

The Protective Effect of Interleukin 1 β and IL-1 α on the Acetaminophen Induced Liver Toxicity

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Summary

The effect of mouse recombinant IL-1 β and its antagonists (IL-1Ra and rabbit polyclonal antibodies to mouse IL-1 β) on survival of animals and concentration of serum aminotransferases (AST and ALT) was investigated in mice intoxicated with acetaminophen (APAP). IL-1 β (1000 IU/mouse) or IL-1 β antibody (0.5 mL/mouse) were given i.p. 3 hours before APAP and IL-Ra (2.5 mg/mouse) ½ hours before APAP, which was administered by gastric lavage. IL-1 β significantly increased the survival mice and decreased plasma level of AST and ALT ($p < 0.05$). Essentially, opposite effect had anti-IL-1 β antibodies and IL-1Ra. RT-PCR analysis has revealed that administration of APAP induces the expression of both IL-1 β and IL-1 α in liver samples already 1 hour after its administration, indicating that the synthesis of these cytokines is part of host defense reaction to toxic effect of APAP. The expression of cytokines at induced by APAP administration could be blocked by pretreatment of mice with aspirin.

Introduction

It is known that inflammatory cytokines influence the effect of hepatotoxic chemicals on liver (1). Previously we have shown that IL-1 β and IL-6 have hepatoprotective effect if given to mice before administration of acetaminophen (APAP) and that this effect is partially mediated by PGE₂ (2, 3). Some data obtained in these experiments indicated that protective effect of exogenously administered cytokines relies on/is dependent on endogenously synthesis of cytokines induced by APAP itself. In these experiments we investigated the influence of IL-1 β in the same model, since we have been able to modulate

its effect *in vivo* with specific antagonists (IL-1Ra and polyclonal anti-IL-1 β antibodies). Using RT-PCR technique we analyzed the expression level of the IL-1 α and IL-1 β in liver samples from APAP intoxicated mice. Also, the possible role of NF- κ B transcription factor in synthesis of endogenous IL-1 β was investigated in mice in which the activity of this transcription factor was blocked by intravenous administration of aspirin.

We report that APAP induces the early synthesis of host's own IL-1, which appears to be a host defense reaction that could be helped by applying appropriate doses of exogenous cytokine.

Materials and methods

Animals. CBA/H Zgr inbred mice were raised in an animal colony unit at the Department of Physiology, School of Medicine, Zagreb. Mice of both sexes aged 12-16 weeks, were kept under standard laboratory conditions and fed with commercially available murine food pellets (K-1, Domzale, Slovenia).

Induction of hepatitis with AAP. The procedure of Guarner et al. (4) was followed with slight modifications. Mice were given phenobarbitone-sodium in drinking water during 7 days (0.3 g/L), in order to induce hepatic drug-metabolizing enzymes. Animals were allowed food 4 h later. Dose of the drug was either 200 or 300 mg/kg as indicated under Results. The dose of 300 mg/kg AAP induces within 48 hours, depending on seasonal variations, between 50% and 80% mortality in control mice and the dose of 200 mg/kg about 10% mortality.

Reagents. Pure AAP substance was donated by the Krka Pharmaceutical Company (Novo Mesto, Slovenia). AAP was dissolved in heated PBS to which 1-2 drops of Tween 80 were added. Recombinant mouse IL-1 β (rmIL-1 β) and IL-1 receptor antagonist (IL-1Ra) were purchased from Peprotech Co (UK) (Cat. No. 200-01B and Cat. No. 200-01RA, respectively). Recombinant mouse IL-1 β (rmIL-1 β ; Cat. No. 1291-01, specific activity sp. act. 3.5×10^5 U/mg) was purchased from Genzyme (TEBU, Paris, France) and IL-1 receptor antagonist (IL-1Ra) from Peprotech Co (UK) (Cat. No. 200-01B and Cat. No. 200-01RA). Cytokines were given before APAP, since previous experiments showed that IL-1 α (2) and IL-6 (3) are not hepatoprotective if given after APAP. Control animals were simultaneously given 0.2 mL pyrogen-free saline.

Polyclonal antibodies to IL-1 β (anti-IL1 β abs) were obtained by three consecutive weekly injections of rmIL-1 β to rabbit, first given s.c. in FCA and remaining i.v. The rabbit was bled 10 days after last immunization, serum separated, aliquoted and frozen until the use. As tested by ability to neutralize the activity of known amount of rmIL-1 β on proliferation of IL-1 dependent line D10s (5), the potency of anti-IL1 β rabbit serum was quite low (4.7×10^3 neutralizing units/per mL).

Plasma ALT and AST concentrations. ALT and AST concentrations were

measured 24 hrs after the AAP administration. Mice were given 250 U heparin i.p. 15 min before bleeding. Blood was obtained by puncture of the medial eye angle with glass capillary tubes. After centrifugation, plasma samples were stored at -20°C for 24 h before aminotransferase determination. AST and ALT levels were determined by standard laboratory techniques.

Statistical analysis. The results are expressed as mean \pm S.E.M. Para-metric variables were compared by Student's t-test. Differences in survival between the groups of mice were compared by chi-square test, using Yates's correction of the test when indicated.

Results

The effect of IL- β on survival mice intoxicated with APAP

The effect of single injection of IL-1 β (1000 U/mouse), given i.p. 3 hours before administration of APAP (300 mg/kg), on survival of mice is shown in Figure 1. As seen, in comparison with control mice given saline, IL-1 β significantly increased survival of mice at 48 hours after administration of APAP (mortality 10% vs. 70%; chi-square test; $p < 0.01$).

The effect of anti-IL- β antibodies on survival mice intoxicated with APAP

Polyclonal α -IL-1 β (0.5 mL per mouse) or the same volume of saline was injected to mice i.p. 3 hours before administration of APAP (300 mg/kg). The survival of mice at within 48 hours is shown in Figure 2. As visible, the mortality of animals was somewhat higher in the group pretreated with α -IL-

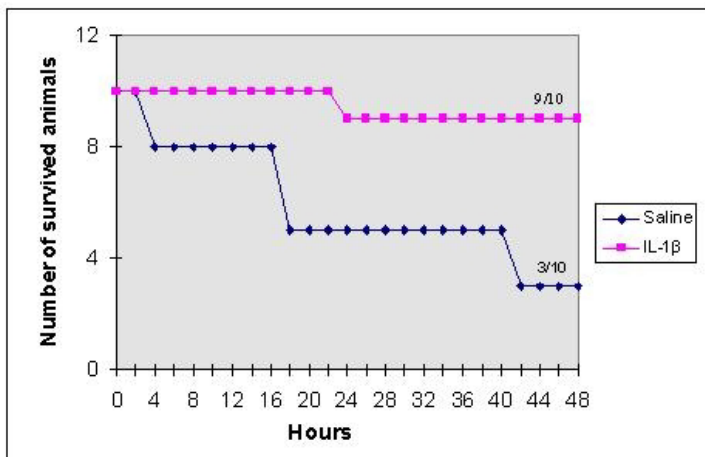


Figure 1. The effect of IL-1 β on survival of mice intoxicated with AAP. IL-1 β (1000 U per mouse) or saline were given i.p. 3 hours before AAP (300 mg/kg). $N=10$ mice per group. (Chi-square test: $p = 0.0062$)

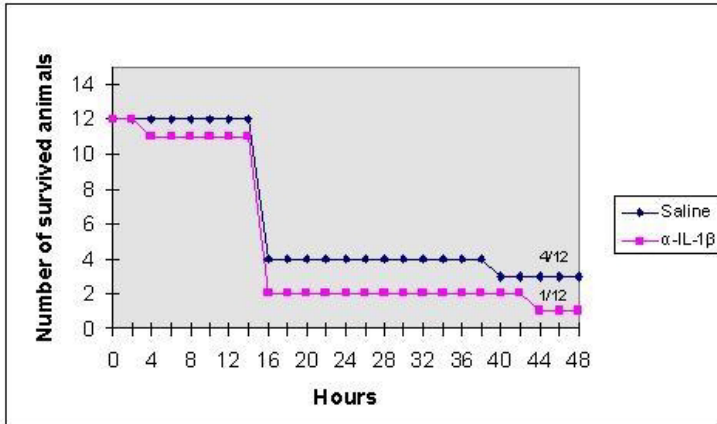


Figure 2. The effect α -IL-1 β antibodies on survival of mice intoxicated with AAP. α -IL-1 β (0.5 ml per mouse) or saline were given i.p. 3 hours before AAP (300 mg/kg). N= 12 mice per group. (Chi-square test: $p = 0.2733$)

1 β , but, most probably due to relatively mortality in control group, the difference was not statistically significant.

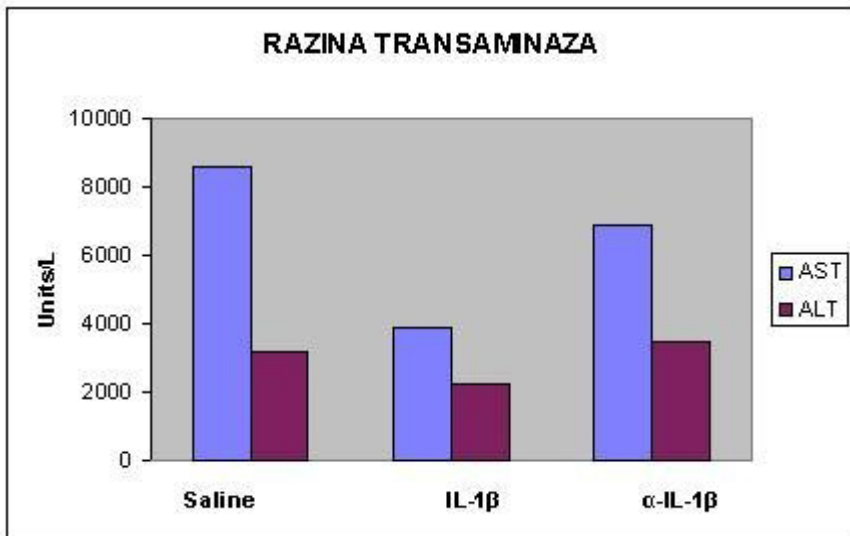


Figure 3. The effect of IL-1 β and α -IL-1 β antibodies on concentration of serum aminotransferases in mice with AAP-induced liver damage. IL-1 β (1000 U/mouse), α IL-1 β (0.5 ml/mouse) or saline were injected to mice i.p. 3 hours before AAP (250 mg/kg). AST and ALT were determined 24 hours after administration of APAP. N = 8 mice per group. (Student's t-test: IL-1 β , $p = 0.0099$ for AST and $p = 0.0222$ for ALT; anti-IL-1 β , $p = 0.17491$ for AST and $p = 0.0682$ for ALT)

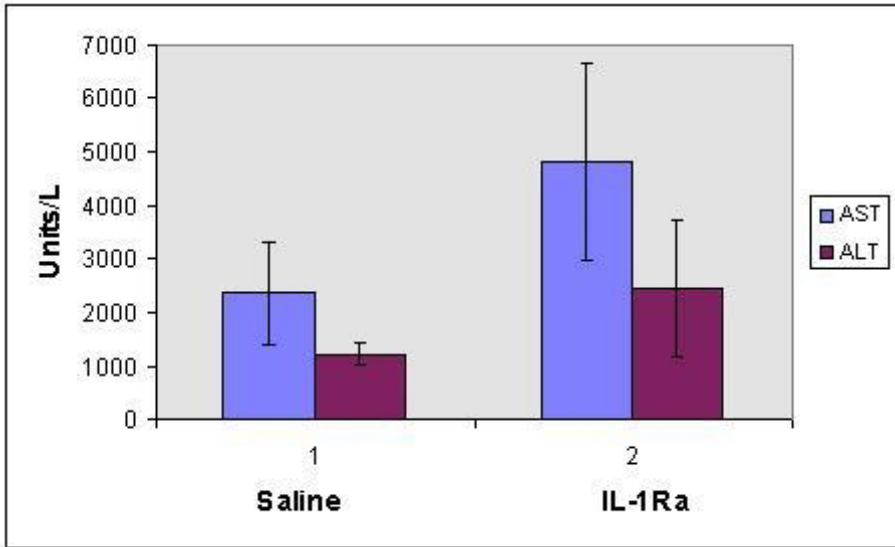


Figure 4. Effect of IL-1Ra on concentration of serum aminotransferases in mice with AAP-induced liver damage. IL-1Ra (2.5 $\mu\text{g}/\text{mouse}$) or saline were injected to mice *i.p.* 30 minutes before APAP (200 mg/kg). AST and ALT were determined 24 hours after administration of APAP. $N = 8$ to 9 mice per group. (Student's *t*-test: $p = 0.0158$ for AST and $p = 0.0286$ for ALT)

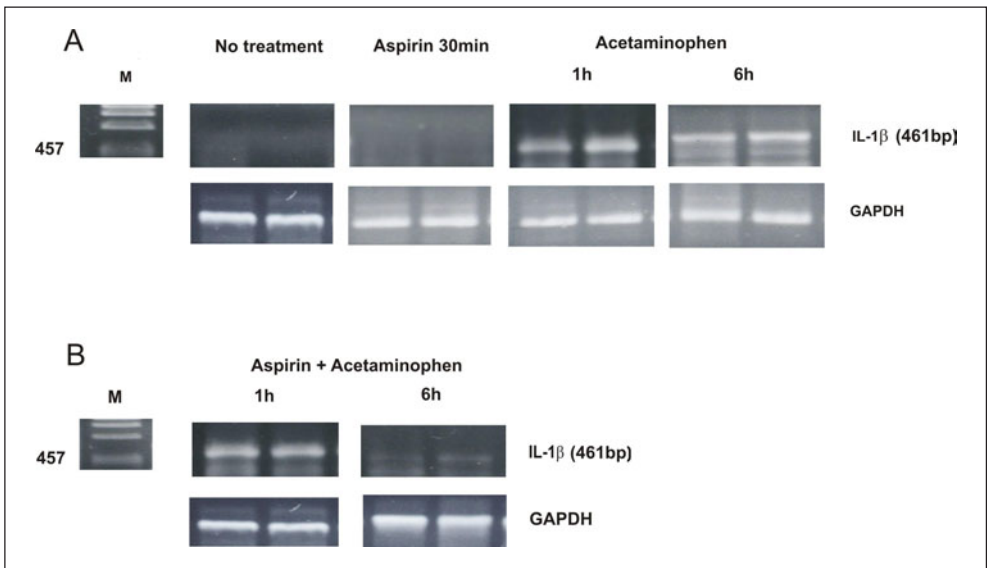


Figure 5. RT-PCR analyzes of the IL-1 β expression level in the liver samples from animals with no treatment at all, animals treated either with 100 mg of aspirin alone or 300mg of acetaminophen for 1h and 6 h (A), and animals pretreated with aspirin for 30 minutes and then treated with acetaminophen for 1 h and 6 h (B). All samples were presented in duplicate representing two different animals.

The effect of IL-1 β anti-IL- β antibodies on plasma aminotransferase level in mice intoxicated with APAP

The concentration of aspartic- and alanin-aminotransferases (AST, ALT) was determined 24 hours after administration APAP (250 mg/kg). In comparison to values in normal mice (AST 215 \pm 4 U/L, ALT 72 \pm 1 U/L), the level of AST and ALT in group of mice were given APAP highly increased (Figure 3). Pretreatment with IL-1 β significantly decreased the concentration of both enzymes ($p < 0.01$ and $p < 0.05$, respectively). Pretreatment with anti-IL-1 β antibodies increased concentration of both enzymes, but the difference was not significant ($p > 0.05$).

The effect of IL-1 receptor antagonist on plasma aminotransferase level in mice intoxicated with APAP

IL-1 receptor antagonist (IL-1Ra, 2.5 μ g/mouse) or the saline was injected to mice half hour before administration of APAP (200 mg/kg). The concentration of serum aminotransferases was determined 24 hours after administration APAP (Figure 4). Pretreatment with IL-1Ra significantly decreased the concentration of both enzymes significantly decreased concentration of both AST and ALT in blood ($p < 0.05$). Similar data were obtained when higher dose of IL-1Ra (5 μ g/mouse) was used, but its effect was less expressed if given 3 hours before APAP (data not shown).

The expression of IL-1 in liver cells of mice intoxicated with APAP

Expression of the IL-1 β and IL-1 α ? was determined by RT-PCR analysis in liver cells of mice intoxicated with APAP (300 mg/kg). As seen in Figure 4, the expression of IL-1 β which is absent in normal mice, becomes readily detectable already 1 hour after administration of APAP. This expression was even higher at 6 hours following APAP administration and could be blocked by intravenous administration of aspirin (100 mg/kg) at the dose which inhibits the activity of NF- κ B transcription factor. Similar results were obtained for IL-1 β (data not shown).

Discussion

Presented data have shown that IL-1 β given before APAP decreased its toxicity, as was evident by significant increased survival of animals and decreased concentration of aminotransferases in plasma. On the contrary, the antagonist of IL-1 receptor (IL-1Ra), which antagonize the action of both IL-1 cytokines (α and β), had just opposite effect; it increased significantly aminotransferase level in comparison to APAP-intoxicated mice given saline. Similarly, polyclonal anti-IL-1 β antibodies, although the data were not significant, appear to decrease the survival of animals and increase plasma aminotransferases concentrations.

The fact that administration of IL-1 receptor (IL-1Ra) and most probably

also anti-IL-1 β antibodies, led us to an assumption that intoxicated host synthesizes its own IL-1 β in an attempt to resist the toxic effect of APAP. Indeed, RT-PCR analysis have shown that liver cells of mice intoxicated with APAP synthesize and express IL-1 β already one hour after APAP administration, which is even more visible at 6 hours after intoxication. This expression could be almost completely blocked if mice were pretreated with aspirin, at the dose which is known to inhibit NF- β B activity (6). Intoxication with APAP induced also the expression of IL-1 α (data not shown)

Since exogenously administration IL-1 α (2) and IL-6 (3, 7) have also protective effect on APAP toxicity, it appears that beneficial effect of exogenously applied cytokines rely (is aided) on the early induction of their endogenous expression by acetaminophen itself.

Literature

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