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# Isoniazid Protects Mice against Endotoxin Lethality without Influencing Tumor Necrosis Factor Synthesis and Release

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**Treatment of NMRI mice with isoniazid (INH; 25 mg/kg) intraperitoneally induced significant protection when it was injected before or after a lethal intravenous challenge with endotoxin. The INH preparation used was not contaminated with endotoxin. Tumor necrosis factor (TNF) was not elevated in sera from NMRI mice 2 h after the injection of INH. INH did not influence TNF synthesis or release determined in human monocytes in vitro. Therefore, it is concluded that the protective effect of INH against lethal endotoxin is not due to a suppressive effect of INH on TNF production.**

Silverstein and colleagues (11) recently reported that hydrazine sulfate pretreatment of mice results in a significant protection against a subsequent lethal endotoxin challenge, while more fatalities were obtained with injection after the endotoxin challenge. The authors suggested that the protective effect might be due to the prevention of the drop in the hepatic phosphoenolpyruvate carboxykinase, although no improvement in plasma glucose was observed. In this context, the interference of hydrazine with tumor necrosis factor (TNF) also was discussed. While TNF has been shown to be an important mediator of the lethal effects of endotoxin (4, 12), it was demonstrated that small concentrations of TNF mediate the beneficial effects of endotoxin, such as endotoxin tolerance (6) and radioprotection and stimulation of hematopoiesis (14). Therefore, it was of interest to study whether the protection elicited by hydrazine (11) may be due to a release of TNF. In the study described here, isoniazid (INH), a precursor of hydrazine sulfate, was used.

INH together with rifampin is one of the most potent antituberculosis drugs (1). The major pathway of INH metabolism is acetylation, which leads, via acetylisoniazid, to isonicotinic acid; cleavage of acetylisoniazid in the human body (5) and in mice (9) results also in the formation of monoacetylhydrazine, an important determinant of the hepatotoxicity of INH. A substantial fraction of the acetylhydrazine passes through a pathway that has been shown to generate in animals highly reactive and hepatotoxic intermediates by the cytochrome P-450 monooxygenase system (9). Up to 14 ng of hydrazine $ml^{-1}$ was detected in the sera of healthy male volunteers receiving INH (300 mg) and rifampin (600 mg) daily for 15 days (3). Because of the different pharmacokinetics of INH in mice compared with those in humans, a relative high dosage of 25 mg/kg of body weight is required in mice, which corresponds roughly to 5 mg of INH per kg in humans (7).

Ten female NMRI mice (age, 11 to 12 weeks; Zentralinstitut für Versuchstiere, Hannover, Federal Republic of Germany) per group were injected intraperitoneally (i.p.) with 25 mg of INH per kg of body weight at different times, as indicated in Fig. 1, before or after a lethal intravenous (i.v.) challenge with 150 μg of endotoxin. The endotoxin was extracted from *Escherichia coli* O111 by the Boivin trichloroacetic acid method. The mice were not fasted before or during the experiment. The 50% lethal dose of the preparation used in these experiments was <125 μg per mouse. In former experiments studying the protective effect of a thromboxane A receptor blocker (15), it was found that saline injection of the control groups of mice at the different times of treatment did not change the lethality rate following endotoxin challenge compared with the lethality rate in untreated controls; therefore, a saline-treated control group was not used in the present study, to avoid the unnecessary use of additional groups of control mice.

In the first experiment, 25 mg of INH per kg was injected i.p. into 10 mice 5 h before (−5 h) the i.v. challenge with 125 μg of endotoxin. No significant protection resulted in this experiment; within 1 day, 60% of the nonpretreated control group of 10 mice died and 30% of the pretreated mice died. In both groups, no additional deaths occurred until the end of the observation period (day 5). These results are in contrast to those described by Silverstein et al. (11), in which protection was induced when hydrazine was given 5 h before the lethal challenge with endotoxin. In those experiments, the mice were fasted and a different endotoxin preparation was used. However, a statistically significant delay in the onset of death in all groups treated and a reduction in lethality rate following treatment at −1 h or +2 h was observed, as indicated in Fig. 1. INH (25 mg) failed to produce protection when it was given at −24 h; however, when 50 mg of INH per kg was injected 24 h before the challenge with endotoxin, the profile of the lethality rate was identical to that shown in Fig. 1 for the pretreatment at −1 h.

The protection of mice to a lethal challenge with endotoxin induced by INH differs from the endotoxin tolerance-inducing capacity of small doses of endotoxin or its mediators. It is well known that minute doses of endotoxin are only potent inducers of endotoxin tolerance when animals are pretreated ≥24 h before the injection of toxic endotoxin concentrations. Endotoxin injected in small doses shortly before or after the lethal challenge has no protective effect. TNF enhanced the lethality rate when it was injected simultaneously with cecal ligation and puncture, resulting in severe septicemia (16).

Since small amounts of endotoxin (5 ng) are capable of inducing endotoxin tolerance in mice when it is injected 24 h

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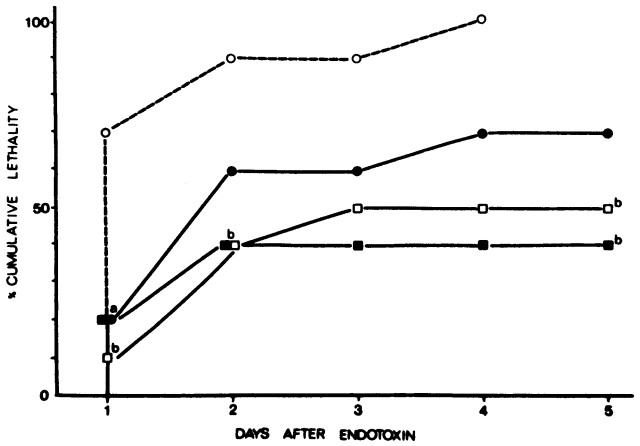


FIG. 1. Protection against endotoxin-induced lethality by INH in NMRI mice ($n$ = 10 mice per group). INH (25 mg/kg) was injected i.p. into mice 24 h (●), or 1 h (■) before and 2 h (□) after a lethal i.v. challenge with 150 μg of trichloroacetic acid endotoxin from *E. coli* O111 (control; ○); mice were pretreated i.p. with 50 mg of INH per kg 24 h before the endotoxin challenge (■). a, $P < 0.05$; b, $P < 0.01$ compared with the nontreated controls (chi-square test).

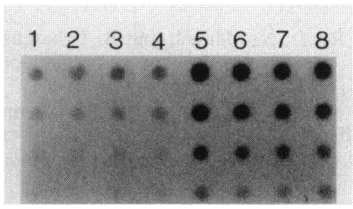


FIG. 2. TNF mRNA expression in human monocytes. Cytoplasmic RNA from the adherent fraction of 4 × $10^6$ peripheral blood mononuclear cells cultured without stimulus (lanes 1 to 4) or with *S. aureus* (10 μg/ml; lanes 5 to 8) in the absence (lanes 1 and 5) or in the presence of 2 μg of INH per ml (lanes 2 and 6) 10 μg of INH per ml (lanes 3 and 7), or 50 μg of INH per ml (lanes 4 and 8) was blotted in serial $\log_2$ dilutions from top to bottom and was hybridized with a TNF cDNA probe.

before the lethal challenge (17), the concentration of INH used in these experiments (Saarstickstoff-Fatol) was tested for contamination with endotoxin. By using an automatic kinetic turbidimetric *Limulus* amebocyte lysate (LAL) microtiter test developed in our laboratory (13), no LAL activity was detectable. In addition, INH interference with the endotoxin-LAL reaction also was excluded.

In order to determine TNF levels after INH injection, female NMRI mice (age, 11 to 12 weeks) were injected i.p. with 25 mg of INH per kg of body weight. Two hours after the injection of INH, these mice and an untreated control group ($n$ = 6 mice per group) were bled by orbital puncture, and the sera were stored at −20°C until they were assayed individually. In these sera, TNF levels were determined in a TNF cytotoxicity assay by using L929 cells (10). The sensitivity of this assay is 0.5 ng of TNF per ml. TNF was not measurable in any of the sera from either group tested.

Recently, it was demonstrated that antibiotics, in addition to their potent antimicrobial activity, also elicit immunomodulatory effects. Ciprofloxacin, for instance, was shown to suppress TNF production by human monocytes (2). In order to test whether INH influences TNF synthesis and production, TNF mRNA expression was measured in cultured human monocytes treated with 2, 10, or 50 μg of INH after 2 h by using dot blot or Northern blot techniques (8). TNF production was measured in supernatants of identical cultures after 16 h in the bioassay or by enzyme-linked immunosorbent assay (10). In the same way, the influence of INH on the capacity of *Staphylococcus aureus*, in contrast to lipopolysaccharide, a very potent stimulator of TNF production in human monocytes (10), to increase TNF mRNA was determined. In all experiments performed, INH did not influence TNF mRNA expression, per se, or *S. aureus*-stimulated TNF mRNA expression (Fig. 2). *S. aureus*-induced values of soluble TNF were 2.95 ± 0.21 ng/ml, which were unchanged in the presence of INH. The TNF levels were 2.78 ± 0.52 ng/ml (2 μg of INH), 2.54 ± 0.35 ng/ml (10 μg of INH), and 2.41 ± 0.14 ng/ml (50 μg of INH). Thus, the capacity of INH to exert protection against a lethal endotoxin challenge under the experimental conditions described here is not due to a suppressive effect of INH on TNF production. Further experiments are necessary to elucidate the mechanisms involved in the enhancement of nonspecific resistance by INH.

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