ISOFLURANE AGGRAVATES THE DECREASE OF PHOSPHATIDYCHOLINE SYNTHESIS IN ALVEOLAR TYPE II CELLS INDUCED BY HYDROGEN PEROXIDE

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SUMMARY

The influence of isoflurane (Iso) on the synthesis and secretion of phosphatidylcholine (PC) of alveolar type II cells (AT II cells) injured by hydrogen peroxide (H_2O_2) was investigated. After primary culturing for 32 h, AT II cells isolated and purified from adult Sprague-Dawley rats were randomly divided into six groups: control group, 02.8 mM Iso group, 2.8 mM Iso group, 75 μM H_2O_2 group, 75 μM H_2O_2 + 0.28 mM Iso group, and 75 μM H_2O_2 + 2.8 mM Iso group. Synthesis and secretion of phosphatidylcholine (PC) were detected by ^{3}H-choline chloride incorporation. It was found that 0.28 mM and 2.8 mM Iso significantly reduced PC synthesis compared with the control group ($p < 0.05$, $p < 0.01$, respectively), but not PC secretion. 75 μM H_2O_2 markedly decreased the synthesis and secretion of PC in AT II cells compared with the control group ($p < 0.01$). 0.28 mM and 2.8 mM Iso aggravated the decrease of PC synthesis induced by H_2O_2 ($p < 0.05$, $p < 0.01$, respectively), but did not affect PC secretion. These findings suggest that Iso itself may inhibit the synthesis of PC of AT II cells *in vitro* and further damage the cells' function under peroxidation.

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KEY WORDS

isoflurane, alveolar type II cells, phosphatidylcholine, hydrogen peroxide

INTRODUCTION

Alveolar type II (AT II) cells play an important physiological role in secreting pulmonary surfactant to decrease pulmonary surface tension, and to prevent end expiratory alveolar collapse and pulmonary edema. Phosphatidylcholine (PC) is the predominant phospholipid component of pulmonary surfactant (PS), and quantitative measurement of the synthesis and secretion of PC might improve our knowledge of the function of AT II cells. A previous study reported that halothane could reduce ATP content, decrease Na^+,K^+-ATPase activity and alter PS biosynthesis in AT II cells, especially after exposure to oxidants /1,2/. Isoflurane (Iso) is a widely used volatile anesthetic, whose effect on the synthesis and secretion of PS in AT II cells has not been demonstrated. The goal of the current study was to evaluate the influence of Iso on the synthesis and secretion of PC in AT II cells.

MATERIALS AND METHODS

Materials

Reagents were obtained from the following sources: ^{3}H-choline from Amersham, isoflurane from Abbott, elastase from Difico. DMEM, DNAse 1 and other chemicals of the highest grade available were purchased from Sigma.

Cell culture

Alveolar type II cells were isolated from the lungs of adult Sprague-Dawley rats (180-220 g) as described by Dobbs *et al.* /3/. Rats were weighed and anesthetized with 40 mg/kg i.p. sodium pentobarbital, and injected i.p. with 4000 U/kg heparin sodium. After tracheotomy, the rats were exsanguinated via the abdominal aorta, and the lungs were perfused via the pulmonary artery with solution II

containing (in mM) 140 NaCl, 5 KCl, 10 HEPES, 2 $CaCl_2$, 1.3 $MgSO_4$, 2.5 phosphate-buffered saline (PBS), pH 7.4, at 22°C, and the lungs were ventilated several times via trachea. The heart and lungs were removed, and the lungs lavaged 4-6 times via the trachea with solution I containing (in mM) 140 NaCl, 5 KCl, 10 HEPES, 0.2 EGTA, 6-D-glucose, 2.5 PBS, pH 7.4, at 22°C, to remove macrophages. The lungs were washed twice with solution II and filled with 10 ml elastase solution, and then incubated and digested in a water bath in air at 37°C for 20 min, after which an additional 10 ml of elastase solution was instilled at intervals of 10 min. The lungs were cut in a DNAse I solution (0.25 mg/ml) and digestion was stopped with 5 ml newborn calf serum. The tissue suspension was filtered through 120 μm and 200 μm stainless steel mesh, and the filtrate was centrifuged at 130 *g* for 8 min at 4°C. The cell pellet was suspended in 10 ml DMEM (containing 10% newborn calf serum, 100 U/ml penicillin, 100 U/ml streptomycin) and transferred to rat IgG-coated culture dishes at 37°C in a 5% CO_2 incubator for 1-2 hours to remove macrophages, lymphocytes and neutrophils by differential adherence. The unattached cells were centrifuged again at 130 *g* for 8 min at 4°C, and the cell pellets were resuspended in DMEM. The resulting cells (>90% purity and >95% viability) were plated at a density of 5×10^5/ml in culture dishes and incubated in 5% CO_2/95% O_2 at 37°C for 24 h. The AT II cells were identified with a tannic and polychrome stain /4/.

Assays

Phosphatidylcholine synthesis

AT II cells were cultured for 24 h in primary medium and digested with elastase. The cells were gently plated at a density of 10^6/ml in 96-well culture dishes and further cultured for 8 h. The cells were washed with DMEM and randomly divided into six groups: control group, 0.28 mM Iso group, 2.8 mM Iso group, 75 μM H_2O_2 group, 75 μM H_2O_2 + 0.28 mM Iso group, and 75 μM H_2O_2 + 2.8 mM Iso group (n=8). After adding the relevant experimental factors and ^{3}H-choline chloride (1 μCi/ml), each group of cells was cultured for 3 h and then removed from the incubation medium, washed 3 times with Hank's solution and digested with elastase. At the end of digestion, newborn calf serum was added to stop the reaction, and the cells were collected

onto glass fiber papers. The papers were washed 5 times with 0.9% NaCl solution to eliminate the unbound ^{3}H-choline chloride, and the cells were fixed with trichloroacetic acid. The papers with cells were transferred to a scintillation bottle, scintillation solution was added, and tracer activity was determined by liquid scintillation counting. ^{3}H-Choline chloride incorporation was expressed in cpm.

Phosphatidylcholine secretion

AT II cells were cultured for 24 h in primary medium and digested with elastase. The cells were gently plated at a density of 10^6/ml in 96-well culture dishes and further cultured for 8 h. After washing with DMEM, the cells were randomly divided into six groups as above (n=8). ^{3}H-Choline chloride (1 μCi/ml) was added to each group and incubated for 24 h. The cells were washed 3 times with fresh incubation medium to eliminate the unbound ^{3}H-choline chloride, and then washed once with Hank's solution. Fresh incubation medium and the relevant experimental factors were added and the cells incubated for 3 h. After incubation, 100 μl medium from each well was transferred to a scintillation bottle, 1 ml of scintillation solution was added, and tracer activity was determined by liquid scintillation counting. PC secretion was expressed in cpm.

Data analysis

Results are presented as means ± SE. Data were analyzed by Student's t-test, and differences were considered significant when $p < 0.05$.

RESULTS

Effect of isoflurane on phosphatidylcholine synthesis

On exposure to 0.28 mM Iso or 2.8 mM Iso, the synthesis of PC in AT II cells was significantly inhibited compared with normal cells ($p < 0.05$, $p < 0.01$, respectively); with exposure to 75 μM H_2O_2 the synthesis of PC in AT II cells was notably inhibited compared with normal cells ($p < 0.01$); 0.28 mM Iso or 2.8 mM Iso markedly aggravated the inhibition by 75 μM H_2O_2 of the synthesis of PC ($p < 0.05$, $p < 0.01$, respectively). Details are shown in Table 1.

TABLE 1

Effects of isoflurane (Iso) on phosphatidylcholine (PC) synthesis

	PC synthesis (cpm)	
	Normal cells	**H_2O_2-treated cells**
Control	4928 ± 1770	605 ± 126[a]
0.28 mM Iso group	2886 ± 1474[b]	470 ± 71[c]
2.8 mM Iso group	2560 ± 1345[bb]	395 ± 66[cc]

[a] $p < 0.01$ compared with normal; [b] $p < 0.05$, [bb] $p < 0.01$ compared with normal controls; [c] $p < 0.05$, [cc] $p < 0.01$ compared with H_2O_2-treated controls.

Effect of isoflurane on phosphatidylcholine secretion

On exposure to 75 μM H_2O_2, the secretion of PC in AT II cells was notably inhibited compared with normal cells ($p < 0.05$); 0.28 mM Iso or 2.8 mM Iso did not inhibit the secretion of PC in AT II cells compared with normal cells ($p > 0.05$) and did not aggravate the inhibition by 75 μM H_2O_2 of the secretion of PC ($p > 0.05$). Details are shown in Table 2.

TABLE 2

Effects of isoflurane (Iso) on phosphatidylcholine (PC) secretion

	PC secretion (cpm)	
	Normal cells	**H_2O_2-treated cells**
Control	488 ± 207	279 ± 61[a]
0.28 mM Iso group	416 ± 228	294 ± 90
2.8 mM Iso group	399 ± 173	250 ± 68

[a] $p < 0.05$ compared with normal.

DISCUSSION

Pulmonary surfactant (PS) synthesized by AT II cells consists of more than 90% phospholipids, of which over 80% is phosphatidylcholine (PC). Thus, the incorporation of ^{3}H-choline chloride as a precursor of PC is commonly used to evaluate the synthesis and secretion of PS /5,6/.

Crim *et al.* /7/ reported that 75 mM H_2O_2 decreased the synthesis of PS by inhibiting the activities of phosphorylcholine cytidyltransferase (CTP) and cholinephosphotransferase (CPT), which are two key enzymes in PS synthesis. H_2O_2 inhibits the activities of CTP and CPT by reducing the ATP content in AT II cells. Molliex *et al.* /1,2/ found that halothane also decreased the synthesis of PS and reduced the ATP content in AT II cells. In addition, halothane and isoflurane increased pulmonary artery endothelial cell sensitivity to oxidant-mediated injury and aggravated lung injury /8/. Isoflurane is a halogenated anesthetic similar to halothane, but the effect of isoflurane on pulmonary surfactant metabolism has not been previously investigated.

In the present study, we found that 75 μM H_2O_2 decreased the synthesis of PC, similar to the results of Crim and Simon /8/, and reduced the secretion of PC. Isoflurane itself decreased only the synthesis of PC, and aggravated the inhibition by 75 μM H_2O_2 of the synthesis of PC. Gao *et al.* /9/ reported that 0.28 mM and 2.8 mM isoflurane is equal to a blood concentration of 1 and 10 MAC (minimal alveolar concentration), respectively. We showed in this study that Iso itself may inhibit the synthesis of PC of AT II cells *in vitro*.

In conclusion, our findings suggest that the application of isoflurane might decrease the synthesis of PS and aggravate existing lung injury, especially under peroxidation conditions, such as ischemia/reperfusion, trauma, shock, intoxication, severe systemic infection, etc. The mechanism of inhibition by isoflurane of the synthesis of PC needs further investigation.

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