Protective Effect of Chlorpromazine on Endotoxin Toxicity and TNF Production in Glucocorticoid-Sensitive and Glucocorticoid-Resistant Models of Endotoxic Shock

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Summary

The present study was designed to define the potential of chlorpromazine (CPZ) as a protective agent against lipopolysaccharide (LPS) toxicity in comparison with glucocorticoids, and to obtain initial correlations with its effects on the levels of tumor necrosis factor (TNF), a pivotal mediator of endotoxic shock. It was found that CPZ protects mice, normal or adrenalectomized, and guinea pigs against lethality of LPS, and inhibited TNF serum levels, like dexamethasone (DEX), a well-known inhibitor of TNF synthesis. CPZ protected against LPS lethality when administered 30 minutes (min) before, simultaneously, or up to 10 min after LPS and was ineffective when given 30 min after LPS, paralleling the inhibitory effect on TNF production. In another experimental model, where mice were sensitized to LPS toxicity by actinomycin D, CPZ significantly inhibited LPS lethality and hepatotoxicity, whereas under these conditions DEX was inactive. These experiments indicate that CPZ has a protective action in both glucocorticoid-sensitive and -resistant models of endotoxic shock.

Tumor necrosis factor (TNF) is a key mediator in the toxic effect of endotoxin (LPS) and in the pathogenesis of septic shock. In fact, serum levels of TNF are high after LPS administered to animals and human volunteers, or in septic patients (1, 2), while administration of anti-TNF antibodies protects against the lethal effect of LPS and live bacteria in a variety of animal models (3, 4).

Anti-TNF antibodies are also protective in a variety of animal models of human diseases, including cerebral malaria (5), bacterial meningitis (6), bleomycin lung toxicity (7), liver ischemia/reperfusion damage (8), and graft-versus-host reaction (9), indicating an involvement of TNF in the pathogenesis of these diseases. Therefore, TNF might be an important target for pharmacological action, and inhibitors of its synthesis might be useful in the therapy of these diseases. The well-known inhibitory effect of glucocorticoids on TNF synthesis is thought to be connected with protective action of glucocorticoids in endotoxic shock (10-12).

Previous data from this laboratory have shown that chlorpromazine (CPZ)[1] protects mice against the lethal effect of LPS and IL-1, but not of TNF (13). The objective of the present study was to define the potential of CPZ to block LPS toxicity in comparison with glucocorticoids and to obtain ini-

tial correlations with its effect on the levels of TNF. For this purpose, we have studied the effect of CPZ on LPS toxicity and induction of TNF in various experimental models, including: (*a*) high-dose LPS (30 mg/kg) in mice, (*b*) administration of LPS (5 mg/kg) in guinea pigs, which are more sensitive to LPS than mice in terms of lethality and TNF induction (14), (*c*) adrenalectomized mice, known to be more sensitive to LPS toxicity and TNF induction (11, 15), and (*d*) mice pretreated with actinomycin D (Act D), which present increased sensitivity to LPS (comparable to that of adrenalectomized mice) through a mechanism that is still not clear (10, 16). In these experimental models, the activity of CPZ was compared with that of dexamethasone (DEX), which inhibits TNF production and protects against LPS toxicity in some animal models (10-12). Since some animal models of endotoxic shock appear to be resistant to glucocorticoids, and clinical studies have indicated that glucocorticoid therapy might not be effective in septic patients (17, 18), the comparison of CPZ and DEX might indicate any possible advantages in the use of CPZ in those situations where glucocorticoids are ineffective.

Materials and Methods

Animals, Drugs, and Cytokines. Male adult (25-28 g) CD1 mice, obtained from Charles River Italia (Calco, Como, Italy) were used,

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[1] *Abbreviations used in this paper:* Act D, actinomycin D: CPZ, chlorpromazine; DEX, dexamethasone; GPT, glutamate pyruvate transaminase.

unless otherwise indicated. All treatments were given by i.p. injection in 0.2 ml of sterile pyrogen-free saline, between 9.00 and 1.00 a.m. LPS (Westphal preparation from *Escherichia coli* 055:B5) was from Sigma Chemical Co. (St. Louis, MO), CPZ (Largactil, Farmitalia Carlo Erba, Milano, Italy) and DEX phosphate (Farmacologico Milanese, Milan, Italy) were given intraperitoneally 30 min before LPS at doses of 4 and 30 mg/kg, respectively (13). Survival was assessed every day on at least 10 animals per group and statistical analysis was done using Fisher's exact test. TNF levels were measured in sera obtained 1 h after LPS (unless otherwise indicated) by cytotoxicity on L929 cells (19), and expressed as ng/ml by comparison with a standard of human recombinant TNF (sp act, 107 U/mg). Liver damage was evaluated by measuring serum glutamate pyruvate transaminase (GPT) using a commercially available kit (Boehringer, Mannheim, Mannheim, Germany).

Adrenalectomized Mice. Mice were bilaterally adrenalectomized under ether anesthesia and were kept for 10 d to recover before the experiment. They were given 0.9% NaCl in drinking water. Mice were treated with 75 μg/kg of LPS, as previously described (11) and survival was assessed daily.

Act D-Sensitized Mice. Mice were treated with Act D (0.6 mg/kg), 10 min before LPS (30 μg/kg), as previously described (16), and hepatotoxicity was assessed on the basis of serum GPT measured 7 h after treatment.
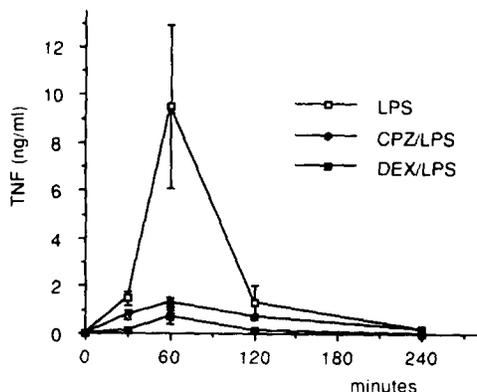
High-Dose LPS Toxicity. Male, adult (22–24 g) Balb/C mice were used (Charles River Italia). They were given LPS at the dose of 30 mg/kg and survival was assessed daily.

Guinea Pigs. Male guinea pigs weighing 275–300 g (Charles River Italia) were used. LPS was administered at the dose of 5 mg/kg and survival was assessed daily.
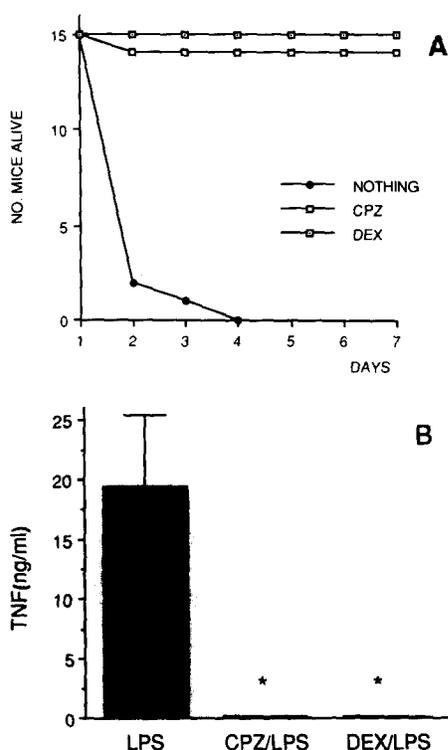
Results

In a first set of experiments, we studied the effect of pretreatment with CPZ (4 mg/kg) or DEX (30 mg/kg) on TNF production in CD1 mice given a nontoxic dose of LPS (75 μg/kg). The time course of TNF production is shown in Fig. 1. Peak TNF levels were observed after 1 h, and both CPZ and DEX almost completely blocked the release of TNF.
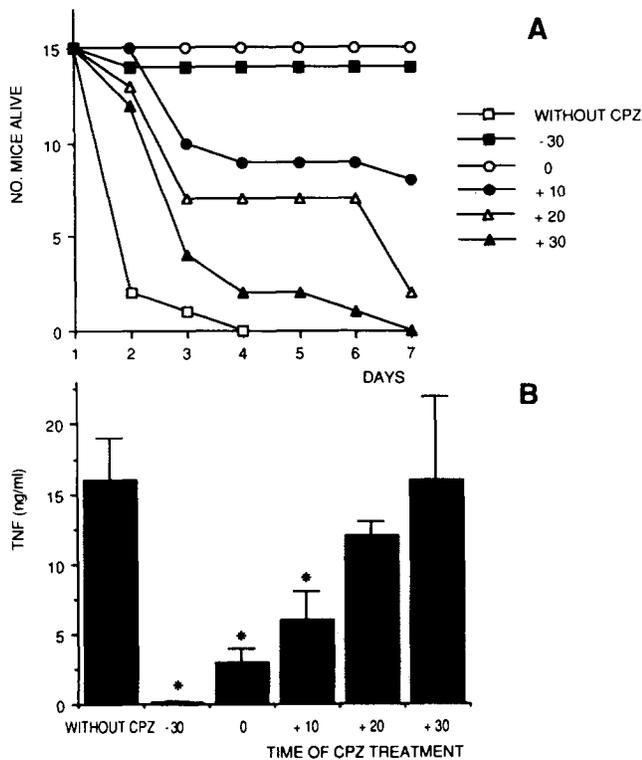
The effect of CPZ and DEX was then studied on the

Figure 2. Effect of CPZ and DEX on LPS toxicity (*A*) and induction of serum TNF (*B*) in Balb/C mice. Mice were treated with 30 mg/kg of LPS, with or without CPZ or DEX pretreatment, as described before. Survival was assessed daily on groups of 15 mice. Serum TNF levels were measured 1 h after LPS treatment. Data are mean ± SD (five mice/group). * $p < 0.01$ vs LPS alone by Student's *t*-test.

lethality of high-dose LPS. Balb/C mice were used since in preliminary experiments CD1 mice were found to be extremely resistant to the toxic effects of high doses of LPS. As shown in Fig. 2 *A*, 30 mg/kg of LPS caused the death of 100% of the mice within 4 d. Both CPZ and DEX completely protected against LPS toxicity. The same figure (*B*), shows serum TNF levels 1 h after LPS in the same experimental conditions. Both drug inhibited TNF production, thus paralleling the data on mortality.

Using this experimental model we have studied the effect of different schedules of CPZ treatment on LPS toxicity and serum TNF levels. As shown in Fig. 3, CPZ had maximal protective effect on LPS toxicity and TNF production when given as a pretreatment (30 min before LPS). The effectiveness of CPZ was still significant when administered simultaneously with or 10 min after LPS both on lethality and TNF production. The efficacy of CPZ disappeared when it was administered as late as 30 min after LPS.

A dose response of the effect of CPZ on LPS toxicity and TNF production is presented in Fig. 4. It can be seen that the optimal dose to give a protection against LPS toxicity is 4 mg/kg. A protective effect was still evident at 1 mg/kg and disappeared below 0.25 mg/kg or when CPZ dose was increased at 8 mg/kg. When the inhibitory effect of different

Figure 1. Effect of CPZ and DEX on serum TNF in LPS-treated mice. Mice were treated with 75 μg/kg of LPS, with or without CPZ or DEX pretreatment, as described in the text. Data are mean ± SD (five mice/group).

Figure 3. Time-dependency of the protective effect of CPZ on LPS lethality and TNF induction in Balb/C mice. Mice were treated with 30 mg/kg of LPS, with or without CPZ. CPZ was given 30 min before, simultaneously with, 10, 20, or 30 min after LPS. Survival was assessed daily on groups of 15 mice. Serum TNF levels were measured 1 h after LPS treatment. Data are mean ± SD (five mice/group). * $p < 0.01$ vs LPS alone by Student's t-test.
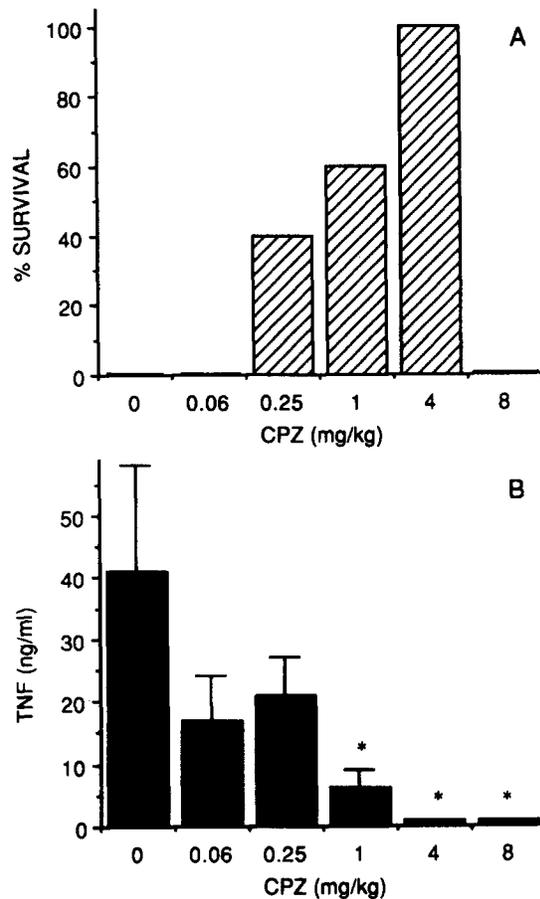

Figure 4. Effect of different doses of CPZ on LPS lethality and TNF production in Balb/C mice. Mice were treated with 30 mg/kg of LPS, with or without CPZ. CPZ was given 30 min before LPS at the indicated dose. Survival was evaluated 5 d after LPS treatment. Serum TNF levels were measured 1 h after LPS treatment. Data are mean ± SD (five mice/group). * $p < 0.01$ vs LPS alone by Student's t-test.
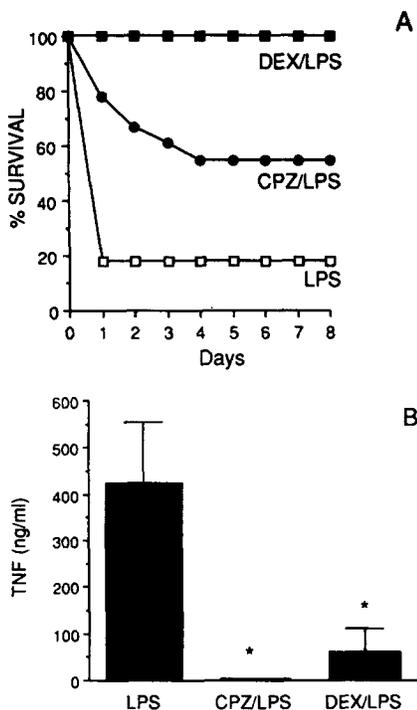
doses of CPZ on TNF production was investigated, an inhibition was also observed with the lowest doses tested but the minimal effective dose to attain an almost complete inhibition was 1 mg/kg. Interestingly enough, at the highest dose (8 mg/kg) CPZ completely inhibited TNF production but did not improve survival of mice.

The effects of pretreatment with CPZ and DEX in guinea pigs given 5 mg/kg LPS are reported in Fig. 5. A high mortality (82%) was observed in guinea pigs within the first day after LPS. Guinea pigs also had high TNF levels, compared to mice, in agreement with previous reports on their high susceptibility to LPS (14). In this case too, CPZ markedly

Figure 5. Effect of CPZ and DEX on LPS toxicity (A) and induction of serum TNF (B) in guinea pigs. Guinea pigs were treated with 5 mg/kg of LPS with or without CPZ or DEX pretreatment as described before. Survival was assessed daily on groups of 16 guinea pigs. Serum TNF levels were measured 1 h after LPS treatment. Data are mean ± SD (five guinea pigs/group). * $p < 0.01$ vs LPS alone by Student's t-test.
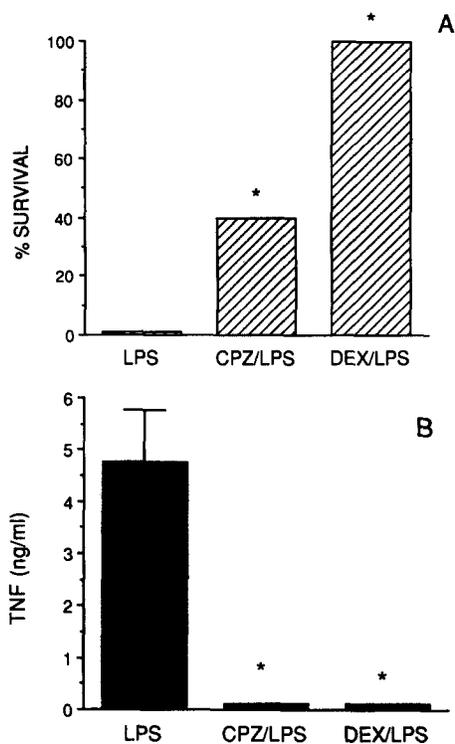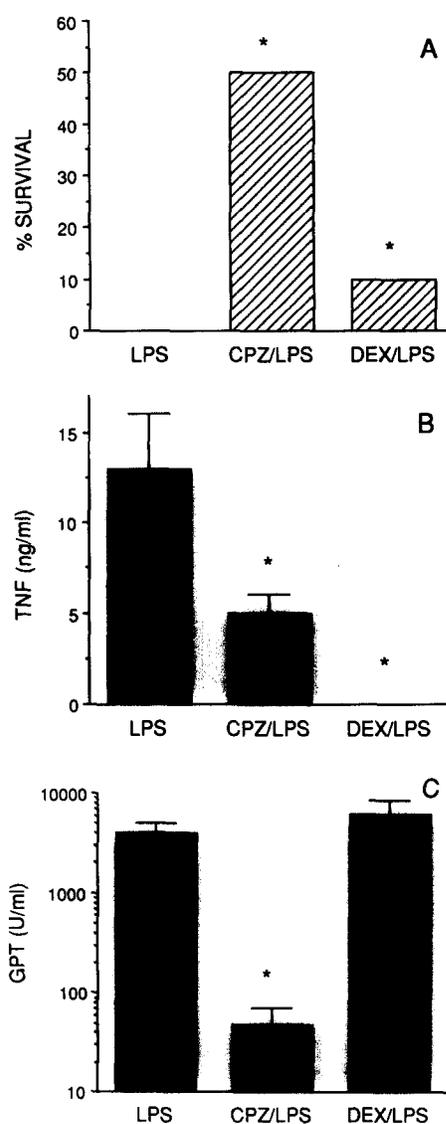
Figure 6. Effect of CPZ and DEX on LPS toxicity (*A*) and induction of serum TNF (*B*) in adrenalectomized mice. Mice were treated with 75 μg/kg of LPS with or without CPZ or DEX pretreatment as described before. Lethality was evaluated on groups of 15 mice 2 d after LPS treatment, and no further deaths were observed up to 8 d. Serum TNF levels were measured 1 h after LPS treatment. Data are mean ± SD (five mice/group). * $p < 0.01$ vs LPS alone by Student's t test (TNF levels) or $p < 0.05$ by Fisher's exact test (lethality).

protected against LPS toxicity and inhibited TNF production, although it was less potent than DEX.

CPZ and DEX were then tested in adrenalectomized mice, and the results are reported in Fig. 6. In this experimental model, 100% toxicity was observed after low doses of LPS (75 μg/kg). CPZ and DEX once again had a significant protective effect, confirming our previous findings (13). Both drugs also inhibited TNF production in adrenalectomized mice.

Finally, we used a murine model where sensitization to LPS is achieved by pretreatment with Act D. LPS toxicity in this experimental model is associated with a marked hepatotoxicity, and serum GPT levels were also measured. As shown in Fig. 7 *A*, 100% lethality was observed when a dose of LPS as low as 30 μg/kg was given after Act D. Pretreatment with CPZ had a marked protective effect against Act D/LPS toxicity, while DEX was only marginally effective. However, as shown in Fig. 7 *B*, both drugs inhibited TNF release in this experimental model. Indeed, the inhibitory effect of DEX was even stronger than that of CPZ. When serum GPT were measured in moribund animals, 7 h after Act D/LPS (Fig. 7 *C*), it was evident that only CPZ, not DEX, protected against Act D/LPS hepatotoxicity.

Figure 7. Effect of CPZ and DEX pretreatment on LPS toxicity and induction of serum TNF in mice treated with 30 μg/kg of LPS and 0.6 mg/kg of Act D (see Materials and Methods). (*A*) lethality was evaluated on groups of 15 mice 2 d after LPS treatment, and no further deaths were observed up to 8 d. * $p < 0.05$ vs LPS alone by Fisher's exact test. (*B*) serum TNF levels were measured 1 h after LPS treatment (data are mean ± SD of five mice/group). * $p < 0.01$ vs LPS alone by Student's t-test. (*C*) serum GPT levels were measured 7 h after LPS treatment (data are mean ± SD of seven mice/group). * $p < 0.01$ vs LPS alone by Student's t-test.

Discussion

The present study indicates that CPZ has a protective effect against the toxicity of LPS in various animal models. In three experimental models, normal and adrenalectomized mice and guinea pigs, CPZ was less effective than DEX. In these experimental models, the protective effect of CPZ in terms of survival was associated with an inhibition of serum TNF levels, similarly to what was observed with DEX. Furthermore, CPZ had a protective effect against LPS toxicity in Act D-sensitized

mice, although in this case the interpretation of the results is more difficult.

Like galactosamine, Act D potentiates the toxicity of LPS (10, 16, 20). It was suggested this was due to inhibition of hepatic protein synthesis, and in fact fulminant hepatitis was observed in galactosamine/LPS-treated mice. The mechanism of this hepatotoxic effect has not yet been clarified, but seems to involve damage similar to that following ischemia/reperfusion (20). This experimental model differs from the other models studied in many respects. In particular, there was no protection by DEX and toxicity (lethality and liver damage) did not correlate with TNF production, which was blocked by DEX. Finally, in all the other experimental models there was no hepatotoxicity, since no transaminase levels were detected in moribund animals (data not shown). Therefore, in Act D-sensitized mice, toxicity seems not to be mediated only by TNF and, more importantly, is resistant to glucocorticoids. The effectiveness of CPZ in an experimental model of DEX-resistant LPS toxicity is particularly interesting if we consider that recent clinical trials indicated that high-dose glucocorticoids are ineffective in the treatment of severe sepsis and septic shock (17, 18).

The mechanism by which CPZ exerts its protective effect is still unclear, but the data reported here indicate that inhibition of TNF production might have a key role. In fact, when different schedules of CPZ administration were used, it was found that the ability of CPZ to protect against LPS lethality paralleled the inhibition of TNF production, further supporting the view of TNF being the key mediator of LPS toxicity. Similar results were obtained with dose-response experiments (Fig. 5), where a marked protective effect on LPS toxicity was obtained only with CPZ doses effective in completely inhibiting TNF production. The optimal dose of CPZ was found to be 4 mg/kg, while higher doses, while

still inhibiting TNF production were not effective on LPS toxicity in the same mice. However it should be noted that CPZ at this dose had a strong hypotensive effect that might well oppose the protective action due to inhibition of TNF production.

As far as the mechanism of the inhibitory effect on TNF production is concerned, previous reports indicated that CPZ inhibits the induction of mRNA for IL-2, IFN-γ and TNF in thymocytes (21) and inhibits the secretion of IL-1 by monocytes (22). It is possible that inhibition of TNF production reported in this paper is mediated by the calcium antagonizing activity of CPZ, as it was suggested for its inhibition of IL-1 secretion.

Although extrapolation of this data to man will obviously require further studies, it should be noted that the plasma levels of CPZ in rats and mice treated with doses of CPZ that inhibit TNF production in this study are well in the range (50–600 ng/ml) observed in patients treated with pharmacologically effective doses of CPZ (23–25).

On the other hand, the protective action of CPZ in Act D-sensitized mice, where inhibition of TNF production was not sufficient to protect against LPS toxicity (as indicated by the ineffectiveness of DEX) indicates that CPZ might act by some mechanisms different from those of DEX and possibly not only by inhibiting TNF production. In this respect it is important to consider that CPZ has a wide range of pharmacological activities, ranging from antipsychotic and antihistaminic action (23) to inhibition of phospholipase A2 (26), all of which could be important for the reported protective effect. It is clear that its mode of action in protecting against LPS toxicity and inhibiting TNF production needs to be elucidated and could provide useful indications for the therapy of septic shock and other cytokine-mediated pathologies.

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