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Biochemical Aspects of Cesium Administration in Tumor-Bearing Mice

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MESSIHA, F S *Biochemical aspects of cesium administration in tumor-bearing mice* PHARMACOL BIOCHEM BEHAV 21: Suppl 1, 27–30, 1984 —The effect of pretreatment with CsCl on mice bearing sarcoma I implants was studied as a function of duration of treatment period, life span and tissue Cs^+ and K^+ levels Treatment with CsCl for 14 consecutive days prior to sarcoma implantation resulted in initial reduction of the tumor-mediated mortality compared to controls and to a one week pretreatment period with identical doses of CsCl A large accumulation of endogenous K^+ was noted in tumor mass compared to nonmalignant tissue of the same animals or to tumor-free controls receiving identical Cs-treatment The entry of exogenously administered Cs^+ into malignant tissue was less than that accumulating in respective controls The accumulation of Cs^+ in tumor mass was dose-dependent The ratio of K^+ C^+ was greater in tumor tissue than in nonmalignant tissue The results suggest that a critical balance between these alkali metals may be required for adequate Cs effect against the tumor studied

Cesium Mortality Potassium Sarcoma Tumor

THE initial finding of the antitumor activity of cesium (Cs) salts has been originally substantiated by Ishiwara in 1927 [8]. He studied the effect of 58 elements on propagable tumors. His findings identified six potential elements, i e., cesium, germanium, selenium, cerium, scandium and ytterbium. Both *in vitro* [14] and *in vivo* [3] experiments with Cs salts followed. The latter [3] evaluated the efficacy of 57 inorganic compounds, derived from 36 elements, on mammary adenocarcinoma-bearing albino mice Administration of two cesium salts at various dosages to three mice resulted in the survival of one mouse receiving 0.05 mg daily dose of CsCl for 78 days This led to the conclusion of the ineffectiveness of Cs salts on the tumor line studied. Shortly thereafter, one study [16] demonstrated that subcutaneous injection of CsCl in mice was only effective when given for nine days preceding Twort carcinoma implantation, a rapidly growing neoplasm of epithelial origin, but not when the Cs-treatment was initiated 24 hr after tumor transplantation. It was then concluded that the rationale employed, i e., the introduction of strong basic ions as Cs^+ into the tumor nuclear matrix to reduce nuclear activity and tumor progression, could not be materialized [16] Nonetheless, over four decades had expired before Brewer [1,2] and our laboratories [9,10] have independently reinitiated experimental studies relevant to the potential of Cs salts as chemotherapeutic agents. In addition, the uptake of Cs^+ by the malignant tissue has been suggested for the use as a diagnostic tool for cancer therapy using ^{131}Cs [6,7] and ^{132}Cs [13] isotopes. These prompted the clinical trials of CsCl in the cancer patient [2,15]. This report evaluates certain biochemical events associated with CsCl treatment of mice-bearing sarcoma (SR) implants which showed a previous beneficial response to the Cs-treatment [9,10].

METHOD

Male albino A/J mice were purchased from Jackson laboratories, Bar Harbor, ME. They were 8 weeks old and were maintained on purina pellet food and distilled water ad lib for one week. A methylcholanthrene derived viable tumor SR-cell suspension was used They were injected introdermally (ID) in the mouse left gluteal region.

In the initial experiment, the effect of CsCl on SR-bearing mice was studied as a function of duration of pretreatment with CsCl Animals were divided into 4 groups of 25 mice each They received intraperitoneal (IP) injection of saline or CsCl, 3.0 mEq/kg, once daily for 7 days or for 14 consecutive days prior to the SR transplantation Five animals from each group were then sacrificed and individual blood specimens were obtained and centrifuged for plasma Cs^+ determinations by atomic spectroscopy. All groups received SR-implants except for 5 mice of each group who remained tumor-free. The daily saline and CsCl injections were continued for a subsequent 33 days post inoculation with SR.

In the second experiment, the effect of pretreatment with smaller daily CsCl dosage on tumor content of endogenous K^+ and exogenously administered Cs^+ was studied in tumor-free and in mice bearing the SR-implants. Three groups of mice received either saline, CsCl, 0.5 or 1.0 mEq/kg IP once daily for 7 days Each group was then divided into 2 subgroups receiving either SR implantation or saline and remained tumor free. The daily saline and CsCl injections were continued for a comparable period as in the first experiment, i e , 33 days. Thereafter, all surviving animals in addition to 5 experimentally naive mice, i.e., receiving neither saline or SR implants, were sacrificed by decapitation. Tumor mass and non malignant tissues were

TABLE 1

EFFECT OF DURATION OF PRETREATMENT WITH CsCl ON PERCENT MORTALITY OF MICE WITH SARCOMA IMPLANTS

Treatment	Days post tumor implants	(n)	Percent Mortality Cs-Pretreatment period (days) 7	14
Saline controls	15	(14)	60	67
	30	(16)	80	89
CsCl	15	(14)	38*	11†
	30	(16)	50*	40*

Data represents mean percent mortality of mice inoculated with viable sarcoma I cell suspension after one or two weeks pretreatment with a daily IP injection of saline or CsCl, 3 0 mEq/kg The results are expressed as percent mortality occurrences from initial number of animals used

Different from saline-controls by χ^2 *$p<0$ 005, †$p<0$ 0005

Different from mice pretreated with CsCl for 7 days prior to sarcoma transplantation by χ^2 ‡$p<0$ 005

individually dissected from the left and the right gluteal regions, respectively They were weighed and individually homogenized in ice cold trichloracetic acid by a waring blender The volume of the resulting homogenate was measured and centrifuged at 22,000×g at 4°C The supernatant obtained was measured for its volume and aliqots served for the determinations of K^+ and Cs^+ concentrations by atomic absorption technique

The mortality score was expressed as percent death occurrences of initial number of animals scored 15 and 30 days post the SR implantation The concentration of blood plasma Cs^+ concentration was given as mean±SEM and expressed as mEq/L while that of tissue K^+ and Cs^+ were given as mEq/mg wet weight. Both student's t-test and χ^2 distribution test were used for the statistical analyses of the data

RESULTS

Table 1 shows the effect of duration of pretreatment with CsCl on mortality score following SR-transplantation in the male mouse Animals receiving CsCl, 3 0 mEq/kg/day, for the 7 day period preceding SR-inoculation exhibited 38% ($p<0$ 005) and 50% ($p<0.005$) death from mean corresponding value of saline controls 15 and 30 days post tumor implantation, respectively Animals receiving the Cs-treatment for 14 days prior to the SR transplantation showed lower death rate initially This was demonstrated by 11% death occurrences 15 days post tumor inoculation compared to 38% and 67% morbidity of animals pretreated with CsCl for 7 days or with saline, respectively The terminal mortality score for the entire period was narrowed to 40% and 50% for both Cs-treated groups which remained significantly lower than the corresponding saline controls ($p<0.005$) Plasma Cs^+ amounted to 0 5±0 1 and 0 8±0 1 mEq/L at time of inoculation with SR as a consequence of daily administration of CsCl, 3 0 mEq/kg IP, for 7 or 14 consecutive days, respectively

Table 2 shows the effect of treatment with CsCl on endogenous tissue content of K^+ of tumor-free and of SR-bearing mice compared to corresponding controls Tissues from gluteal region of mice were used They were derived from tumor-free animals receiving saline or identical CsCl treatment for comparison with these obtained from both non-malignant and tumor tissues of SR-bearing mice. Short-term administration of daily CsCl dosage of 0 5 or 1 0 mEq/kg/IP for 40 consecutive days decreased endogenous tissue K^+ content from saline control of tumor-free mice The malignant tissue showed at least a two fold increase in K^+ concentration from non-malignant tissue of the same mouse or from tumor-free controls receiving identical Cs-treatment Little change in tissue K^+ levels were noted by CsCl in non-malignant tissue of SR-bearing mice compared to tumor-free controls Non-malignant tissue of tumor bearing mice receiving saline showed decreased K^+ concentration compared to tumor-free saline controls

Table 3 shows concentration of exogenously administered Cs^+ assayed in the same tissue preparations used for the K^+ determination listed in Table 2 No measureable Cs^+ was found in tumor-free or in non-malignant tissue of mice with the SR-implants This is compared to small but measurable levels of Cs^+ in tumor mass of saline-controls. The amounts of Cs^+ measured in the tumor preparation were decreased by approximately 65% ($p<0$ 05) and 75% ($p<0$ 05) from non-malignant tissue of the same animals receiving the 0 5 or the 1 0 mEq/kg CsCl dose, respectively Likewise, the Cs^+ content of tumor tissue was 48% to 57% lower than that assayed in tumor-free mice receiving identical CsCl treatment Blood plasma Cs^+ at time of sacrifice was 0 3±0 1 mEq/L for mice injected CsCl, 0 5 mEq/kg/day compared to 0 5±0 03 mEq/L assayed in mice receiving daily injection of CsCl at 1 0 mEq/kg IP

The results also show that the ratio of K^+·Cs^+ in tumor tissue of mice treated with CsCl, 1 0 mEq/kg, accounted to 12 1 This was greater than the 2 2 and 1 2 ratios determined in tissue derived from tumor-free and from non-malignant tissues of tumor-bearing mice, respectively

DISCUSSION

The present study demonstrates that short-term administration of CsCl prior to SR implanatation exerted a protect-

TABLE 2

EFFECT OF CESIUM CHLORIDE ON TISSUE CONTENT OF POTASSIUM IN SARCOMA-BEARING MICE

Treatment			Tissue K^+ Content (mEq/mg wet weight)	
			Sarcoma-bearing mice	
Drug	Daily Dosage (mEq/kg, IP)	Tumor-free Controls	Malignant tissue	Non-malignant tissue
Saline	—	3 9 ± 0 6 (4)	3 6 ± 0 8* (8)	1 5 ± 0 3 (8)
CsCl	0 5	2 1 ± 1 0 (4)	4 5 ± 1 0* (5)	1 8 ± 0 2 (5)
	1 0	2 2 ± 0 1 (5)	5 2 ± 0 6†‡ (7)	2 1 ± 0 4 (6)

Mice were injected CsCl 0 5 or 1 0 mEq/kg, IP, once daily for 7 consecutive days prior to inoculation with 0 5 ml of viable sarcoma cell suspension The Cs-treatment was continued for 33 days post tumor implantation and the controls received saline Additional group of mice received identical CsCl treatment for the same duration of time but remained tumor-free Values are mean ± SE of the mean of tissue K^+ levels for the number of independent determinations given between parenthesis

Different from non-malignant tissue of sarcoma-bearing mice by Student's t-test *$p<0$ 05, †$p<0$ 01

Different from tumor-free mice by Student's t-test ‡$p<0$ 05

TABLE 3

TISSUE CONTENT OF EXOGENOUSLY ADMINISTERED Cs^+ IN SARCOMA-BEARING MICE

Treatment			Tissue Cs^+ Content (mEq/mg wet weight)		
				Sarcoma-bearing Mice	
Drug	Daily Dosage (mEq/kg, IP)	(n)	Tumor-free Controls	Malignant tissue	Non-Malignant tissue
Saline	—	(4)	NM	0 018 ± 0 007	NM
CsCl	0 5	(5)	0 60 ± 0 17	0 29 ± 0 07*	0 83 ± 0 21
	0 1	(5)	0 99 ± 0 28	0 43 ± 0 05*	1 72 ± 0 28

Values are means ± SE of mean tissue content of exogenously administered Cs^+ as detailed in legend to Table 2

Non-measurable (NM) Cs^+ concentration is indicated

*$p<0$ 05

ive action against SR toxicity. This is consistent with previous reports from this laboratory [9,10] and extends them to indicate that prolonged treatment with CsCl, i e., for 2 weeks, prior tumor implantation markedly decreased SR-mediated mortality for the initial 15 days compared to the one week pretreatment period with identical dosages of CsCl and to controls. However, this difference, in mortality as a function of duration of pretreatment with CsCl, was abolished towards the end of the experiment This suggests a probable interference in initial uptake of the inoculant by CsCl analogous to that proposed by Wright and Graham [16] Also, the possibility of Cs-evoked alteration of certain critical biological factors responsible for providing optimal condition for tumor's implantation and progression can not be excluded This agrees with the conclusion made in a study in which the CsCl effectiveness was only apparent when it was given for 9 days preceding carcinoma implantation and not when it was initiated thereafter [16] Moreover, it should be noted that CsCl was devoid of antitumor activity on Novikoff hepatoma-bearing mice (see this issue) and prolonged pretreatment with CsCl potentiated the hepatoma toxicity Thus, CsCl appears to exert beneficial effect on certain tumors, i e., sarcoma and Twort carcinoma, if administered prior to tumor-implantation. Paradoxically, CsCl may accelerate toxicity of other tumors, i e , Novikoff-hepatoma This indicates the need for detailed experimental studies to identify the chemotherapeutic spectrum of Cs salts.

The chemotherapeutic efficacy of CsCl and RbCl salts on tumor-bearing animals has been explained by possible Cs-mediated alteration of pH of tumor tissue, i.e., towards alkalinity, which may hinder and/or delay the transplanted tumor from establishing itself [2]. However, another mechanism may be operating under these circumstances which is related

to the physiological and metabolic resemblances between Cs^+ and K^+ and the implication of another alkali metal as Na^+ in cell transmembrane potential which may be functionally involved in mitotic control and oncogenensis [4,5] The present study shows that the malignant tissue accumulated K^+ in greater amounts that the non-malignant tissue of the same animal or of tumor-free mice. Conversely, tumor-tissue of SR-inovulated mice showed low concentration of exogenously administered Cs^+ compared to that obtained from non-malignant tissue or from corresponding controls This tissue Cs^+ level was increased as a function of CsCl dose administered. This suggests that the ratio between K^+ and Cs^+ in tumor tissue may be critical for the host survival Therefore, it is conceivable that certain concentration of Cs^+ is required for antitumor action and to induce biological changes adversely affecting tumorigenesis The slow accumulation of Cs^+ in malignant tissue may be enhanced by other means as has been recommended [2,15], i e , coadministration of selected compounds such as Vitamins, certain essential fatty acids, electron donor radicals and/or by the use of specific synthetic ionophore compounds to facilitate transport of certain alkali metals across cellular membranes [12]

In conclusion, further studies are needed to delineate the antitumor specificity of Cs salts. Nonetheless, CsCl may find a place as a diagnostic tool for cancer screening tests due to the preferential uptake of ^{131}Cs and ^{132}Cs by certain tumors [7, 8, 13] Noteworthy, the antidepressant property of Cs^+ reported in animals experiments [11] which may provide a dual function for Cs, i e , anticancer action combined with modulation of the depressive mood of the terminal cancer patient.

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