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Protective effects of *S*-methylisothiourea sulfate on different aspiration materials-induced lung injury in rats

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Summary

Objectives: The aim of this study was to evaluate the efficiency of inducible nitric oxide synthase (iNOS) specific inhibitor, *S*-methylisothiourea sulfate (SMT) in preventing lung injury after different pulmonary aspiration materials in rats.
Material and methods: The experiments were performed in 80 Sprague–Dawley rats, ranging in weight from 220 to 250 g, randomly allotted into one of the eight groups ($n = 10$): normal saline (NS, control), Biosorb Energy Plus (BIO), sucralfate (SUC), hydrochloric acid (HCl), NS + SMT treated, BIO + SMT treated, SUC + SMT treated, and HCl + SMT treated. NS, BIO, SUC, HCl were injected in to the lungs in a volume of 2 ml/kg. The rats received twice daily intraperitoneal injections of 20 mg(kg day) SMT (Sigma Chemical Co.) for 7 days. Seven days later, rats were killed, and both lungs in all groups were examined immunohistochemically and histopathologically.
Results: Our data show that SMT inhibits the inflammatory response significantly reducing ($p < 0.05$) peribronchial inflammatory cell infiltration, alveolar septal infiltration, alveolar edema, alveolar exudate, alveolar histiocytes, interstitial fibrosis, granuloma, and necrosis formation in different pulmonary aspiration models. Furthermore, our data suggest that there is a significant reduction in the activity of iNOS and arise in the expression of surfactant protein D in lung tissue of different pulmonary aspiration models with SMT therapy.

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Conclusion: It was concluded that SMT treatment might be beneficial in lung injury, therefore shows potential for clinical use.

1. Introduction

The most serious complication of enteral nutrition is pulmonary aspiration. Aspiration of gastric contents continues to be a concern of physicians involved in the care of critically ill patients [1]. Gastric contents aspiration is one of the major causes of acute respiratory distress syndrome. The severity of the histopathologic changes resulting in lung injury based on several factors such as pH value, volume and content of the aspirated material, the response of the patients and presence of particulate gastric matter [2,3]. Several animal models have been developed to investigate the mechanisms, characteristics, and pathophysiology of aspiration lung injury. The mechanism of lung injury owing to pulmonary aspiration is not definetely understood. Several inflammatory mediators have been implicated in the development of lung inflammation such as nitrite and nitrate. At the same time, little knowledge is present about the histopathological benefits of nitric oxide synthase (NOS) inhibitors on the development of different gastric contents induced lung injury [4–6].

The overproduction of nitric oxide (NO) seems to be an important factor in the pathology of the inflammatory process in lung injury. Selective inhibition of the inducible nitric oxide synthase (iNOS) has been effective in reducing tissue damage in several models of inflammation [7]. The authors hypothesize that NO generated by iNOS plays a key role in lung injury due to aspiration pneumonia [8]. We examined, with the use of selective iNOS inhibitor, *S*-methylisothiourea sulfate (SMT), whether NO contribute to the development of lung injury in different aspiration materials. We compared the histopathologic and inflammatory effects of different materials during the period of aspiration in rats.

2. Material and methods

The Ethical Committee of Trakya University approved all animal procedures and the experimental protocol. Efforts were made to minimize animal suffering and reduce the number of animals used in experimental groups.

2.1. Animals

The experiments were performed in 80 Sprague–Dawley rats, ranging in weight from 220 to 250 g. Rats were provided by the Experimental Research Center of the Medical Faculty of Trakya University. The rats were kept in a windowless animal quarter where temperature ($22 \pm 2\ ^{\circ}C$) and illumination were automatically controlled (light on at 07 a.m. and off at 09 p.m. 14 h light/10 h dark cycle). Humidity ranged from 50% to 55%. Animals were given free access to diets and water until the night before the experiment, when they were fasted.

2.2. Experimental protocol

The experiments were performed in 80 Sprague–Dawley rats, ranging in weight from 220 to 250 g, randomly allotted into one of the eight groups ($n = 10$): normal saline (NS, control), Biosorb Energy Plus (BIO; Nutricia, Zoetermeer, The Netherlands), sucralfate (SUC; 0.7%; pH 5.3 [1], Antepsin, Bilim İlaç Sanayi ve Ticaret A.Ş, Istanbul, Turkey), hydrochloric acid (HCl; 0.1N, pH 1.25), NS + SMT treated, BIO + SMT treated, SUC + SMT treated, and HCl + SMT treated. NS, BIO, SUC, HCl were injected into the lungs in a volume of 2 ml/kg. The rats received twice daily intraperitoneal injections of 20 mg(kg day) SMT (Sigma Chemical Co.) for 7 days. Seven days later, all rats were sacrificed with intraperitoneal injection of ketamine hydrochloride, and both lungs were examined immunohistochemically and histopathologically for lung injury. After instillation of NS, BIO, SUC and HCl, the tuberculin syringe was removed, and the tracheal incision was repaired with a 6–0 Ethilon suture. Animals were observed until they recovered from anesthesia.

2.3. Surgical procedure

The rats were anesthetized with an intraperitoneal injection of ketamine hydrochloride (100 mg/kg) and allowed to breathe spontaneously throughout the entire experimental protocol. The animals were placed in a supine position with the extremities pulled caudally to facilitate exposure of the trachea. The trachea was exposed through an anterior neck incision and a direct puncture with a 20-gauge needle on a 1-mL tuberculin syringe was performed two to four tracheal rings below the larynx.

2.4. Histopathologic evaluation

The lung specimens were individually immersed in 10% neutral buffered formalin, dehydrated in alcohol