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FREE RADICALS SCAVENGERS ATTENUATE PLATELET-ACTIVATING FACTOR (PAF)- AND ENDOTOXIN-INDUCED INTESTINAL MYOELECTRIC DISTURBANCES IN RATS

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Pretreatment with radical scavengers significantly reduced the intestinal myoelectric disturbances following either *E. coli* endotoxin or platelet-activating factor (PAF) injection in the rat indicating that free radicals might be involved in the intestinal motor alterations observed in endotoxin shock and that PAF acts partially via free radical production. Moreover, dimethylsulfoxide (DMSO) was found to be more effective in inhibiting the endoxotin-induced intestinal motor alterations, than superoxide dismutase (SOD) and allopurinol. BN 52021, a specific PAF antagonist, was able to reduce the effects of endotoxin on intestinal motility. However, when BN 52021 was combined with free radical scavengers, no additive effect was observed. It is concluded that free radicals involved in endotoxin-induced intestinal motility alterations are at least in part produced in response to PAF.

KEY WORDS: Free Radicals, platelet-activating factor, endotoxic shock, intestinal motility, free radicals scavengers, PAF antagonist

INTRODUCTION

Platelet-Activating Factor (PAF) is thought to be involved in the pathophysiology of endotoxic shock. Indeed, it has been shown to be implicated in the genesis of intestinal motor disturbances induced by systemic administration of *E. coli* endotoxin.[1] PAF is known to be produced by and act on a range of cell types including neutrophils, eosinophils, monocytes, macrophages, platelets and endothelial cells. It has been demonstrated to be a potent chemotactic agent for neutrophils inducing superoxide release, aggregation and degranulation.[2] It has been shown that free radicals are involved in inflammatory processes and gastro-intestinal lesions.[3-5] Thus, we investigated whether free radicals are involved in intestinal myoelectric disturbances induced by systemic administration of *E. coli* endotoxin in rats. We also examined the role of PAF in the endotoxin-induced production of free radicals.

MATERIAL AND METHODS

Under halothan (Fluothan N.D.) anesthesia, male Wistar rats (280-320 g) were

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chronically implanted with nichrome electrodes in the duodeno-jejunum. After one week recovery, the experiments were performed in animals fasted for at least 8 hours, with free access to water. They were administered the following drugs:

— *E. coli* endotoxin (S.0111:B4, Sigma, La Verpillière, France), injected intravenously in 0.2 ml saline at a dose of 50 μg/kg,
— PAF (Sigma, La Verpillière, France), injected intraperitoneally in 0.2 ml saline at a dose of 25 μg/kg,
— BN 52021, a gift from IHB Laboratory (Le Plessis-Robinson, France), dissolved in 0.2 ml solvent and injected intravenously at a dose of 50 mg/kg,
— Allopurinol (Sigma, La Verpillière, France), given *per os* (50 mg/kg) in 0.2 ml saline added with 20 μl NaOH (1N) in order to facilitate its dissolution,
— Superoxide Dismutase (Bovine Liver, Sigma Chemical Co St. Louis Mo) injected intravenously in 0.2 ml saline at a dose of 15000 U/kg,
— Dimethylsulfoxide (Prolabo, Paris, France) given *per os* by gastric intubation at a dose of 50 mg/kg.

Effects on motility were evaluated by measuring the time elapsed from the injection to the recovery of a normal fasted pattern i.e. MMCs on integrated electromyographic records. These values were compared using one or two way ANOVA followed by Student's "t" test for fasted values.

RESULTS

The myoelectric activity of conscious 8h-fasted rats is organized into migrating myoelectric complexes (MMCs) recurring at 16.2 ± 5.8 min intervals. Each MMC consisted of irregular spiking activity (phase II) lasting 4–6 min followed by a short

TABLE I

Comparative effects of different free radicals scavengers combined or not with the PAF antagonist BN 52021 on *E. coli* endotoxin- and PAF-induced intestinal myoelectric alterations of the duodenum in fasted rats (values are means ± SD, $n = 8$).

Dose	Interval between 2 MMCs[a] (min)			
	Control	Allopurinol (50 mg/kg PO)	SOD (15000 U/kg PO)	DMSO (50 mg/kg PO)
Vehicle (0.2 ml)	16.3 ± 3.1	15.9 ± 2.3	16.8 ± 1.9	16.4 ± 2.6
E. coli endotoxin (50 μg/kg IV)	114.8 ± 22.0*	42.5 ± 6.5*†	45.7 ± 9.9*†	38.2 ± 6.4*†
PAF (25 μg/kg IP)	146.2 ± 16.3*	68.5 ± 10.6*†	72.9 ± 10.4*†	31.7 ± 6.1*†
BN 52021 (50 mg/kg IV) + endotoxin (50 μg/kg IV)	43.1 ± 12.2*	38.9 ± 11.0*	45.2 ± 9.6*	39.7 ± 9.9*

*significantly different from vehicle values ($p < 0.01$).
†significantly different from control values ($p < 0.01$).
[a]interval measured at duodenal level from the last phase III before to the first phase III after treatment.

(4–5 min) period of intense activity called regular spiking activity or phase III. These two periods of activity were separated by a quiescent period (phase I).

Intravenous administration of *E. coli* endotoxin (50 μg/kg) resulted in a disruption of the MMCs pattern which was replaced by a continuous spiking activity for 114.8 ± 22.0 min (Table I). Such a disruption appeared within 20 min after injection. PAF administered IP at a dose of 25 μg/kg, induced a transient inhibition of the spiking activity followed by a long-lasting disruption of the MMC pattern during 146,2 ± 16.3 min (Table I). Control animals injected with 0.2 ml saline solution showed normal myoelectric activity.

When administered orally, allopurinol (50 mg/kg) had no effect *per se* on the intestinal MMC pattern; administered PO 6 hours before administration of either *E. coli* endotoxin (50 μg/kg IV) or PAF (25 μg/kg IP), it significantly ($p < 0.01$) shortened the MMC inhibition to 42.5 ± 6.5 min and to 68.5 ± 10.6 min respectively (Table I). Similarly, SOD injected IV at a dose of 15000 U/kg 45 min before endotoxin administration (50 μg/kg IV) also reduced the intestinal MMC inhibition induced by endotoxin, with no influence *per se* on the intestinal motor profile. Injected 45 min before PAF (25 μg/kg IP), SOD (15000 U/kg IV) also reduced the duration of PAF-induced intestinal myoelectric alterations (Table I). DMSO given orally at a dose of 50 mg/kg 1 hour before IP injection of PAF (25 μg/kg) or IV injection of *E. coli* endotoxin (50 μg/kg) diminished the duration of MMC inhibition (Table I), injected alone, DMSO did not affect the intestinal MMC pattern. At the dosages used, DMSO appeared more potent than SOD and allopurinol in antagonizing PAF or endotoxin effects on intestinal motility (Figure 1).

BN 52021 injected IV (50 mg/kg), 5 min prior to endotoxin or PAF was found to

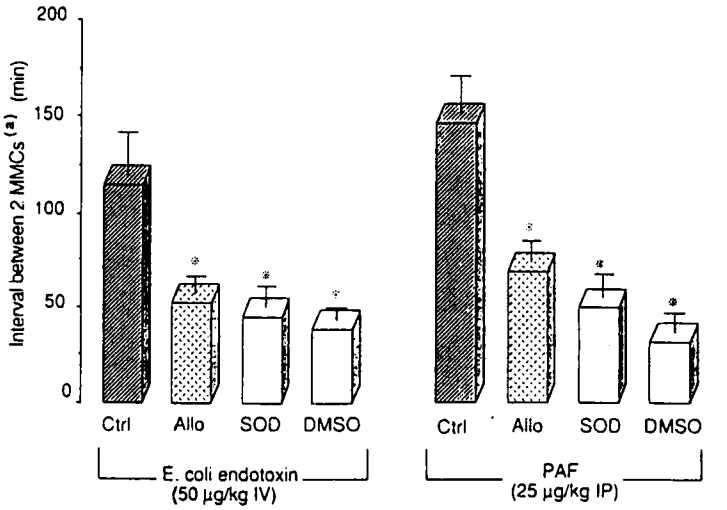

FIGURE 1 Protective effect of different free radicals scavengers on endotoxin- and PAF-induced suppression of the duodenal migrating myoelectric complexes in fasted rats (values are means ± SD, $n = 6$). *significantly different from control values ($p < 0.01$). (a) interval measured at duodenal level from the last phase III before to the first phase III after treatment. Ctrl: control values; Allo: allopurinol (50 mg/kg *per os*); SOD: superoxide dismutase (15000 U/kg IV); DMSO: dimethylsulfoxide (50 mg/kg *per os*).

abolish the effects of PAF on intestinal motility, and reduce significantly those induced by *E. coli* endotoxin to 43.1 ± 12.2 min. When combined with either allopurinol, SOD or DMSO, BN 52021 did not exhibit an improved protective effect upon endotoxin-induced intestinal motor disturbances (Table 1).

CONCLUSION

These results suggest that 1) free radicals are partly responsible for intestinal myoelectric disturbances observed in endotoxin shock, 2) PAF acts partially via free radical production, 3) free radicals involved in endotoxin-induced intestinal disturbances appear to be induced by PAF, and 4) DMSO appears to have a more potent effect upon endotoxin-induced intestinal myoelectric alterations than SOD and allopurinol.

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