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Depletion of glutathione by the radioprotective agent *S*-2-(3-aminopropylamino)ethyl phosphorothioic acid (WR2721)

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S-2-(3-Aminopropylamino)ethyl phosphorothioic acid (WR2721*) is a free radical scavenger currently in limited clinical use as an adjunct in cancer radio- and chemotherapy [1, 2]. We have previously determined the pharmacokinetics and protein binding characteristics of WR2721 in rabbits and in humans [3, 4]. We have also shown that WR2721 is an effective mucolytic agent in patients with cystic fibrosis. *In vivo*, WR2721 is converted to its free thiol analogue, *N*-2-mercaptoethyl-1,3-diaminopropane (MDP), and, as such, reacts with disulfide bonds in the mucin molecule, altering its rheology [3, 5].

In the course of experiments designed to examine the spectrum of its activity as a free radical scavenger, we have observed that WR2721 in fact exacerbates the toxicity of those free radical-generating agents, such as acetaminophen and 6-hydroxydopamine, which depend upon glutathione for their detoxication. For this reason, we examined the glutathione content of the livers of mice treated with WR2721.

Materials and methods

Chemicals. WR2721 was supplied by Dr. K. Borah of Organon, Inc. (West Orange, NJ). Dithionitrobenzoic acid, NADPH, and glutathione reductase were obtained from the Sigma Chemical Co. (St. Louis, MO).

Animal studies. Determinations of hepatic glutathione (GSH + GSSG) levels were performed on 5- to 7-week-old female CD-1 mice obtained from Charles River Laboratories (Cambridge, MA) and on 7-week-old male A/J mice obtained from Jackson Laboratories (Bar Harbor, ME). There were five mice in each treatment group. Mice were given access to food and water *ad lib.*

WR2721 was administered by orogastric or intraperitoneal instillation as a solution in distilled water. The concentration of the solution was such that a 20 g mouse received 0.2 ml/dose. Control mice (0 mg/kg) received an equal volume of distilled water alone.

Assay for hepatic glutathione (GSH + GSSG). Hepatic glutathione content was determined upon protein-free supernatant fractions of tissue homogenates prepared by mechanical homogenization of weighed samples of mouse liver in 1 ml of 5% trichloroacetic acid, 0.01 M hydrochloric acid in a Tenbroeke glass tissue grinder at 4°. Residual trichloroacetic acid was removed from the resulting supernatant fraction by three ether extractions, and the samples were assayed for GSH + GSSG by the method of Tietze [6]. Glutathione levels for the mice in each group were averaged and compared using a one-tailed *t*-test. Control and treated paradigms were conducted simultaneously to obviate the problem of diurnal variation in glutathione levels.

Results

CD-1 female mice receiving doses of WR2721 up to 400 mg/kg via orogastric tube were clinically indistinguishable from normal animals. The relationship between the dose of WR2721 and the GSH + GSSG content of their livers 6 hr after dosing is shown in Fig. 1. Six hours was chosen because this was the point at which animals given WR2721 in conjunction with acetaminophen or 6-hydroxydopamine were demonstrably clinically impaired, but had not yet succumbed to the treatment (Schor, unpublished observations). Treatment with 200 mg/kg WR2721 resulted in a 30–40% decline in total hepatic glutathione content ($P < 0.05$). Treatment with higher doses did not result in further reduction of the glutathione content. That this is not a strain- or sex-specific effect is also shown in Fig. 1. Male A/J mice showed virtually identical decreases in glutathione content in response to WR2721. The difference between these two strains was significantly only at 100 mg/kg, with a $P < 0.05$. Intraperitoneal injection of WR2721 into A/J male mice also resulted in a similar dose–response curve of glutathione depletion (data not shown), including the inability to drive glutathione below 60% of control values.

Discussion

WR2721 is a thiophosphate which has been developed as a radioprotective agent by the Walter Reed Army Insti-

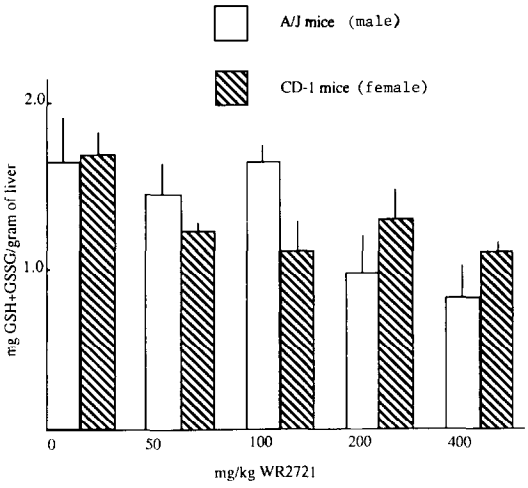

Fig. 1. Total glutathione levels in liver of control mice and mice treated with 50, 100, 200, and 400 mg/kg WR2721 via orogastric tube. Assays were performed 6 hr after dosing. There were five mice in each treatment group.

* Abbreviations: WR2721, *S*-2-(3-aminopropylamino)-ethyl phosphorothioic acid; (GSH + GSSG), total (reduced plus oxidized) glutathione; and MDP, *N*-2-mercaptoethyl-1,3-diaminopropane.

tute of Research. It has been given as a single agent to adult humans at doses of up to 70 mg/kg/day without apparent toxicity [7]. It represents an inactive "prodrug" which is cleaved to the active thiol *in vivo* [4]. Clinical and animal studies have shown that WR2721 can protect subjects from the toxic effects of cyclophosphamide, mechlorethamine, and *cis*-platinum [8–10]. It has been proposed for use with other chemotherapeutic agents as well [11].

The present studies demonstrate a depletion of hepatic glutathione by orally and intraperitoneally administered WR2721 in mice. The relationship of this finding to the clinical toxicity we observed when WR2721 was combined with certain free radical-generating agents is not known. However, several of these agents themselves deplete glutathione [12–14]. These results may indicate the need for prudence in the adjunctive use of WR2721 with free radical-generating agents. Such agents have been classified with regard to their effect upon glutathione levels and to the generation of oxygen or organic free radicals. These characteristics may ultimately prove helpful in predicting which adjunctive treatments are likely to be problematic [15].

The relationship of the dose of WR2721 to the magnitude of the decline in total glutathione content indicates that there is a level below which the glutathione content cannot be driven, implying either the existence of a non-depletable pool of glutathione, compensatory synthesis induced increasingly by increasing consumption of glutathione, or a saturable enzyme-mediated mechanism for glutathione consumption. Lauterburg *et al.* [16] have demonstrated that, especially in young animals, compounds that deplete hepatic glutathione markedly enhance the synthesis of new glutathione. Preliminary studies in our laboratory showed that MDP did not alter the hepatic content, K_m, or V_{max} of glutathione reductase or γ-glutamylcysteine synthetase. Moreover, incubation of MDP or MDP disulfide with reduced or oxidized glutathione for 24 hr *in vitro* at pH 7.4 and 37° did not lead to the formation of a glutathione adduct of MDP. The formation of such an adduct *in vivo* or in the presence of glutathione-*S*-transferase cannot be ruled out by these preliminary studies. Blackburn and Peterson [17] have demonstrated the non-enzymatic preferential formation of the mixed disulfide of MDP and cysteine *in vitro*. Such a reaction could form the basis for cysteine depletion and a subsequent decrease in the availability of this precursor for glutathione synthesis. Alternatively, if some degree of anorexia is induced by WR2721, this might limit the availability of cysteine to treated animals. Studies of the mechanism of glutathione depletion by WR2721 are currently ongoing.

In summary, WR2721 is a radioprotective compound currently in limited clinical use. It is activated by conversion to its free thiol analogue *in vivo*. WR2721 has been used adjunctively with chemotherapeutic agents which generate toxic free radicals in an effort to obviate some of their toxicity. Many of these agents depend upon glutathione for their detoxication. The present study demonstrates that WR2721 depleted hepatic glutathione in mice. These results may place limitations upon the potential adjunctive use of WR2721.

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