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The Influence of Antagonists of Poly(ADP-Ribose) Metabolism on Acetaminophen Hepatotoxicity

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ABSTRACT. An array of therapeutically used analgetic and antirheumatic drugs causes severe liver damage. The present study investigates the hepatoprotective effects of inhibitors of NAD-dependent adenoribosylation reactions in analgesics-induced hepatic injury. Male NMRI mice were treated perorally with 500 mg/kg of acetaminophen, and the activities of both glutamate-oxaloacetate transaminase (GOT) and glutamate-pyruvate transaminase (GPT) were determined in serum. In addition, the activity of poly(ADP-ribose)polymerase (PARP) was quantified in liver cell nuclei. While the PARP-activity remained essentially unchanged, the acetaminophen-induced release of both GOT and GPT from injured liver cells could be inhibited by 90–99%, when mice were injected additionally with the selective PARP-inhibitors nicotinic acid amide, benzamide, caffeine, theophyline, and thymidine, respectively. We see the main application of inhibitors of adenoribosylation reactions as for the combinational use in pharmaceutical preparations of analgesics and antirheumatic drugs in order to avoid hepatic damage.
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KEY WORDS. Poly(ADP-ribose), acetaminophen, hepatotoxicity, mice

INTRODUCTION

It is well-known that a large number of therapeutically used drugs cause liver damage (Ludwig and Axelsen, 1983; Peters *et al.*, 1993). Our previous studies showed that the hepatotoxic effects of various nonsteroidal slow-acting antirheumatic drugs (NSARDs; SAARDs) involve a process which interferes with NAD-dependent adenoribosylation reactions in liver (Kröger *et al.*, 1989). The hepatotoxic effects of NSARDs and SAARDs were prevented by inhibitors of poly(ADP-ribose) polymerase. The ubiquitous nuclear enzyme poly(ADP-ribose) polymerase (PARP; EC 2.4.2.30) catalyzes the synthesis of the nucleic-acid-like homopolymer poly(ADP-ribose). While the N-terminus of the enzyme contains two zinc finger motifs, which assemble to nicks of DNA, NAD^+ is bound by the C-terminal domain. In the course of the catalytic reaction, ADP-ribosyl-moities are transferred from NAD^+ to nuclear proteins with an accompanying release of nicotinic acid amide. Preferred acceptor proteins are histones (H1, H2A, H2B, H3, H4, and H5), whose poly-ADP-ribosylation induces local alterations in the architecture of chromatin domains. The induction of free DNA domains by removing histones from specific nucleosomes is a prerequisite step for all major chromatin functions, including replication, transcription, and repair of DNA (Althaus *et al.*, 1982). Excessive activation of PARP, due to numerous DNA strand breaks, depletes the cell from its intracellular NAD^+-pools and initiates apoptosis (Hoshino *et al.*, 1993).

Acetaminophen (AAP), an analgesic of widespread use, displays profound hepatotoxic effects, and its overdose causes fulminant hepatic and renal failure (Davidson and Eastham, 1966; Volans, 1976; Boyer and Rouff, 1971; Meredith *et al.*, 1981).

The toxicity of acetaminophen is enhanced by simultaneous ethanol consumption (Seeff *et al.*, 1986). Despite an increasing number of publications on the hepatotoxicity of acetaminophen, its precise mechanisms of liver damage are still obscure. The present study analyzes the question whether acetaminophen influences the metabolism of NAD-dependent-adenoribosylation and whether inhibitors of poly(ADP-ribose)polymerase can abolish the toxic effects of acetaminophen.

MATERIALS AND METHODS

Chemicals

Unless otherwise indicated, all reagents were purchased from Sigma-Aldrich, Munich.

Animals

Male NMRI mice were housed under specific pathogen-free conditions on a standard laboratory diet *ad libitum* in a 12-h light/darkness cycle. Two hundred mice, weighing 25–30 g, were starved for 12 h. Five hundred mg/kg of acetaminophen were given by gavage. Then, nicotinic acid amide (300 mg/kg), benzamide (75 mg/kg), caffeine (100 mg/kg), theophylline (100 mg/kg), thymidine (100 mg/kg), nicotinic acid (100 mg/kg), and L-tryptophan (300 mg/kg), respectively, were administered by intraperitoneal injection to acetaminophen-treated mice. Saline (10 ml/kg) was given i.p. to untreated animals. Sixteen hours later, 180 animals were sacrificed and the activity of GOT and GPT determined in serum. For the determination of poly(ADPR)polymerase activity, 20 animals were sacrificed 3 h after the application of substances, livers removed, pooled (5 per group), and nuclear extracts prepared as described by Blobel and Potter (1966).

Enzyme Determinations

Serum glutamate-oxaloacetate transaminase (GOT; EC 2.6.1.1) and glutamate-pyruvate transaminase (GPT; EC2.6.1.2.) activities were determined photometrically according to Bergmeyer (1974).

The activity of poly(ADPR) polymerase was determined in liver cell nuclei using ^{14}C adenosine-labelled NAD as described by Kidwell and Burdette (1974).

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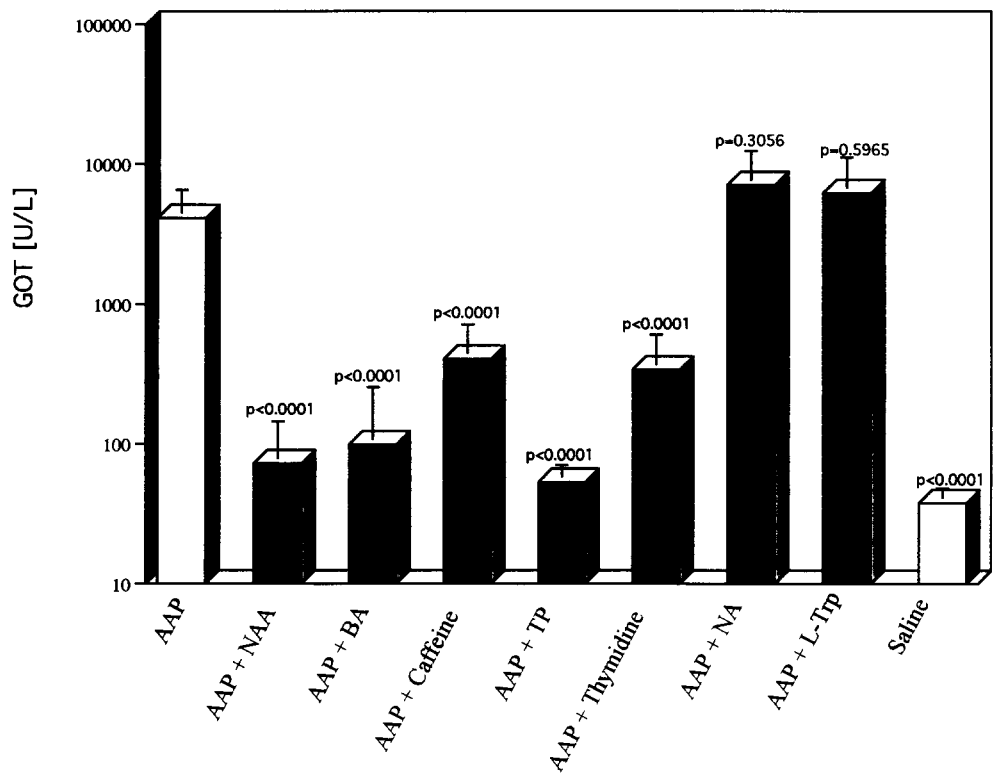

FIGURE 1. Hepatoprotective effects of inhibitors of poly(ADPR) polymerase in mice suffering from acetaminophen-induced liver damage, quantified as serum GOT activity. Male NMRI mice were starved for 12 h. Five hundred mg/kg acetaminophen (AAP) were given by gavage. Then nicotinic acid amide (NAA) 300 mg/kg, benzamide (BA) 75 mg/kg, caffeine 100 mg/kg, theophylline (TP) 100 mg/kg, thymidine 100 mg/kg, nicotinic acid (NA) 100 mg/kg, and L-tryptophan (L-Trp) 300 mg/kg, respectively, were administerd intraperitoneally to 20 AAP-treated mice each per group. Sixteen hours later, the animals were sacrificed and the activity of GOT determined spectrophotometrically in serum. The data are presented as means ± standard deviations. The statistical analysis was performed using Mann-Whitney's nonparametric test. $P < 0.05$ was considered significant.

Statistical Analysis

All data were analyzed with Mann-Whitney's nonparametric test using the Instat 2.01 statistics program (GraphPad, San Diego, CA) for Apple/Macintosh and presented as means ± standard deviations; $P < 0.05$ was considered significant.

RESULTS

GOT Activity in Serum of Mice Treated with Acetaminophen and Inhibitors of Poly(ADPR)Polymerase

When compared to saline-treated controls (29 U/L), a single peroral application of 500 mg/kg of acetaminophen (AAP) to male NMRI mice caused a 136-fold increase of serum GOT activity (Fig. 1). The AAP-induced elevation of serum GOT activity was almost completely inhibited by antagonists of poly(ADPR) polymerase ($P < 0.0001$): Thus, nicotinic acid amide, benzamide and theophylline caused a 97–99% inhibition of GOT release (54, 108, and 37 U/L compared to 3946 U/L for AAP-treated animals). The PARP-inhibitors caffeine and thymidine inhibited the GOT release by 92% ($P < 0.0001$). Both nicotinic acid and L-tryptophan, which are known not to inhibit PARP, had no protective effects on the AAP-induced liver damage, and even caused a slight increase of GOT activity.

GPT Activity in Serum of Mice Treated With Acetaminophen and Inhibitors of Poly(ADPR)Polymerase

Similar to the effects observed with GOT, the AAP-induced hepatic release of GPT was also inhibited by 90-98% with the PARP-inhibitors nicotinic acid amide, benzamide, caffeine, and theophylline ($P < 0.0001$) (Fig. 2). The inhibitory influence of thymidine on the GPT release was less pronounced (81%; $P = 0.0006$), when compared to the 92% inhibition observed with GOT.

Activity of Poly(ADPR)Polymerase in Liver Cells of Mice Treated With Acetaminophen, Nicotinic Acid Amide, and L-Tryptophan

Mice were treated perorally with 500 mg/kg of AAP (Table 1). After 3 h, the animals were sacrificed, the livers removed, and the PARP activity determined by liquid scintillation counting using ^{14}C adenosine-labeled NAD as substrate. When compared to saline-treated controls, no significant changes of nuclear PARP activity was detectable in mice treated with AAP, AAP/nicotinic acid amide, or AAP/L-tryptophan.

DISCUSSION

The present study reports the profound hepatoprotective effects of inhibitors of poly(ADPR) polymerase in mice suffering from acetaminophen-induced liver damage. These results add to our earlier findings of hepatoprotection by PARP-inhibitors in mice with liver damage induced by various antirheumatic drugs (Kröger *et al.*, 1989). Further, our results support the hypothesis that the liver damage caused by these drugs involves an adenoribosylation step which can be blocked by PARP-inhibitors.

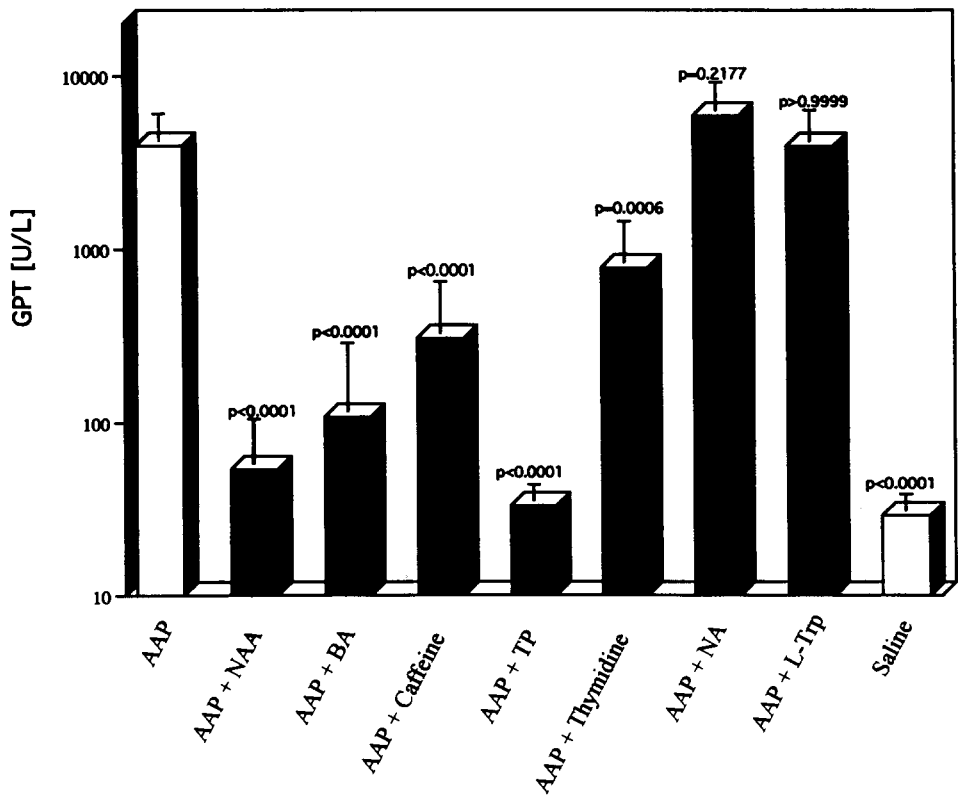

FIGURE 2. Hepatoprotective effects of inhibitors of poly(ADPR) polymerase in mice suffering from acetaminophen-induced liver damage, quantified as serum GPT activity. Male NMRI mice were starved for 12 h. Five hundred mg/kg acetaminophen were given by gavage. The application of nicotinic acid amide, benzamide, caffeine, theophylline, thymidine, nicotinic acid, and L-tryptophan, respectively, to 20 AAP-treated mice each per group followed the modus described in the legend to Fig. 1. Sixteen hours after the induction of liver damage by acetaminophen, the animals were sacrificed and the activity of GPT determined spectrophotometrically in serum. The data are presented as means ± standard deviations. Statistical analysis was performed using Mann-Whitney's nonparametric test. $P < 0.05$ was considered significant.

The analgesic drug acetaminophen has found increasing use in recent years as a substitute for aspirin. Unlike aspirin, it does not irritate the stomach lining. However, its poor anti-inflammatory activity tempt many patients to overdose (Flower *et al.*, 1985). Acetaminophen is a powerful inducer of cytochrome P-450, and the simultaneous consumption of alcohol or the combinational use of drugs, which additionally induce the P-450 system, diminish dramatically the tolerated concentrations of acetaminophen (Seeff *et al.*, 1986). The action of the P-450 system on acetaminphen produces a highly reactive quinone-imine which combines to sulfhydryl-groups of proteins and causes the rapid depletion of intracellular glutathione (Jollow *et al.*, 1974). Even at the recommended dose of up to 40 mg/kg of the analgetic, a 20% reduction of intracellular glutathione levels is observed (Jollow *et al.*, 1974). A complete depletion of hepatic GSH is achieved with 500 mg/kg of acetaminophen in mice.

GSH contributes significanlty to the intracellular antioxidant defense system as it is a powerful consumer of superoxide, singlet oxygen, and hydroxyl radicals (Miesel and Zuber, 1993). GSH is also a substrate of the H_2O_2-removing enzyme glutathione peroxidase and cofactor for several enzymes in widely different metabolic pathways, such as maleylacetoacetate isomerase, prostaglandin endoperoxide synthetase, and DDT dehydrochlorinase (Halliwell and Gutteridge, 1989). The major function of GSH, however, is the biotransformation of xenobiotics to mercapturic acids which are excreted into bile (Forth *et al.*, 1983). The acetaminophen-induced depletion of GSH does not only reduce the liver's capacity to cope with hydrogen peroxide and other oxyradicals, which are continuously produced by mitochodrial respiration and cytochrome P-450 metabolism, but increases also the intracellular levels of oxidized GSH (GSSG) (Murphy and Brand, 1987). Accumulation of GSSG inactivates an array of enzymes, including adenylate cyclase, fatty-acid synthetase, phosphofructokinase, and phosphorylase phosphatase, thereby blocking major catabolic and anabolic pathways and diminishing both intracellular NADH and ATP levels (Halliwell and

TABLE 1. Activity of Poly(ADPR) Polymerase in Hepatic Nuclei from Mice Suffering from Acetaminophen-Induced Liver Damage

Applicated Drugs	Nucleic Activity of Poly(ADPR) Polymerase (cpm/mg DNA)
Acetaminophen (500 mg/kg p.o.)	7559
Acetaminophen (500 mg/kg p.o.) + L-Tryptophan (300 mg/kg i.p.)	8547
Acetaminophen (500 mg/kg p.o.) + Nicotinic Acid Amide (300 mg/kg i.p.)	8160
Saline (10 ml/kg i.p.)	7597

Male NMRI mice were starved for 12 h prior to the application of substances: acetaminophen (500 mg/kg) was given by gavage, and L-tryptophan (300 mg/kg), nicotinic acide amide (300 mg/kg), and saline (10 ml/kg), respectively, were administered by intraperitoneal injection. Three hours later, 10 animals were sacrificed, livers removed, and the activity of poly(ADPR)polymerase determined in pooled nucleic extracts.

Gutteridge, 1989). More importantly, GSSG is a powerful inhibitor of intracellular antioxidases, such as superoxide dismutase. The breakdown of the GSH-dependent antioxidant defense system and the simultaneous inhibition of intracellular antioxidases increase the flux of oxygen free radicals. Oxygen free radicals have come to the fore as powerful inducers of poly(ADPR) polymerase-dependent adenoribosylation of nuclear proteins (Cerutti, 1985). The biological function of ADP-ribosylation reactions are still obscure but are increasingly recognized to play a crucial role in DNA repair (Nduka *et al.*, 1980), malignant transformation (Borek *et al.*, 1984), DNA replication (Lönn and Lönn, 1985), cellular differentiation (Althaus *et al.*, 1982), and sister chromatid exchange (Oikawa *et al.*, 1980).

Excessive adenoribosylation of nuclear protein initiates apoptosis and causes liver cell necrosis that can be completely prevented by specific inhibitors of poly(ADPR) polymerase including nicotinic acid amide (Ki = 13 mM), benzamide (Ki = 1 mM), thymidine (Ki = 13.3 mM), and theophylline (Ki = 29.8 mM) (Shall, 1983). A stable loop may form, in which the acetaminophen-induced depletion of the antioxidant/antioxidase defense system increases the intracellular oxidative stress, and the elevated generation of oxygen radicals goes on to further stimulate poly(ADPR) polymerase, subsequently resulting in liver cell necrosis via apoptotic cell death.

We see the main application of inhibitors of poly-adenoribosylation as for the combinational therapeutic use in pharmaceutical preparations of GSH-depleting analgetics in order to avoid liver injury. Further studies are warranted that thoroughly address the multitude of events both at the biochemical and molecular-biological level, which result in acetaminophen-induced hepatic damage and shed light on the precise mechanism of hepatoprotection by inhibition of poly(ADPR) polymerase.

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