corrosive alkali (pH $>$ 13) [1] and are capable of penetrating deeply into tissues to cause prolonged destruction, the direct effects were judged to be insufficient to cause death even in industrial accidents with second to third degree chemical burns covering up to 29% of TBSA [5,6].

The contribution of hydroxide ion to the toxicity of TMAH is seldom studied. After a literature search in the PubMed database, we found only a few published reports addressing the effects of dermal TMAH exposure. Using a rat model, Lee et al. found that the 4-h lethal dose (LD_{50}) of dermal exposure was 85.9 mg/kg for 2.38% TMAH and 28.7 mg/kg for 25% TMAH [5]. Observing the lower values of LD_{50} in comparison with that administered by subcutaneous injection (12.9 mg/kg and 11.9 mg/kg, respectively), the authors proposed that the skin serves as an effective barrier against the uptake of TMAH. When administered in the same way, either intravenous or subcutaneous injection, 25% TMAH had an LD_{50} much lower than that of 2.38% TMAH. This might result from the higher concentration of TMA alone or its combination with the corrosive effect of hydroxide ion, which may destroy the skin barrier and significantly increase the uptake of TMA.

In industrial TMAH accidents, the reported clinical presentations included salivation, weakness, dyspnea, and death [3,5,6], which were more severe than those observed in cases eating food containing TMA [12–15]. Because the skin has a polar nature and hydrophobilicity, it seems difficult for TMA to pass through the intact skin. Therefore, we suspected that the toxic effects of dermal exposure to TMAH can be attributable to the corrosive effect of hydroxide ion in the first stage and the systemic effects of TMA in the second stage. In order to clarify the effects of hydroxide ion and TMA and explore the effective first-aid management, we conducted an animal study consisted of two-step treatments with dermal exposure to NaOH and TMACl. The mortality and effects on the mean blood pressure (MBP) and heart rate (HR) were compared among the groups exposed to different levels of NaOH and TMACl. In addition, we compared the effects between the groups with and without decontamination.

2. Materials and methods

2.1. Experimental animals and preparation

Adult male Wistar rats (Laboratory Animal Center NCKU, Tainan, Taiwan) were housed at an ambient temperature at 22 ± 1 °C, with 12-h light/dark cycles. The animals weighted between 270 and 330 g (9–12 weeks old) at the time when the experiment started. On the day before the experiment, we shaved the back of the rat using a small clipper carefully to avoid abrading the epidermis and produce a bare area of at least for 6 cm $\times$ 6 cm. On the experiment day, the rats were assigned into different treatment groups randomly. We defined death as a MAP less than 10 mmHg without any pulse lasting for more than 5 s and euthanized the animals using a CO2 chamber at the end of the study. This study has been reviewed and approved by the Institutional Animal Care and Use Committee of the National Cheng Kung University.

2.2. Drugs

We obtained industrial grade 25% TMAH from the Chang-Chun Petro-Chemical Company, Taiwan. NaOH and TMACl were obtained from the Sigma–Aldrich Chemical Co., USA. In order to simulate the concentrations in industrial exposure, NaOH and TMACl were dissolved in distilled de-ionized water to produce stock solutions of 2.75 M and 0.26 M, which are molar equivalent to 25% and 2.38% of TMAH, respectively. In the industry, TMAH is mostly used at these two concentrations.

2.3. Catheterization, cardiovascular parameters recording

The rats were anesthetized using ketamine (Ketalart, Pfizer, Taiwan) 100 mg/kg intraperitoneally and then placed on a wooden plate with a warming light. The right femoral artery was cannulated using a polyethylene tube (PE 50) for blood pressure monitoring. Heparinized saline was injected into the catheter to avoid thrombosis and catheter obstruction without the intention of fluid replacement. After a stabilization period of about 5 min, the blood pressure was recorded and analyzed using the MP35 system and AcqKnowledge software (BIOPAC Systems Inc., CA, USA). The blood pressure is reported as MBP (mmHg), and the HR is counted from the blood pressure signals and expressed as beats per min (bpm). The monitoring was terminated at death or 100 min, whichever came first.

2.4. Treatment procedure for NaOH and TMACl

In order to compare the effects of dermal exposure between NaOH and TMACl, we used a two-step experiment design in which NaOH or saline was administered in the gauze on the shaved back for 5 min and then washed off by irrigation in the first step, and TMAH, TMACl, or saline was treated in a similar way in the second step. The animals were randomly divided into one of seven groups: ''TMAH,'' ''NaOH 2.75 + TMACl 2.75,'' ''NaOH 0.26 + TMACl 2.75,'' ''saline + TMACl 2.75,'' ''NaOH 2.75 + TMACl 0.26,'' ''NaOH 2.75 + TMACl 0.26 WI,'' and ''NaOH 2.75 + saline'' (Fig. 1). We used two layers of hospital-standard