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ORIGINAL ARTICLE

Assessing the therapeutic and toxicological effects of cesium chloride following administration to nude mice bearing PC-3 or LNCaP prostate cancer xenografts

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Abstract
Purpose The purpose of this study was to assess the therapeutic and toxicological effects of cesium chloride (CsCl) administration in mice bearing prostate cancer tumors.
Methods Three CsCl dose titration studies were completed in tumor-bearing and non-tumor-bearing athymic nude mice. All mice were administered either vehicle (controls), 150, 300, 600, 800, 1,000, or 1,200 mg/kg of CsCl once daily by oral gavage for 30 consecutive days. Body mass was measured daily, food and water consumption were measured every 2 days, and tumor volume was measured twice weekly. Histopathological analysis was conducted on tissues collected from each of the studies. Serum AST/ALT and creatinine were also measured.
Results Administration of 800–1,200 mg/kg CsCl reduced PC-3 tumor growth but had no effect on LNCaP tumors. Administration of 800–1,200 mg/kg CsCl also resulted in increased water consumption, bladder crystal development, and higher prevalence of cardiac fibrin clots. An observed loss in body mass was dependent on the xenograft type and concentration of CsCl administered. CsCl did not affect serum AST/ALT and creatinine levels.
Conclusions CsCl may have a therapeutic effect against prostate cancer, but one cannot overlook the acute toxicities also described.

Keywords Cesium chloride · Prostate cancer · PC-3 tumor · LNCaP tumor · Acute toxicity

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Introduction

The search for alternative strategies to treat prostate cancer remains elusive. Although the Food and Drug Administration has not approved the use of cesium chloride (CsCl) as a cancer treatment, individuals seeking this therapy need only search the internet to purchase CsCl solutions or tablets [1–3]. Most of the companies that advertise on the internet, promote their products by citing the most current CsCl cancer research, the majority of which is outdated or incomplete.

There have only been a limited number of studies conducted evaluating the therapeutic effect of CsCl. In the sole-published clinical study, 25 out of 50 terminal patients with generalized metastatic disease were still alive after 3 years of receiving 6–9 g of CsCl each day [4]. However, there was no control group in this study; every patient was treated with CsCl.

In vivo CsCl studies reported to date, which were conducted in the 1980s, have used mice with surgically implanted murine sarcoma I or colon tumors. In these studies, a reduction in tumor volume was reported in animals that were administered daily intraperitoneal injections of CsCl [5–7].

Recently, our laboratory conducted the first CsCl study in a human-derived cancer xenograft mouse

model. Mice bearing a LNCaP prostate cancer xenograft treated with 150 mg/kg CsCl daily via oral gavage had no significant reduction in tumor volume compared to control mice [8]. A reduction in tumor volume was only observed when CsCl was administered in combination with vitamin D [8].

In terms of toxicity, there appear to be some inherent side affects attributed to CsCl treatment, although the severity of these effects has not been resolved. Historically, the only adverse effects reported by patients were nausea, diarrhea, paresthesia, and hypokalemia [4]. However, animal studies analyzing the effects of CsCl on cardiac function have observed detrimental effects contributed to CsCl administration. It has been reported that canines dosed with CsCl experienced long QT syndrome, torsades de pointes, and various cardiac arrhythmias [9–12]. In addition, a few recent case studies have reported observing these cardiac problems in patients taking CsCl [13–19].

The lack of CsCl research is detrimental to the safety of consumers taking CsCl products, because the efficacy and toxicity of CsCl has not been fully delineated. In the present study, three animal experiments were conducted to assess the therapeutic and toxicological effects of CsCl treatment in mice bearing either a PC-3 or LNCaP prostate cancer xenograft.

Materials and methods

Husbandry of athymic nude mice

On arrival to The Prostate Center, athymic nude mice were placed in microisolater cages, three mice per cage, and were allowed to acclimatize to their new environment for 1 week. Following the acclimatization process, mice from the PC-3 and LNCaP studies were inoculated subcutaneously with prostate tumor cells and tumors were allowed to propagate.

Approximately 1 week before the initiate on of each study, mice were transferred into individual cages, and the husbandry duties (e.g., the changing of the cages, food and water) were passed from the animal care staff onto the researcher. The purpose of this exercise was to decrease the level of disturbance to each cage, in order to accurately assess water and food consumption as measures of acute toxicity for each mouse.

Dose preparation of CsCl solutions

Cesium chloride dosing solutions were prepared once weekly by dissolving CsCl in milli-Q water. The final concentrations achieved were 150, 300, 600, 800, 1,000, and 1,200 mg/kg CsCl to be dosed via oral gavage. The range of CsCl dosages was determined based on several factors—150 mg/kg appears to exhibit no toxicity in the nude mouse model based on previous studies in our laboratory; the oral LD_{50} of CsCl in mice is an acute dose of 2,306 mg/kg [20]; the biological half-life of CsCl is ~3–6 days in mice [21, 22]; a CsCl distribution study in our laboratory found that CsCl is retained in major bodily organs 24 h after administration, indicating a potential additive accumulation of CsCl in these organs through chronic dosing [8]; companies advise patients to take 3–9 g of CsCl per day, which in terms of body surface area translates to 527–1,581 mg/kg in mice (for a 70-kg human). The oral administration route was chosen to stay consistent with the route of administration of most CsCl products.

The administration volume of CsCl was 100 µl for a 30-g mouse. All mice were administered either vehicle (controls), 150, 300, 600, 800, 1,000, or 1,200 mg/kg of CsCl once daily by oral gavage for 30 consecutive days.

PC-3 solid tumor xenograft study

The PC-3 cell line was established from bone metastases [23]. This cell line is androgen insensitive and does not secrete prostate-specific antigen (PSA). Upon injection into athymic nude mice, PC-3 cells form subcutaneous tumors which have a consistent shape and rapid growth rate. The PC-3 xenograft model was employed to determine the effects of oral CsCl administered as part of a dose escalation study against an advanced prostate cancer model.

Sixty male athymic nude mice were subcutaneously inoculated with 2×10^6 PC-3 cells in a single dorsal fat pad. Tumors appeared in 56 mice 2 weeks after inoculation and were measurable by the third week. The homogeneous growth of the PC-3 tumors allowed for all of the animals to be integrated into the experiment on the same day, which was 3 weeks after inoculation.

Mice were randomly distributed into the seven treatment groups according to their tumor volume and body mass ($n = 9$ for control group, $n = 8$ for the 150-, 300-, 600-, 800-, and 1,000-mg/kg groups and $n = 7$ for the 1,200-mg/kg group). Each treatment group had an average tumor volume of 87–107 mm^3 and an average body mass of 28.4–31.4 g. Statistical analysis by one-way ANOVA showed no statistical difference between any of the treatment groups in regard to average tumor volume or average body mass on day 1 of the study ($P > 0.05$).

LNCaP solid tumor xenograft study

The LNCaP cell line originates from a metastatic lesion [24]. Unlike PC-3 cells, the LNCaP cells are androgen sensitive and do secrete PSA. Circulating PSA levels from LNCaP tumors are often correlated with tumor growth in studies in this xenograft model. An oral CsCl dose escalation study was conducted using the LNCaP xenograft model.

Seventy athymic nude mice were subcutaneously inoculated with 2×10^6 LNCaP cells in two dorsal bilateral fat pads. Two tumor sites were used in this study, because the success rate of LNCaP tumor propagation is lower than PC-3 tumor propagation. In addition, LNCaP cells must be inoculated in a BD matrigel basement membrane matrix:RPMI solution to ensure an adequate tumor success rate.

The LNCaP tumors have a highly variable growth rate, and thus, mice were integrated into this experiment in a staggered fashion. Tumor volumes were monitored rigorously, until volumes of >70 mm^3 were observed, at which time the animals were allocated into one of the seven treatment groups. Body mass was also taken into consideration in determining the distribution of the animals into the treatment groups. The first group of mice was integrated into the study 3 weeks after inoculation and the last group of mice was integrated 6 weeks after inoculation. From the 70 mice that were originally inoculated with LNCaP cells, 53 animals were used in this study as these were the only animals that had large enough tumors during the time range indicated ($n = 8$ for the control, 150-, 800-, and 1,000-mg/kg groups, and $n = 7$ for the 300-, 600-, and 1,200-mg/kg groups). It should be noted that total tumor burden was not measured in this study; only one tumor was tracked on each mouse. Although the second tumor would sometimes appear on each animal, it was usually significantly smaller and showed slower growth characteristics than the tumor of interest.

Each treatment group had an average tumor volume of 94–114 mm^3 and an average body mass of 25.8–28.5 g on day 1 of the experiment. Statistical analysis by one-way ANOVA showed no statistical difference between any of the treatment groups in regard to average tumor volume or average body mass on day 1 ($P > 0.05$).

Non-tumor-bearing nude mouse study

To determine the toxicity of CsCl alone without any contribution from either of the two xenograft models, the final study was conducted in nude mice not bearing any tumors. Mice in this study were integrated into the experiment 4 weeks after their arrival at The Prostate Center, in order to maintain a consistent starting age with the mice used in the PC-3 and LNCaP studies.

The 41 animals were randomly distributed into the seven treatment groups according to body mass ($n = 6$ for the control, 150-, 300-, 600-, 800-, and 1,000-mg/kg groups, and $n = 5$ for the 1,200-mg/kg group). The average body mass in each treatment group was 26.3–27.6 g. Statistical analysis by one-way ANOVA showed no statistical difference between any of the treatment groups in regard to average body mass on day 1 of the study ($P > 0.05$).

Analysis of CsCl on tumor growth and acute toxicity

For the duration of the three 30-day studies, all animals were administered either vehicle (H_2O) or CsCl treatment via oral gavage daily (7 days a week). CsCl efficacy was monitored in the PC-3 and LNCaP studies via tumor volume measurement, which was conducted twice weekly using calipers. Three acute toxicity measurements were taken in each study—body mass was measured daily using a top loading balance prior to dosing, and food and water consumption were measured every 2 days using a top loading balance. Substantial decreases in body mass (>20% initial mass), shakiness, lethargy, and loss of food and water consumption were used as criteria for acute toxicity. Any animals observed with these traits were euthanized to minimize suffering, and their associated treatment group was promptly removed from any further study.

On the last day of each study, mice were euthanized. Tumors, brains, hearts, lungs, livers, spleens, kidneys, small intestines, femurs, quadriceps muscles, prostates, and serum were removed from each animal for further analysis.

Histopathology

Brains, hearts, lungs, livers, spleens, kidneys, small intestines, and quadricept muscles were collected from each mouse and transferred to 10% formalin. Using conventional tissue processing steps, tissues were processed and paraffin blocks were made. For morphological analysis, paraffin blocks were cut into 5 μm sections and mounted on the slides. The slides were subsequently stained with hematoxylin and eosin. Each slide was analyzed for organ-specific changes by a pathologist (L.F.) who was blinded to the treatment groups.

Serum analysis

Serum from each animal was analyzed for changes in AST (aspartate aminotransferase), ALT (L-alanine:

2-oxoglutarate aminotransferase), and creatinine levels. Serum AST and ALT analysis was conducted in 96-well plates using Infinity™ AST Liquid Stable Reagent and Infinity ALT Liquid Stable Reagent (Thermo Electron, Pittsburgh, PA), respectively. Plates were scanned using a Power Wave X microplate reader linked with KC4 Kineticalc (Biotek Instruments Inc., Winooski, VT).

Creatinine levels in serum were quantified using an HPLC method developed on a Waters 2695 Separations Module paired with a Waters 996 Photodiode Array Detector (Waters Limited, Mississauga, ON). The column chosen for this assay was a 3-μm 2.1 × 50 mm Waters Atlantis™ HILIC Silica column. The mobile phase used was a 97.5% acetonitrile solution containing 0.025% ammonium formate. Creatinine standards (0, 5, 10, 20, 40, and 80 μg/ml) were created in 94.5% acetonitrile from a creatinine 2 mg/ml stock solution (Cayman Chemical Company, Ann Arbor, MI). For sample preparation, 10 μl of serum was added to 190 μl of 99.5% acetonitrile, vortexed, and centrifuged at 15,000 *g* at 4°C for 5 min. The supernatant (~94.5% acetonitrile) from each sample was collected and placed in a HPLC autosample vial for injection. Duplicate injections of 80 μl were performed for each standard and sample. The flow rate was maintained at 1 ml/min resulting in the creatinine peak to elute at ~2 min, which was monitored with a UV absorbance of 238 nm. After every ten injections a gradient elution was run for 10 min to clean the column and a QC sample was injected to monitor the consistency of the method.

Statistical analysis

All of the data presented was analyzed using the SigmaStat 3.0 software. Groups were first compared by one-way ANOVA. If statistical significance was observed ($P < 0.05$), a subsequent Tukey test was implicated to determine which groups were significantly different from each other.

Results

PC-3 solid tumor xenograft study

Figure 1 illustrates the body growth rate of the animals in the PC-3 study. Mice administered 1,200 and 1,000 mg/kg CsCl had significant reductions in % average initial body mass compared to the control animals, beginning on day 5 and day 8, respectively. However, the reductions in body mass observed were not great enough to consider the effects of CsCl as too toxic (% initial body mass was not reduced below 80% for any of the animals); thus, euthanization was not performed.

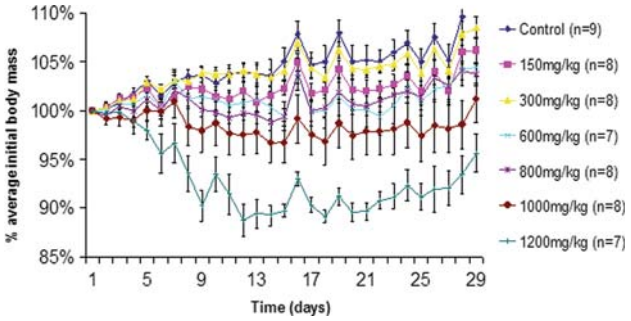

Fig. 1 Graph illustrating changes in % average initial body mass of nude mice bearing a PC-3 tumor xenograft for control (vehicle) and treatment groups (150–1,200 mg/kg CsCl). All mice were administered vehicle or CsCl daily for 30 days. Results are represented as mean value ± standard error of the mean. Statistical analysis using one-way ANOVA indicates a significant difference between the control and treatment groups beginning on day 5 and extending to the end of the study ($P < 0.05$). Pair-wise multiple comparison via Tukey test indicates a difference between the 1,200-mg/kg group and the control from day 5 until the end of the study ($P < 0.05$), and a difference between the 1,000-mg/kg group and the control from day 8 until the end of the study ($P < 0.05$)

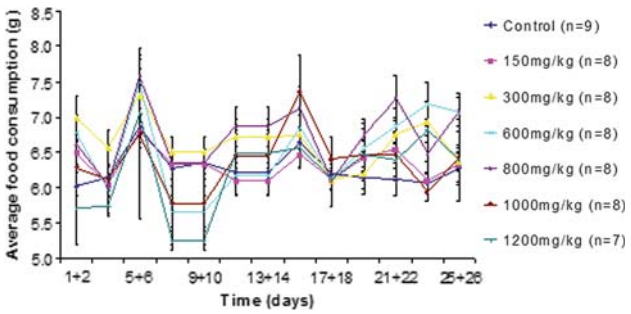

Fig. 2 Graph illustrating changes in average food consumption of nude mice bearing a PC-3 tumor xenograft for control (vehicle) and treatment groups (150–1,200 mg/kg CsCl). All mice were administered vehicle or CsCl daily for 30 days. Results are represented as mean value ± standard error of the mean. There was no statistical difference between any of the groups ($P > 0.05$)

In terms of food consumption, there was no difference between any of the treatment groups and the control (Fig. 2). Alternatively, water consumption increased significantly in the 1,200-, 1,000-, and 800-mg/kg groups compared to the control, beginning on day 3 for the 1,200- and 1,000-mg/kg groups and on day 17 for the 800-mg/kg group (Fig. 3). The 300-mg/kg group also displayed a significant elevation in water consumption compared to the control, beginning on day 3 and ending on day 10 (Fig. 3).

Cesium chloride administration appears to have a therapeutic response in the PC-3 xenograft. A significant

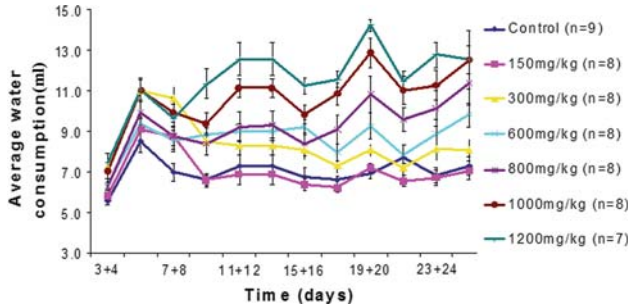

Fig. 3 Graph illustrating changes in average water consumption of nude mice bearing a PC-3 tumor xenograft for control (vehicle) and treatment groups (150–1,200 mg/kg CsCl). All mice were administered vehicle or CsCl daily for 30 days. Results are represented as mean value ± standard error of the mean. Statistical analysis using one-way ANOVA indicates a significant difference between the control and treatment groups beginning on day 3 and extending to the end of the study ($P < 0.05$). Pair-wise multiple comparison via Tukey test indicates a difference between the 1,200-mg/kg group and the control from day 3 until the end of the study ($P < 0.05$), a difference between the 1,000-mg/kg group and the control from day 3 until the end of the study ($P < 0.05$), a difference between the 800-mg/kg group and the control from day 17 until the end of the study ($P < 0.05$), and a difference between the 300-mg/kg group and the control from day 3 to day 10 ($P < 0.05$)

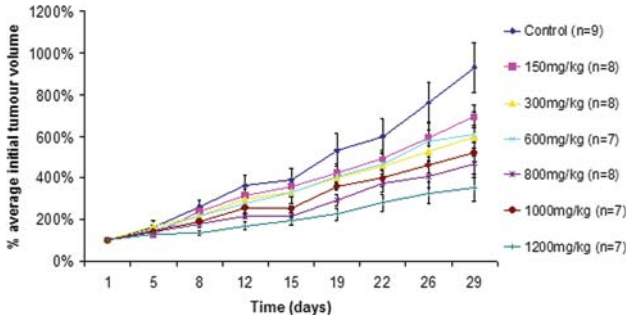

Fig. 4 Graph illustrating changes in % average initial tumor volume of nude mice bearing a PC-3 tumor xenograft for control (vehicle) and treatment groups (150–1,200 mg/kg CsCl). All mice were administered vehicle or CsCl daily for 30 days. Results are represented as mean value ± standard error of the mean. Statistical analysis using one-way ANOVA indicates a significant difference between the control and treatment groups beginning on day 5 and extending to the end of the study ($P < 0.05$). Pair-wise multiple comparison via Tukey test indicates a difference between the 1,200-mg/kg group and the control from day 5 until the end of the study ($P < 0.05$), a difference between the 1,000-mg/kg group and the control from day 26 until the end of the study ($P < 0.05$), and a difference between the 800-mg/kg group and the control from day 12 to the end of the study ($P < 0.05$)

reduction in % average initial tumor volume compared to the control was observed in the three highest concentration groups. The 1,200-mg/kg group had significantly inhibited tumor growth beginning on day 5, the 1,000-mg/kg group had significantly inhibited tumor growth beginning on day 26, and the 800-mg/kg group had significantly inhibited tumor growth beginning on day 12 (Fig. 4).

LNCaP solid tumor xenograft study

Administration of CsCl appeared to be quite toxic in nude mice bearing LNCaP tumors. The 1,200-, 1,000-, and 800-mg/kg treatment groups were removed from the study as one or more animals in each group had a drop in body mass below 80% of their initial body mass (Fig. 5). The 1,200- and 1,000-mg/kg groups were both removed on day 15 and the 800-mg/kg group was removed on day 19 (Fig. 5). Until these animals were removed, each of these groups showed a significant reduction in % average initial body mass compared to the control; this occurred in 1,200-mg/kg group beginning on day 6, the 1,000-mg/kg group beginning on day 7, and the 800-mg/kg group beginning on day 16 (Fig. 5).

There was no significant difference in food consumption or water consumption between any of the treatment groups and the control except on day 3 + 4 where the 600-mg/kg group had a significant increase in food consumption (data not shown).

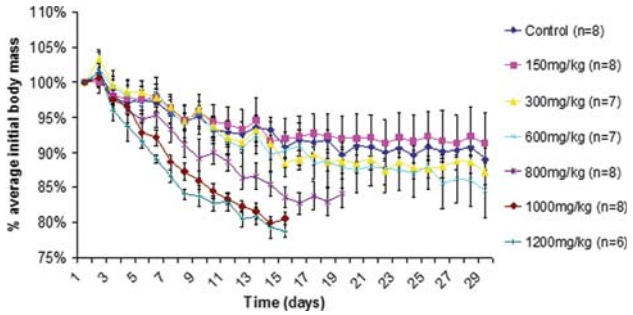

Fig. 5 Graph illustrating changes in % average initial body mass of nude mice bearing a LNCaP tumor xenograft for control (vehicle) and treatment groups (150–1,200 mg/kg CsCl). Most mice were administered vehicle or CsCl daily for 30 days; the 800-, 1,000-, and 1,200-mg/kg treatment groups were removed halfway through the study because the % average initial body mass of the mice dropped below 80%. Results are represented as mean value ± standard error of the mean. Statistical analysis using one-way ANOVA indicates a significant difference between the control and treatment groups beginning on day 6 and extending to day 19 ($P < 0.05$). Pair-wise multiple comparison via Tukey test indicates a difference between the 1,200-mg/kg group and the control from day 6 to day 15 ($P < 0.05$), a difference between the 1,000-mg/kg group and the control from day 7 to day 15 ($P < 0.05$), and a difference between the 800-mg/kg group and the control from day 16 to day 19 ($P < 0.05$)

Unlike the PC-3 xenograft, the LNCaP xenograft was less responsive to the CsCl treatment. Only on day 5 did any of the treatment groups have a significant reduction in % average initial tumor volume compared to the control group (1,000-, 800-, and 600-mg/kg

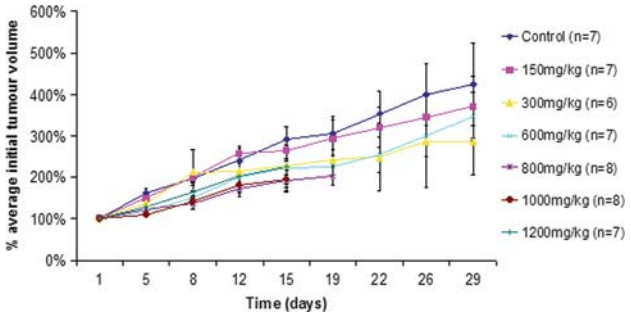

Fig. 6 Graph illustrating changes in % average initial tumor volume of nude mice bearing a LNCaP tumor xenograft for control (vehicle) and treatment groups (150–1,200 mg/kg CsCl). Most mice were administered vehicle or CsCl daily for 30 days; the 800-, 1,000-, and 1,200-mg/kg treatment groups were removed halfway through the study because the % average initial body mass of the mice dropped below 80%. Results are represented as mean value ± standard error of the mean. There was no statistical difference between any of the groups ($P > 0.05$)

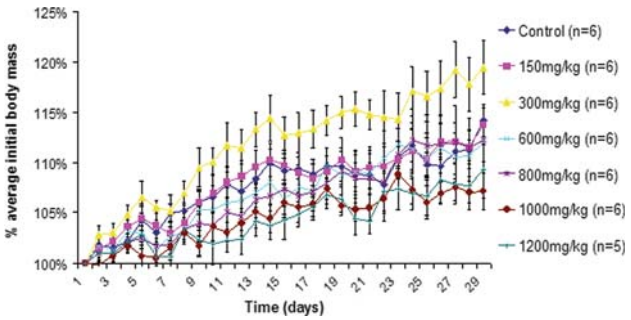

Fig. 7 Graph illustrating changes in % average initial body mass of tumor-free nude mice for control (vehicle) and treatment groups (150–1,200 mg/kg CsCl). All mice were administered vehicle or CsCl daily for 30 days. Results are represented as mean value ± standard error of the mean. Statistical analysis using one-way ANOVA indicates no difference between the control and treatment groups for the duration of the study ($P > 0.05$)

groups) (Fig. 6). Since there was no difference in tumor volume, PSA tests were not conducted.

Non-tumor-bearing nude mouse study

The final study carried out in non-tumor-bearing mice provides insight into the toxicity of CsCl alone without the contributing factors of tumor burden. When CsCl was chronically administered to these animals, it was discovered that none of the treatment groups showed any significant difference in % average initial body mass when compared to the control (Fig. 7). Even mice receiving the highest concentrations of CsCl, showed a similar increase in body mass compared to the control mice, which was not observed in the PC-3 or LNCaP studies.

There was no difference in food consumption between any of the treatment groups and the control mice (data not shown). However, the water consumption results were similar to those observed in the PC-3 study. Animals receiving 1,200 and 1,000 mg/kg CsCl had significantly elevated water consumption compared to the control beginning on day 15 (data not shown). Water consumption was also elevated in animals receiving 800 mg/kg CsCl compared to the control from day 21 to 27 (data not shown).

Histopathology

Of the eight tissues examined, changes were only observed in the hearts and small intestines. Prostates and tumors were not analyzed in this study. Instead, tissue microarrays will be constructed using these tissues, and will be stained with antibodies for markers of cellular proliferation and apoptosis for inclusion in follow-up work.

Fibrin clots present in the aorta, atrium, or ventricles were found in animals from all three studies (Fig. 8a). Although fibrin clots can form post-mortem, there appears to be a higher incidence of fibrin clots in animals receiving CsCl compared to control animals in both the PC-3 and LNCaP studies (Fig. 8b). Interestingly, this same trend was not observed in mice without tumors; fibrin clots were present in 33% of the animals within each treatment group (data not shown).

Atrophy of the mucosal epithelium and villi, as well as the presence of inflammatory infiltrate of the submucosa of the small intestine were observed in both control and CsCl treated animals from the three studies. There appeared to be no evident trend in the incidence of these abnormalities across the different treatment groups and the control (data not shown).

Serum AST/ALT and creatinine

In conjunction with the tissue histopathological analysis, serum AST, ALT, and creatinine were analyzed as secondary measures of organ damage; specifically, damage to the heart, liver, skeletal muscle, and kidney. Serum from the CsCl treated PC-3-bearing mice had no differences in the levels of these markers as compared to the control animals (data not shown). Similar results were also observed in the LNCaP and non-tumor study (data not shown).

Bladder crystals and viscous mucosal fluid

During the excision of prostates from the PC-3 animals, it was observed that animals in the higher

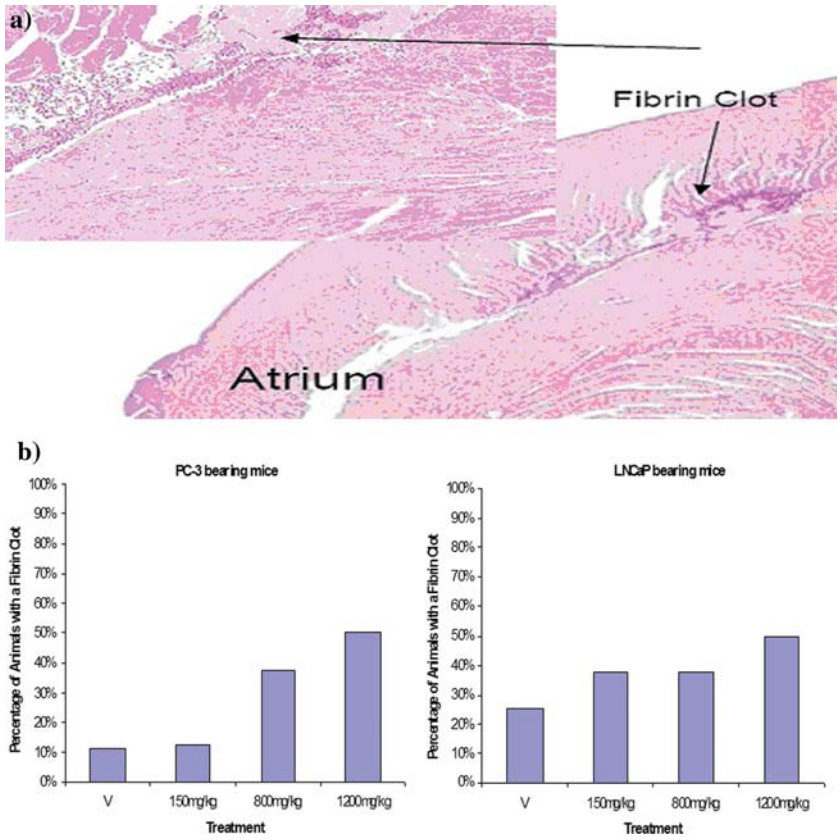

Fig. 8 **a** Image of a cardiac fibrin clot discovered post-mortem in a nude mouse of the PC-3 study. **b** Graphs illustrating the percentage of nude mice bearing a PC-3 or LNCaP xenograft with the presence of post-mortem fibrin clots for control (vehicle) and treatment groups (150, 800, and 1,200 mg/kg CsCl). Most mice were administered vehicle or CsCl daily for 30 days; the 800-, 1,000-, and 1,200-mg/kg treatment groups of the LNCaP study were removed halfway through the study because the % average initial body mass of the mice dropped below 80%

treatment groups had significant inflammation within and surrounding their bladders. Further investigation revealed the presence of crystals or viscous mucosal fluid in the bladders of these animals (Fig. 9a).

Upon completion of LNCaP and non-tumor studies, it was apparent that these crystals and viscous mucosal fluid were not conserved solely to the PC-3 study, but were present in animals from all three studies. Interestingly, these bladder abnormalities only existed in animals that had been administered CsCl at concentrations of 600 mg/kg and higher (Fig. 9b).

Discussion

In the present study we have shown that CsCl can slow the growth of the PC-3 tumor model when administered at 800–1,200 mg/kg. Although there was no therapeutic effect observed in the LNCaP model, it should be noted that the animals receiving the highest dosages (800–1,200 mg/kg) of CsCl were euthanized halfway through the study, and thus, did not receive the same number of doses as the PC-3-bearing animals.

We speculate a possible mechanism for CsCl's therapeutic effect may be similar to its mechanism for disrupting cardiac function. In the heart, CsCl has been shown to block a number of potassium channels including Kir2.1, Kir3.1/Kir3.4 and channels involved in transient outward potassium current [25–31]. CsCl has also been shown to slow the rate of inactivation of the HERG potassium channel, which is involved in the onset of long QT syndrome [25, 32]. Each of the described channels play a role in repolarizing cardiac cells back to their resting potential during an action potential [30, 32, 33]. Thus, the affects of CsCl on these channels are likely linked to the propagation of cardiac arrhythmias which have been observed in animal models and in some humans receiving the CsCl treatment [9–19]. Like cardiac cells, some of these inward rectifying potassium channels have been described in cancer cells, where they maintain the resting potential of these cells at depolarized levels to ensure unlimited tumor growth [34, 35]. Although, these channels have not yet been described in prostate cancer cells, their existence could provide a mechanism for the observed effects of CsCl in this study.

Cardiac function was only indirectly monitored in the three animal studies via AST measurement. Thus, it is unknown whether the concentrated dosages of CsCl administered, which slowed tumor growth, had any negative impact on cardiac output. That being stated, we did observe a higher prevalence of fibrin clots in CsCl treated animals bearing PC-3 or LNCaP tumors. However, mice without tumors displayed no

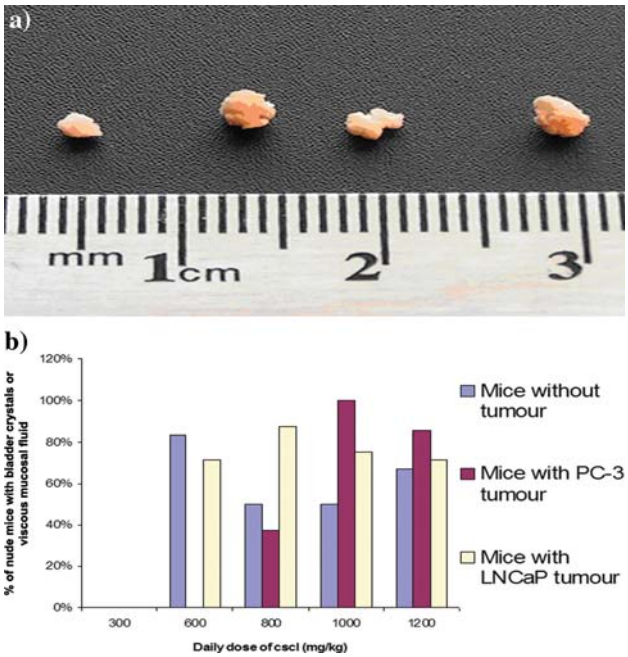

Fig. 9 **a** Image of crystal structures removed from the bladders of nude mice treated with 600–1,200 mg/kg CsCl daily. **b** Graph illustrating the % of nude mice with the presence of bladder crystals or viscous mucosal fluid in the PC-3, LNCaP, and non-tumor studies. In the PC-3 and non-tumor studies, these crystals were removed from the animals on day 30 during tissue collection. However, in the LNCaP study these crystals were observed as early as day 15 in animals that were euthanized (1,000- and 1,200-mg/kg treatment groups). Results are represented as percentage of mice per treatment group with the presence of bladder crystals or viscous mucosal fluid. Bladder crystals or viscous mucosal fluid may have been present in the 600-mg/kg group from the PC-3 study, but unfortunately were overlooked in these animals

difference in fibrin clot prevalence between the controls and the treated animals. Based on this observation, it appears that fibrin clot formation shows some dependence on both the presence of a tumor and the dose of CsCl administered. Interestingly, the cardiac abnormalities in the human clinical case studies, were caused by CsCl doses that were similar to the doses in which we observed a greater prevalence of fibrin clots in mice (if human to mouse body surface area dose conversion is implemented) [13–16, 18].

There appeared to be other toxicological effects from CsCl in athymic nude mouse model, specifically, increased water consumption, the presence of bladder crystals and viscous mucosal fluid, and a decrease in body mass. Animals receiving the three highest dosages of CsCl in both PC-3 and non-tumor study demonstrated a significant increase in water consumption. These results were not observed in the LNCaP study, perhaps due to the early removal of the animals of interest. It appears that CsCl is dehydrating the animals, possibly leading to the observed loss in body mass of animals which received the highest doses of CsCl. However, this claim cannot be confirmed as urination was not measured. Future experiments will need to be conducted to investigate this further.

The presence of bladder crystals or viscous mucosal fluid in animals from all three studies was an unexpected finding. The formation of these crystals appears to be directly dependant on the concentration of CsCl administered; crystals were only observed in animals that were administered 600–1,200 mg/kg CsCl. Although it is not known when these crystals first began to form in the mice, their presence was observed as early as day 15 in euthanized animals of the LNCaP study. It will be of interest to analyze the composition of these crystals to determine the role of CsCl in their formation; however, it was not possible to complete this analysis within the constraints of this study.

A significant decrease in body mass was observed in animals receiving the higher concentrations of CsCl in both the PC-3 and LNCaP studies (Figs. 1, 5). However, mice not representing tumors appeared to tolerate these concentrated doses. Although there was a dose-dependant trend in regard to loss in body mass of these animals, no groups were statistically different from the control group by the end of the study (Fig. 7). This result indicates that the significant decreases in mass of the nude mouse model, as observed in the PC-3 and LNCaP studies, is from an additive stress and/or toxicity of both tumor burden and CsCl (Figs. 1, 5). In addition, the impact of the two tumor types on body mass is not equal. Only in the LNCaP study, did body mass fall below the ethical level (Fig. 5); alternatively, each treatment group in the PC-3 study had a % average initial body mass above 90% (Fig. 1). These results indicate that the LNCaP tumor may elicit a greater degree of stress on the nude mouse model than the PC-3 tumor. This higher order of stress likely sensitizes LNCaP-bearing nude mice to the toxicological effects of CsCl.

Conclusions

This manuscript is the first to provide a detailed investigation into the therapeutic and acute toxicological effects of CsCl in human xenograft models. Although CsCl appears to have some therapeutic benefit in treating prostate cancer, a number of toxicological effects were also observed linked to its administration, including loss of body mass, increase of water consumption, fibrin clot formation, and bladder crystal formation. Interestingly, one of most life threatening of these toxicological effects from CsCl, specifically, a loss in body

mass, was not observed in disease-free animals receiving the CsCl treatment. This observation indicates some interplay between CsCl administration and the presence of a tumor compounding to culminate this effect.

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