Published in final edited form as:

J Environ Pathol Toxicol Oncol. 2013 ; 32(3): 241–244.

Hydration with Saline Decreases Toxicity of Mice Injected With Calcitriol in Preclinical Studies

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Abstract

The effectiveness of saline injection in reducing the toxicity profile of calcitriol when coadministered in mice was evaluated. Mortality was used as an end point to study the toxic effects of calcitriol; the relative risk of mortality in mice injected with saline was evaluated from our previously published animal experiments. We discovered that coadministration with 0.25 mL normal saline solution injected intraperitoneally is associated with a lower mortality rate than calcitriol given alone. The estimated relative risk of mortality was 0.0789 (95% confidence interval, 0.0051–1.22; $z = 1.82$; $P = 0.070$) when saline is administered with calcitriol compared to calcitriol alone. There was a reduction in serum calcium levels in mice that received saline (11.4 ± 0.15 mg/dL) compared to mice that did not receive saline (12.42 ± 1.61 mg/dL). Hydration with saline seems to reduce mortality and toxicity in mice receiving calcitriol. Given the decrease in mortality rates, intraperitoneal injections of saline should be considered in studies involving mice receiving injections of calcitriol.

Keywords

calcitriol; saline hydration; toxicity

I. INTRODUCTION

Calcitriol (1,25-dihydroxyvitamin D₃) is the most biologically active form of vitamin D¹ and it comes from both diet and synthesis in the skin via a photochemical reaction.² The originally recognized role of calcitriol in the human body is to maintain calcium regulation through its action on the intestines, bones, kidneys, and parathyroid glands.¹ Given its

physiologic role, calcitriol is used to treat a wide array of human conditions such as osteoporosis, hypocalcemia, and rickets.^{3–5}

There also is increasing evidence in the literature that calcitriol possesses anticancer properties. This has been demonstrated in both human and animal subjects. Several mechanisms for the antitumor activities of calcitriol have been postulated, including cell cycle arrest, induction of apoptosis, and inhibition of angiogenesis via anti-vascular endothelial growth factor actions.^{1,6} The dose of calcitriol that is required to obtain these antitumor effects, however, is many fold higher than the dose required to treat more common ailments such as osteoporosis.^{7,8} It is well known that calcitriol induces toxicity in both human and animal subjects, which is evident particularly at these higher doses. Some of the calcitriol-associated toxicities in humans are hypercalcemia; hyperphosphatemia; primary renal failure; pancreatitis; and calcification of the kidney, aorta, heart, and lung.^{9–11} In mice, calcitriol and its derivatives are known to induce hypercalcemia, kidney calcifications, weight loss, lethargy, and death.^{12–13}

In our preclinical animal studies, we observed—but have not previously included in published reports—a decrease in mortality in mice treated with calcitriol when normal saline solution was injected intraperitoneally. This parallels the general practice by physicians who routinely administer intravenous (IV) fluids when treating patients with IV calcitriol and/or chemotherapeutic agents.^{14,15} As a consequence we performed a retrospective analysis of our previously published data to better understand the effectiveness of hydration in reducing mortality and toxicity in mice injected with calcitriol.

II. MATERIALS AND METHODS

We screened our previously published mouse experiments, which were performed in accordance with the Association for Research in Vision and Ophthalmology statement for the use of animals in ophthalmic and vision research and were approved by our institutional ethics committee. We identified 7 experiments involving calcitriol injection in mice with data on mortality and serum calcium levels. All experiments were designed and performed to study the effect of calcitriol on retinoblastoma tumor in comparable mice models. Mice that were used in the experiments were either transgenic models for retinoblastoma (LHb-Tag) or athymic nude mice injected with human retinoblastoma cells. The calcitriol dose used in our experiments ranged from 0.025 to 0.2 µg. The duration of calcitriol treatment was once daily for 5 weeks, and animals were killed via carbon dioxide inhalation at the end of the 5-week period. Mortality and the serum calcium levels were recorded for all animals. The relative risk was estimated using MedCalc statistical software (available at http://www.medcalc.org/calc/relative_risk.php), which uses methods described by Sheskin.¹⁶ A Student *t* test was used to compare the serum calcium levels in mice injected with saline versus those injected with calcitriol only.

III. RESULTS

The characteristics of each study are summarized in Table 1. A total of 91 mice receiving calcitriol injections were ultimately tabulated. Relative risk of mortality for the group

receiving saline versus groups not receiving saline was calculated from data presented in Table 1. Fifteen mice received intraperitoneal saline injections at the time of calcitriol administration, and no mortality was observed. However, a 39.8% mortality rate ($n = 30$) was observed in the group of mice that did not receive saline injections. The relative risk of mortality in mice injected with saline was 0.0789 (95% confidence interval, 0.0051–1.22; $z = 1.82$; $P = 0.070$). The mean serum calcium level for mice receiving saline was 11.4 ± 0.15 mg/dL, and it was lower than the serum calcium level (12.42 ± 1.61 mg/dL) in the mice that did not receive saline, although the difference was not significant.

IV. DISCUSSION

We included the results of 7 experiments that were published in 4 articles.^{2,7,12,17} The studies included in this retrospective analysis used different calcitriol dosages, which limits the validity of the statistical inference. Nevertheless, this analysis seems to suggest that when saline is coadministered with calcitriol, the risk of mortality is lowered. This is consistent with common practice by oncologists who hydrate patients with IV fluids while administering chemotherapeutic agents, including calcitriol, to reduce chemotherapy-related complications.¹⁸ We suggest that giving saline injections in addition to calcitriol likely decreases mortality because of a reduction of the toxic effects of hypercalcemia.

Our observations indicated a trend toward increased survival with saline injection; therefore, we conclude that saline injection may decrease calcitriol-induced mortality. The serum calcium level was lower in the saline-injected group compared to the group that did not receive saline. Although this difference was not significant, it may have led to the reduced toxicity that was observed in the saline-injected animals.

Given the findings of our analysis, hydration with intraperitoneal saline injection should be considered in mice given calcitriol intraperitoneally and/or intravenously to decrease mortality rates. Future prospective studies using a greater number of mice and different animals and tumor types may prove to be beneficial. In addition, further animal testing complemented by histopathological studies should be performed to reach an appropriate dosage of coadministration of calcitriol and saline. Finally, some further investigations may be needed to prove that the potency of calcitriol is not altered by increased kidney clearance during hydration. Extrapolating the findings in our analysis, we propose that hydration techniques should be used when other potentially toxic drugs, such as chemotherapeutic agents, are used in mice. The findings here could possibly be extended to other studies of chemotherapeutic agents and other animal models as well.

V. CONCLUSIONS

The results of preclinical studies form the basis for decisions about drug use in patient trials. Decreasing mortality in animal experiments will prove to be a meaningful contribution to the field of research. In early studies of vitamin D analogs to treat cancer in animals, a large proportion of mice died because of the toxic effects of calcitriol. To avoid or decrease such calcitriol-induced mortality from toxicity in mice, a common practice for investigators was administering less than the target doses or even skipping doses. The combination of high

mortality in mice and administration of a suboptimal dose in certain situations may impede evaluation of the potential therapeutic effects of calcitriol. The same problem is likely to exist with other drugs as well (e.g., chemotherapeutic agents). This retrospective analysis indicates that supplemental hydration should be kept in mind when evaluating drug toxicology.

Acknowledgments

The authors thank Dr. Hector F. Deluca and Dr. Julie A. Mares for their advice and encouragement in this project.

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TABLE 1**VARIOUS STUDIES OF MICE MORTALITY IN ASSOCIATION WITH CALCITRIOL INJECTION**

Article	Case No.	Calcitriol Dose (μ g)	Mortality	Saline Injection (mL)	Serum Calcium After Treatment (mg/dL)
Albert et al., 2002 ²	15	0.025	1	0	13.0
	23	0.05	4	0	13.0–14.03
	10	0.1	5	0	NA
	8	0.2	4	0	NA
Kulkarni et al., 2009 ⁷	15	0.05	0	0.25	11.4 $\pm$ 0.15
Sabet et al., 1999 ¹²	10	0.05	9	0	7.50 $\pm$ 0.90
Cohen et al., 1988 ¹⁷	10	0.1	7	0	10.35

NA, not available.