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Research article

Effects of xenon and isoflurane on apoptosis and inflammation in a porcine myocardial infarction model

Sasa Sopka[a], Christine Mertens[b], Anna Bettina Roehl[a], Katharina Schiffl[c], Rolf Rossaint[a], Irmgard Classen-Linke[b,*]

[a] Department of Anaesthesiology, RWTH Aachen University, Pauwelsstraße 30, 52074 Aachen, Germany
[b] Institute of Molecular and Cellular Anatomy, RWTH Aachen University, Wendlingweg 2, 52057 Aachen, Germany
[c] Institute of Medical Statistics, RWTH Aachen University, Pauwelsstraße 30, 52074 Aachen, Germany

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SUMMARY

Volatile anaesthetics can reduce the infarction size in myocardial tissue when administered before and during experimentally induced ischaemia. The aim of this study was to investigate whether xenon is beneficial compared to isoflurane in limiting myocardial tissue apoptosis and inflammation induced by experimental ischaemia–reperfusion injury in a porcine right ventricular infarction model.

Twenty-one animals used for this study randomly received isoflurane, xenon or thiopental, (n = 6–8 per group). Myocardial infarction was induced for 90 min, followed by reperfusion for 120 min. Tissues from the left and right ventricles were removed from the sites of infarction, reperfusion and remote areas, and processed for immunohistochemistry. Apoptosis (caspase-3 staining) and neutrophilic infiltration (naphthol AS-D chloroacetate-specific esterase) were assessed and evaluated. Statistical analysis was performed using an ANOVA of repeated measures.

Density of apoptotic cells were higher in tissues from animals that were anesthetized with xenon. This effect was significant in comparison to isoflurane (p = 0.0177). Neutrophilic infiltration was significantly higher in the right compared to the left ventricle (p < 0.001), whereas no significant differences in the number of granulocytes based on the anaesthetic regime or the different tissue areas were found.

We conclude that xenon, in the early phase of ischaemia and reperfusion, induces a significant increase in apoptosis compared to isoflurane. Therefore, clinical use of this anaesthetic in cardiocompromised patients should be taken with care until more long-term studies have been carried out. The increased neutrophilic infiltration in the right vs. the left ventricle indicates the right ventricle being more susceptible to ischaemia–reperfusion injury.

1. Introduction

It is well known that apoptosis and inflammation are common events during ischaemia and can be used as marker for acute ischemic injury. Different studies have shown that apoptosis arises in myocardial infarction, plays an important role in ventricular remodeling and in the clearance of dying neutrophils and other inflammatory cells, as well as in scar formation (Anversa et al., 1998; Dorn, 2009a; Kossmehl et al., 2006; Rodriguez-Calvo et al., 2001; Saraste et al., 1997; Wang et al., 2009b; Zidar et al., 2007). Apoptosis rarely occurs in the vital myocardium (Narula et al., 1996). It can be detected in myocytes in the site of infarction as early as two hours after ischaemia (Anversa et al., 1998). During the early phase of myocardial infarction (i.e., less than 24 h), mainly cardiomyocytes undergo apoptosis, whereas 48 h after ischaemia, also neutrophil granulocytes are affected (Zidar et al., 2006).

Myocardial ischaemia provokes an inflammatory response in the cardiac tissue, which is important for healing, remodeling and scarring (Frangogiannis et al., 2002). Reperfusing the myocardium after ischemic injury improves tissue repair by enhancing the inflammatory response (Frangogiannis et al., 2002), but reperfusion itself may also result in tissue injury (Gottlieb et al., 1994; Jugdutt, 1997; Reimer et al., 1993; Richard et al., 1995). The size of myocardial infarction and reperfusion injury were shown to be influenced by the anaesthetic regime (Hein et al., 2008b). When administered prior to ischaemia, several volatile anaesthetics and xenon can limit infarction size in the left ventricle (Baumert et al., 2007; Cason et al., 1997; Symons and Myles, 2006). Xenon has been found to limit myocardial infarction size in rabbits (Preckel et al., 2000) and in a porcine model of left and right ventricular infarction (Baumert et al., 2005, 2007; Hein et al., 2008a,b). Due to its minimal hemodynamic side effects and its potential role in cardio protection (Hecker et al.,

* Corresponding author. Tel.: +49 (0) 241 80 88 924; fax: +49 (0) 241 80 82 508.
E-mail address: iclassen-linke@ukaachen.de (I. Classen-Linke).

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2004; Reyle-Hahn and Rossaint, 2000; Rossaint et al., 2003; Schroth et al., 2002), xenon has been suggested to be an ideal anaesthetic for patients with compromised cardiac function.

For clinical usage in anaesthesia it is of high relevance to further investigate the effects of xenon for cardiac compromised patients in relation to apoptosis and inflammatory reaction. The purpose of the present study has been to test the hypothesis that xenon is beneficial compared to isoflurane in limiting myocardial tissue apoptosis and inflammation induced by experimental ischaemia–reperfusion injury in both ventricles of the heart.

2. Materials and methods

The experimental protocols and procedures used in this study were approved by the local animal care committee and the governmental animal care office (No. 50.203.2-AC 38, 4/05; Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen, Recklinghausen, Germany) and were followed according to the "Guide for the Care and Use of Laboratory Animals," National Academy Press, Washington D.C., 1996.

We collected samples from 24 German land-race pigs (separate cohorts, $n = 8$ per group) weighing 35.03 ± 2.25 kg (mean ± SD). Three animals, one of the xenon- and two of the isoflurane-anesthetized group, could not be analyzed because of failed tissue fixation and embedding. The general anaesthetic and surgical procedures were performed as described previously and functional parameters have been assessed (Hein et al., 2008b, 2010).

2.1. Experimental protocol

Using a sealed envelope system, the animals were randomly assigned to one of three groups: a control group, which was subjected to myocardial ischaemia with only thiopental application; and two treatment groups, which were subjected to myocardial ischaemia with the additional application of 0.55 MAC xenon (equal to 70 vol%) or isoflurane (equal to 0.9 vol%) beginning 1 h before the induction of ischaemia and until the end of the protocol with a 50% reduced thiopental dosage (Fig. 1).

After 60 min of adjunct therapy with xenon or isoflurane, the right coronary artery (RCA) was occluded for 90 min. After 120 min of reperfusion, the RCA was reoccluded to stain perfused myocardium with Evans Blue. Upon cardiac arrest, both atria were opened and the heart was dissected. The ventricle was immediately cut into six 5–8 mm slices from the apex, perpendicular to the long axis. The slices of myocardium were placed in 1% triphenyl-tetrazolium chloride at 37 °C for 10 min to stain viable tissue (Fishbein et al., 1981) and differentiate the regions of interest: infarcted, reperfused and remote (non-ischemic) myocardium (Fig. 2).

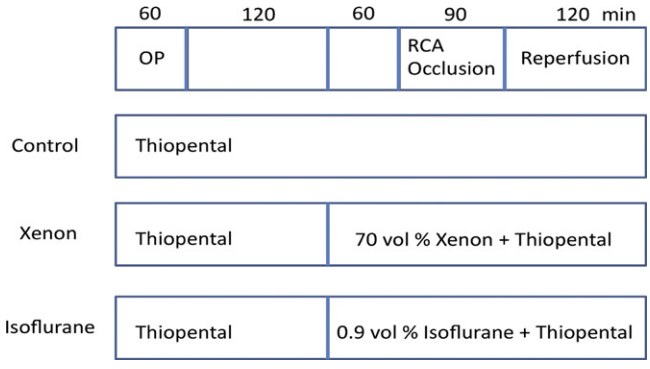

Fig. 1. Diagram of study design. Treatment of groups related to procedures. The isoflurane and xenon group received 0.55 MAC of the drug with a 50% decremental thiopental infusion.

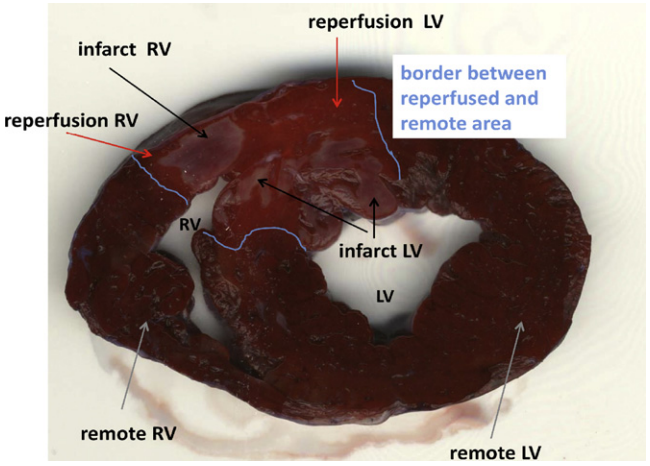

Fig. 2. Midpapillary cross-sectional slice of the heart showing the site of infarction and the reperfusion and remote areas in the right (RV) and left ventricles (LV). The area at risk for infarction was demarked after injection of Evans Blue dye. Non-ischemic, remote regions appear blue. The infarcted site within the area at risk remained bright after incubating the tissue sections with triphenyltetrazolium chloride. Viable cells appear red, indicating the presence of enzymatic dehydrogenase activity. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

2.2. Immunohistochemistry

Tissue samples from the infarcted, reperfused and remote myocardium were collected from the left and right ventricles and fixed overnight at 4 °C in neutral buffered 3.7% formalin. After fixation, the tissues were washed for 1 h in PBS, washed again for 1 h in 0.86% sodium chloride and dehydrated with increasing concentrations of isopropanol before embedding in paraffin. Tissue samples from each of the above mentioned areas were routinely cut, mounted and stained with haematoxylin–eosin for histological evaluation.

2.3. Quantification of apoptotic cells by detection of caspase-3

For immunohistochemistry, 5-μm thick sections were mounted on SuperFrost®/Plus glass slides (Menzel, Braunschweig, Germany), deparaffinized with xylene and rehydrated. For epitope retrieval, the slides were heated with citrate buffer in a steamer. Immunohistochemical staining was performed using the streptavidin–biotin-peroxidase Histostain-Plus® Kit (Zymed® Laboratories, Carlsbad, CA, USA). Endogenous peroxidase activity was blocked by incubating the tissue sections with 0.3% H_2O_2/methyl alcohol for 30 min. The sections were then washed in PBS, and non-specific binding sites were blocked using serum supplied with the kit. The sections were incubated at 4 °C with primary rabbit polyclonal antibody against cleaved caspase-3 (Cell Signaling Technology, Danvers, MA, USA) diluted 1:400 in PBS/1.5% BSA overnight, washed with PBS and incubated at room temperature with secondary antibody according to the manufacturer's instructions. Following three washes in PBS, the sections were placed in streptavidin-peroxidase conjugate solution. The cleaved caspase-3 staining on the tissue sections was visualized by treating the sections with peroxidase, which catalyzes the conversion of the chromogen aminoethylcarbazole (Zymed Laboratories Inc., Berlin, Germany) into a red deposit. Finally, the sections were washed with deionized water and mounted in glycerol gelatin. One section was counterstained with hemalum. For the negative controls, the tissues were incubated with normal mouse