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In vivo inhibition of plasma protein leakage and *Salmonella enteritidis* - induced mortality in the rat by a specific paf-acether antagonist: BN 52021

A. ETIENNE, F. HECQUET, C. SOULARD, B. SPINNEWYN, F. CLOSTRE and P. BRAQUET*

I.H.B. – IPSEN Institute for Therapeutic Research, 17, Avenue Descartes, F-92350 Le Plessis Robinson (France)

Abstract

The effects of BN 52021, a new specific paf-acether receptor antagonist and the total Ginkgo biloba extract (GBE 761) from which this product was isolated, were studied in the rat on paf-acether-induced permeability and cell number changes and on endotoxin-induced lethality.

Their activities were compared to those of cycloxygenase, 5-lipoxygenase and phospholipase A_2 inhbitors. BN 52021 given s.c. or orally exerted a dose-related inhibition of paf-acether deleterious effects as well as of endotoxin lethality whereas the other drugs tested were poorly effective. These results strongly suggest paf-acether involvement in endotoxic and septic shock.

Introduction

Endotoxemia and sepsis are clinically important problems since the classical treatments (steroids, dobutamide, aprotinin) do not improve sufficiently the clinical outcome of the patients whose mortality remains high [1].

The pathophysiology of endotoxemia and gram negative sepsis is still unclear. The clinical symptoms are characterized by profound haemodynamic disturbances such as systemic hypotension associated with a decrease in peripheral vascular resistances, pulmonary hypertension, bronchoconstriction and lung oedema. In addition, disturbances in leukocyte and platelet function also occur which contribute to increase the harmful role of circulatory disorders. The increase of permeability is also a major point for the prognosis and evolution of endotoxemia because it plays an essential role in the loss of fluid from the circulatory system and contributes to the lung failure observed in such pathology [2].

Previous investigations suggest a participation of arachidonic acid (AA) metabolites such as leukotrienes (LTs) and/or thromboxane A_2 (TxA_2) in the early phase of shock but this remains controversial since certain TxA_2 synthetase inhibitors were demonstrated to be ineffective [3].

Platelet activating factor (paf-acether, 1-0-alkyl-2-0-acetyl-sn-glycero-3-phosphoryl choline) was first found to be released from IgE-sensitized rabbit basophils. Paf-acether is not only one of the most potent platelet activators but also mimicks the symptomatology of endotoxic shock: a single injection of this mediator in animal cause pulmonary hypertension, bronchoconstriction, thrombocytopenia and leukopenia, acute circulatory collapse associated with extravasation, decrease in splanchnic flow and finally death. Therefore, paf-acether could play a key role in the pathogenesis of endotoxic shock. In order to verify this assumption, we investigated the effects of BN 52021, a new specific paf-acether receptor antagonist [4] on Salmonella enteritidis (SE) – induced endotoxic shock in the rat. The total standardized Ginkgo biloba (GBE 761) from which BN 52021 is isolated, dexamethasone (DXM), indomethacin (IND) and nordihydroguaiaretic acid (NDGA) were also studied in our experiments. The activities of these drugs were previously determined on the systemic permeability changes induced in the rat by acute i.v. injection of paf-acether in order to compare them to those exerted on endotoxin-induced lethality.

Materials and Methods

Materials

Paf-acether (1-0-hexadecyl-2-0-acetyl-sn-glycero-3-phosphoryl choline) was purchased from Bachem (Budendorf, Switzerland). A stock solution (0.5 mg/ml) in a 0.5% albumin normal saline stabilizing solution was kept frozen at −70°C.

The standardized Ginkgo biloba extract (GBE 761) and BN 52021 [3-(1, 1-dimethylethyl) hexahydro-1, 4 7b-trihydroxy-8-methyl-9H-1, 7 α (epoxy methanol-1H, 6αH-cyclopenta (c) furo (2, 3-b) furo (3′, 2′:3, 4) cyclopenta (1, 2-d) furan-5, 9, 12 (4H)-trione] were prepared by IHB-IPSEN Institute for Therapeutic Research, Le Plessis (France). EP 10045 [methyl-2 butyl (mercapto)-4 catechol] a 5-lipoxygenase inhibitor was synthetized by EXPANSIA chem. (Aramon, France). Dexamethasone acetate was obtained from ROUSSEL-UCLAF (Paris, France), Ketoprofene from SPECIA (Paris, France) and methyl prednisolone sodium succinate from UPJOHN (Paris, France). Nordihydroguaiaretic acid (NDGA) was purchased from FLUKA (FRG), Indomethacin and Endotoxin (Salmonella Enteritidis endotoxin, LO11) from SIGMA (St-Louis, Mo, USA).

Systemic vascular permeability increase and blood cell number changes induced by i.v. injection of paf-acether

Male Wistar rats (200–220 g) (Janvier Breeding Lab.)

*Reprint requests to P. Braquet.

were used in this experiment. They received, under light diethyl oxide anaesthesia a saline solution (i.v. injection, penis vein) of paf-acether (1 μg/kg) and trypan blue (1%). The following parameters: hematocrit (H), dye concentration (DC), white (WBC) and red (RBC) cell numbers, were determined in blood samples. The compounds tested were administered either by the subcutaneous (sc) route 15 min or orally (p.o.) 1 hour before challenge. Eight animals per group were used. The variations in the different parameters were expressed as per cent compared to a normal group (animals receiving only the saline solution).

Endotoxic shock in conscious rat

Male Sprague Dawley rats (200–220 g Charles River Breeding Lab.) were used in this experiment. They received, under light diethyl oxide anaesthesia, a saline solution of Salmonella Enteritidis Endotoxin (5 mg/kg i.v., penis vein). Ten animals per group were used. The death rate was noted after 24 hours. Drugs were given either by i.v. injection or s.c. route 15 min before the challenge or p.o. 1 hour before it.

Results

Effects of drugs on systemic vascular permeability increase and blood cell number changes induced by paf-acether

Preliminary dose-range and time-course studies have been performed on the effects of systemic acute injection of paf-acether (0.25 → 7.50 μg/kg, i.v.) in the rat. Following this challenge, a dose-dependent increase in blood cell (WBC and RBC) numbers and in hematocrit value was observed, associated with a disappearance from the plasma of trypan blue. These alterations were maximal about 15 min after the paf-acether injection. Immediately after the dose of 1 μg/kg, the variations were highly significant: RBC, cells. $mm^{-3} \times 10^{-6} = 7.7 \pm 0.20$ vs. 6.0 ± 0.15; $p < 0.001$ – WBC, cells $mm^{-3} \times 10^{-3} = 26.70 \pm 3.35$ vs 16.90 ± 1.27; $p < 0.001$ – H (%) = 51.4 ± 1.0 vs 43.4 ± 0.6; $p < 0.001$ – DC ($mg\ l^{-1}$) = 377 ± 6 vs 439 ± 14; $p < 0.001$.

Therefore, the effects of drugs were examined at this dose: as shown in the table, BN 52021 given either s.c. (1, 2 and 4 mg/kg) or p.o. (4, 8 and 16 mg/kg) exerted a dose-related inhibition of the paf-acether-induced disorders.

GBE 761 was also effective orally (table) but at higher doses and to a lower extent than BN 52021. Surprisingly, methyl prednisolone (30 mg/kg, s.c.) was ineffective; a similar result was obtained with NDGA (25 mg/kg, s.c.) and ketoprofene (5 mg/kg, s.c.) (data not shown).

Effects of drugs on endotoxin-induced shock in the rat

Pre-treatment (15 min) of rats with BN 52021 either i.v. (2.5 mg/kg) or s.c. (5–10 mg/kg) decreased the death rate induced by endotoxin. The protective effect was dose-related. For example, at 5 mg/kg, s.c., BN 52021 led to a 45% drop in lethality. This effect was greater at higher doses and was almost total at 20 mg/kg (data not shown). At a non-active dose (2 mg/kg, s.c.) BN 52021 exerted a synergistic effect on indomethacin treatment (1 mg/kg, s.c.) giving a 47% decrease in lethality vs 25% with indomethacin alone. Dexamethasone (5 mg/kg, s.c.) provided total protection in this model.

Discussion

In this report, we describe the effects in the rat of BN 52021, a new specific paf-acether receptor antagonist on paf-acether – induced permeability and blood cell number changes, and on Salmonella Enteritidis endotoxin-induced lethality.

Due to the lack of specific inhibitors, there is not yet complete evidence of paf-acether involvement in endotoxic and septic shock, although its participation in such a pathology was strongly suggested.

Our results seem to confirm this assumption, since

Table

Activity of BN 52021 and GBE 761 on the effects of a single i.v. injection of paf-acether 1 μg/kg. Variations of red and white cell numbers, plasma dye concentration and hematocrit, compared to a group of animals receiving only saline, were measured 15 min after paf-acether injection. Drugs were given to 60 min or sc 15 min before paf-acether.

Treatment	Dose mg/kg route	Percentage variation of parameters			
		Red cell number	White cell number	Hematocrit	Plasma dye concentration
Control	—	+28.5	+50.8	+25.2	−16.4
BN 52021	1 sc	+19.0*	+43.5	+14.7***	−9.7
BN 52021	2 sc	+10.6***	+39.3	+11.1***	−2.4***
BN 52021	4 sc	+7,1***	+21.8	+5.6***	−1.8***
Control	—	+22.9	+87.3	+23.7	−17.5
BN 52021	4 PO	+14.4	+49.1**	+11.5***	−6.5***
BN 52021	8 PO	+14.8	+28.6***	+10.6***	−4.2***
BN 52021	16 PO	0***	+26.7***	+4.4***	0***
Control	—	+20.2	+35.1	+20.0	−13.9
GBE 761	100 PO	+19.8	+13.0*	+11.9***	−10.5
GBE 761	200 PO	+14.3***	+1.4***	+8.1***	−1.4***
BN 52021	8 PO	+7.5***	+14.4***	+5.6***	−2.2***

Statistical significance; student's *t*-test compared to paf-acether control group. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

BN 52021 not only prevented fluid escape and blood cell increase but also inhibited Salmonella Enteritidis-induced endotoxic shock. In these models, indomethacin, a cyclo-oxygenase inhibitor, only provided partial protection. NDGA and the 5-lipoxygenase inhibitor EP 10045 were practically inactive both on the systemic extravasation model and on endotoxic shock. Therefore, these results suggest that although thromboxane intervention cannot be ruled out, the participation of leukotrienes and other lipoxygenase products appear to be less evident. Since dexamethasone totally inhibits endotoxic shock, its action may be related to the inhibition of phospholipase A_2.

In this respect, paf-acether antagonists could provide a new class of drugs for shock treatment without having the side effects of corticosteroids.

References

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