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ACUTE EFFECTS OF PENTOBARBITAL, THIOPENTAL AND URETHANE ON LUNG OEDEMA INDUCED BY ALPHA-NAPHTHYTHIOUREA (ANTU)

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This study was designed to investigate the possible participation of urethane, pentobarbital sodium and thiopental sodium anaesthesia in the lung oedema induced by alpha-naphthylthiourea (ANTU), which is a well known noxious chemical agent in the lung. ANTU when injected intraperitoneally (i.p.) into rats (10 mg kg^{-1} i.p.) produced lung oedema as indicated by an increase in lung weight/body weight (LW/BW) ratio and pleural effusion (PE) reaching a maximum within 4 h. Administration of urethane prior to ANTU, at doses of 100 and 200 mg $(100\ g)^{-1}$, elicited a significant and dose-dependent inhibition in LW/BW ratio and PE. Thiopental sodium at doses of 25, 50 mg kg^{-1}, also produced a significant and dose-dependent inhibition of both parameters. Prior i.p. injection of pentobarbital sodium at a dose of 40 mg kg^{-1} elicited a significant inhibition in both parameters. These results suggest that i.p. urethane, thiopental sodium and pentobarbital sodium pretreatment have a prophylactic effect on ANTU-induced lung injury in rats. The possible role of the anaesthetics in lung oedema induced by ANTU and the possible underlying mechanisms are discussed.

KEY WORDS: α-naphthylthiourea, thiopental, urethane, pentobarbital, lung oedema, pleural effusion.

INTRODUCTION

Alpha-naphthtylthiourea (ANTU) is a chemical agent largely used as a rodenticide which produces a dose- and time-dependent inflammatory reaction characterized by pulmonary oedema secondary to permeability changes in the lung microvasculature [1]. Morphological studies with light and electron microscopy indicate that the capillary endothelial cell of the lung is the primary cellular target of ANTU toxicity [2, 3]. Injury to the endothelium appears as blebbing and scalloping of the cell surface with eventual loss of the endothelial barrier. This loss of endothelial barrier integrity results in increased capillary permeability and the production of an interstitial and alveolar oedema [4].

It has been speculated that vasoactive substances originating from the pulmonary vascular bed and airways or affected by them may contribute to the pulmonary oedema induced by ANTU. Such damage has been shown to be partially mediated through arachidonic acid metabolites [5]. We have recently shown that the oedema producing effect of ANTU is effectively prevented by intravenous (i.v.) bolus injection of endothelin-1 (ET-1) [6]. Also we have presented evidence indicating participation of L-arginine/nitric oxide (NO) pathway in this pathological event [7].

Many anaesthetic agents affect the vascular endothelium and muscle functions and modulate the lung inflammatory responses [8–14]. While there are numerous scientific publications on the influence of anaesthetics on the function of the pulmonary vascular system, effects on pleural effusion (PE) and pulmonary oedema are unknown. The aim of this study was to investigate the effect of thiopental sodium, pentobarbital sodium and urethane on the lung inflammatory responses induced by ANTU.

MATERIALS AND METHODS

Male albino rats weighing 180–250 g were used in this study. They were housed under standard laboratory conditions with a 12 h light/12 h dark cycle and allowed food and water *ad libitum*. The procedures and protocols of the study were in accord with our institutional guideline, which is similar to *Guide for the care and use of laboratory animals* (US National Institute of Health, revised 1985).

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During the experimental procedure, the animals were placed in separate cages and kept at room temperature (22 °C). ANTU (suspended in olive oil 4 mg kg^{-1}) was injected intraperitoneally (i.p.) at the dose of 10 mg kg^{-1}. The control group received the same volume of olive oil. Four hours later, the animals were anaesthetized with urethane (1.5 g kg^{-1} s.c.) and were bled by cutting the carotid artery. The thorax was opened and PE was carefully collected by suction and measured volumetrically. Care was also taken to eliminate blood contamination with PE. The lungs were then removed and all surrounding tissues were dissected and weighed with an analytical balance. The volume of PE (as ml), the lung weight/body weight (LW/BW) and PE/body weight (PE/BW) ratios were calculated and considered as three parameters for pulmonary oedema.

The animals were divided into 11 groups. The first group of animals received only olive oil (i.p.) and the second group ANTU (10 mg kg^{-1} i.p.). Both groups were kept as controls. The third, fourth and fifth groups were injected with urethane at doses of 50, 100 and 200 mg $(100\ g)^{-1}$ (i.p.) 15–30 min before ANTU. The sixth, seventh and eighth groups were injected with thiopental sodium at doses of 5, 25, 50 mg kg^{-1} (i.p.) and the following three groups received pentobarbital sodium at the doses of 10, 20 and 40 mg kg^{-1} (i.p.) 15–30 min before ANTU.

For histopathological examination of control groups (olive oil and ANTU), the lungs were immersed in 10% formalin and allowed to fix for 2–3 days. All lobes of each lung were examined. 10 μm cross-sections were processed for standard haematoxylin and eosin staining [15]. These sections were then examined via a light microscope and photographed.

The following drugs were used in this study: ANTU (Interchim) was a gift from Dr Schillinger, Schering AG, Berlin, Germany. Olive oil was purchased from Sigma (St Louis, MO, USA). Urethane, thiopental sodium, pentobarbital sodium (Abbott) were dissolved just before use.

Results were expressed as mean ± SEM. Comparisons between groups were made using a Kruskall–Wallis non-parametric analysis of variance, followed in case of significance by a Mann–Whitney U-test. $P < 0.05$ was accepted as significant.

RESULTS

On microscopic examination ANTU-treated rats were shown to have severe lung injury associated with perivascular, peribronchial, alveolar septal oedema, loss or destruction of interstitial cellular elements and deposition of eosinophilic oedema fluid in alveoli [Fig. 1(b)], while no changes were observed in olive-oil-treated rats [Fig. 1(a)]. Protective effects of urethane, thiopental and pentobarbital on lung damage induced by ANTU were observed obviously by macroscopic examination.

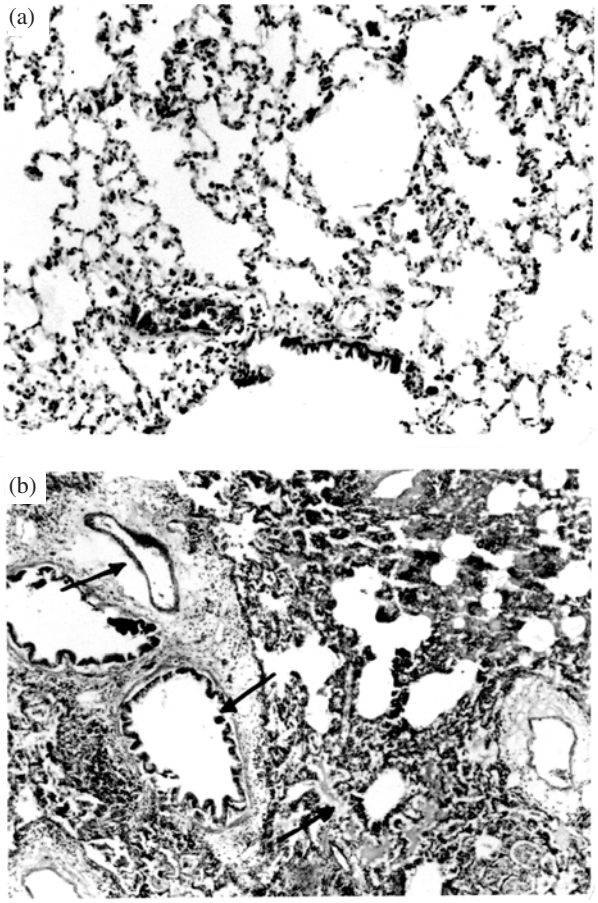

Fig. 1. (a) Normal histological appearance of olive-oil-treated rat lungs. The section was taken from the middle lobe of the right lung. Haematoxylin–eosin (H–E) stain ×200. (b) Prominent perivascular (indicated with upper and lower arrows), peribronchial oedema (middle arrow), thickening of alveolar septa and eosinophilic oedema fluid deposition after ANTU treatment (H–E ×400). The section was taken from the same place.

A significant lung oedema was observed 4 h after i.p. injection of ANTU at the dose of 10 mg kg^{-1} as indicated by an increase in LW/BW ratio and PE when compared with olive-oil-injected rats. LW/BW ratio was calculated as $140.96 \pm 7.35 \times 10^{-4}$ for ANTU-treated rats while it was found to be $58.2 \pm 2.8 \times 10^{-4}$ for olive-oil-injected rats ($P < 0.001$) [Fig. 2(a)]. Although PE was measured as 3.11 ± 0.48 ml in ANTU-treated rats, no detectable PE was observed in vehicle-injected rats [Fig. 2(b)].

Intraperitoneal injection of urethane before ANTU treatment significantly reduced LW/BW ratio, PE and PE/BW ratio at 100 mg $(100\ g)^{-1}$ ($P < 0.01$) and 200 $mg\ (100\ g)^{-1}$ ($P < 0.05$) doses but not at the dose of 50 $mg\ (100\ g)^{-1}$. Thiopental sodium at the doses of 25 and 50 mg kg^{-1} also significantly reduced LW/BW ratio, PE and PE/BW ratio ($P < 0.05$) except the dose of 5 mg kg^{-1}. Pentobarbital sodium was significantly reduced all parameters only the dose of 40 mg kg^{-1} ($P < 0.01$) and did not induce significant changes at the doses of 10 and 20 mg kg^{-1} [Fig. 2(a, b)].

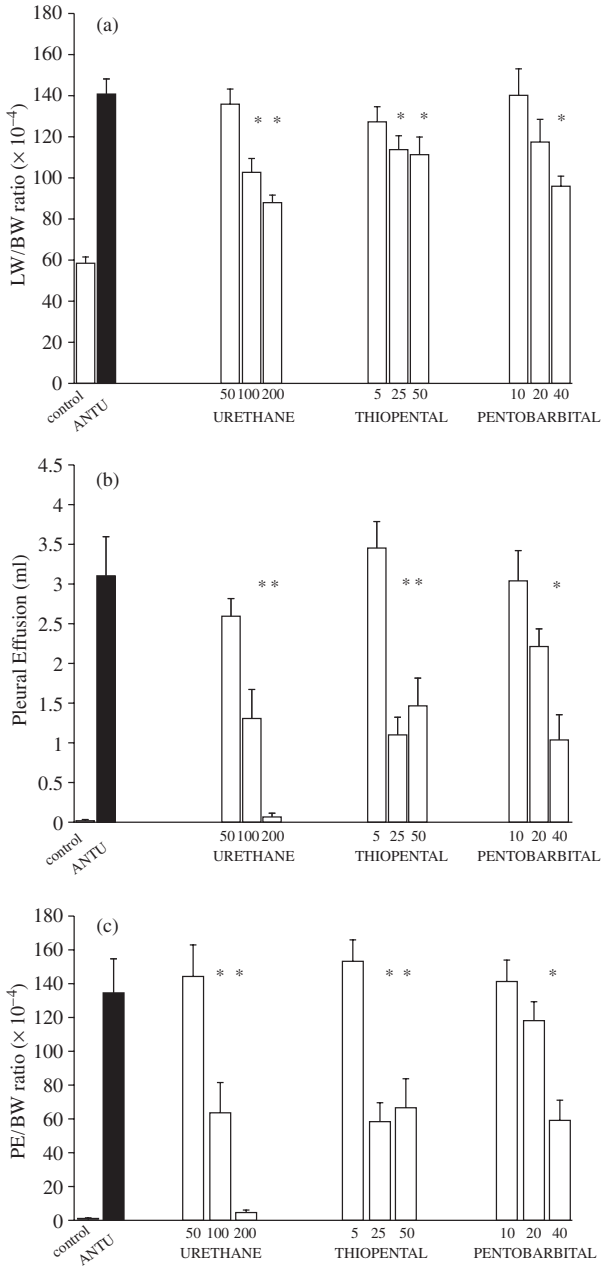

Fig. 2. The calculated results of lung oedema induced by ANTU and alterations by urethane (50, 100 and 200 mg 100 g^{-1}), thiopental sodium (5, 25 and 50 mg kg^{-1}) and pentobarbital sodium (10, 20 and 40 mg kg^{-1}), as evaluated by the changes of LW/BW ratio ($\times 10^{-4}$) (a), PE (ml) (b) and PE/BW ratio ($\times 10^{-4}$) (c). Each column [except columns for olive oil control (c) ($n = 10$) and ANTU ($n = 10$)] shows the mean value of eight experiments, vertical bars on the columns represent SEM. * $P < 0.05$.

DISCUSSION

The present experiments show that urethane produced significant and dose-dependent inhibition in LW/BW ratio and PE induced by ANTU, which is a well known noxious chemical agent in the lung. Urethane is an anaesthetic agent that, although never used in humans, is frequently the drug of choice for acute animal experimentation because it results in deep, surgical level anaesthesia with minimal physiological changes of the cardiovascular system [16]. In respiratory-related research its usefulness is based on the fact that it provides surgical level analgesia, with no effect on the control of breathing [17–19].

Aside from its anaesthetic properties, this drug has been shown to induce physiological changes that suggest a powerful anti-inflammatory effect. These changes include fever inhibition [20, 21], inhibition of carrageenan-induced inflammation [22], attenuation of lipopolysaccharide- (LPS-) induced lung inflammation and leukopenia [23]. The mechanism responsible for the anti-inflammatory effects of urethane is unclear. Previous investigators suggested that it could be the result of the drug-induced epinephrine and cortisone level increase [24]. Recent evidence indicates that following endotoxemia in rats, urethane suppresses tumour necrosis factor-alpha (TNF-α), a known mediator of the inflammatory response [23]. Previous investigators also suggested that urethane reduced the basal constitutive and inducible cyclooxygenases and inducible nitric oxide synthase mRNA levels in the rat lung [17]. We have recently presented evidence indicating participation of the L-arginine–NO pathway in lung oedema induced by ANTU. Administration of N^{G}-nitro-L-arginine methyl ester, a NO synthase inhibitor, prior to ANTU, produced a significant inhibition of PE and LW/BW ratio in a dose-dependent manner [7]. It can be thought that ANTU causes pulmonary oedema and consequently up-regulates inducible NO synthase, for which the mechanisms are not clearly known, and urethane may decrease the pathology by reducing the effect on inducible NO synthase mRNA levels in the rat lung like N^{G}-nitro-L-arginine methyl ester in our previous study [7].

We have recently observed that the oedema producing effect of ANTU is effectively prevented by a single i.v. bolus injection of ET-1 [6]. This is probably due to an acute potent and long-lasting vasoconstrictor effect of the peptide. Urethane may have a preventive effect via increasing the levels of epinephrine, which is a potent vasoconstrictor like endotelin-1. The results of this study indicate that the oedema producing effect of ANTU is effectively prevented by i.p injection of urethane. This may be due to down-regulation of inducible NO synthase and increasing the epinephrine level by urethane.

We have shown that thiopental sodium at the doses of 25 and 50 mg kg^{-1} also significantly reduced LW/BW ratio and PE ($P < 0.05$) except the dose of 5 mg kg^{-1} (LW/BW ratio was reduced but not statistically significantly). Pentobarbital sodium significantly reduced both parameters only at the dose of 40 mg kg^{-1} ($P < 0.01$); at a dose of 20 mg kg^{-1} both parameters reduced but not statistically significantly ($P > 0.05$) and no changes were induced at the dose of 10 mg kg^{-1} [Fig. 2(a, b)]. Thiopental administrated i.v. is frequently used in clinical anaesthesia as an inductor agent because of its rapid onset and short duration of action [25]. Recently, it has been shown that endothelium

functional alterations may also be related to the exposure to thiopental, at doses that did not produce endothelium damage [26]. Rich *et al.* [9] demonstrated that thiopental is a direct pulmonary vasoconstrictor on the pulmonary vasculature of the isolated rat lung and produced a transient, dose-dependent increase in pulmonary vascular resistance (PVR) independent of baseline PVR or endothelial injury. Andreasen and Christensen [27] also demonstrated that thiopental caused a gradual increase in tension in rabbit pulmonary artery rings. Lischke *et al.* [26] showed a selective inhibition by thiopental of the synthesis of endothelium-derived hyperpolarizing factor (EDHF) in the rabbit carotid artery. On the other hand, Terasako *et al.* [28] suggested that thiopental may be related to an inhibition of the NO-dependent relaxation response to acetylcholine in rat aorta. Recently, it was found that thiopental inhibits both endothelium-dependent relaxation and nitrite production elicited by acetylcholine and histamine in rat aortic rings; therefore, an interference with the synthesis of NO provoked by the thiopental was suggested [29]. In a dog study, Hirota *et al.* [30] showed that the mechanism of thiopental bronchospasm may result from cholinergic nerve stimulation. However, it was found that thiopental augments the interleukin-1βeta-stimulated expression of the inducible form of NOS in vascular smooth muscle cells [31]. In contrast, Castillo *et al.* [25] suggested that thiopental inhibits the constitutive form of NOS in both renal and aortic homogenates. Hence, thiopental seems to have differential effects on constitutive and inducible NOS isoforms. Pentobarbital anaesthesia has been clearly shown to modify control of the systemic circulation in response to a variety of stimuli [10, 11]. It has been shown that, like thiopental, pentobarbital inhibits the relaxation and cGMP formation induced by endothelium-dependent relaxants and sodium nitroprusside (SNP) [28]. Therefore, the inhibitory action of barbiturates on endothelium-dependent relaxation is mediated not by inhibition of endothelial NO synthesis but by inhibiting the action of NO, or by inactivating it in vascular smooth muscle. NO probably plays a role in the preventive effect of these barbiturates in the oedema producing effect of ANTU.

As we have shown before [7], on microscopic examination ANTU-treated rats were shown to have severe lung injury associated with perivascular, peribronchial, alveolar septal oedema, loss or destruction of interstitial cellular elements and deposition of eosinophilic oedema fluid in alveoli [Fig. 1(b)]. Polimorphonuclear eosinophilic leucocytes (PME) produce cytokines which influence acute and chronic inflammatory responses. They modulate wound healing processes by migrating into wound sites and liberating transforming growth factor-alpha and beta. Moreover, eosinophils synthesize several lipid mediators such as leukotriene C4, lipoxins and platelet-activating factor. During asthmatic attacks, major basic proteins and eosinophil peroxidase enhance bronchoconstriction. Krumholz *et al.* [32] have recently shown that chemotaxis, one of the most important functions of PME, was inhibited by high concentrations of thiopental *in vitro*. The sulphur atom of thiopental is responsible for this phenomenon [33]. Therefore the inhibition of PME chemotaxis or vasocontrictor effects of thiopental may play a role in the prevention of lung damage induced by ANTU. The contributions of these effects of thiopental to the lung pathology are still under investigation.

In conclusion, the results of the present study indicate that the oedema producing effect of ANTU is effectively prevented by i.p. injection of urethane, thiopental and pentobarbital prior to ANTU. It has been shown that they have vasoconstrictor effects on the pulmonary vascular system. These effects of the anaesthetics may be responsible for their preventive effect and mediated by their various effects on NO synthase, inflammation, vascular smooth muscle and endothelium. As we determined before [7], in this study, it seems that NO is involved in the mechanisms of ANTU-induced lung oedema and PE and urethane, thiopental and pentobarbital may show their effects partially by inhibition of NO. The preventive mechanisms of these anaesthetics on ANTU-induced pathology and especially interaction between anaesthetics and NO are still under investigation.

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