IMMUNOMODULATORY EFFECTS OF SEVOFLURANE ANESTHESIA IN AN EXPERIMENTAL MODEL OF CHRONIC ASTHMA

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ABSTRACT

Background: Sevoflurane is a volatile agent routinely used in anesthesia and recent investigations have reported its anti-inflammatory effects in acute lung injury experimental models. However, there are no studies, so far, examining the immunomodulatory effects of sevoflurane in a chronically inflamed and remodeled airway, such as that found in asthma. The aim of this study is to define the immunomodulatory effects of sevoflurane in a murine model of chronic asthma. For this purpose, lung histology and gene expression of pro- and anti-inflammatory cytokines were analyzed. Methods: Twelve BALB/c mice (20-25 g) were randomly divided into four groups. In OVA groups, mice were sensitized with ovalbumin and exposed to repeated ovalbumin challenges. In SAL groups, mice received saline using the same protocol. Twenty-four hours after the last challenge, the animals were anesthetized with pentobarbital sodium (PENTO, 20 mg/kg i.p.) or sevoflurane (SEVO). We measured interleukin (IL)-6, tumour necrosis factor (TNF)-α, tumor growth factor (TGF)-β, interferon (IFN)-γ, and macrophage migration inhibitory factor (MIF) mRNA expression in right lung tissue using ribonuclease protection assay (RPA). Results: Detailed quantitation by densitometry analysis showed that OVASEVO group presented lower levels of IL-6 (35%), TNF-α (46%), and TGF-β (39%) than OVAPENTO group ($P < 0.001$). No significant differences were detected in INF-γ and MIF levels. Conclusion: Sevoflurane anesthesia reduced the inflammatory response in chronic allergic asthma.

INTRODUCTION

- The use of volatile agents have been recommended for general anesthetics techniques in patients with obstructive airway diseases and even to treat status asthmaticus.
- Sevoflurane is a inhalational anesthetic largely used in clinical practice since it provides faster induction and awakening, and causes less airway irritation than other inhaled agents.
- Asthma is a chronic inflammatory disease that compromises not only central airways but also distal airways and lung parenchyma.
- Recently, we observed that sevoflurane anesthesia induced dilation in central and distal airways, lessening alveolar collapse in experimental chronic allergic asthma.
- These changes may be attributed to the effects of sevoflurane anesthesia modulating the inflammatory process.
- Although there are some reports describing the anti-inflammatory effects of sevoflurane in sepsis, so far, no study examined these effects in a chronically inflamed and remodeled airway, such as that found in asthma.

AIM

- To determine the effects of sevoflurane anesthesia on gene expression of pro- and anti-inflammatory cytokines in an experimental model of chronic allergic asthma.
- For this purpose, cytokine RNAm levels were determined.

METHODS

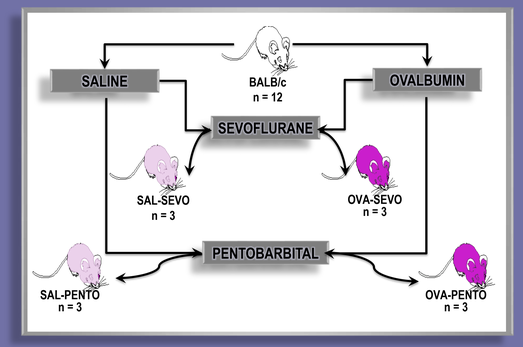


Figure 1. Experimental Groups

METHODS

Animal Preparation

- Twelve BALB/c mice (20-25 g) were randomly divided into four groups (Figure 1).
- In OVA groups, mice were sensitized with ovalbumin and exposed to repeated ovalbumin challenges (Figure 2).
- In SAL groups, mice received saline using the same protocol.
- Twenty-four hours after the last challenge, three mice of OVA group (OVA-SEVO group) and three of SAL group (SAL-SEVO group) were anesthetized with sevoflurane.
- Similarly, three animals of OVA group (OVA-PENTO group) and three of SAL group (SAL-PENTO group) were sedated with diazepam (1 mg i.p.) and anesthetized with pentobarbital sodium (20 mg/kg i.p.).
- Animals were then tracheotomized, paralyzed with vecuronium bromide (100 µg/kg i.v.), and mechanically ventilated during 30 minutes.
- At the end of experiments, lungs were removed *en bloc*.

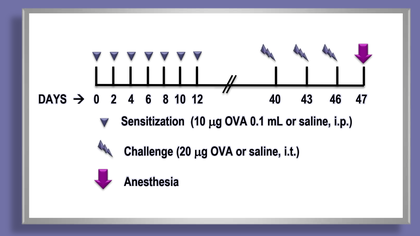


Figure 2. Asthma Protocol

Ribonuclease Protection Assay

- RNA was isolated from lung tissue and hybridized with a mCK-3b Multi-Probe Template Set containing DNA templates for mouse mRNAs (Figure 3).
- The *in vitro* transcription kit and a customized template set [containing mouse IL-6, TNF-α, TGFβ, INFγ, MIF, the housekeeping gene glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) and L32 (ribosomal RNA)] were used to synthesize a radiolabeled probe set using [α-32P]UTP.
- The quantity of each mRNA was determined by optical densitometry and density of each cytokine mRNA was expressed relative to that of the housekeeping gene GAPDH.

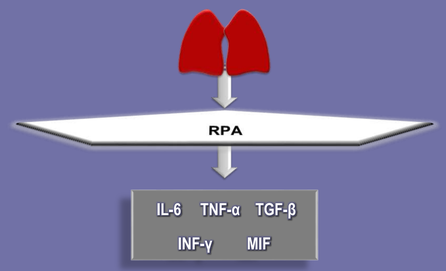
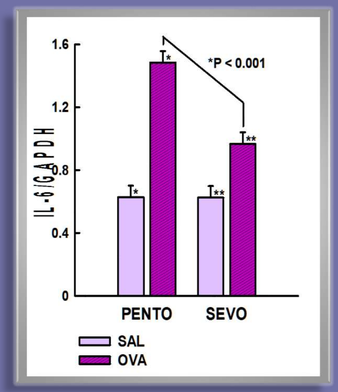


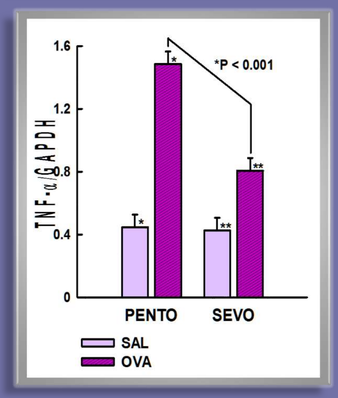
Figure 3. Ribonuclease Protection Assay to determine cytokines mRNA expression.

Statistical Analysis

- SigmaStat 3.1 statistical software package (Jandel Corporation, San Raphael, CA) was used for data analysis.
- Differences among the groups were assessed by two-way ANOVA followed by Holm-Sidak test.
- A P value < 0.05 was considered significant.

RESULTS





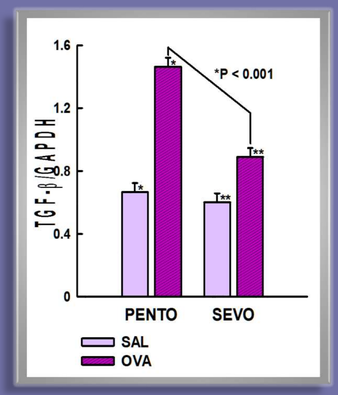
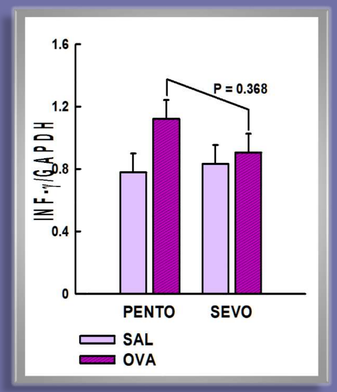


Figure 4. Cytokine mRNA expression. Bars are means + SEM of 3 animals in each group. Mice were sensitized and exposed to repeated challenges with saline (SAL) or ovalbumin (OVA). In PENTO groups, animals were anesthetized with pentobarbital sodium. In SEVO groups, animals were anesthetized with sevoflurane. Density of each cytokine mRNA was expressed relative to that of the housekeeping gene GAPDH. IL-6, TNF-α, and TGF-β were higher in OVA than in SAL groups (*$P < 0.001$ and **$P < 0.05$). OVA-SEVO mice presented lower values of IL-6 (35%), TNF-α (46%), and TGF-β (39%) than OVA-PENTO (*$P < 0.001$).

RESULTS



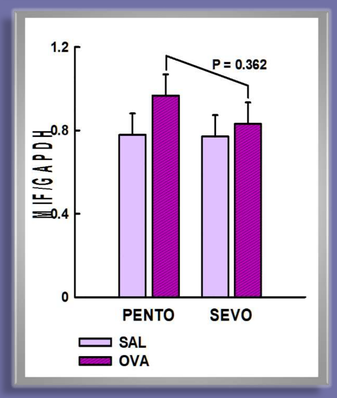


Figure 5. Cytokine mRNA expression. Bars are means + SEM of 3 animals in each group. Density of each cytokine mRNA was expressed relative to that of the housekeeping gene GAPDH. No significant difference were detected in INF-γ and MIF levels between asthmatic (OVA) and non-asthmatic (SAL) animals. Besides, INF-γ and MIF values did not vary after sevoflurane anesthesia ($P > 0.05$).

CONCLUSION

The present experiment disclosed that sevoflurane anesthesia reduced cytokine RNAm expression levels in experimental chronic asthma. Our findings suggest that sevoflurane could be beneficial in reducing lung inflammation in chronic asthma.

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