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Dose and Time Relationships of the Radioprotector WR-2721 on Locomotor Activity in Mice[1]

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LANDAUER, M R , H D DAVIS, J A DOMINITZ AND J F WEISS *Dose and time relationships of the radioprotector WR-2721 on locomotor activity in mice* PHARMACOL BIOCHEM BEHAV 27(3) 573–576, 1987 —The effects of the radioprotector S-2-(3-aminopropylamino)ethylphosphorothioic acid (WR-2721) on locomotor activity were evaluated in CD2F1 male mice Separate groups of animals (N=10/group) received an IP injection of vehicle, 25, 50, 100, 200, or 400 mg/kg of WR-2721 immediately before testing Horizontal and vertical activity were measured using a Digiscan automated animal activity monitor The latency to onset and duration of action of each dose of the radioprotector were recorded For both behavioral measures, a significant reduction was observed in activity at doses of 200 and 400 mg/kg A dose of 200 mg/kg had a 12- to 14-min latency to onset and significantly reduced behavioral activity for 3 hr Mice injected with 400 mg/kg exhibited locomotor deficits within 8–10 min and were affected for up to 9 hr The ED50 for horizontal and vertical activities at 1 hr postinjection were determined to be 271 and 105 mg/kg, respectively The results demonstrate that significant reductions in locomotor activity are exhibited at doses of 200 mg/kg or more and that vertical activity was more sensitive to the disruptive effects of WR-2721 than was horizontal activity

Locomotor behavior WR-2721 Ethiofos Radioprotection Digiscan

S-2-(3-aminopropylamino)ethylphosphorothioic acid, also known as WR-2721, ethiofos, or gammaphos, is the most effective radioprotector available today [4,10] Among the proposed mechanisms of action is scavenging of radiation-induced free radicals [4] WR-2721 has been reported to have one of the highest (2 7) dose reduction factors (DRF) known [9] (The DRF is defined as the radiation LD50/30 dose of mice treated with the radioprotector divided by the radiation LD50/30 of nontreated animals) A variety of species, including rodents, dogs, and monkeys, have been shown to be protected against ionizing radiation-induced lethality when pretreated with WR-2721 [4] This compound was originally designed to serve as a military radioprotector, but it is now undergoing clinical trials and has been found to be an effective adjunct to radiotherapy and chemotherapy in cancer patients [1,10]

Although it is effective as a radioprotective agent, WR-2721 has been demonstrated to produce behavioral side effects that could limit its usefulness It produces nausea, vomiting, hypotension, and somnolence in humans [1], and it results in conditioned taste aversions [3,7] and decreased motor performance [2] in rats In this paper, the onset and duration of decrements in locomotor activity as a function of WR-2721 dose are examined in mice in order to further characterize the behavioral toxicity of this compound

METHOD

Subjects

Sixty adult male (BALB/c × DBA/2)F1, hereafter referred to as CD2F1 mice (Charles River, Wilmington, MA), weighing 27–34 g, served as subjects Animals were maintained on a 12-hr light-dark cycle in a temperature-controlled room (21°C) Immediately following arrival from the supplier, animals were quarantined for 2 weeks They were determined to be free of *Pseudomonas* and showed no signs of histologic lesions of common murine diseases Mice were individually housed and provided with Wayne Rodent Blox diet and water

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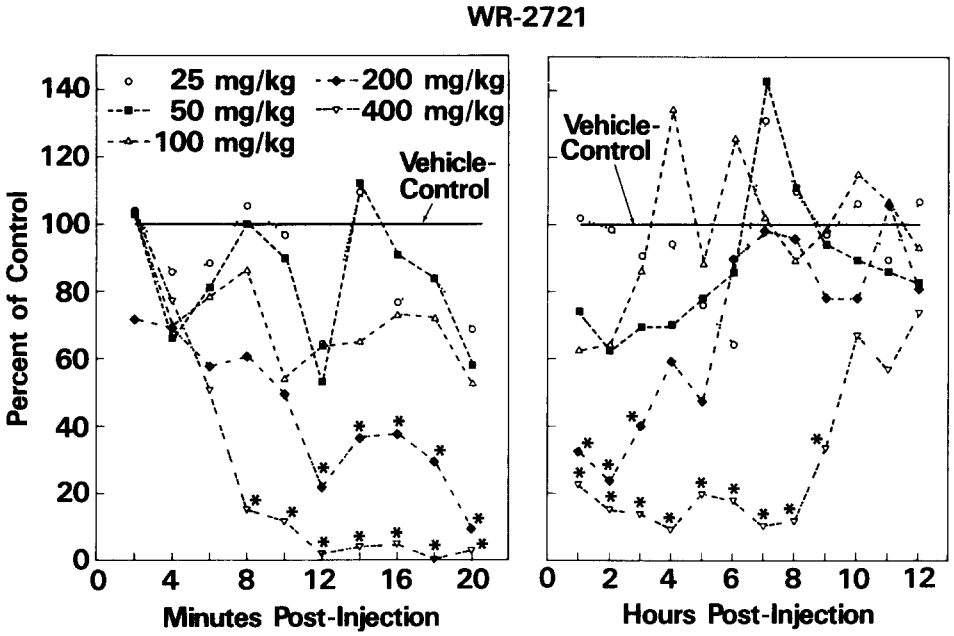

FIG 1 Effect of dose of WR-2721 on the latency to onset of alterations in vertical activity Activity was measured at 2-min intervals (left) Duration of the suppression of vertical activity as a function of WR-2721 dose The numbers of vertical movements at 1 hr intervals are illustrated (right) Data are presented as percent of the vehicle control group *Significantly ($p<0$ 05) different from vehicle control group

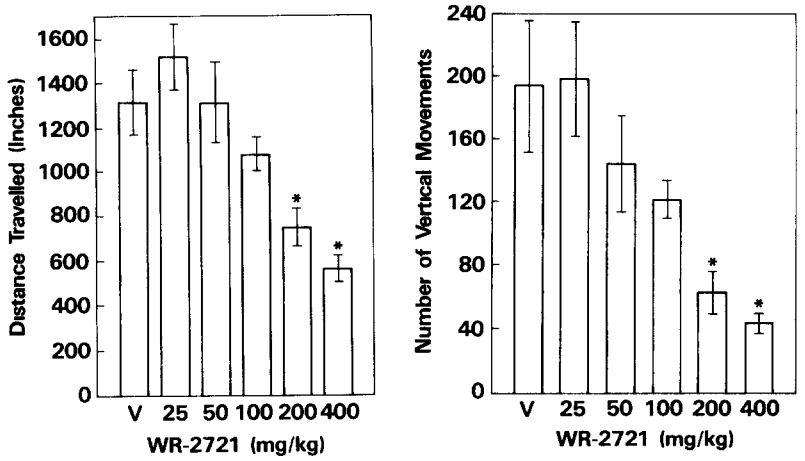

FIG 2 Dose-response determinations for the effects of WR-2721 on horizontal activity expressed as total distance travelled (left) and vertical activity expressed as the number of vertical movements (right) Data represent the means±SEM at 1 hr after testing (N=10/group) The drug was administered IP immediately prior to assessment of locomotor activity V refers to the vehicle control group *Significantly ($p<0$ 05) different from vehicle control group

Experimental Procedure

A computerized Digiscan Animal Activity Monitor (Model RXYZCM-16, Omnitech Electronics, Columbus, OH) was used to quantitate locomotor behavior The apparatus used an array of 16×16 infrared photodetectors to determine total distance travelled in inches (horizontal activity) and an additional 16 vertical sensors to record the number of vertical movements (vertical activity) The test chamber consisted of a 40×40×30 5 cm Plexiglas arena into which two 20×20×30 5 cm Plexiglas cages were placed in diagonal quadrants This allowed two animals to be tested simultaneously The horizontal and vertical detectors were positioned 1 3 and 6 3 cm, respectively, above the floor of the arena

All animals were habituated to the test chamber for 2 hr on the day before the experiment The next day, mice were weighed and received either a saline vehicle injection or 25,

50, 100, 200, or 400 mg/kg WR-2721 (N=10/group) Injections were administered IP in a volume of 10 ml/kg WR-2721 was obtained from the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD Just prior to injection, it was dissolved in 0 9% saline solution with a pH of 7 2–7 5

Immediately following injection, animals were placed into the activity monitor where horizontal and vertical activities were recorded every 2 min for 2 hr to ascertain the behavioral onset of the drug For the next 10 hr, locomotor activity was monitored every 60 min to determine the duration of action of WR-2721 After each test, the apparatus was cleaned with a 50% alcohol solution Animals were weighed weekly for 4 weeks

Statistical Analysis

Two-way ANOVAs with repeated measures were used to determine significance levels for the latency and duration of drug action as well as the effect of drug dose on body weight Dose-response determinations for total horizontal and vertical activities at 1 hr after testing were subjected to one-way ANOVAs Post hoc comparisons were made using Dunnett's test The effective dose of WR-2721 that disrupted horizontal and vertical activity by 50% from vehicle control levels (ED50) at 1 hr postinjection was calculated by linear regression

RESULTS

Horizontal and vertical activities showed similar patterns Therefore, only one parameter, vertical activity, is illustrated (Fig 1) A significant drug × time interaction was seen for latency of onset, $F(145,1566)=1\ 33$, $p<0\ 0007$, and duration, $F(55,594)=1\ 37$, $p<0\ 045$, of drug action A dose of 200 mg/kg resulted in decrements in behavior within 12–14 min and significantly reduced activity for 2 hr At 400 mg/kg, degradation of motor activity occurred within 8–10 min and was effective for up to 9 hr The dose-response functions for the effects of WR-2721 on total horizontal and vertical activity at 1 hr after administration are presented in Fig 2 ANOVAs revealed significant effects of dose on horizontal activity, $F(5,54)=8\ 29$, $p<0\ 0001$, and vertical activity, $F(5,54)=5\ 72$, $p<0\ 0003$ For both parameters, doses of 200 mg/kg and above significantly disrupted locomotor behavior The ED50s for horizontal and vertical activities at 1 hr postinjection were calculated to be 271 and 105 mg/kg, respectively No significant effect of WR-2721 was seen on body weight

DISCUSSION

WR-2721 produced a dose-dependent decrease in both horizontal and vertical activities Only the two highest doses yielded significant reductions in activity At these doses (200 and 400 mg/kg), behavioral deficits were observed within 12–14 and 8–10 min, and they remained depressed for 3 and 9 hr, respectively Rasey *et al* [8] reported that blood levels of the radioprotector, WR-2721, reach peak levels 10 min after IP injection of 400 mg/kg, and therefore are well correlated with the onset of behavioral toxicity The results of the present study are in agreement with Bogo *et al* [2], who reported that doses of 200 and 400 mg/kg WR-2721 disrupt the motor performance of rats trained to maintain their balance on an accelerating rotarod

ED50s were determined to be 271 and 105 mg/kg, respectively, for horizontal and vertical activities Therefore, at 1 hr postinjection, vertical activity appears to be more sensitive to the disruptive effects of WR-2721 than is horizontal activity The LD50 for WR-2721 for outbred CD2F1 mice is 980 mg/kg (unpublished) This compares to an LD50 of 950 mg/kg for outbred ICR mice [4] and an LD50 range of 510–784 for several inbred mouse strains [9]

The doses of WR-2721 that resulted in the greatest behavioral decrements are doses that, in our laboratory, have yielded the best radioprotection The DRFs in CD2F1 mice for doses of 100, 200 and 400 mg/kg are 1 2, 1 6, and 2 0, respectively (unpublished) Only the lowest dose did not significantly affect locomotor activity A similar relationship between radioprotector efficacy and behavioral toxicity has been observed for 16,16-dimethyl prostaglandin E2 [5]

The present study involved analysis of the behavioral toxicity of the drug WR-2721 when administered alone Future studies should include an analysis of locomotor behavior following combinations of WR-2721 and radiation exposure Because the irradiation of mice results in locomotor decrements [6], WR-2721 treatment may potentiate the behavioral toxicity [2,3] Chemical compounds administered in conjunction with WR-2721 may be able to maximize its radioprotective qualities while mitigating its behavioral side effects

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