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European Journal of Pharmacology 350 (1998) 223–228

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Effects of MK-886, a leukotriene biosynthesis inhibitor, in a rabbit model of endotoxic shock

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Received 2 October 1997; revised 12 February 1998; accepted 31 March 1998

Abstract

Leukotrienes are one of the biological mediators that play a role in endotoxic shock. In this study, we investigated the effects of a leukotriene biosynthesis inhibitor, MK-886, in a rabbit model of endotoxic shock. Lipopolysaccharide (*Escherichia coli* serotype 055:B5) infusion (1 mg kg^{-1} h^{-1}) to rabbits caused a biphasic decline in arterial blood pressure and decreased the vasoresponsiveness to phenylephrine, potassium chloride, sodium nitroprusside and acetylcholine in abdominal aortic rings. Oral administration of MK-886 (3-(1-(4-chlorobenzyl)-3-*t*-butyl-thio-5-isopropylindol-2-yl(-2,2-dimethylpropanoic acid) (5 mg/kg) 3 h prior to lipopolysaccharide infusion significantly inhibited the decline in arterial blood pressure and enhanced the responsiveness to phenylephrine and acetylcholine, whereas the changes in sodium nitroprusside and potassium chloride responses were not significant. However, the pD_2 ($-\log EC_{50}$) values for sodium nitroprusside in this group were higher than those of the group that received lipopolysaccharide alone. Neither the administration of the vehicle alone to endotoxemic rabbits, nor MK-886 administration to control animals, caused significant changes. These data suggest that MK-886 attenuates the hypotension and partially reverses the impaired vascular responsiveness observed in endotoxic shock.

Keywords: Endotoxic shock; (Rabbit); Hypotension; Leukotriene; MK-886

1. Introduction

Endotoxic shock is a syndrome characterized by hypotension, low systemic vascular resistance and a diminished response to vasoconstrictor agents (Suffredini et al., 1989). It is generally accepted that many biological activities brought about by lipopolysaccharide are secondary to the production or release of different mediators such as tumor necrosis factor-α (TNF-α), platelet activating factor (PAF), leukotrienes, interleukin 1, 2 and 6 from polymorphonuclear leucocytes, macrophages or endothelial cells (Bone, 1991).

Leukotrienes, the biologically active metabolites of arachidonic acid produced in the 5-lipoxygenase pathway are synthesized in response to inflammatory and immunologic stimuli (Samuelsson, 1983). Leukotriene B_4 which is known to be a powerful chemotactic agent, stimulates the adhesion of leucocytes to the endothelium and their emigration across the endothelial wall (Dahlen et al., 1981). The cysteinyl leukotrienes, leukotriene C_4, leukotriene D_4 and leukotriene E_4 have been shown to alter vascular permeability and induce macromolecular leakage (Hua et al., 1985). These mediators were shown to play an important role in endotoxic shock (Feuerstein and Hallenbeck, 1987; Jansen et al., 1991). Recently, potential beneficial effects of leukotriene biosynthesis inhibitors have been reported following endotoxin administration in vivo (Kuratomi et al., 1993; Klabunde and Calvello, 1995), whereas others reported that a side specific 5-lipoxygenase inhibitor, MK-886 was not effective in endotoxic shock (Shade et al., 1992).

MK-886 (3-(1-(4-chlorobenzyl)-3-*t*-butyl-thio-5-isopropylindol-2-yl(-2,2-dimethyl-propanoic acid) is a highly potent inhibitor of leukotriene formation in vivo and in vitro (Gillard et al., 1989). This compound inhibits leukotriene biosynthesis indirectly by a mechanism through the binding of a membrane bound 5-lipoxygenase-activating pro-

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0014-2999/98/$19.00

tein, thereby inhibiting the translocation and activation of 5-lipoxygenase (Dixon et al., 1990; Rouzer et al., 1990).

On the basis of these observations we aimed to investigate whether the inhibition of leukotriene synthesis is able to ameliorate the symptoms associated with endotoxic shock. With this purpose, we observed the effects of MK-886 in vivo on blood pressure and ex vivo on vascular responses in a rabbit model of endotoxic shock.

2. Materials and methods

2.1. Experimental protocol

Thirty male New Zealand rabbits weighing 1700–2200 g were studied. Animals were anesthetized by urethane (1.5 g kg^{-1} i.p.). Mean arterial blood pressure was monitored from the ear artery by a pressure transducer (MAY 9601, COMMAT Iletişim, Ankara, Turkey) connected to the computerized direct blood pressure recording system. Rectal temperature was monitored continuously by a probe and thermal variations were prevented by an infraruge lamp placed over the body.

Endotoxemia was induced by infusion of lipopolysaccharide, (*Escherichia coli* serotype 055:B5, Sigma-L-2880) in a 5 ml saline solution via the marginal ear vein (1 mg kg^{-1} h^{-1}) by a perfusion pump (perfusor secura FT, Braun, Germany) for 1 h. Arterial blood pressure was recorded for 120 min after the beginning of lipopolysaccharide infusion. Then, animals were sacrificed by cervical dislocation and the first segment of the abdominal aorta was removed carefully, cleared of adhering fat and connective tissue and cut into transverse rings of approximately 4–5 mm long each. In some of the rings, endothelium was removed by gently rubbing the intimal surface with a forceps. The rings were suspended horizontally in 50 ml organ chambers containing Krebs–Henseleit solution of the following composition (mM): NaCl, 118.30; KCl, 4.70; $MgSO_4$, 1.20; KH_2PO_4, 1.22; $CaCl_2$, 2.50; $NaHCO_3$, 25.00; glucose, 11.10; ph, 7.4 when gassed with a 95% O_2, 5% CO_2 mixture and maintained at 37°C.

Each ring was connected to a force displacement transducer (MAY-COM FDT 10-A, COMMAT Iletişim) for the measurement of isometric force which was continuously displayed and recorded on-line on a computer via an eight channel transducer data acquisition system (TDA 94, COMMAT Iletişim) using a software (Polywin 95 ver. 1.0 COMMAT Iletişim) which also had the capacity to analyse the data.

The vessels were systematically stretched to 1, 1.5, 2 g tension and after a 15 min equilibration period for each tension rings were contracted with 20–40 and 60 mM KCl, respectively, and washed repeatedly with Krebs–Heinselheit solution following each KCl contraction. After the initial equilibration period, the contractile responses were determined in endothelium denuded rings for increased concentrations of phenylephrine (3×10^{-8}–10^{-5} M) or for a single concentration of KCl (120 μM) that was prepared by equimolar replacement of sodium. The absence of endothelium in these rings was confirmed by the failure of the ability to relax to acetylcholine (10^{-6} M) after contraction with phenylephrine (10^{-5} M). Relaxant responses were determined using cumulative concentrations of either acetylcholine (3×10^{-8}–10^{-5} M) or sodium nitroprusside (3×10^{-8}–10^{-5} M) on endothelium intact rings precontracted with a submaximal concentration of phenyephrine (3×10^{-6} or 10^{-5} M), and were expressed as the percentage of phenylephrine precontraction. In order to maintain similar precontractile tension in all preparations, submaximal concentrations were determined using cumulative phenylephrine concentration–response curves for each ring.

2.2. Study design

The rabbits were divided into five groups each containing six rabbits.

Group 1 (Lipopolysaccharide) received 1 mg kg^{-1} h^{-1} lipopolysaccharide as described above. Group 2 (MK-886 + lipopolysaccharide) were given MK-886 (5 mg/kg body weight) (Gillard et al., 1989) in a 0.1% methylcellulose solution by gastric gavage 3 h prior to the beginning of the lipopolysaccharide infusion. Group 3 (methylcellulose + lipopolysaccharide) were given the vehicle alone 3 h prior to lipopolysaccharide infusion to determine whether the vehicle alone had any effect on aortic responses or arterial pressure. Group 4 (MK-886) were given the same dose of MK-886, but instead of lipopolysaccharide solution they

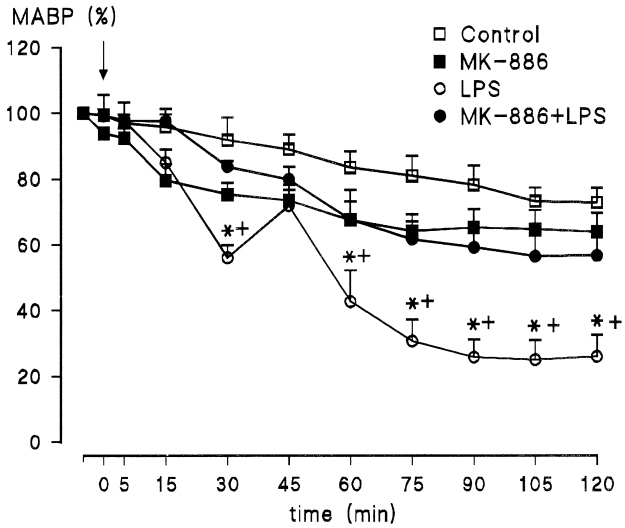

Fig. 1. Mean arterial blood pressure (MABP) of the groups expressed as % of the basal value (Data are means ± S.E.M of the groups; $n = 6$ in each group). Values marked (*) show significantly different values in endotoxemic animals when compared to controls. (+) denotes significance between endotoxemic rabbits (LPS) and MK-886-treated endotoxemic animals (MK-886 + LPS). Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Student–Neuman–Keuls test between groups on each timepoint.

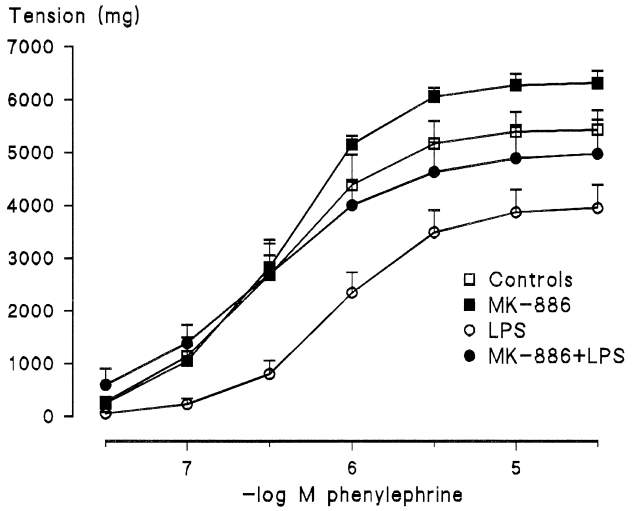

Fig. 2. Vasoconstrictor dose response curves for phenylephrine in endothelium denuded rabbit abdominal aortic rings. (Data are means ± S.E.M of the groups; $n = 6$ in each group.) Two-way ANOVA for repeated measures indicates significant difference between the curves of endotoxemic rabbits (LPS) and saline infused animals (controls) ($P < 0.05$) and between those of MK-886 administered endotoxemic rabbits (MK-886 + LPS) and endotoxemic animals (LPS) ($P < 0.05$). Comparison of the curves from MK-886 treated normal rabbits (MK-886) with those of controls showed no significant differences.

were infused with 5 ml of saline in order to detect the effects of MK-886 in normal rabbits. Group 5 consisted of six animals, which were infused with saline for 60 min and served as the control group.

2.3. Data analysis

Results were expressed as mean ± S.E.M. of the groups. Concentration–response curves were fitted by non-linear regression with simplex algorithm and E_{max} and pD_2 ($-\log EC_{50}$) were calculated using the software of transducer data acquisition system. Comparison of the dose–response curves were evaluated by two-way analysis of variance (ANOVA) for repeated measures. Differences between KCl responses, pD_2 and E_{max} values were analysed by Kruskal–Wallis one-way ANOVA followed by Mann–Whitney *U*-test. One-way ANOVA with a post-hoc Student–Neuman–Keuls test was used for the comparison of mean arterial blood pressure values of groups on each time point. Results were considered statistically significant when $P < 0.05$.

2.4. Reagents

Phenylephrine HCl, acethylcholine HCl and sodium nitroprusside were purchased from Sigma Chemical (USA) and KCl from E. Merck (Darmstadt, Germany). MK-886 was a kind gift from Merck Frosst, Canada. Stock solutions of the drugs were prepared in distilled water and concentrations were expressed as final molar concentrations in the bath solution.

3. Results

3.1. Blood pressure

Lipopolysaccharide infusion produced a severe and biphasic decline in mean arterial blood pressure (26 ± 6.5% of the basal value on 120 min) (Fig. 1). This decline was significant as compared with the values of control animals on different timepoints (72.8 ± 4.4% of the basal value on 120 min). MK-886 pretreatment reduced this decline significantly (56.7 ± 6.4% of the basal value on 120 min). Administration of MK-886 to normal animals caused a decrease in arterial blood pressure, but this decrease was not significant at any timepoint when compared to the controls. Oral administration of the vehicle to lipopolysaccharide infused rabbits resulted in a decline that is similar to lipopolysaccharide infused group (data not shown).

3.2. Contractile responses

The basal tonus of the aortic rings from lipopolysaccharide infused rabbits was significantly reduced (875.8 ±

Table 1
The pD_2 ($-\log EC_{50}$) values of concentration–response curves for acetylcholine, phenylephrine and sodium nitroprusside, E_{max} values for phenylephrine concentration–response curves and responses to potassium chloride (120 mM)

	ACh (pD_2)	PE (pD_2)	SNP (pD_2)	PE (E_{max}) (tension mg)	KCl (tension mg)
Controls	6.72 ± 0.2	6.53 ± 0.1	6.15 ± 0.1	5429.3 ± 375	4910.6 ± 315
LPS	6.51 ± 0.1	6.15 ± 0.1[a]	5.32 ± 0.3[a]	3950.5 ± 435[a]	2788.0 ± 632[b]
MK-886 + LPS	6.84 ± 0.1	6.66 ± 0.1[d]	6.16 ± 0.1[c]	4974.5 ± 649	3706.0 ± 826
MK-886	7.10 ± 0.1	6.51 ± 0.1	5.94 ± 0.3	6311.6 ± 233	5725.0 ± 247

LPS, lipopolysaccharide; Ach, acetylcholine; PE, phenylephrine; SNP, sodium nitroprusside; KCl, potassium chloride.
[a] $P < 0.05$ compared to controls.
[b] $P < 0.01$ compared to controls.
[c] $P < 0.05$ compared to lipopolysaccharide group.
[d] $P < 0.01$ compared to lipopolysaccharide group.
Data are expressed as mean ± S.E.M. of the groups; $n = 6$ in each group.
Statistical analysis was performed by one-way analysis of variance followed by Mann–Whitney *U*-test.

51.9) as compared with those of control animals (1083.3 ± 63.2) ($P < 0.05$). MK-886 administration significantly increased the basal value (1135.8 ± 27.6) ($P < 0.01$) in shocked animals. Phenylephrine (0.03–100 μM) induced dose-dependent contractions in endothelium denuded aortic rings are shown in Fig. 2. The pD_2 and E_{max} values elicited by phenylephrine and KCl-induced contractile responses are shown on Table 1. The contractile responses to phenylephrine were reduced in aortas from lipopolysaccharide-treated rabbits when compared to the vessels from control animals ($P < 0.05$). The pD_2 and E_{max} values were significantly decreased ($P < 0.05$, $P < 0.05$, respectively) and the dose–response curve shifted to right. The contractile responses to KCl were also decreased in these animals ($P < 0.01$). Pretreatment with MK-886 enhanced the phenylephrine responses significantly ($P < 0.05$) and shifted the dose–response curve to the left with significantly higher pD_2 values ($P < 0.01$) in endotoxemic aortas, but did not alter the E_{max} values. The increase in KCl induced contractions in this group was not significant. Administration of MK-886 to saline infused rabbits caused no significant changes in contractile responses when compared with those of control animals. Likewise, the vehicle did not alter the contractile responses in endotoxemic aortas (data not shown).

3.3. Relaxant responses

On endothelium intact rings precontracted with submaximal concentrations of phenylephrine determined for each ring, acetylcholine (0.03–10 μM) and sodium nitroprusside (0.03–10 μM) induced relaxation in all groups (Figs. 3 and 4, respectively). The pD_2 values are given in Table 2. Relaxations to both acetylcholine and sodium nitroprusside were attenuated in aortas from lipopolysaccharide treated rabbits as compared to the control group ($P < 0.01$ and $P < 0.05$, respectively). The pD_2 values for sodium nitroprusside curves were decreased ($P < 0.05$), whereas the values for acetylcholine showed no significant changes.

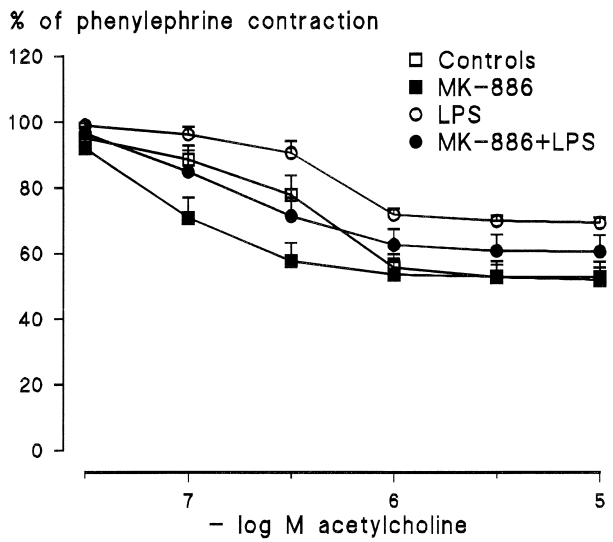

Fig. 3. Vasorelaxant dose–response curves for acetylcholine in endothelium intact aortic rings precontracted with a submaximal concentration of phenylephrine. (Data are means ± S.E.M of the groups; $n = 6$ in each group). Statistically significant difference is present between the curves of saline infused controls (control) and endotoxemic rabbits (LPS) ($P < 0.01$). Two-way ANOVA for repeated measures indicates significant difference between MK-886 treated endotoxemic animals (MK-886 + LPS) and endotoxemic rabbits (LPS) ($P < 0.05$). Comparison of the curves from MK-886 treated normal rabbits (MK-886) with those of controls showed no significant differences.

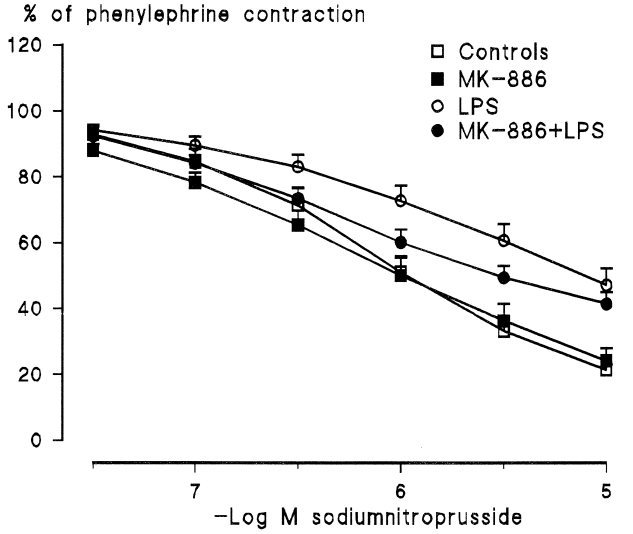

Fig. 4. Vasorelaxant dose–response curves for sodiumnitroprusside in endothelium intact aortic rings precontracted with a submaximal concentration of phenylephrine. (Data are means ± S.E.M of the groups; $n = 6$ in each group). Two-way ANOVA for repeated measures indicates significant difference between the curves of endotoxemic rabbits (LPS) and saline infused animals (controls) ($P < 0.05$). No significant differences were observed between the curves of MK-886 administered endotoxemic rabbits (MK-886 + LPS) and endotoxemic animals (LPS) ($P < 0.05$). Comparison of the curves from MK-886 treated normal rabbits (MK-886) with those of controls showed no significant differences.

Pretreatment with MK-886 augmented the relaxations induced by acetylcholine ($P < 0.05$) without altering the pD_2 values. The pD_2 values for sodium nitroprusside induced relaxations were increased ($P < 0.05$), but the comparison of the concentration–response curves for this agent showed no significant differences. Neither acetylcholine, nor sodium nitroprusside induced relaxations altered with MK-886 administration alone in saline treated rats when compared to the controls. The vehicle alone did not cause significant changes in relaxant responses in lipopolysaccharide infused rabbits (data not shown).

4. Discussion

In the present study, infusion of lipopolysaccharide to anesthetized rabbits caused a biphasic fall in mean arterial blood pressure which has previously been described by different authors (D'Orio et al., 1987; Wakabayashi et al., 1987). Administration of MK-886 prior to lipopolysaccha-

ride infusion prevented the fall in mean arterial blood pressure significantly.

Adhesion of activated neutrophiles to endothelial cells is a key event in development of endothelial cell damage in sepsis (Harlan, 1985; Bone, 1991). Leukotrienes, promote neutrophil chemotaxis and adhesion of neutrophils to endothelium (Dahlen et al., 1981; Tonnesen et al., 1989). They also increase vascular permeability causing contraction of adjacent endothelial cells and a resulting increase in the diameter of interendothelial cell pores (Bone, 1991). The increased vascular permeability results in extravasation of intraluminal plasma and a subsequent fall in blood pressure. MK-886 has been shown to reduce the extravasation of plasma (Fernandez-Gallardo et al., 1992) and prevent the leucocyte adhesion to the endothelium (Lehr et al., 1991) in experimental animals. Thus, it is considerable that MK-886 prevents the endothelial damage and the subsequent fall in blood pressure by these effects. These actions also bring an explanation to the MK-886 induced enhancement of the endothelium dependent responses to acetylcholine, a vasodilator which has been shown to be entirely endothelium dependent (Furchgott and Zawadski, 1980). The diminished aortic responses to acetylcholine in endotoxemia are in accord with previous reports (Beasley et al., 1990; Parker and Adams, 1993; Umans et al., 1993), but the attenuated responses to the endothelium independent vasodilator, sodium nitroprusside are not consistent with those of others who suggested that lipopolysaccharide spares the ability of vascular smooth muscle to respond to agents that act through guanylyl cyclase-cGMP pathway (Parker and Adams, 1993; Umans et al., 1993). However, a similar impairment of vasodepressor responsiveness to sodium nitroprusside in endotoxemia has previously been reported by others (Güç et al., 1991; Peters and Lewis, 1996). It is known that the tissue catalysed reduction of nitroprusside results in nitric oxide (NO) production (Bates et al., 1991) and NO directly activates the cytosolic fraction of vascular smooth muscle guanylyl cyclase causing intracellular increase of endogenous vasodilator cGMP. Thus, such a generalized vasodepressor impairment can be explained by a defect in the ability of NO to activate guanlyl cyclase, presumably due to a physical barrier such as vessel wall edema or a decreased sensitivity of vascular smooth muscle to cGMP mediated actions. It seems plausible that inhibition of leukotriene synthesis abolishes the vascular edema resulting in enhanced ability of NO to reach the vascular smooth muscle.

The impairment of α-adrenoceptor agonist induced contractions has previously been described in the rat (Wakabayashi et al., 1987; Beasley et al., 1990) and rabbit (Umans et al., 1993) aortas. Taken together with the decreased KCl responses, which are induced by direct depolarization of vascular smooth muscle, the generalized contractile defect suggests basic alterations either in Ca^{2+} mobilization or in the contractile apparatus within the vascular smooth muscle. This arterial hyporeactivity has recently been demonstrated to be mediated by the endotoxin promoted induction of a calcium-independent NO synthase, which leads to increased synthesis of the potent vasodilator NO (Julou-Schaeffer et al., 1990; Salvemini et al., 1989). MK-886 administration to lipopolysaccharide infused rabbits shifted the dose–response curves of phenylephrine contractions to the left with significantly higher pD_2 values, but failed to restore the decreased responsiveness to KCl. The mechanisms mediating this selective amelioration of vasoconstrictor responses and a possible relationship between leukotriene biosynthesis inhibition and NO synthesis remain to be determined.

There is, on the other hand, strong evidence for a relationship between leukotrienes and other mediators that participate in endotoxic shock. Leukotriene B_4 has been reported to amplify the production of interleukin-1 and interleukin-2 (Rola-Pleszczynski et al., 1986), TNF-α (Horiguchi et al., 1989; Mohri et al., 1990) and PAF (Mizoguchi et al., 1991). The hypothesis that inhibition of leukotriene biosynthesis might also prevent the effects of these substances is supported by the evidence for an inhibitory effect of 5-lipoxygenase inhibitors on PAF (Glaser et al., 1991), but this is not compatible with the findings of others who reported that a 5-lipoxygenase inhibitor, MK-886 was not effective in inhibiting TNF production in endotoxic shock (Shade et al., 1992).

5. Conclusion

The results of our study demonstrate that inhibition of leukotriene biosynthesis by MK-886 attenuates the severe hypotension and partially reverses the impaired vascular responsiveness seen in endotoxic shock, but the mechanisms underlying these effects remains to be cleared by further investigations.

Acknowledgements

The experiments had been approved by the ‘Animal Care Ethics Committee’ of our faculty. We wish to thank Dr. A.W. Ford-Hutchinson and Merck Frosst Centre for Therapeutic Research (Quebec, Canada) for their generous gift of MK-886.

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