Veterinary Immunology and Immunopathology 165 (2015) 93–97

Contents lists available at ScienceDirect

Veterinary Immunology and Immunopathology

journal homepage: www.elsevier.com/locate/vetimm

Short communication

Effects of isoflurane and sevoflurane on the neutrophil myeloperoxidase system of horses

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ARTICLE INFO

Article history:
Received 18 November 2014
Received in revised form 10 February 2015
Accepted 26 February 2015

Keywords:
Volatile anaesthetics
Isoflurane
Sevoflurane
Neutrophil
Myeloperoxidase
Oxidative stress

ABSTRACT

Volatile anaesthetics have shown to modulate the oxidative response of polymorphonuclear neutrophils (PMNs). We investigated the effects of isoflurane and sevoflurane on the degranulation of total and active myeloperoxidase (MPO) from horse PMNs and their direct interaction with MPO activity. Whole blood from horse was incubated in 1 and 2 minimal alveolar concentrations (MAC) of isoflurane or sevoflurane for 1 h and PMNs were stimulated with cytochalasin B (CB) plus N-formyl-méthionyl-leucyl-phenylalanine (fMLP). After stimulation, the plasma was collected to measure total and active MPO by enzyme-linked immunosorbent assay (ELISA) and specific immunological extraction followed by enzymatic detection (SIEFED) respectively. The effects of 1 and 2 MAC of isoflurane and sevoflurane on the peroxidase and chlorination activity of pure MPO were assessed by fluorescence using Amplex red and 3′-(p-aminophenyl) fluorescein (APF) respectively and in parallel with a SIEFED assay to estimate the potential interaction of the anaesthetics with the enzyme. Although isoflurane and sevoflurane had inconsistent effects on total MPO release, both volatile agents reduced active MPO release and showed a direct inhibition on the peroxidase and the chlorination activity of the enzyme. A persistent interaction between MPO and anaesthetics was evidenced with isoflurane but not with sevoflurane.
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1. Introduction

Myeloperoxidase (MPO) is a pro-oxidant haemic enzyme from azurophilic granules of polymorphonuclear neutrophils (PMNs) which represent a pivotal component of the neutrophil machinery involved in the clearance of host damaged tissues and exogenous invading microorganisms (Deby-Dupont et al., 1999). During neutrophil phagocytosis, most of these processes are confined to the phagolysosome, leading to effective intracellular destruction of tissue debris and microbial pathogens. However, activated PMNs also release MPO in the extracellular space, which results in collateral damages to healthy host structures. The cytotoxic potential of MPO is attributable to its dual peroxidase and chlorination activities able to inactivate multiple biological molecules including proteins, lipids and nucleic acids. It is now recognized that extracellular MPO not only represents a marker of neutrophil activation but also plays a role in collateral injuries encountered during the course of inflammatory diseases (Davies

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http://dx.doi.org/10.1016/j.vetimm.2015.02.010
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et al., 2008; Klebanoff, 2005). Hence, our group demonstrated that MPO has a pathophysiological significance in various clinical conditions associated with acute PMNs stimulation like *i.e.* intestinal ischaemia (Grulke et al., 2008), laminitis (de la Rebiere et al., 2008) or sustained physical exercise (Serteyn et al., 2010).

Volatile anaesthetics such as sevoflurane and isoflurane are commonly used in veterinary anaesthesiology to provide anaesthesia during surgical procedures. Numerous studies have shown that these volatile anaesthetics have anti-inflammatory and organ protective effects partly attributable to an inhibition of neutrophil activation (Hu et al., 2005). We developed *in vitro* experimental models to study the effect of volatile anaesthetics on the degranulation of total and active MPO in whole blood and their interaction with the activity of pure MPO. We previously demonstrated that sevoflurane inhibits the degranulation and the activity of equine MPO (Minguet et al., 2013). However, recent investigations have suggested that the immunomodulatory properties of volatile anaesthetics are pharmacological class effects imputable to their common halogenated carbon groups (Urner et al., 2011). Therefore, we hypothesized that isoflurane, a halogenated anaesthetic chemically and pharmacologically related to sevoflurane, could similarly inhibit the horse MPO system. The objective of this study was to evaluate the effects of isoflurane and sevoflurane on the degranulation of total and active MPO from unstimulated and stimulated horse PMNs in a whole blood model. In addition, we evaluated the potential interaction of the volatile agents with the peroxidase and chlorination cycles of pure solubilized MPO.

2. Material and methods

2.1. Blood samples

Samples of whole blood were collected from adult healthy horses ($n = 14$) by jugular venipuncture in EDTA tubes in compliance with institutional ethical guidelines for animal investigations. Blood samples were put in a six-well (2 mL per well) cell culture plate (Nunclon delta surface; Nunc, Denmark) before exposure to isoflurane or sevoflurane.

2.2. Exposure to volatile anaesthetics

Isoflurane and sevoflurane (Forene® and Sevorane®, Abbott Laboratories, Wavre, Belgium) were administered to reproduce duration and doses susceptible to be used in the practice of clinical anaesthesia. According to a minimal alveolar concentration (MAC) of 1.3% for isoflurane and 2.3% for sevoflurane, the volatile anaesthetics were administered at 1 and 2 MAC for 1 h at 37 °C as previously described (Minguet et al., 2013). Briefly, treated blood samples were placed in a tight incubator chamber (Billups-Rothenberg, Del Mar, CA) and isoflurane or sevoflurane was delivered *via* a specific calibrated vaporizer (Dräger, Lübeck, Germany) supplied with air as the carrier gas and anaesthetic concentrations were measured and adjusted with a Capnomac Ultima® multigas analyzer (Datex Ohmeda, Helsinki, Finland). Untreated control blood samples were placed in a tight incubator filled with air at 37 °C and without volatile anaesthetics.

2.3. Activation of neutrophils in whole blood

Polymorphonuclear neutrophils were stimulated as already described with solutions of cytochalasin B (CB) in 100% dimethylsulfoxide (DMSO) and N-formylmethionyl-leucyl-phenylalanine (fMLP) in 10% DMSO to reach the final concentration of 5 μg/mL CB and 10^{-6} mol/L fMLP in whole blood (Ceusters et al., 2012). The effects of the vehicle DMSO solutions were also studied as controls. Our method for PMNs activation consisted of an incubation of whole blood with CB (5 μg/mL) as a priming agent under 1-h exposure to isoflurane, sevoflurane or air, followed by an additional activation of 30 min with fMLP (10^{-6} mol/L). Control tests were performed in parallel with unstimulated blood samples. Samples were poured in culture plates with large well diameter (5 cm) and all experiments were conducted on a rotating base allowing a slight stirring of the content to improve the diffusion of the volatile anaesthetics and stimulants, and to avoid cell sedimentation.

2.4. Measurement of total and active MPO released in plasma

After the 90-min anaesthetic incubation and neutrophil stimulation, blood samples were centrifuged ($450 \times g$, 10 min) and plasma was collected to measure the total and active MPO. An ELISA assay (Equine MPO ELISA, BioPtis, Belgium) was used to measure total equine MPO in plasma (Franck et al., 2005). Before the ELISA assays, the plasma was diluted 100-fold with 20 mmol/L PBS buffer at pH 7.4 and added with 5 g/L BSA and 0.1% Tween 20. The active MPO released by neutrophils in plasma was measured with a Specific Immunological Extraction Followed by Enzymatic Detection (SIEFED) developed for the specific quantification of active equine neutrophil MPO (Franck et al., 2006). Briefly, this technique consists of the extraction of MPO from plasma by its capture on specific immobilized antibodies, followed by a washing step to eliminate non-specifically bound compounds and interfering substances. Finally, the peroxidase activity of MPO is detected *in situ* by Amplex red as a fluorogenic substrate in the presence of a nitrite-based amplifier system. For the SIEFED assay, plasma samples were not diluted.

2.5. Measurement of the activity of purified MPO

To study the direct effect of the volatile anaesthetics on the peroxidase and chlorination activity of MPO, we added the specific substrates required to detect enzymatic reactions immediately after exposure of a purified MPO solution to isoflurane or sevoflurane. Purified equine MPO was diluted in phosphate buffer (50 mmol/L, pH 7.5) to a final concentration of 230 ng/mL and incubated in microplate wells (100 μL per well) in air or in air plus 1 and 2 MAC of isoflurane or sevoflurane for 1 h at 37 °C and under a slight stirring. Thereafter, the peroxidase activity of MPO was directly measured by adding 100 μL of a 40 mmol/L Amplex red solution freshly prepared

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