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Effect of Cesium and Potassium Salts on Survival of Rats Bearing Novikoff Hepatoma

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MESSIHA, F. S. AND D. M. STOCCO *Effect of cesium and potassium salts on survival of rats bearing Novikoff hepatoma* PHARMACOL BIOCHEM BEHAV **21**: Suppl 1, 31–34, 1984 —The effect of CsCl on the life span of female Sprague-Dawley rats inoculated with Novikoff's hepatoma was studied as a function of both pre- and post-treatment with CsCl and as a function of the inoculant dose. The effect of KCl on the CsCl treatment was also studied. Rats treated with CsCl for 12 consecutive days prior to or immediately after inoculation with 1.0 ml of viable hepatoma cell suspension showed an increase in mortality score from corresponding controls. Conversely, increases in the dose of the inoculant resulted in delaying the onset of toxicity in rats receiving the Cs-treatment after inoculation as evidenced by a decrease in mortality. Availability of KCl in drinking water ad lib further decreased total mortality when given alone but not when combined with CsCl The results indicate a dose-dependent paradoxical effect of CsCl on Novikoff hepatoma cell toxicity and suggest a critical intercellular balance requirement between Cs^+ and K^+ on the effect studied

Cesium Hepatoma Mortality Potassium

ONE investigative approach to mechanism(s) underlying tumorigenesis resides in the evaluation of the relationship between transmembrane potential and changes in subcellular elemental ionic concentration [1–4]. This approach advanced the hypothesis that a correlation may exist between the degree of mitotic activity and electrical transmembrane potential of somatic cells and involves alteration of certain alkali metal levels, particularly those of Na^+ and K^+, e.g., high Na^+ concentration is considered mitogenic [9]. The other alkali metals Li^+ and Cs^+ resemble Na^+ and K^+ and can displace them in certain biological functions, respectively [7]. Lithium salts have an application in chemotherapy [8], and initial experiments with Cs^+ on mice bearing Sarcoma-I implants suggested some cytostatic activity of this ion as well [5]. Hepatoma cell lines often induce enlargement of liver and produce an increase in mitotic activity of the tumor-bearing host [10]. The present study evaluates the effect of CsCl and KCl on survival rate of rats inoculated with Novikoff hepatoma (NH) transplanted in sexually immature Sprague-Dawley female rats [6]

METHOD

The subjects were experimentally naive Sprague-Dawley rats which were obtained from Holtzman Farm, Inc., Madison, WI. Animals were caged in groups of three in a room with 12 hr light/dark cycles They had access to Purina pellet food and distilled water unless otherwise specified.

In the first set of experiments, the effect of pretreatment with CsCl and its continued administration on NH morbidity was studied. The 30-day old female rats were housed for a 4-day acclimatization period and were divided into two groups of 8 animals each. They received either water (water-controls) or 0.4% (w/v) CsCl solution as the sole drinking fluid for 12 consecutive days preceding intraperitoneal (IP) injection of 1.0 ml of viable NH cell suspension. Thereafter, the concentration of the CsCl solution was decreased to 0.2% and remained unchanged throughout the 32 day observation period that followed. The experiment was repeated twice resulting in 24 animals for each treatment group.

In the second experiment, 33-day-old female rats had access to food and distilled water for 12 consecutive days before they were similarly inoculated with 1 0 ml of the NH cell suspension. Thereafter, they were divided into two groups of 8 rats each receiving distilled water or 0.4% CsCl solution as the sole drinking fluid throughout the 32-day period of the study

In the third experiment, the male rats used were 35 days old at the time of inoculation with 2.0 ml of NH cell suspension. They were then placed immediately on either 0.2% CsCl solution, 0.02% KCl alone or combined, and the controls received distilled water as the sole drinking fluid for a subsequent 32 day observation period.

Animals were weighed at various time intervals and their fluid consumption was recorded and expressed as mg fluid

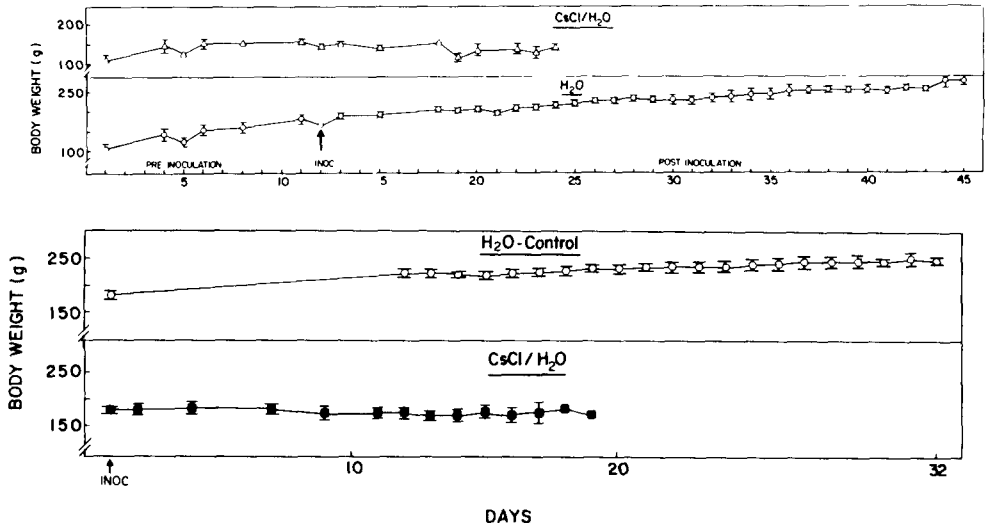

FIG 1 The effect of CsCl on the body weight of female rats inoculated with Novikoff hepatoma cell suspension Rats were inoculated intraperitoneally with 1 0 ml of Novikoff hepatoma (NH) cell suspension The CsCl was dissolved in distilled water to obtain 0 4% CsCl drinking solution for the pre-inoculation period and was decreased to 0 2% CsCl for the post-inoculation period (upper panel) The 0 2% CsCl solution was also made available to separate group of rats immediately after inoculation with NH (lower panel) Distilled water was the drinking fluid for the inoculated controls (H_2O-controls) Drinking fluids were available ad lib Arrows indicate inoculation time Each value represents the mean±SE of the mean for 24 rats (upper panel) or 8 rats (lower panel) The absence of data for the Cs-treatment is due to death of the majority of animals initially inoculated with the NH

consumed per kg body weight per 24 hr The death rate was expressed as percent mortality occurring from initial number of inoculated animals and presented cumulatively Student's *t*-test was used for the evaluation of the statistical significance of the results.

RESULTS

Figure 1 shows changes in body weight of CsCl-treated rats compared to water-controls prior to and subsequent to inoculation with NH as a function of time. A moderate decrease in body weight of Cs-treated rats from water-controls was noted for the period monitored At the time of sacrifice, the majority of the animals of the water-control group displayed enlargement of the stomach and an increase in volume of abdominal fluidity compared to the Cs-treated rats. The pattern of body growth observed in Experiment 1 (Fig. 1, upper panel) resembled that of Experiment 2 (Fig. 1, lower panel) for rats not exposed to CsCl before the NH inoculation.

Figure 2 compares mean changes in body weight obtained for a 12-day period prior to inoculation with that obtained 24 hr prior to the death (terminal phase) of both Cs-treated rats and water controls. The initial body weight difference between the groups was not statistically significant The average mean body weight throughout the 12-day period preceding inoculation declined by 14% ($p<0.05$) in the injected animals This decrease was even greater, i e., 34% ($p<0.01$), at the terminal phase

There was a moderate decline in daily fluid intake for a short period after inoculation with the NH The amounts of Cs^+ consumed prior to tumor transplantation amounted to a daily range between 6.5 to 8.0 mEq/kg which declined rapidly after 5 days of the NH injection to approximately 3 7

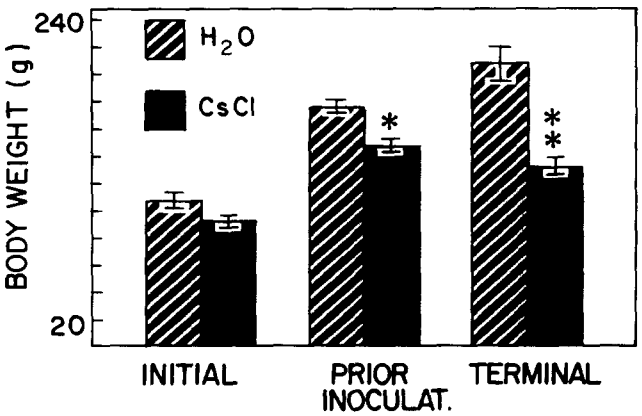

FIG 2 The effect of CsCl intake in drinking fluid on body weight of female rats pre- and post-inoculation with 1 0 ml of Novikoff hepatoma cell suspension Each bar graph represents the mean±SE of the mean of 24 rats receiving 0 4% CsCl solution as the only drinking fluid for a 12-day period preceding inoculation with Novikoff hepatoma (NH) The CsCl concentration was then decreased to 0 2% immediately after the NH injection for a subsequent 12 days (terminal weight) $*p<0.05$, $**p<0$ 02

mEq/kg/24 hr until death of the animals Likewise, the amounts of water consumed declined after inoculation with NH but stabilized between 1 5 to 1.8 mg/kg/day until the end of the experiment

Figure 3 shows mortality scores of rats maintained on water or on CsCl as a function of the inoculant dose and duration of the Cs-treatment. In Experiment 1 (upper left

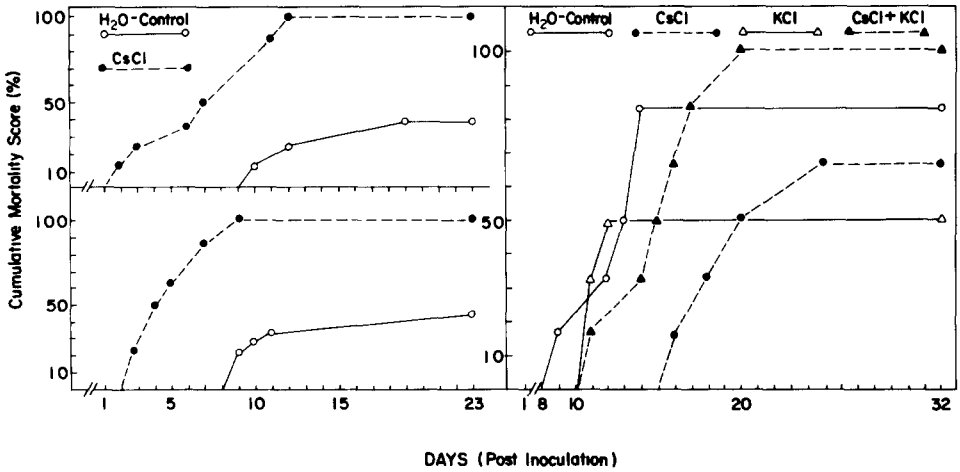

FIG 3 The effect of CsCl treatment on survival time of female rats inoculated with various doses of a Novikoff hepatoma cell suspension The upper left panel (Experiment 1) shows cumulative mortality score of rats receiving 0 4% CsCl as the sole drinking fluid for 12 days preceding inoculation with 1.0 ml of Novikoff hepatoma (NH) preparation Thereafter, the concentration of CsCl was reduced to 0 2% solution for the post-inoculation period shown (n=24). In Experiment 2 (lower left panel) a 0.2% CsCl drinking fluid was initially made available immediately after inoculation with 1.0 ml of the NH (n=8) In Experiment 3 (right panel) rats were inoculated with 2.0 ml of the NH preparation and various drinking regimens were then instituted This consisted of distilled water (controls), 0 2% CsCl, 0 02% KCl alone or combined with 0 2% CsCl as the sole drinking fluid (n=8) The results were expressed as percent mortality occurring from the initial number of animals used for each treatment group They were recorded daily and plotted cumulatively as a function of time

panel) and in Experiment 2 (lower left panel), animals received 1.0 ml of the inoculant compared to 2.0 ml in Experiment 3 (right panel). There was a shift in the toxicity curve to the left in rats inoculated with 1 0 ml of NH (left panel) before or after initiation of the Cs-treatment compared to corresponding controls. The Cs-treatment resulted in the earlier onset of death and in greater mortality counts in both experiments (left panel). This is indicated by 98% to 100% mortality of Cs-treated rats compared to 12% to 30% of the corresponding water-controls at 10 days post-inoculation with 1 0 ml of NH. Cesium intake increased the total death score by 1.5 fold from controls at the end of the observation period.

The effect of KCl intake alone and combined with CsCl on mortality score of rats inoculated with twofold larger dose of NH is given in the right panel of Fig. 3 (Experiment 3). The Cs-treatment resulted in a shift of the mortality curve to the right compared to water-controls. This is indicated by a 5 day period delay in the onset of death of tumor bearing rats. Cumulative death scores at the end of 32 days post-inoculation were 50% for the KCl-group and 67% for the Cs-treated rats compared to 83% of the water-controls. The combined treatment with KCl and CsCl gave rise to 100% morbidity within 20 days of inoculation with NH

DISCUSSION

The present results indicate that ad lib intake of 0.4% or 0.2% solution of CsCl prior to or immediately post NH-inoculation resulted in an early onset of mortality and in its enhancement, compared to water-controls. This is indicated in both Experiments 1 and 2 when 1.0 ml of NH was injected Conversely, Cs-treatment resulted in protection against NH when a greater dose of the inoculant was used, i.e., 2.0 ml This paradoxical effect of Cs^+ action on NH may be due, at least in part, to the amounts of KCl in the inoculation medium used (RPMI 1640 synthetic media, Gibco Co.). A 1.0 ml inoculum contains 0.4 mg KCl and appears to increase mortality in the animals which are exposed to CsCl whereas 2.0 ml doubles the KCl content and may exert protective action on tumor bearing animals which received identical Cs-treatment. This assumption is supported by the effect of K^+ on NH noted in which KCl intake for 32 days post-inoculation with NH decreased mortality by 39% and by 23% compared to controls and to Cs-treated rats, respectively. However, it appears that the addition of KCl to CsCl regimens acted antagonistically. This suggests that a critical balance between Cs^+ and K^+ may be required to modify some of the toxic effects of the NH studied. It is conceivable, however, that the tumor studied is more sensitive to K^+ than to Cs^+ at certain cellular concentrations and increase of K^+ levels will then lead to toxic manifestations

The data presented indicate some of the experimental variables which may influence NH growth and development. The close resemblance between K^+ and Cs^+ in various physiological parameters [7] and their capacity to displace intercellular Na^+ may contribute to the phenomenon observed. The implication of alteration in ion transport across cell membrane in tumorigenesis [1–4] suggests that these ions may conceivably have changed intercellular ionic environment resulting in changes in cell surface membranes of the malignant cells. Moreover, modulation of certain intracellular elements as Na^+, K^+, Mg^{++} may interfere in processes regulating active transport mechanisms across cell membranes and the electrochemical gradients involved, i e , Na^+-K^+-Mg^{++}-ATPase. In addition, the results suggest lack of antitumor action by Cs^+ on NH used as contrasted with that

reported earlier with the Sarcoma-I implants in mice [5] More experiments are required to evaluate the efficacy of Cs-salts on various experimental tumors before a cyto-static effect for Cs^+ can be established.

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