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Short communication

Quinine inhibits release of tumor necrosis factor, apoptosis, necrosis and mortality in a murine model of septic liver failure

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Abstract

We investigated the effect of quinine on liver injury induced by lipopolysaccharide in mice sensitized with D-galactosamine. This model is characterized by high systemic release of tumor necrosis factor, which mediates hepatic apoptosis and necrosis. Pretreatment with quinine, a K^+ channel blocker, prevented formation of tumor necrosis factor (TNF) as well as the subsequent hepatic DNA fragmentation and liver enzyme leakage. Thus, inhibition of K^+ channels may be a novel therapeutic approach in cytokine-related organ damage.

Keywords: Endotoxin; Septic shock; DNA fragmentation; K^+ channel; Systemic inflammatory response syndrome; Cerebral malaria

1. Introduction

Lipopolysaccharide is widely used to initiate an experimental systemic inflammatory response syndrome, which is characterized by overexpression and subsequent release of high concentrations of circulating proinflammatory cytokines such as tumor necrosis factor-α (TNF). Therefore, pharmacological approaches to the amelioration of the fatal outcome of clinical complications evoked by the systemic inflammatory response syndrome have focused on drugs that reduce the excessive release of this distal mediator. Recently it was reported that lipopolysaccharide-induced TNF release from human alveolar macrophages involves the activation of K^+ channels. This study showed that quinine, a potent K^+ channel blocker, inhibited TNF production in vitro (Marayuma et al., 1994). This prompted us to investigate quinine in a murine in vivo model of the systemic inflammatory response syndrome, i.e. the D-galactosamine/lipopolysaccharide-induced liver failure, in which the central role of TNF is well documented (Tiegs et al., 1989) and which is characterized by early apoptosis and subsequent necrotic liver cell lysis (Leist et al., 1995).

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2. Materials and methods

D-Galactosamine was purchased from Roth Chemicals (Karlsruhe, Germany), lipopolysaccharide (*Salmonella abortus equi* endotoxin) was from Sebak (Aldenbach, Germany). Quinine was from Sigma Chemicals (Deisenhofen, Germany).

Specific pathogen-free male BALB/c mice weighing 25–30 g from the animal house of the University of Konstanz, Germany, were kept in a 12 h day/night rhythm and received humane care in adherence to legal requirements. D-Galactosamine (700 mg/kg) was injected i.p. in saline. Lipopolysaccharide (1 μg/kg) was given together with D-galactosamine in a total volume of 200 μl. Quinine (23 mg/kg or 230 mg/kg, respectively) or vehicle (corn oil) was administered i.p. in 300 μl per animal 30 min before injection of D-galactosamine or D-galactosamine plus lipopolysaccharide.

Blood was collected from the tail vein 90 min after D-galactosamine/lipopolysaccharide administration for plasma TNF determination by an ELISA specific for murine TNF. Further blood samples were obtained after lethal anesthesia of mice (150 mg/kg pentobarbital) or from animals that died during the experiments.

After termination of the experiments, blood was

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withdrawn by cardiac puncture into heparinized syringes. Livers were perfused for 10 s with cold buffer (50 mmol/l phosphate, 120 mmol/l NaCl, 10 mmol/l EDTA, pH 7.4) before the large median lobe was excised and a 20% liver homogenate (w/w) in phosphate buffer was prepared using an Elvehejm-type homogenizer. Liver homogenates were centrifuged at 13000 × *g* for 15 min, supernatant was further diluted 250-fold and used for determination of DNA fragmentation by a commercially available ELISA kit designed to quantify cytosolic oligonucleosome-bound DNA (Boehringer, Mannheim, Germany). The cytosolic fraction (13000 × *g* supernatant) from approximately 75 μg liver tissue was employed as an antigen source in a sandwich ELISA with a primary anti-histone antibody and a secondary anti-DNA antibody coupled to peroxidase (Leist et al., 1994). From the absorbance values of the ELISA the percentage of fragmentation of hepatic DNA from livers of D-galactosamine/lipopolysaccharide-challenged mice was calculated in comparison to D-galactosamine controls, which were not significantly different from saline-treated controls (not shown). Liver cell lysis was assessed by measuring plasma enzyme activities of aspartate aminotransferase, and the liver specific enzymes alanine aminotransferase and sorbitol dehydrogenase according to Horder and Rej (1984), using an automated procedure.

Data were analyzed by non-parametric analysis of variance (Kruskal-Wallis), and where there were differences among the groups ($P > 0.05$) data were subjected to one-sided non-parametric multiple comparisons of the D-galactosamine/lipopolysaccharide group against all other groups (Zar, 1984). $P < 0.05$ was considered to be significant.

3. Results

Injection of D-galactosamine-sensitized mice (700 mg/kg) with lipopolysaccharide (1 μg/kg) resulted in severe liver failure, i.e. the presence of high plasma activities of alanine aminotransferase, aspartate aminotransferase and sorbitol dehydrogenase 8 h after injection of lipopolysaccharide (Fig. 1). Approximately 75% of the animals died within 8 h (Table 1). Pretreatment of mice with quinine prior to D-galactosamine/lipopolysaccharide injection gave a partial protection at a dose of 23 mg/kg and a complete prevention of liver failure at the 10-fold dose (Fig. 1). Thus quinine provided a dose-dependent prophylactic protection from liver injury and from lethality at the high dose tested (Table 1). The behavior of the quinine-pretreated animals appeared normal. Since TNF is known to be a critical mediator of D-galactosamine/lipopolysaccharide-induced liver failure, we measured TNF concentrations in the circulation of the animals 90 min after injection of D-galactosamine/lipopolysaccharide, i.e. at the peak time. High amounts of TNF were released into plasma following injection of D-galactosamine/lipopolysaccharide, whereas no TNF was detectable in the circulation after quinine pretreatment (230 mg/kg). These mice were completely protected against D-galactosamine/lipopolysaccharide-induced DNA fragmentation.

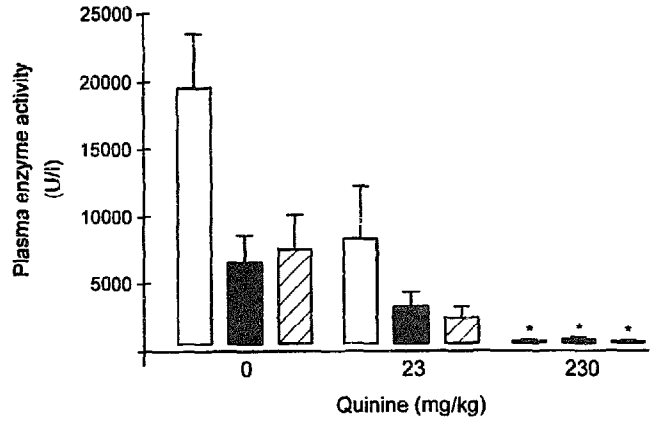

Fig. 1. Protection of D-galactosamine/lipopolysaccharide-induced liver failure by quinine. Quinine (23 ($n = 3$), or 230 mg/kg ($n = 6$), respectively) was injected 30 min before D-galactosamine (700 mg/kg) and lipopolysaccharide (1 μg/kg). Control animals received the corresponding volume of vehicle (corn oil, 300 μl per mouse, $n = 9$). Eight hours after challenge, animals were killed and plasma enzyme activities were determined: alanine aminotransferase (open bars), aspartate aminotransferase (solid bars), sorbitol dehydrogenase (striped bars). Data represent mean ± S.D.; $^*P < 0.05$ vs. disease control.

Table 1
Effects of quinine on TNF production, hepatic DNA fragmentation and mortality of mice challenged with D-galactosamine/lipopolysaccharide

Treatment	TNF (pg/ml)	DNA fragmentation (% Control)	*m*/*n*
D-Galactosamine control	< 10 *	100 ± 20 *	0/3
D-Galactosamine + lipopolysaccharide	920 ± 330	1030 ± 54	7/9
Quinine (230 mg/kg) + D-galactosamine + lipopolysaccharide	< 10 *	174 ± 108 *	0/6

Blood for determination of TNF was withdrawn 90 min after injection of D-galactosamine (700 mg/kg) and lipopolysaccharide (1 μg/kg). Detection limit of the assay: ≤ 10 pg/ml. From the absorbance values of the enzyme-linked immunosorbent assay, the percentage of DNA fragmentation was calculated in comparison to D-galactosamine controls, which were not significantly different from saline controls (data not shown). *n* = number of animals per group; *m* = number of animals that died before the end of the experiment. Data represent mean ± S.D.; * $P < 0.05$ vs. disease control (D-galactosamine/lipopolysaccharide).

4. Discussion

The present findings demonstrate that quinine is an inhibitor of lipopolysaccharide-induced TNF production in vivo. These observations demonstrate that previous in vitro findings, i.e. that quinine inhibits TNF release from lipopolysaccharide-stimulated alveolar macrophages (Marayuma et al., 1994), translate to an in vivo situation of septic liver failure. The mechanism of action of quinine is believed to be due to its K^+ channel blocking activities, which is in line with the observation that lipopolysaccharide activates K^+ channels in vitro (Marayuma et al., 1994) and that macrophages were identified as the primary cellular source of TNF in the D-galactosamine/lipopolysaccharide model (Freudenberg et al., 1986).

The protective effect of quinine was shown for all of the different parameters of lipopolysaccharide-induced toxicity in D-galactosamine-sensitized mice that were investigated: D-galactosamine/lipopolysaccharide-induced mortality, necrotic liver injury as characterized by plasma enzyme leakage and apoptotic liver damage as assessed by DNA fragmentation were completely suppressed by quinine pretreatment. All these effects can be explained by inhibition of TNF production, since this cytokine has been shown to be a critical mediator of these pathologic events when evoked by lipopolysaccharide administration in D-galactosamine-sensitized mice (Lehmann et al., 1987; Tiegs et al., 1989; Leist et al., 1995). Whether quinine has additional direct actions on TNF-mediated processes on hepatocytes as target cells remains to be elucidated.

Amazingly, the long-known ability of quinine to suppress fever still awaits a mechanistic explanation. The fact that it suppresses TNF secretion now suggests a rationale for the antipyretic action of the drug. A further connection to pathophysiology is worthwhile mentioning: experimental cerebral malaria in mice has been shown to be mediated by TNF (Grau et al., 1987) and dynamics of fever and serum TNF levels are closely associated in patients suffering from malaria (Karanuweera et al., 1992). Pharmacological textbooks list quinine as an anti-malarial drug: our suggestion is that attenuation of TNF secretion might be the major mechanism of quinine action against cerebral malaria.

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