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Short communication

Effect of radioprotective and chemoprotective drug (WR-2721) on toxicity of acetaminophen in mice

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Abbreviations:
WR-2721 – [S-2-(3-aminopropylamino)ethylposphorothioate];
APAP – acetaminophen,
AST – aspartate aminotransferase;
ALT – alanine aminotransferase

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Abstract

Background and Purpose: *It is known that WR-2721 WR-2721 – [S-2-(3-aminopropylamino)ethylposphorothioate] has radioprotective and chemoprotective effects on non-tumor tissues, and is now introduced into clinical tumor therapy protocols. We investigated whether WR-2721 have a protective role in acute liver injury induced with acetaminophen (APAP).*

Materials and Methods: *CBA/H Zgr inbred mice of both sexes aged 12–16 weeks, weighing 20–25 g were used. Mice were given phenobarbitone-sodium in drinking water during 7 days (300 mg/kg) in order to induce hepatic drug-metabolizing enzymes. Thereafter, mice were fasted overnight and WR-2721 was given i.p. (50, 100 or 200 mg/kg). After 15–30 minutes they received acetaminophen (APAP; Paracetamol) 200 mg/kg by gastric tube. Animals were allowed food 4 hours later. The mortality of mice was followed for 3 days and serum aminotransferase levels were determined 24 hours after APAP administration.*

Results: *Survival of mice was prolonged after all doses of WR-2721, but significantly only after the dose of 100 mg/kg of WR-2721. Similarly, the pretreatment of mice with 100 mg/kg of WR-2721 highly significantly reduced serum aspartate aminotransferase levels (AST, $p<0.0005$) and serum alanine aminotransferase levels (ALT, $p<0.005$). The doses of 50 and 200 mg/kg of WR-2721 also reduced AST and ALT, but not significantly.*

Conclusions: *These data showed that WR-2721 has definitive hepatoprotective effect which is significant in very restricted dose range.*

INTRODUCTION

Since acetaminophen (N-acetil-p-aminophenol, APAP), when used in very high dose, can cause heavy damage of liver and death of recipients, it is frequently used for experimental study of the mechanisms of hepatotoxicity. It is not toxic *per se* but its electrophilic metabolite, most probably N-acetil-p-benzoquinone imine which is inactivated by binding to sulphydryl (thiol) groups of glutathione. After consumption of glutathione, the metabolite binds to liver cell macromolecules and causes liver necrosis *(1, 2)*. Alkylation of glutathione or DNA seems to be one of major mechanisms by which the active APAP metabolite causes liver necrosis *(3, 4)*. Since WR-2721 – [S-2-(3-aminopropylamino)ethylposphorothioate] with its active aminothiol group protects cells against toxicity of ionizing radiation and alkylating chemotherapeutic drugs *(5, 6)*, we tested possible protective effect of WR-2721 on acute hepatotoxicity induced with APAP in mice.

MATERIALS AND METHODS

Induction of toxic hepatitis with APAP was described previously *(7, 8)*. Briefly, to induce drug metabolizing enzymes, mice were given phenobarbitone-sodium for 7 days in drinking water and, after overnight fasting, APAP was administered *per os* by stomach tube in doses between 200 and 350 mg/kg (LD50 in phenobarbitone treated CBA mice about 350 mg/kg). WR-2721 was given *i.p.* 2 hours before APAP in doses from 50 to 200 mg/kg (LD50 > 400 mg/kg). Control mice obtained 0.2 ml of saline before APAP. The survival of mice was followed for 72 hours and concentration of aminotransferases (AST and ALT) in plasma was determined 24 hours after APAP administration by standard laboratory methods.

The concentration of aminotransferases was statistically evaluated by Student's t-test, and survival of mice by Kaplan-Meier method and by χ^2-test. The differences at $p \leq 0.05$ level were considered significant.

RESULTS

The cumulative survival of mice, obtained in two different experiments after administration of APAP in a dose of 350 mg/kg is shown in Figure 1. In the control group, mice started to die already 8 hours after APAP and most deaths occurred within 48 hours after administration of APAP. At the end of observation period (72 hours), 12 out of 23 mice (52%) survived (further mortality in this model is usually not observed after this time). Pretreatment of mice by either dose of WR-2721 (50, 100 or 200 mg/kg) delayed the morality of mice (by Kaplan-Mayer analysis of survival, survival was longer in all groups and significantly higher than in control group mice, data not shown). However, final survival, as tested in by χ^2-test was significantly higher ($p < 0.05$) than in the control group only in the group of mice given 100 mg/kg WR-2721 (17 out 20 mice survived, or 85%).

Essentially, similar results were obtained by measuring serum aminotransferase level (ALT and AST) in mice given WR-2721 or saline before APAP (given in a dose of 200 mg/kg in this experiment) (Figure 2). In all groups pretreated with WR-2721, plasma concentrations of ALT and AST decreased in comparison to saline-treated controls. Again, the dose of 100 mg/kg WR-2721 was the most effective – the decrease in both aminotransferases was highly significant in comparison to the control group ($P < 0.01$ for ALT and $P < 0.001$ for AST). Serum concentration of ALT also significantly decreased in the group of mice given 50 mg/kg WR-2721 ($P < 0.01$).

DISCUSSION

The presented results showed that WR-2721 had definitive protective effect on toxicity of liver induced by APAP. All applied doses of WR-2721 (50 to 200 mg/kg) manifested some kind of protection (as judged by increased survival of mice and decrement in serum aminotransferase levels); however, the changes in these parameters were significant only after application of the dose

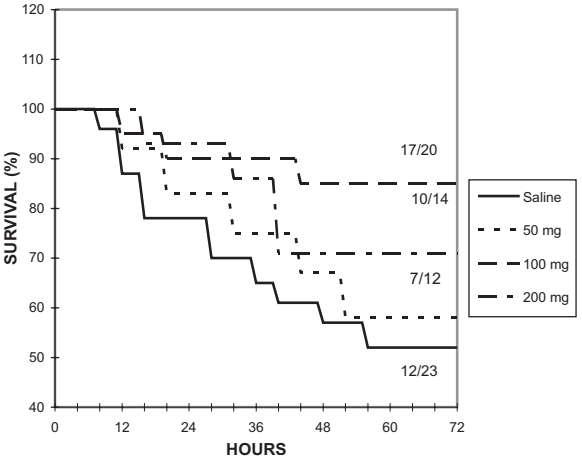

Figure 1. *Cumulative survival of mice given different doses of WR-2721 before administration of APAP (350 mg/kg). Data obtained in two different experiments.*

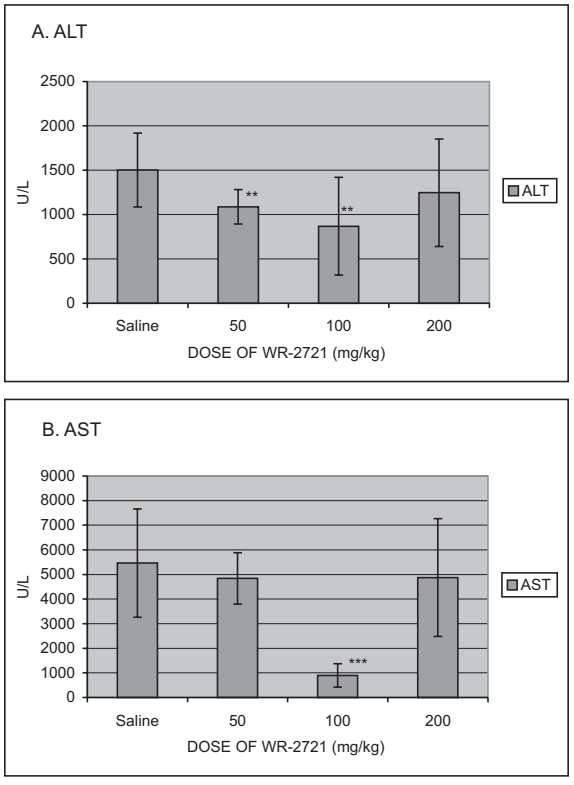

Figure 2. *Plasma concentration of aminotransferases in mice intoxicated with APAP treated with various doses of WR-2721 before administration of APAP. Mean ± SD; Values in normal (untreated mice) 75 ± 4 (AST) and 23 ± 1 (ALT)*
** $P < 0.01$; *** $P < 0.001$

of 100 mg/kg. The dose of 200 mg/kg WR-2721, which was usually most protective for normal tissues against toxicity of X-irradiation or alkylating chemotherapeutic agents *(5, 9),* may itself be hepatotoxic.

WR-2721 is a selective protector against toxicity of X-irradiation or chemotherapeutic agents (primarily alkylating agents but also against other anti-cancer drugs)

in a manner that it essentially protects normal but not cancer tissue *(10, 11)*. The main reason for this selectivity is that the membranes of normal cells (or surrounding capillaries) have, contrary to the membranes of tumor cells, the enzyme alkaline phosphatase which removes from the compound hydrophilic phosphate group and allows entrance into the cell to the active protective metabolite – WR-1065. The protection of normal tissues enables the use of a higher dose of X-rays or chemotherapeutic agents to achieve greater anti-tumor effect without increasing side effects of the anti-tumor agent. This was the reason why WR-2721 was approved for use in clinics in cancer therapy (under the commercial name of Amifostine).

The biochemical mechanisms of APAP liver toxicity are still not precisely defined. Active metabolites (N-acetil--p-benzoquinone imine) covalently bind to various important cellular proteins and induce oxidative stress in mitohondria *(2)*. Alkylation of glutathione and protein thiols appears to be important consequence of the toxic effect of active metabolite *(4)* and for the protective effect of WR-2721. Various mechanisms of the protective effect of WR-2121 against X-irradiation *(10, 12),* of which best proven is dealkylation of important cell proteins (enzymes) and reduction in the formation of DNA-DNA interstrand cross links as well as scavenging of free radicals with its free thiol group *(13, 14)*. Also, its disulphide bonds may of important for detoxification of sulfur containing compounds, such as cisplatin. Essentially, WR-2721 may act in a similar way as a basic thiol-containing protective compound, acetylcysteine, which is clinically used as an antidote for acetaminophen (Paracetamol) poisoning or adverse reactions *(15)*.

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