INHIBITORY EFFECT OF BISBENZYLISOQUINOLINE ALKALOIDS ON THE QUICK DEATH OF MICE TREATED WITH BCG/LPS

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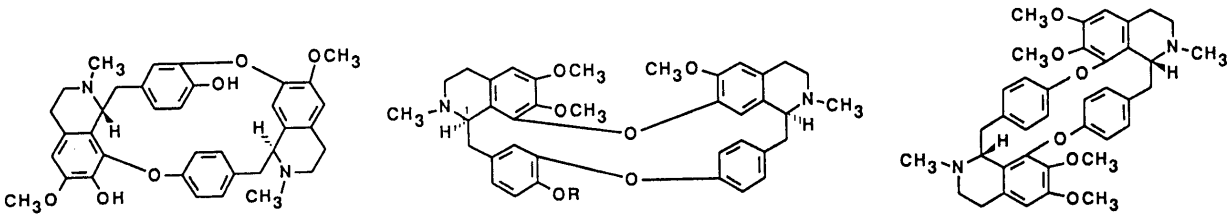
Three bisbenzylisoqinoline alkaloids, chondocurine (Chon), berbamine (Ber), and cycleanine (Cyc) were tested for their protective effect on the quick death of mice primed with bacillus Calmette Guerin (BCG) and elicited with lipopolysaccharide (LPS). Seven-consecutive treatments with Chon or Cyc at a dose of 10 mg/kg following BCG priming resulted in significant improvement in the survival rate. A single dose of Chon also protected the BCG/LPS-treated mice from death if it was given immediately after, not 4 h after, LPS elicitation. These data show that bisbenzylisoquinoline alkaloids can protect mice from the lethal toxicity induced by the BCG/LPS combination treatment by inhibiting the priming with BCG or the elicitation with LPS.

KEYWORDS bisbenzylisoquinoline alkaloid; BCG/LPS treatment; chondoculine; cycleanine; quick death

The susceptibility to lethal action of lipopolysaccharide (LPS) is increased by treatment or infection with some bacteria such as the bacillus Calmette Guerin (BCG)[1] or *Coxiella burnettii*.[2] Recently, Morisawa et al.[3] reported that the mice primed with bacteria and elicited with LPS showed an acute fulminant hepatic injury due to hepatic microvascular thrombosis. This treatment provides an experimental model of fulminant hepatitis.



chondocurine berbamine cycleanine

Chart 1

Bisbenzylisoquinoline alkaloids, a common component in some plant remedies, have been used for diseases with inflammation such as chonic rheumatism, silicosis, neuralgia, acute hepatitis, and some infections. In this study, three bisbenzylisoquinoline alkaloid analogues, chondoculine (Chon), berbamine (Ber) and cycleanine (Cyc), were tested for their protective effect on the mortality of the BCG/LPS-treated mice. These agents were obtained from the plant families Menispermaceae and Ranunculaceae (genus Thalictrum).[4)]

Inbred BALB/c male mice, 6 to 8 weeks of age, (SLC Co., Shizuoka) were injected intravenously (iv) (day 0) with 1 mg of BCG (Nippon BCG Co., Tokyo), and seven days after the BCG treatment, LPS (50 μg) from *E. coli* 0111:B4 strain (Difco, Detroit) was injected iv. The mortality of the test mice was periodically examined until 24 h later. Bisbenzylisoqunoline alkaloids were injected intraperitoneally (ip) for 7 consecutive days (day 0 to day 6) starting 12 h after the BCG priming and ending 24 h before the LPS elicitation. Six of 7 control mice died and all of the mice given 10 mg/kg of Chon survived (Fig.1). The survival time of the Cyc-treated mice was significantly increased compared with the control mice, although all of the mice in both groups died within 20 h. The Ber treatment did not increase the survival of the mice (Fig.2). The mechanisms underlying the high sensitivity to LPS in animals treated with bacteria such as BCG or *Propionibacterium acnes* are not fully understood, but apparently macrophages, including Kuppffer cells, are increased and stimulated in the liver and then these cells are fully activated upon LPS injection. In this context, the data obtained here suggest that if most of the agents administered were excreted in 24 h before the LPS treatment, the Chon and Cyc protected the BCG/LPS-treated mice by inhibiting the increase of stimulated macrophages in the liver.

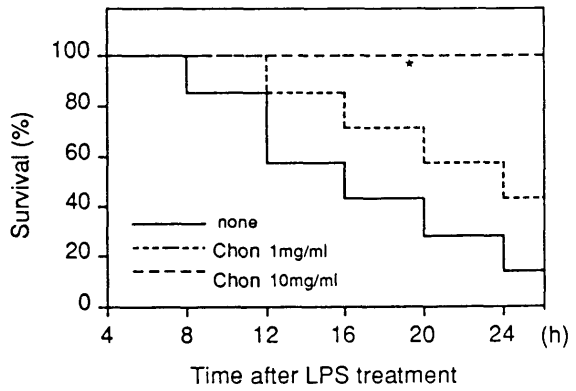


Fig. 1. Effect of Chon on the Survival of Mice Primed with BCG and Elicited with LPS

Mice were primed iv with 1 mg of BCG (day 0) and treated ip with Chon for 7-consecutive days (day 0 to day 6). Seven days after BCG priming (day 7), LPS (50 ug) was injected iv into the mice. Values in each group were obtained from 7 mice. Differences in survival between experimental and control animals were statistically compared by the Cox and Mantel method. Significantly different from control, *p<0.01.

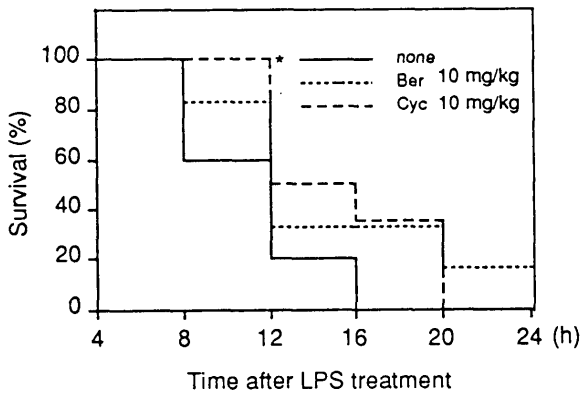


Fig. 2. Effects of Ber and Cyc on the Survival of Mice Primed with BCG and Elicited with LPS

Treatment and comparison of survival were as described in Fig.1. Values in the control group were obtained from 5 mice and these in the Ber- or Cyc-treated group were obtained from 6 mice. Significantly different from control, *p<0.05.

The effect of Chon on the eliciting phase triggered with LPS is shown in Fig.3. In this experiment, a single dose (10 mg/kg) of Chon was given immediately after, or 4 h after the LPS elicitation. The survival time of mice treated immediately with Chon was significantly longer than that of the control mice, but the

delayed treatment of Chon did not improve survival. This result indicates that Chon can suppress the triggering process, but can not suppress the lethal process after it is triggered.

The interaction between LPS and macrophages may play a major role in the eliciting step. Triggering of macrophages with LPS results in the cell activation and the release of various mediators including TNF and IL1. TNF was originally found in the serum of mice receiving the BCG/LPS combination treatment[5] and was shown to be identical to cachectin.[6] This molecule has been reported to have pleiotropic biological activites including the promotion of clot formation[7] and to be a probable factor responsible for the death accompanying hepatic damage.[8] We can postulate these mechanisms: the bisbenzylisoquinoline alkaloids protect BCG/LPS-treated animals by inhibiting the activation of macrophages, followed by the release of toxic mediators such as TNF, and by inhibiting the accumulation of stimulated macrophages . Seow et al.[9] reported that tetrandrine inhibited IL1 production by macrophages in vitro, and we[10] also obtained some evidence that macrophages from Chon-treated mice produced less IL1 than the control macrophages did. The present data suggest that Chon and Cyc are promising agents for fulminant hepatitis, in which activated macrophages play a key role.

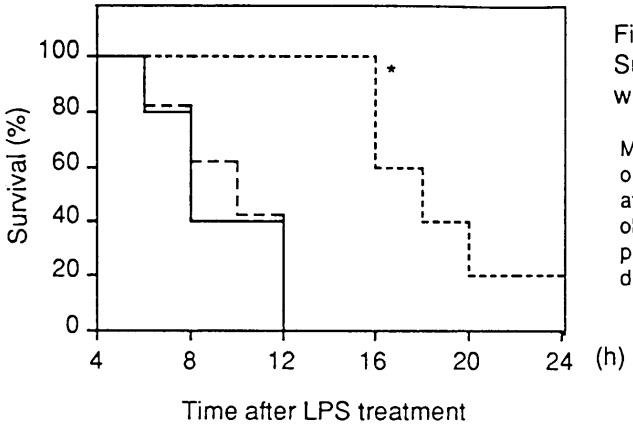


Fig.3. Time-Dependent Effect of Chon on the Survival of Mice Primed with BCG and Elicited with LPS

Mice primed iv with 1 mg of BCG were treated ip once with Chon (10 mg/kg) immediately after, or 4 h after LPS elicitation. Values in each group were obtained from 5 mice. Comparison of survivial was performed as described in Fig. 1. Significantly different from control, *$p<0.01$.

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