Toxicity study in rats of a tellurium based immunomodulating drug, AS-101: A potential drug for AIDS and cancer patients

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Abstract. Male and female Sprague Dawley rats were injected intraperitoneally for 4 weeks with ammonium trichloro (dioxylene-0-0'-) tellurate, an immunomodulating drug at doses ranging from 3 to 24 mg/kg/week. Routine laboratory examinations included body weight, food consumption, clinical chemistry and hematological examinations. At termination of the experiment, all rats were sacrificed and subjected to a detailed necropsy. Few mortalities were recorded during the course of the study. Clinical signs included hind limb paresis and paraphimosis. A garlic odor pervaded the room. Body weight and food consumption were adversely affected in a dose-related manner. Effects were elicited on the hematological system; changes being noted in the platelet and leukocyte counts as well. Clinical chemistry evaluation revealed signs of hepatotoxicity, especially in the female treated groups. The level of beta-globulin was increased. At necropsy organs were found to have a grayish-blue discoloration. Tellurium related histopathological changes were observed in the eyes, liver, thymus, bone marrow, heart and kidneys. An attempt has been made to compare the toxicity of this drug with other tellurium-containing compounds. A good correlation was found. Novel effects of the drug were retinopathy and replacement of bone marrow by bony or fibrous tissue. The possibility that some of the effects may have been elicited due to selenium-vitamin E deficiency has been considered.

Key words: Tellurium – Rat – Toxicology – Immunomodulator – Drug

Introduction

Tellurium is the 52nd element in the periodic table. It belongs to the VIA group along with elements like sulfur and selenium. In the metal, rubber, glass and ceramic industries tellurium has found a number of commercial applications as an additive element. Tellurium compounds have been used in bacteriological procedures for a rapid diagnostic test, especially for Corynebacterium diphtheriae, by virtue of the fact that the bacteria reduce a tellurite solution to black amorphous tellurium. Addition of tellurium permits the growth of Staphylococcus aureus from cultures contaminated with other staphlococci, micrococci and gram negative bacillae, by suppressing their growth (Sandratskaya 1967).

An interest in the harmful effects of tellurium has stemmed from its potential toxicity in the industry. A number of reviews relating to the toxicological aspects of tellurium are available (Klevay 1976; Sandratskaya 1976). A new and novel application of tellurium has been proposed as an immunomodulating drug, coded AS-101 (Sredni et al. 1987, 1988a). The immunomodulating and anti-tumor effects of AS-101 have made this a potentially useful drug without any significant toxic side-effects (Laporte et al. 1988; Ruiz-Palacios et al. 1988; Sredni et al. 1988b).

AS-101 induces human lymphocytes to proliferate and to produce IL-2 in vitro; it also enhances the production of IL-2, colony stimulating factor by mouse spleen cells. Splenocytes of BALB/c mice injected with AS-101 increased their production of IL-2 and CSF in vitro in the presence of mitogen. After incubation with AS-101, mononuclear cells from normal donors became responsive to recombinant IL-2 and bound monoclonal antibody to IL-2 receptors. Splenocytes of mice treated in vivo with AS-101 expressed high levels of IL-2 receptor. AS-101 administered systemically to mice mediated anti-tumor effects which could be attributed to its immunomodulatory properties. AS-101 also enhanced the ratio of OKT4 to OKT8-positive cells in cultured mononuclear cells from AIDS (Acquired Immune Deficiency Syndrome) patients. Current phase I clinical trials with AIDS and cancer patients show that no side effects were seen at the dosages of 2, 3, and 5 mg/m² injected 3 times weekly. An enhancement of CD4 cells, and CD4/CD8 cell ratio and a simultaneous clinical and biological improvement occurred in most of the patients (Laporte et al. 1988; Ruiz-Palacios et al. 1988; Sredni et al. 1988b).

In the present study we report the toxicological effects of AS-101 administered intraperitoneally to rats for a period of 4 weeks. Detailed clinical, clinicopathological and pathological studies were carried out in groups of rats treated with increasing concentrations of AS-101. The highest concentration administered to the rats was about 120 times that of the proposed therapeutic dose for humans.

Materials and methods

Chemical. Ammonium trichloro(dioxylene-0-0')tellurate, coded AS-101, was provided as a solution dissolved
in phosphate buffered saline (PBS), by Bar Ilan University, Ramat Gan, Israel. Concentrations of 5, 10, and 20 mg/100 ml of AS-101 was supplied.

Animals and treatment. One hundred and fifty Sprague Dawley rats (Crl: CD BR) equally divided by sex were obtained from Charles River Laboratories, England. The rats were about 6 weeks of age on arrival. During the acclimatization and experimental periods the rats were held under barrier conditions. The room in which they were maintained was kept at a positive pressure in relation to the outside. Fresh, uncirculated air was supplied to the room, which was monitored for temperature (target range 21 ± 2°C) and humidity (target range 55 ± 15%). There were about 15 air changes per hour, and a 12 h dark:12 h light cycle.

On arrival the rats were randomized into five experimental groups of males and females, respectively. The rats were housed in groups of five, in modified type RC1 cages from North Kent Plastics (Kent, England). The cages were distributed randomly throughout the cage-racks in order to avoid any spatial effects.

Four groups were injected intraperitoneally (i.p.) with ascending concentrations of AS-101 for a period of 4 weeks. The first group (group 1) served as a control, and was injected intraperitoneally with PBS. The four remaining groups received doses of 3 (group 2), 6 (group 3), 12 (group 4), and 24 (group 5) mg/kg/week AS-101. Those rats receiving 3, 6, and 12 mg/kg/week, were dosed three times per week; the control and high dose groups were injected six times per week. The frequency at which the rats were dosed was based on the results of preclinical and clinical trials, in relation to the induction of lymphokine production, and induction of IL-2 receptors (Sredni et al. 1987, 1988a). Each animal received the appropriate quantity of test material in PBS at a volume dosage of 20 ml/kg body weight.

A commercially available pelleted laboratory animal diet (Altromin 1324N) (Altromin Spezialfutterwerke GmbH, West Germany) was fed ad lib. during the acclimatization and study period. Drinking water was supplied to each cage in two polythene bottles. Water was routinely tested at the laboratory at 6-monthly intervals for physical, chemical and biological characteristics.

Routine laboratory examinations. Animals were examined for their general condition once every day during the treatment period. Body weight, food consumption, and water intake were measured weekly throughout the treatment period.

Clinical pathological examinations. Blood chemistry, hematology and urinalysis were carried out prior to termination of the experiment.

Blood samples were taken from the retro-orbital sinus with the rat held under ether anesthesia. EDTA (for hematology) and lithium heparin (for blood chemistry) were used as anticoagulants. Blood chemistry was carried out on the Cobas Bio (Roche) Centrifugal analyzer; hematology on the Coulter ZF counter. Protein electrophoresis was performed on cellulose acetate gels using equipment of Helena Laboratories.

Pathological investigations. On completion of the 4 weeks of treatment all rats were sacrificed by CO₂ inhalation and a detailed post mortem was performed on each animal. Selected organs were weighed shortly after death. Relative organ weights were calculated as a percentage of the body weight.

Samples of all tissues were preserved in 4% formaldehyde saline with the exception of the eyes which were preserved in Davidson’s solution. Tissues were sectioned at 4–5 μ thickness, stained with hematoxylin and eosin and examined microscopically.

Statistical analyses. Continuous data was tested for homogeneity of variances using the Bartlett’s test (Sokal and Rohlf 1981). Where the samples proved to be homogeneous, a two-tailed t-test was used, using a pooled within error variance. Where the variances were found to be heterogeneous, the Mann-Whitney U-test was applied. Incidence tables of pathology data were analyzed using the Chi-squared test.

Results

Clinical signs

Two males and two females of the high dose groups died during the course of the study. Clinical signs relating to treatment with AS-101 were darkening of the eyes, dark colored urine, hind limb paresis and paraphimosis. A garlic odor pervaded the room in which the experiment was performed throughout the dosing period.

Body weight

Body weight gain was adversely affected in all treatment groups in a dose-related manner. Statistically significant differences from their controls were detected in males and females receiving 12 and 24 mg/kg/week AS-101 throughout most of the study period, and in females receiving 6 mg/kg/week during the last week of the study.

Food consumption

Food consumption was depressed in a dose-related manner throughout the study period. Statistically significant differences from the controls were demonstrated in rats of both sexes receiving 12 and 24 mg/kg/week AS-101.

Hematology

The results of the hematological examination carried out prior to the termination of the experiment are presented in Table 1.

It was apparent that the response of the rats to the compound was more severe in female than in male rats. Effects noted included decreases in pack cell volume (PCV), hemoglobin concentration (Hb), erythrocyte counts (TRBC), and thrombocyte numbers (PLT). These declines were seen in female rats receiving 12 and 24 mg/kg/week AS-101. A decrease in the above parameters was also evident in the male groups, however, with the exception of the thrombocyte count, statistical significance was not achieved.

An increased (p < 0.05) corpuscular volume (MCV) was evident in the two higher-dose female groups. The immaturity of the erythrocytes was borne out by the increased (p < 0.01) reticulocyte count in both these groups, as compared to their controls.