ELSEVIER

European Journal of Pharmacology 345 (1998) 285–287

ejp

Short communication

Monophosphoryl lipid A reduces both arrhythmia severity and infarct size in a rat model of ischaemia

Wu Song, Brian L. Furman, James R. Parratt *

Department of Physiology and Pharmacology, University of Strathclyde, Glasgow G1 1XW, UK

Received 16 December 1997; revised 9 February 1998; accepted 13 February 1998

Abstract

A non-toxic derivative of the active lipid A component of the endotoxin molecule (monophosphoryl lipid A) when given to rats in a dose of 5 mg kg^{-1} by intraperitoneal injection 24 h prior to anaesthesia and coronary artery occlusion, markedly decreased the severity of ischaemia-reduced ventricular arrhythmias (ventricular fibrillation reduced from 60 to 21%; $P < 0.05$) and reduced myocardial infarct size (from 35.8 ± 1.6% of the area at risk to 22.7 ± 2.0%; $P < 0.05$). It did not modify blood pressure or heart rate either before or during the period of ischaemia. © 1998 Elsevier Science B.V.

Keywords: Monophosphoryl lipid A; Myocardial ischaemia; Ventricular arrhythmias; Infarct size; Ischaemic preconditioning

1. Introduction

In sub-lethal doses, the administration to rats of *Escherichia coli* endotoxin enhances the recovery of contractility from a period of ischaemia and reperfusion (Brown et al., 1989) and markedly reduces the severity of ischaemia-induced ventricular arrhythmias, and the extent of myocardial necrosis, following coronary artery occlusion 24 h later (Wu et al., 1994, 1996). Recently, a relatively non-toxic monophosphoryl derivative of the lipid A component of the endotoxin molecule (monophosphoryl lipid A) has been developed. This is non-pyrogenic in rabbits and is more than 1000 times less toxic than endotoxin in the embryonic chicken egg test (Dr. P. Weber, RIBI personal communication). In dogs and rabbits, monophosphoryl lipid A reduces myocardial infarct size (Yao et al., 1993; Baxter et al., 1996) and enhances recovery of contractile function following a period of ischaemia and reperfusion (Nelson et al., 1991). We now show that monophosphoryl lipid A, like endotoxin itself, also reduces the severity of the life-threatening arrhythmias which result from coronary artery occlusion and reperfusion and which represent a particularly important manifestation of ischaemic injury.

2. Methods and materials

The experimental model was similar to that used in the previously described endotoxin study (Wu et al., 1996). Sprague–Dawley male rats weighing between 250 and 320 g were given monophosphoryl lipid A in a dose of 5 mg kg^{-1} (22 rats), or an equivalent volume (2 ml) of its vehicle (40% propylene glycol, 10% ethanol, 50% water for injection) by intraperitoneal injection; 21 rats. After 24 h, the rats were anaesthetised with pentobarbitone (60 mg kg^{-1} by intraperitoneal injection). The rats were then respired with room air through an intratracheal cannula at a rate and volume sufficient to maintain blood gases, after thoracotomy, within the normal range (Clark et al., 1980). Blood pressure was measured from a carotid artery with a Statham transducer (Gould, USA) and the electrocardiogram recorded from standard limb leads; the heart rate was calculated from the electrocardiogram. Blood pressure and the electrocardiogram were recorded on a Grass polygraph (Grass Instruments, Quincy, MA, USA). After a left thoracotomy and a suitable (20 min) stabilisation period, the left

* Corresponding author. Tel.: +44-141-548-2858; fax: +44-141-552-2562; e-mail: j.r.parratt@strath.ac.uk

coronary artery was occluded for a period of 30 min and arrhythmias assessed as previously described (Clark et al., 1980; Wu et al., 1996) using the Kruskal–Wallis non-parametric test (for ventricular ectopic beats) or the χ^2 test (with Yates correction) for comparing the incidence of ventricular fibrillation. In seven rats in each of the two groups, the coronary artery was occluded for 30 min, the myocardium reperfused for 3 h and the area at risk and infarct size assessed with Evans blue dye (3%) and 2,3,5-triphenyltetrazolium chloride (1%), respectively (Clark et al., 1980; Wu et al., 1996).

3. Results

There was no significant difference between vehicle controls and rats treated with monophosphoryl lipid A with respect to mean systemic arterial blood pressure and heart rate immediately prior to coronary artery occlusion. These were 115 ± 2 mmHg (mean $\pm$ S.E.M.) in the controls and 112 ± 5 mmHg (in the monophosphoryl lipid A-treated rats) and, respectively, 432 ± 5 and 422 ± 9 beats min^{-1}. Coronary occlusion resulted in an immediate decrease in arterial pressure, maximal at 2 min, and to levels of 65 ± 3 mmHg and 54 ± 2 mmHg in the vehicle control and treated animals, respectively ($P < 0.05$). Over the occlusion period pressure returned towards normal and at the time of reperfusion (at 30 min), the pressures were 106 ± 5 mmHg in both control and monophosphoryl lipid A-treated rats. Coronary occlusion also led to a reduction in heart rate, again similar in both groups, to a value at 30 min of 404 ± 10 beats min^{-1}.

In the vehicle controls coronary artery occlusion resulted in considerable ventricular ectopic activity (Table 1). All the animals exhibited periods of ventricular tachycardia which was prolonged, nearly two-thirds had periods of ventricular fibrillation and 27% of the animals succumbed during the occlusion period in non-reversible ventricular fibrillation (Table 1). Arrhythmia severity was much less marked in those rats given monophosphoryl lipid A 24 h previously (Table 1). Ventricular tachycardia, which occurred in 93% of rats, was shortened and the number of ectopic beats that occurred as ventricular tachycardia was reduced from 1255 ± 185 to 353 ± 124; $P < 0.05$). The incidence of ventricular fibrillation (both reversible and non-reversible) was significantly reduced (Table 1) and no animal succumbed from irreversible VF during the occlusion period.

Table 1
Effect of pretreatment (24 h prior to coronary occlusion) with monophosphoryl lipid A (MLA; 5 mg kg^{-1}) on the severity of ischaemia-induced arrhythmias during a 30 min coronary artery occlusion in anaesthetised rats

Treatment (n)	VPBs	VT duration (s)	% VF	Mortality %
Vehicle (15)	1537 ± 194	126 ± 21	60	27[c]
MLA (5 mg kg^{-1}; 14)	588 ± 129[a]	37 ± 16[a]	21[b]	0[b]

VPBs = ventricular premature beats; VT = ventricular tachycardia; VF = ventricular fibrillation (both reversible and irreversible).
[a] $P < 0.05$ compared to vehicle treated group.
[b] $P < 0.05$.
[c] All rats died in irreversible VF.

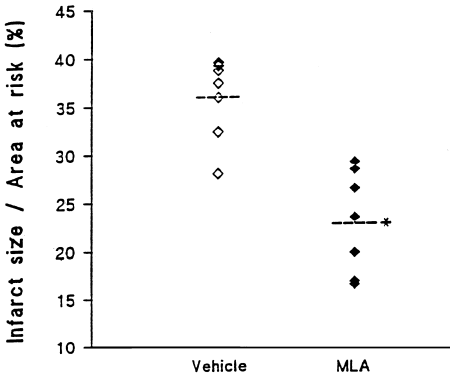

Fig. 1. Effect of pretreatment with monophosphoryl lipid A (MLA), 5 mg kg^{-1} given 24 h prior to coronary artery occlusion, on the relationship between infarct size and area at risk. There was no difference in area at risk between vehicle and monophosphoryl lipid A-treated groups; however, monophosphoryl lipid A significantly reduced infarct size ($P < 0.05$). Symbols refer to individual observations with the horizontal broken lines indicating the group means.

Following monophosphoryl lipid A administration, the area of necrosis following coronary artery occlusion was also markedly reduced (Fig. 1) from 102 ± 6 mg in the controls to 68 ± 8 mg ($n = 7$; $P < 0.05$) this represents $35.8 \pm 1.6\%$ (controls) and $22.7 \pm 2.0\%$, respectively, of the area at risk (which was 286 ± 11 mg and 296 ± 12 mg, respectively, in the controls and monophosphoryl lipid A-treated rats, n.s.). There was no difference between the groups in respect of total heart weight (1064 ± 30 mg and 1030 ± 39 mg), or body weight (236 ± 4 g and 225 ± 4 g), or between blood pressure (114 ± 2 and 113 ± 4 mmHg) and heart rate (432 ± 7 and 421 ± 9 beats min^{-1}) prior to coronary occlusion.

4. Discussion

These results are rather somewhat similar to those obtained with *E. coli* endotoxin (Wu et al., 1996) although at a somewhat higher dose. The dose of monophosphoryl lipid A in the present study (5 mg kg^{-1}) is considerably higher than that required to reduce myocardial infarct size in dogs (30 and 100 μg kg^{-1}; Yao et al., 1993) or in rabbits (35 μg kg^{-1}; Baxter et al., 1996) and parallels the relative toxicity of endotoxin in these species; rats are rather resistant to the toxic, as well as the protective, effects of bacterial endotoxin. The dose required to achieve cardioprotection against arrhythmias and infarct size in the present study is about 100 times more than that required to protect the canine myocardium (Yao et al., 1993).

The mechanisms of this marked cardioprotective effect are unclear but, as with bacterial endotoxin (Wu et al., 1994, 1996) are likely to involve the induction of a nitric oxide synthase (Zhao et al., 1997) or cyclooxygenase-2, perhaps through the generation of cytokines such as interleukin-I and tumour necrosis factor (TNF-α). These cytokines, which are certainly released by endotoxin, have also been shown to induce myocardial tolerance to ischaemia (Dinarello, 1989; Brown et al., 1990). Whatever the mechanism, we show for the first time that monophosphoryl lipid A not only decreases infarct size (which it does in all species so far examined) but also markedly reduces the severity of ischaemia-induced ventricular arrhythmias. As has been suggested elsewhere (Parratt and Szekeres, 1995; Przyklenk et al., 1996), parallels can be drawn between the protection afforded by monophosphoryl lipid A and that by ischaemic preconditioning. The results now show that this relatively non-toxic lipid A derivative has potential for reducing *all* the major manifestations of ischaemic injury, including the suppression of ischaemia-induced, life-threatening ventricular arrhythmias.

Acknowledgements

Monophosphoryl lipid A was a gift from Dr. G.T. Elliott, RIBI Immunochem. Research, Hamilton, MT, USA.

References

Baxter, G.F., Goodwin, R.W., Wright, M.J., Cerac, M., Heads, R.J., Yellon, D.M., 1996. Myocardial protection after monophosphoryl lipid A: studies of delayed anti-ischaemic properties in rabbit heart. Br. J. Pharmacol. 117, 1685–1692.

Brown, J.M., Grosso, M.A., Terada, L.S., Whitman, G.J.R., Banerjee, A., White, C.W., Harken, A.H., Repine, J.E., 1989. Endotoxin pretreatment increases endogenous myocardial catalase activity and decreases ischaemia-perfusion injury of isolated rat heart. Proc. Natl. Acad. Sci. USA 86, 2526–2530.

Brown, J.M., White, C.W., Terada, L.S., Grosso, M.A., Shanley, P.F., Mulvin, D.M., Banerjee, A., Whitman, G.J.R., Harken, A.H., Repine, J.E., 1990. Interleukin-I pretreatment decreases ischemia/reperfusion injury. Proc. Natl. Acad. Sci. USA 87, 5026–5030.

Clark, C., Forman, M.I., Kane, K.A., McDonald, F.M., Parratt, J.R., 1980. Coronary artery ligation in anaesthetised rats as a method for the production of experimental dysrhythmias and for the determination of infarct size. J. Pharmacol. Methods 3, 357–368.

Dinarello, C.A., 1989. Interleukin-I and its biologically related cytokines. Adv. Immunol. 44, 153–205.

Nelson, D.M., Brown, J.M., Banerjee, A., Bensard, D.D., Rodgers, K.B., Locke-Winter, C.R., Anderson, B.O., Harken, A.H., 1991. Pretreatment with a non-toxic derivative of endotoxin induces functional recovery against cardiac ischemia/reperfusion injury. Surgery 110, 365–369.

Parratt, J.R., Szekeres, L., 1995. Delayed protection of the heart against ischaemia. Trends Pharmacol. Sci. 16, 351–355.

Przyklenk, K., Zhao, L., Kloner, R.A., Elliott, G.T., 1996. Cardioprotection with ischemic preconditioning and MLA: role of adenosine-regulating enzymes?. Am. J. Physiol. 271 (Heart Circ. Physiol. 40), H1004–H1014.

Wu, S., Furman, B.L., Parratt, J.R., 1994. Attenuation by dexamethasone of endotoxin protection against ischaemia-induced ventricular arrhythmias. Br. J. Pharmacol. 113, 1083–1084.

Wu, S., Furman, B.L., Parratt, J.R., 1996. Delayed protection against ischaemia-induced ventricular arrhythmias and infarct size limitation by the prior administration of *Escherichia coli* endotoxin. Br. J. Pharmacol. 118, 2157–2163.

Yao, Z., Auchampach, J.A., Pieper, G.M., Gross, G.J., 1993. Cardioprotective effects of monophosphoryl lipid A, a novel endotoxin analogue, in the dog. Cardiovasc. Res. 27, 832–838.

Zhao, L., Weber, P.A., Smith, J.R., Comerford, M.L., Elliott, G.T., 1997. Role of inducible nitric oxide synthase in pharmacological 'preconditioning' with monophosphoryl lipid A. J. Mol. Cell. Cardiol. 29, 1567–1576.