CHANGES IN BIOMECHANICAL PARAMETERS DURING HEART PERFUSION AND AFTER MIDAZOLAM PRE-MEDICATION - EXPERIMENTAL PILOT STUDY

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Key words: Heart ischemia-reperfusion/Midazolam

Background: Midazolam is a frequently used benzodiazepine in anaesthesiology and intensive care.

Aim: The aim of pilot study was to monitor its effect during heart perfusion in the laboratory rat.

Methods: The same groups of animals ($n = 10$). The 1st group was treated with midazolam in a dose of 0.5mg/kg i.p. The 2nd group was a placebo. After i.p. administration of heparine injection of 500 IU dose, the hearts were excised and perfused (modified Langendorf's method). Working schedule: stabilization/ischaemia/reperfusion proceed at intervals of 20/30/60 min. Monitored parameters in isolated heart: left ventricle pressure (LVP), end-diastolic pressure (LVEDP), contractility ($+dP/dt_{\max}$).

Results: The treated hearts showed improved postischemic recovery, reaching LVP values of $92 \pm 6\%$ at the end of the reperfusion, placebo only $61 \pm 7\%$. In placebo hearts LVEDP rose from 10.0 ± 0.5 mmHg to 43 ± 4 mmHg after, in treated animals only about 25 mmHg. The treated hearts improved $+dP/dt_{\max}$ recovery during reperfusion to $91 \pm 8\%$. These values were significantly greater than those obtained from the placebo hearts.

Conclusions: Positive changes in monitored parameters were found in this experimental pilot study. We conclude that the administration of midazolam in laboratory rats has a cardioprotective potential against ischemia-reperfusion induced injury.

INTRODUCTION

Midazolam is a benzodiazepine derivative. It has powerful anxiolytic, amnestic, hypnotic, anticonvulsant, skeletal muscle relaxant and sedative properties. It is considered a fast-acting benzodiazepine, with a short elimination half-life. It is therefore a very useful drug to use for short minor procedures such as dental extraction. Midazolam was first synthesized in 1976 by Fryer and Walser¹.

Like other benzodiazepines, midazolam acts on benzodiazepine receptors to enhance the binding of GABA to the GABA_A receptor which results in inhibitory effects on the central nervous system².

Midazolam is indicated for the acute management of aggressive or delirious patients and also is sometimes used for the acute management of seizures such as status epilepticus. Long term use for the management of epilepsy is not recommended however, due to the significant risk of tolerance which renders midazolam and other benzodiazepines ineffective and as well the significant side-effect of sedation³. In mice given chronic midazolam a slowly evolving tolerance developed to the anticonvulsant properties of midazolam over 15 days, although some anticonvulsant effects were still apparent after 15 days of continued administration⁴.

Midazolam is occasionally used as a hypnotic, especially in hospitals. Like other benzodiazepines, it produces a decrease in delta activity, though the effect of

benzodiazepines on delta may not be mediated via benzodiazepine receptors. Delta activity is an indicator of depth of sleep within non-REM sleep; it is thought to reflect sleep quality, with lower levels of delta sleep reflecting poorer sleep. Thus midazolam and other benzodiazepines cause deterioration in sleep quality. Cyproheptadine may be superior to nitrazepam in the treatment of insomnia as it enhances sleep quality based on EEG studies⁵.

Midazolam is frequently used benzodiazepine in anaesthesiology and intensive care, too.

The aim of the study was to monitor the effects of midazolam during heart perfusion of laboratory rat.

METHODS

The study and its experimental protocol were approved and monitored by the Ethics Committee of Palacký University. The state of health of all animals was inspected regularly several times a day both during the acclimation of the animals and in the course of the whole experiment performed by the working group whose members are holders of the Eligibility Certificate issued by the Central Commission for Animal Protection pursuant to Section 17 of the Czech National Council Act No 246/1992 Coll. on animal protection against maltreatment.

This study was performed on 20 male Wistar SPF (AnLab, Germany) laboratory rats of the same age (6 months) and comparable weight (345 ± 15 gr). The

animals were housed in a standard controlled temperature, fed the standard diet for small laboratory animals, and given water ad libitum. After a recovery period, the animals were divided randomly into 2 groups ($n = 10$).

The first group – treated group – received midazolam in a single dose 0.5 mg/kg by i.p. injection. The second group – the placebo group – received only normal saline solution also by i.p. injection.

The rats were anesthetized with an i.p. injection of anaesthetical mixture (2% Rometar 0.5 ml + 1% Narkamon 10 ml, dose 0.5 ml solution/100 g body weight). After the i.p. heparine injection of 500 IU dose, the hearts were excised and perfused. In all experiments, the modified Langendorff method and the universal apparatus Hugo Sachs Electronic UP 100 (Germany HSE) were used. Schedule: stabilization/ischaemia/reperfusion proceeded at intervals of 20/30/60 min. Biomechanical parameters from isolated heart: left ventricle pressure (LVP), end-diastolic pressure (LVEDP), contractility (dP/dt_{max}) were measured using a ball filled with liquid (8–12 mmHg), inserted through the left atrium in the left ventricle connected to the analog convertor (Isotec HSE, DIF modul HSE)⁶.

RESULTS

See Fig. 1–3.

DISCUSSION

A variety of laboratory and clinical studies clearly indicate that exposure to anaesthetic agents can lead to a marked protection of the myocardium against ischaemia-reperfusion injury. Several changes in the protein structure of the myocardium that may mediate this cardioprotection have been indentified. Ischaemia-reperfusion of the heart occurs in a variety of clinical situations including transplantations, coronary artery bypass grafting and vascular surgery. Ischaemia may also occur during stressful anaesthetic induction. Early restoration of arterial blood flow and measures to improve the ischaemic tolerance of the tissue are the main therapeutic options (i.e. cardioplegia and betablockers). There is increasing evidence that anaesthetic agents interact with the mechanisms of ischaemia-reperfusion injury and protect the myocardium by a preconditioning and postconditioning mechanism^{7–10}.

In 1996, specific protection against myocardial reperfusion injury by halothane was described¹¹. While all previous studies had been unable to discriminate between anti-ischemic effects and effects against reperfusion injury, this study demonstrated for the first time that modification of the reperfusion conditions by administration of a common volatile anaesthetic specifically reduced reperfusion damage. A similar cardioprotective effect was confirmed for enflurane, isoflurane, sevoflurane and desflurane and the noble gas xenon under a variety of ex-

Fig. 1. Left ventricle pressure.

In hearts from placebo animals, LVP recovered up to 61 ± 7 % of pre-ischemic values at the end of the reperfusion. In the midazolam pretreated animals, the hearts showed significantly better post-ischemic recovery, reaching LVP values of 92 ± 6 % at the end of the reperfusion.

Fig. 2. End-diastolic pressure.

In hearts from placebo animals LVEDP rose from 10.0 ± 0.5 to 43 ± 4 mmHg after 60 min of reperfusion. This increase was diminished in the hearts from the midazolam pretreated animals at the end of reperfusion.

Fig. 3. Contractility.

The pretreatment with midazolam improved $+dP/dt_{max}$ recovery during reperfusion to 91 ± 8 % after 60 min of reperfusion. These values were significantly greater than those obtained from placebo hearts.

perimental conditions *in vitro* and *in vivo*; cardioprotection against reperfusion damage was also maintained when the heart was already protected against ischaemic damage by cardioplegic solutions^{12, 13}.

The benzodiazepines have hardly been investigated for myocardial protection. Whether these intravenous anesthetics interfere with neutrophil-endothelium interaction, Ca^{2+} influx and free radical production still needs to be elucidated¹⁴⁻¹⁸. It is known, that peripheral benzodiazepine receptors are present in peripheral tissues such as adrenals, kidney and heart, as well as in the brain^{19, 20}. The peripheral benzodiazepine receptor is different from the central benzodiazepine receptor, which is coupled to GABA receptors and responsible to the classical sedative, anxiolytic and anticonvulsant effect^{21, 22}. Peripheral benzodiazepine receptor is a 169-amino acid protein with five trans-membrane domains associated with the mitochondrial outer membrane^{23, 24} which has been suggested to be involved in the control of several mitochondrial functions including the respiratory chain and ion channel activities, in the regulation of apoptosis, which occurs during cardiac injury^{25, 26} and in the modulation of immune functions, steroidogenesis and neurodegenerative process^{24, 27}.

Midazolam is extensively used as hypnotic and anesthetic in clinical situations²⁸. Midazolam has been reported to impair memory retention at sedative doses in human studies, and retrograde amnesia after recovery from anesthesia with anesthetic doses is often observed in humans²⁹. Midazolam is used in pediatric practice too³⁰. Midazolam benzodiazepines have not been investigated extensively in the adult population and no references were found in the literature to their myocardial effects in pediatric cardiac surgical patients.

In isolated perfused guinea pig hearts subjected to ischaemia midazolam reduced neutrophil adhesion to non-ischaemic control levels. Adhesion of polymorphonuclear neutrophils to the coronary endothelium is a crucial step in the development of ischemic myocardial injury¹⁴.

Midazolam may interfere with Ca^{2+} influx and free radical production but the data are contradictory in this respect³¹. In the study of Papaioannou et al.³² some interesting anesthetic effects on mitochondrial and sarcolemmal $\text{K}^{+}_{\text{ATP}}$ channels were described. This study has showed that barbiturates inhibit mitochondrial $\text{K}^{+}_{\text{ATP}}$ channels while propofol, etomidate and midazolam have no effect on the above proteins and final myocyte survival in rat models. The effect of midazolam on sarcolemmal $\text{K}^{+}_{\text{ATP}}$ channels activity still needs to be elucidated³³.

An important regulator of cardiac function is adenosine. Its cardiac effects are predominately mediated by A_1 and $\text{A}_{2\text{A}}$ receptors. Activation of A_1 receptors reduces oxygen demand by causing a negative chronotropic and dromotropic effect, and by inhibiting catechoamine-stimulated increases in ventricular contractility, whereas activation of $\text{A}_{2\text{A}}$ receptors increases oxygen supply by causing coronary vasodilatation. The physiologic properties of adenosine convey cardioprotection during myocardial ischemia³⁴. Some drugs that weakly inhibited adenosine metabolism reduced the incidence of early cardiac death and myocardial infarction after coronary artery bypass

grafting³⁵. Benzodiazepines reduce adenosine degradation by weakly inhibiting the nucleoside transporter, the major mechanism whereby the effect of adenosine is terminated through reuptake into cells and reincorporation into the intracellular nucleoside pool. In the functional studies, benzodiazepines augmented the negative inotropic effect of exogenous adenosine in guinea pig atrial strips^{36, 37}. Binding and functional studies have been conducted in cardiac tissue for the most commonly used benzodiazepine in the perioperative setting, midazolam. Seubert et al.³⁸ showed that midazolam selectively potentiates the $\text{A}_{2\text{A}}$ receptor-mediated effects of adenosine.

From the results of our experiment it can be deduced that the administration of midazolam in the laboratory rats has cardioprotective potential against ischemia-reperfusion induced injury in rat hearts. This effect is demonstrated in the functional parameters of hearts, LVP, LVEDP and $+\text{dP}/\text{dt}_{\text{max}}$.

From recent studies there is increasing evidence that cardioprotection by anaesthetic agents can be elicited in the clinical setting and may add to other organ protection strategies. Thus, it is conceivable that the choice of anaesthetic drug may have an impact on patient outcome in ischemia-reperfusion situations. However, this still has to be confirmed by large studies that examine definitive outcome parameters.

REFERENCES

1. Walser A, Zenchoff G, Fryer RI. Quinazolines and 1,4-benzodiazepines. 7,5,7-hydroxyaminobenzodiazepines and derivatives. *J Med Chem* 1976; 19:1378-81.
2. Skerritt JH, Johnston GA. Enhancement of GABA binding by benzodiazepines and related anxiolytics. *Eur J Pharmacol* 1983; 89:193-98.
3. Isojärvi JI, Tokola RA. Benzodiazepines in the treatment of epilepsy in people with intellectual disability. *J Intellect Disabil Res* 1998; 42:80-92.
4. Garratt JC, Gent JP, Feely M, Haigh JR. Can benzodiazepines be classified by characterising their anticonvulsant tolerance-inducing potential? *Eur J Pharmacol* 1988; 145:75-80.
5. Tokunaga S, Takeda Y, Shinomiya K, Hirase M, Kamei C. Effects of some H1-antagonists on the sleep-wake cycle in sleep-disturbed rats. *J Pharmacol Sci* 2007; 103:201-6.
6. Kozlovski VI, Vdovichenko VP, Chlopicki S, Malci SS, Praliyev ZD, Zcilikbayev OT. Antiarrhythmic profile and endothelial action of novel decanhydroquinoline derivatives. *Pol J Pharmacol* 2004; 56:767-74.
7. Yellon DM, Downey JM. Preconditioning the myocardium: from cellular physiology to clinical cardiology. *Physiol Rev* 2003; 83:1113-51.
8. Vaage J, Valen G. Preconditioning and cardiac surgery. *Ann Thorac Surg* 2003; 75:S709-14.
9. Mullenheim J, Ebel D, Bauer M, Otto F, Heinen A, Frassdorf J, Preckel B, Schlack W. Sevoflurane confers additional cardioprotection after ischemic late preconditioning in rabbits. *Anesthesiology* 2003; 99:624-31.
10. Zaugg M, Lucchinetti E, Uecker M, Pasch T, Schaub MC. Anaesthetic and cardiac preconditioning. Part I. Signalling and cytoprotective mechanisms. *Br J Anaesth* 2003; 91:551-65.
11. Schlack W, Hollmann M, Stunneke J, Thämer V. Effect of halothane on myocardial reoxygenation injury in the isolated rat heart. *Br J Anaesth* 1996; 76:860-67.

12. Preckel B, Schlack W. Effect of anesthetic on ischemia-reperfusion injury of the heart. In: Vincent JL, ed. Yearbook of intensive care and emergency medicine. Berlin: Springer; 2002.
13. Oguchi T, Kashimoto S, Yamaguchi T, Nakamara T, Kumazawa T. Comparative effects of halothane, anflurane, isoflurane and sevoflurane on function and metabolism in the ischemic rat heart. *Br J Anaesth* 1995; 74:569-75.
14. Szekely A, Heindl B, Zahler S, Conzen PF, Becker BF. Nonuniform behavior of intravenous anesthetics on postschismic adhesion of neutrophils in the guinea pig heart. *Anesth Analg* 2000; 90:1293-1300.
15. Buljubasic N, Marijic J, Berczi V, Supan DF, Kampine JP, Bosnjak ZJ. Differential effects of etomidate, propofol, and midazolam on calcium and potassium channel currents in canine myocardial cells. *Anesthesiology* 1996; 85:1092-99.
16. Galley HF, Dubbels AM, Webster NR. The effect of midazolam and propofol on interleukin-8 from human polymorphonuclear leucocytes. *Anesth Analg* 1998; 86:1289-93.
17. Nishina K, Akamatsu H, Mikawa K, Shiga M, Maekawa N, Obara H, Niwa Y. The inhibitory effects of thiopental, midazolam and ketamine on human neutrophil functions. *Anesth Analg* 1998; 86:159-65.
18. Nonaka A, Kashimoto S, Imamura M, Furuya A, Kumazawa T. Mechanism of the negative inotropic effect of midazolam and diazepam in cultured foetal mouse cardiac myocytes. *Eur J Anaesthesiol* 1997; 14:481-87.
19. Anholt RR, De Sousa EB, Oster-Granite ML, Snyder SH. Peripheral-type benzodiazepine receptors: autoradiographic localization in whole-body section of neonatal rats. *J Pharmacol Exp Ther* 1985; 233:517-26.
20. De Sousa EB, Anholt RR, Murphy KMM, Snyder SH, Kuhar MJ. Peripheral-type benzodiazepine receptors in endocrine organs: autoradiographic localization in rat pituitary, adrenal, and testis. *Endocrinology* 1985; 116:567-73.
21. Gavish M, Katz Y, Bar-Ami S, Weizman R. Biochemical, physiological and pathological aspects of the PBR. *J Neurochem* 1992; 58:1589-1601.
22. McEnery MW, Snowman AM, Trifiletti RR, Snyder SH. Isolation of the mitochondrial benzodiazepine receptor: association with the voltage-dependent anion channel and the adenine nucleotide carrier. *Proc Natl Acad Sci USA* 1992; 89:3170-74.
23. Liazun EJ, Delmas P, Shire D, Ferrara P. Topological analysis of the peripheral benzodiazepine receptor in yeast mitochondrial membranes supports a five-transmembrane structure. *J Biol Chem* 1998; 273:2146-52.
24. Li J, Wang J, Zeng Y. Peripheral benzodiazepine receptor ligand, PK 11195 induces mitochondria cytochrome c release and dissipation of mitochondria potential via induction of mitochondria permeability transition. *Eur J Pharmacol* 2007; 560:117-22.
25. Bono F, Lamarche I, Prabonnaud V, Le Fur G, Herbert JM. Peripheral benzodiazepine receptor agonists exhibit potent antiapoptotic activities. *Biochem Biophys Res Commun* 1999; 265:457-61.
26. Leducq N, Bono F, Sulpice T, Vin V, Janiak P, Le Fur G, O'Connor SE, Herbert JM. Role of peripheral benzodiazepine receptors in mitochondrial, cellular and cardiac damage induced by oxidative stress and ischemia-reperfusion. *J Pharmacol Exp Ther* 2003; 306:828-37.
27. Galiegue S, Tinel N, Casellas P. The peripheral benzodiazepine receptor: a promising therapeutic drug target. *Curr Med Chem* 2003; 10:1563-72.
28. Semba K, Adachi N, Arai T. Facilitation of serotogenic activity and amnesia in rats caused by intravenous anesthetics. *Anesthesiology* 2005; 102:616-23.
29. Veselis RA, Reinsel RA, Feshchenko VA, Wronski M. The comparative amnestic effects of midazolam, propofol, thiopental, and fentanyl at equisedative concentrations. *Anesthesiology* 1997; 87:749-64.
30. Malagon I, Hogenbirk K, Pelt J, Hazekamp MG, Bovill JG. Effect of three different anaesthetic agents on the postoperative production of cardiac troponin T in pediatric cardiac surgery. *Brit J Anaesth* 2005; 94:805-9.
31. Szekely A, Heindl B, Zahler S, Conzen PF, Becker BF. Nonuniform behaviour of intravenous anesthetics on postschismic adhesion of neutrophils in the guinea pig heart. *Anesth Analg* 2000; 90:1293-1300.
32. Nishina K, Akamatsu H, Mikawa K. The inhibitory effect of thiopental, midazolam and ketamine on human neutrophil functions. *Anesth Analg* 1998; 86:159-65.
33. Papaioannou V, Dragoumanis CH. Molecular mechanisms of cell adaptation to hypoxia and the role of ischemic and anesthetic preconditioning. *Greek Eur J Perioperative Med* 2007; 5:1-10.
34. Zaugg M, Lucchinetti E, Spahn DR, Pasch T, Garcia C, Schaub MC. Differential effects of anesthetics on mitochondrial KATP channel activity and cardiomyocyte protection. *Anesthesiology* 2002; 97:15-23.
35. Shryock JC, Belardinelli L. Adenosine and adenosine receptors in the cardiovascular system: Biochemistry, physiology, and pharmacology. *Am J Cardiol* 1997; 79:2-10.
36. Mullane K. Adenosine: The prototype adenosine regulating agent for reducing myocardial ischaemic injury. *Cardiovasc Res* 1993; 27:43-7.
37. Barker PH, Chanachan AS. Inhibition of adenosine accumulation into guinea pig ventricle by benzodiazepines. *Eur J Pharmacol* 1982; 78:241-44.
38. Kenakin TP. The potentiation of cardiac responses to adenosine by benzodiazepines. *J Pharmacol Exp Ther* 1982; 222:752-58.
39. Seubert CN, Morey TE, Martynyuk AE, Cucchiara RF, Dennis DM. Midazolam selectively potentiates the A2A- but not A1-receptor-mediated effects of adenosine. *Anesthesiology* 2000; 92:567-77.