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PHENYL-T-BUTYL-NITRONE IS ACTIVE AGAINST TRAUMATIC SHOCK IN RATS

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Oxygen free-radicals appear to be involved in the pathogenesis of shock; therefore trapping of these radicals would modify the evolution of experimental shock. Experiments were performed on rats submitted to 100% lethal whole body trauma (rotating drum) and their survival, pathology, acid-base status and hematocrit level observed.

The spin trapping agent phenyl-t-butyl-nitrone (PBN) was administered before trauma (50, 100, 150 mg/kg i.p.) or at various intervals (30, 60 minutes) after establishment of a severe traumatic shock. It appeared that PBN administration was highly effective both in prevention and in reversion of traumatic shock in rats.

Key words: Shock; Trauma; Oxyradicals; Spin-trapping; Phenyl-t-butyl-nitrone.

INTRODUCTION

Circulatory shock is a syndrome of generalized tissue hypoperfusion and cell hypoxia, consequent to hypovolemia, trauma, sepsis, endotoxin, myocardial insufficiency, etc. Some prominent events in circulatory shock are increase of cell membrane rigidity and permeability, lipoperoxidations, decrease of transmembrane potentials, increase of capillary permeability with destabilization of the interstitium, etc. Therefore, the hypothesis of an involvement of oxygen free radicals in the pathogenesis of circulatory shock appears to be feasible.

In fact, some reports indicate that the evolution of experimental shock in rats is ameliorated by previous administration of large doses of α-tocopherol or reduced glutathione[1,2] or by a very large quantity of superoxide-dismutase[3]. Other experiments have been performed by Lefer *et al.*[4] with a drug with scavenging activity. Allopurinol, also, was reported to be effective in increasing survival after hemorrhage[5].

Data from our laboratory indicated that both the inhibition of scavenging enzymes provoked by diethyldithiocarbamate and the increased oxygen free radical generation associated with hyperoxia worsen the evolution of circulatory shock in rats[2].

Endotoxin increases lipoperoxidation and decreases superoxide dismutase (SOD) activity in the liver of rats and these effects were prevented by allopurinol, α-

tocopherol or reduced glutathione[6]. Diffuse intravascular coagulation induced by endotoxin appeared to be prevented by SOD and catalase[7]. A decrease of superoxide-dismutase content of muscle tissue associated with functional damage to mitochondrial function has been reported by Corbucci *et al.*[8] in shocked patients.

In summary, it seems that the role of the scavenging systems in shock have been studied more than the role of oxygen free radicals *per se* and that there is the need for their more direct study.

The capture of free radicals by the spin-trapping agent seems to suggest a model of more direct approach to the problem, offering elimination of the reactive species of oxygen before their impact on scavenging systems. We report the results of experiments designed to determine if phenyl-t-butyl-nitrone is effective in prevention and in reversion of traumatic shock in rats.

MATERIALS AND METHODS

Experiments were performed on male Wistar strain rats (200 g) caged on a standard balanced diet, fasted 12 hours before experiments and allowed water "ad libitum" until 2 hours before experiments. No water was permitted during the first 8 hours after experiment. They were submitted to a lethal traumatic shock with the aim to observe if a single dose of PBN was effective in preventing or reverting it.

Traumatic shock was provoked by the method of Noble and Collip[9] using a drum of 380 mm internal diameter, with two opposite smooth edged internal bars, 300 mm height, rotating at 58 revolutions/min. Rats were lightly anesthetized (pentobarbital 10 mg/kg i.p.) before being introduced in the rotating drum in groups of five and submitted to 1200 revolutions (100% lethal dose). The rats dead in the drum during rotation were not included in experiments as their death was presumed to be due to an acute event, different from shock (i.e. cranial trauma), as confirmed by the absence of its typical pathology. Survival, pathology, acid-base status and hematocrit were accepted as parameters of the evolution of circulatory shock. Survival was measured at 3, 6, 12 and 24 hours; surviving animals were considered permanent survivors and were sacrificed on the third day.

Pathology indicative of shock was a liver congestion associated with intestinal blood stasis in absence of other events lethal "per se" (i.e. hemorrhage). Metabolic

TABLE I
Survival after whole body trauma of rats pretreated with phenyl-t-butyl-nitrone or with olive oil alone.

		Hours after the end of the rotation			
		3	6	12	24
Olive oil (n = 13)	survivors	8/13	5/13	1/13	0/13
PBN 150 mg/kg (n = 14)	survivors	14/14	14/14	14/14	14/14
	p <	0.01	0.0001	0.00001	∞
PBN 100 mg/kg (n = 14)	survivors	14/14	14/14	14/14	14/14
	p <	0.01	0.0001	0.00001	∞
PBN 50 mg/kg (n = 12)	survivors	10/12	10/12	9/12	9/12
	p <	NS	0.02	0.001	0.0001

acidosis and hemoconcentration were accepted as signs of traumatic shock. To measure acid-base status and hematocrit (Hct), three control and three treated rats were sacrificed at each experimental time. A sample of blood was taken by cardiac puncture. pH and acid-base (BE) were measured in duplicate using a micromethod (IL 1312, Instrumentation Laboratories, Milano, Italy). Hct was measured in duplicate using a micromethod (Bayropharm, Milano, Italy).

The spin trapping agents phenyl-t-butyl-nitrone (Kodak, Ltd) was dissolved in olive oil immediately before experiments, so to avoid the risk of its hydrolysis in aqueous solutions, with formation of aldehydes and hydroxylamines[10]. Caution was taken to avoid exposure of solutions to air and light.

The following experiments were performed:

1) experiments directed to search if various doses of PBN were effective in preventing traumatic shock in rats;

2) experiments directed to search if a single dose of PBN was effective in reverting an established traumatic shock;

3) experiments directed to search for unfavourable effects of repeated PBN administration.

In experiments directed to prevent shock, PBN (50, 100 or 150 mg/kg in 1 ml solution) was injected into the peritoneal cavity ten minutes before rotation. Blood samples were taken only in the group injected with the largest dose of PBN. In experiments directed to search for reverting a well established shock, PBN was injected 30 or 60 minutes after the end of rotation. Control experiments were performed on rats receiving olive oil (1 ml i.p.) alone.

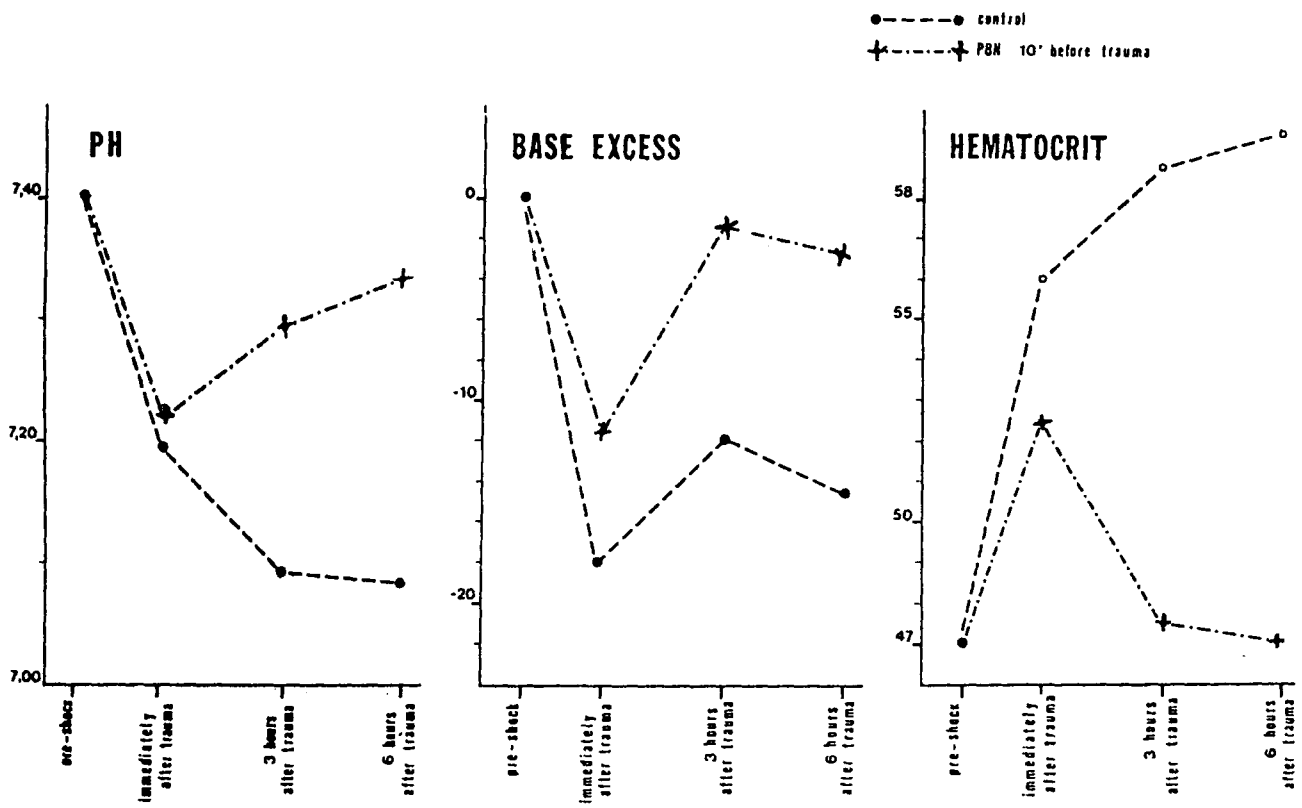
Statistical analysis were performed by the "chi square" test comparing rats receiving PBN with control ones at each experimental time. Because the effects of PBN in living animals are unknown, ten rats were submitted for seven days to a daily injection of this compound (125 mg/kg i.p.) comparing them to control rats receiving the oil alone. Alertness, general behaviour, eating, drinking and movement were examined, although not quantitated. Moreover, at the end of the experimental period, rats were sacrificed and main organs (heart, lung, liver and intestine) were examined, without finding gross pathological changes.

RESULTS

In the experiments carried out, PBN was employed in a single dose and no adverse effect was apparent. Although the method of observation was a rather crude one, no difference was apparent between PBN treated and untreated rats in alertness, eating, movement, etc.; after killing there was no macroscopical evidence of pathology of heart, liver, intestine and lungs.

Trauma provoked by the rotating drum was lethal in about 12 hours to all the untreated rats. Death was due to traumatic shock as demonstrated by typical pathology, by progressively increasing metablic acidosis and hemoconcentration. At the end of rotation pH and BE were 7.18 and - 19 respectively and progressively worsened until at the sixth hour; hematocrit was 56% and arrived to 59.6% in the last sample.

All rats receiving PBN before rotation survived in a dose related percentage (Table I). However, the degree of trauma appeared to be relevant: in fact, immediately after



RE 1 pH, base excess and hematocrit of blood taken before and after trauma from untreated rats or from rats pretreated with PBN (150 mg/kg are reported as mean values of three animals.

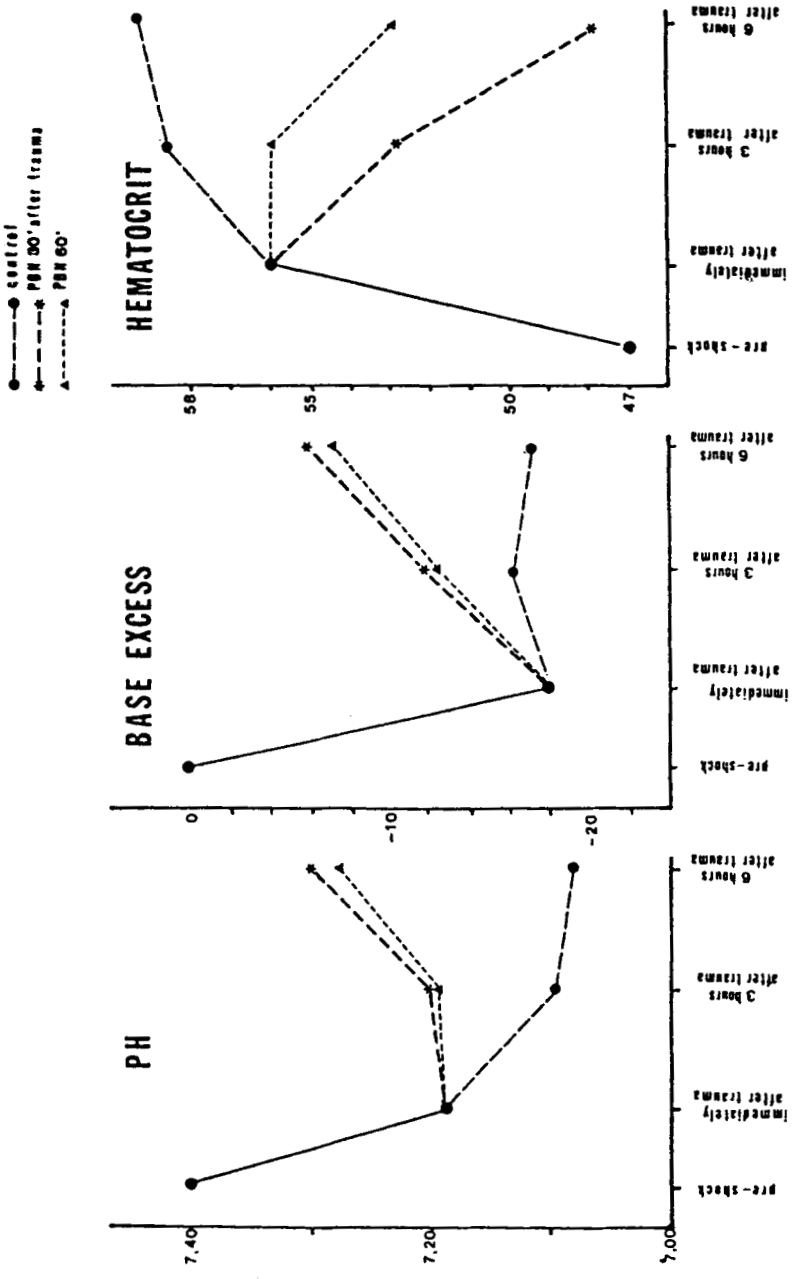


FIGURE 2 ph, base excess and hematocrit of blood taken before and after rotating drum trauma from untreated rats or from rats receiving PBN (150 mg/kg i.p.) 30 or 60 minutes after the end of rotation.
Data are reported as mean values of three animals.

TABLE II
Survival of rats submitted to lethal whole body trauma and treated with phenyl-t-butyl-nitrone at 30 or 60 minutes after trauma.

		Hours after the end of the rotation			
		3	6	12	24
Olive oil (n = 16)	survivors	8/16	3/16	0/16	0/16
PBN after 30′ (n = 14)	survivors p <	14/14 0.001	14/14 0.0001	14/14 ∞	14/14 ∞
PBN after 60′ (n = 16)	survivors p <	16/16 0.001	16/16 0.0001	16/16 ∞	16/16 ∞

rotation they had metabolic acidosis and hemoconcentration, although at smaller levels than those of the controls, progressively returning without any therapy toward normal values (Figure 1).

In another series of experiments, PBN was injected 30 or 60 minutes after the end of rotation, that is when shock was severe as shown by acidosis, high hematocrit and poor survival (Table II, Figure 2). All the rats receiving PBN 60 minutes after rotation survived and their acid-base status and hematocrit returned progressively toward normal values without any therapy.

The correction of shock induced derangements was slower when PBN was administered 60 minutes after the end of rotation. If PBN was given later it failed to reverse the more advanced traumatic shock.

DISCUSSION

The above reported results indicate that the administration of phenyl-t-butyl-nitrone is effective in preventing or reverting lethal traumatic shock in rats.

The main argument to be discussed is the effective mechanism of action of the spin trapping agent. The mechanism of action of PBN in living animals are completely unknown, because that compound is used mostly "in vitro" and only scattered reports concern its use "in vivo" to "capture" carbon centered free-radicals, different from those of oxygen.

However, the spin trapping on oxygen centered radicals is well known because only nitrones are able to detect superoxide and hydroxyl radicals at room temperature[10]; moreover, these radicals are more reactive than others and therefore are more prone to be trapped. A critical point seems to be the diffusion of the compound from the site of injection (peritoneal cavity) to the sites of free-radical generation. There are no data available on this question, although here reported results demonstrate that compound is arriving to the sites of free radical generation in a short period of time. In fact, the brief interval (ten minutes) elapsing between the intraperitoneal injection and the beginning of trauma were sufficient to reduce the degree of metabolic acidosis and hemoconcentration provoked by trauma.

In the successive samples (3 and 6 hours after rotation) both parameters returned toward normal values.

The reverting of established traumatic shock, also, was progressive as shown by the

progressive return toward normal acid-base status and hematocrit. When PBN was injected later than 60 minutes after trauma, its effectiveness decreased and survival was not significantly affected, probably due to the presence of too advanced structural damages.

The experiments reported neither exclude a mechanism of the anti-shock action of the PBN different from a "trapping" effect against oxygen free radicals nor demonstrate it. Therefore, in absence of different hypothesis it might be suggested that the action of PBN of prevention and reversion of traumatic shock in rats is related to the scavenging effect and to the consequent breaking of peroxidation chain reactions.

References

1. R. Ogawa, E. Miygawa and T. Fujita, *Jap. J. Anaesth.*, **27**, 1054, (1978).
2. G.P. Novelli and A.R. De Gaudio, in *Shock Research*, ed. D. Lewis and H. Haglund (Elsevier: Amsterdam, 1983) p. 31.
3. J.C. Saez, P.H. Ward, B. Gunter and E. Vivaldi, *Circ. Shock*, **12**, 229, (1984).
4. A.M. Lefer, H. Araki and S. Okamatsu, *Circ. Shock*, **8**, 273, (1981).
5. S.K. Cunningham and T.V. Keaveny, *Eur. Surg. Res.*, **10**, 302, (1978).
6. R. Ogawa, T. Morita, F. Kunimoto and T. Fujita, *Circ. Shock*, **9**, 369, (1982).
7. T. Yoshikawa, M. Murakami, N. Yoshida, O. Seto and M. Kondo, *Thromb. Haemostas.*, **50**, 869, (1983).
8. G.G. Corbucci, A. Candiani, G. Crimi, M. Antonelli, R.A. De Blasi and A. Gasparetto, *Circ. Shock*, **15**: 15, (1985).
9. R.L. Noble and J.B. Collip, *Quart. J. Exp. Physiol.*, **31**, 187, (1942).
10. G.M. Rosen and E.J. Rauckman, *Methods in Enzymology*, **105**, 198, (1984).

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