Effect of Cholecalciferol Overdosage on Pregnancy Outcome in White Albino Mice

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Abstract

Background: Irrational prescription and use of vitamins is rife in our society today. This practice is very common among pregnant women without adequate need assessment and concern for over dosage. Cholecalciferol has remained a component of some routine antenatal drugs despite the fears that have been expressed concerning its safety.

Objectives: This study was set to determine the effect of cholecalciferol over dosage on pregnancy outcome in white albino mice.

Method: High doses of vitamin D were given to three groups of female white albino mice from the day copulation plugs were seen. A fourth group was given normal saline as control. Drug exposure period was 22 days. Parameters studied included number of litters per delivery, average weight and length of the litters.

Results: The oral LD_{50} was 9,747 IU/kg. No death occurred during teratogenic studies. There was statistically significant reduction in the number of litters delivered by animals given medium (1200 IU) and high (1800 IU) doses compared to controls (P=0.041 and 0.0002 respectively). Their litters also had reduced average length and weight (P =0.0001).

Conclusion: High doses of vitamin D negatively affected pregnancy outcome in white albino mice.

Key words: Cholecalciferol, over dosage, pregnancy outcome, litters, white albino mice.

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Introduction

Vitamin D (cholecalciferol) is one of the fat-soluble vitamins occurring naturally in plants and animals. The physiologically active form (1, 25-dihydrocholecalciterol) is formed by sequential action of 1- and 25-hydroxylases in the liver and kidney respectively.

Manifestations of vitamin D toxicity include weakness, constipation, cardiac arrhythmias, muscle pains, loss of appetite.[1,2] These clinical manifestations are usually as a result of disruption of calcium metabolism leading to hypocalcemia even though a review by Chung *et al*.,2009[3] concluded that relationship between vitamin

supplementation and toxic outcome were inconsistent.

Irrational prescription and use of vitamin is rife in our society today. Being fat-soluble, vitamin D easily accumulates in the body when the recommended daily allowance is exceeded. Daily production of vitamin D from ultra violet light irradiation in the skin is 10,000 IU.[4] This, together with any irrational intake easily overshoots the daily requirement of 200-400 IU resulting in toxicity.[5] Occult vitamin D intoxication was detected in patients who were using dietary supplements containing high levels of vitamin D.[6]

This practice of multivitamin supplementation is very common among pregnant women. There is therefore need to determine the effect of this vitamin over dosage in animals and possibly extrapolate the findings to human beings. The general objective of this study therefore is to determine the teratogenic effect of cholecalciferol over dosage on white albino mice.

Materials and Methods

Animal Source

Fifty three (53) white albino mice weighing 18-35g each were obtained from the animal house at Faculty of Agriculture, University of Nigeria, Nsukka. They were housed at the Pharmacology laboratory of Nnamdi Azikiwe University Teaching Hospital (NAUTH) Nnewi and acclimatized for one week before the commencement of the study. They were divided into four groups of five animals per group and housed in a cage maintained under laboratory conditions of temperature and humidity in accordance with standard guide for care and use of laboratory animals. Food and drinking water was provided.

Drug Source

Vitamin D gel capsules 1000 IU (Mason Vitamins Inc; USA).

Acute Toxicity Studies

Acute toxicity test (LD_{50}) was estimated in mice using Locke's method (1983).The evaluation was done in two phases. In phase one, 3 groups of three mice were treated with 10, 100 and 1000 units/kg orally in order to determine the range in which the LD_{50} falls. The mice were observed for clinical signs of toxicity within 24hours and for death within 72hours. No death was observed at these doses. In the second phase 9,500; 10,000; 10,500; 11,000 units/kg were administered to four mice respectively. LD_{50} was calculated as the square root of the product of the lowest lethal dose and the highest non-lethal dose.

Teratogenic Studies

The animals were divided into four groups of five animals. The test groups were labelled A, B, C, and control group labelled D. In each group four females were randomly selected and one male added. The three test groups received different doses 600; 1200 and 1800units/kg corresponding to groups A, B and C respectively. The control group was given equivalent volume of normal saline. All drugs were given orally through a canula connected to calibrated syringe to females only. Commencement of drug administration was from the day copulation plugs were seen. Food and water consumptions as well as physical appearance and activities of the mice were observed throughout the exposure period of 22 days.

Each mouse that gave birth is separated from the group with its litters. The litters were counted,

physically examined and compared with those from the control group.

Statistical Analysis

Results of the continuous variables (number of litters per delivery, weight and length of the litters) were compared using analysis of variance (ANOVA) with significant value further analyzed using student's t-test. A $P<0.05$ value was considered statistically significant. Values for weight and length of litters were expressed as mean± standard deviation.

Results

Test for LD_{50}

The oral LD_{50} was 9,747 iu/kg. There was no death occurred during the study on teratogenicity.

Average Number, Weight and Length of Litters

Average number, weight and length of the litters for different groups of mice who received different doses of vitamin D are shown in table I. For number of litters/delivery, there was no statistically significant difference between the group that received 600 IU/kg and the controls (P=0.32). However, there was statistically significant difference between the controls and the groups that received 1200 IU/kg and 1800 IU/kg respectively (P =0.041 and 0.0002 respectively). For the weight and length of litters, there was statistically significant difference between the controls and all groups that received increasing doses of vitamin D as shown by P values of 0.012, 0.0001, 0.0001 and 0.029, 0.0001, 0.0001 respectively.

Table I: **Effects of increasing doses of vitamin D on the number of litters/delivery, average weight and length of litters of white albino mice ($p<0.05$)**

Weight of mice (g)	Drug concentration (IU/kg)	Number of litters/delivery	Av. Weight of the litters (g) SD	Av. Length of litters in cm (tip of snout to tip of tail) SD
Control	Normal saline			
19.50	1.00	13	0.99+/- 0.09	6.13+/-0.09
19.00	1.00	12	1.03+/-0.10	6.05+/-0.11
20.00	1.00	14	1.00+/-0.10	6.06+/-0.12
24.00	1.00	16	0.9+/-0.09	6.02+/-0.12
Low	**Drug Concentration**			
20.50	600.00	15	0.91+/-0.10	5.87+/-0.15
29.50	600.00	16	0.69+/-0.09	5.91+/-0.12
21.50	600.00	14	0.76+/-o.07	6.01+/-0.08
22.00	600.00	-	-	-
Medium	**Drug Concentration**			
29.50	1200.00	10	0.63+/-0.18	5.52+/-0.10
33.00	1200.00	12	0.50+/-0.07	5.59+/-0.07
28.50	1200.00	9	0.55+/-0.05	5.55+/-0.07
22.50	1200.00	-	-	-
High	**Drug Concentration**			
35.00	1800.00	4	0.35+/-0.06	5.20+/-0.08
27.00	1800.00	-	-	-
22.50	1800.00	-	-	-
18.00	1800.00	3	0.40+/-0.10	5.13+/-0.06

Discussion

There was no significant difference between the number of litters delivered by the mice treated with low dose (600 IU/kg) of cholecalciferol and that of the controls, but there was significant reduction in the number of litters delivered by animals given medium (1200IU/kg) and high (1800 IU/kg) doses compared to controls.

The study also showed significant reduction in the average weight and length of the litters of mice given cholecalciferol when compared with that of the controls. This could be attributed to intra-uterine growth retardation as a result of inhibitory effect of vitamin D on trophoblastic tissues.[7,8,9] Synthesis of fibroblast growth factor 23 (FGF 23) in bone is regulated by 1, 25-dihydrocholecalciterol.[10,11] High doses of vitamin D as in this study can down-regulate vitamin D receptors and decrease FGF 23 synthesis which will decrease bone growth and result in reduced weight and length of litters of vitamin D treated mice. Also, vitamin D stimulates growth and development by promoting the expression of growth hormone-insulin-like growth factor (GH-

IGF).[12] In very high doses as used in this study, vitamin D can have negative feedback effect on expression of GH-IGF thereby reducing growth and development of the foetuses.

Tryfonidou *et al*., 2002 and 2003 had demonstrated that high dose cholecalciferol supplementation depresses intestinal calcium absorption and severely disturbs endochondrial ossification in growing dogs.[13,14] It does this by increasing plasma calcitonin level. Thus, subnormal levels of calcium and phosphate are made available to developing foetuses which could explain the decrease in length of litters whose mothers were exposed to high doses of vitamin D compared to controls.

Conclusion

From the above findings, it has been demonstrated that high doses of vitamin D negatively affects pregnancy outcome in white albino mice. However, extrapolating these findings to humans will be difficult because of high doses of drug used in this study. Further studies are needed to find the effect of high doses of vitamin D on female reproductive organs and functions. Specifically, we need to know the effect on placental function as this profoundly affects foetal growth and development.

References

1. Wimsatt J, Johnson JD, Wrigley RH, Biggins DE and Godbey JL. Dietary vitamin toxicity in a household of pot-bellied pigs (sus scrota). *J Vet Int Med* 1998; 12: 42-4.
2. Brown LF and Wilson DE. Gastroduodenal ulcers: Causes, diagnosis, prevention and treatment. *Comp Ther* 1999; 25: 30-1.
3. Chung M, Balk EM, Brendel M, Lau J, Lee J, Lichtenstein A *et al*. Vitamin D and calcium; a systematic review of health outcomes. *Evid Rep Technol Assess* (Full Rep) 2009; 183:1-420.
4. Vieth R. Vitamin D supplementation, 25-hydroxyvitamin D concentrations and safety. *Am J Clin Nutr* 1999; 69(5): 842-56.
5. Holick MF. The cutaneous photosynthesis of pre-vitamin D_3: a unique photoendocrine system: J Invest Dermatol 1981; 77(1): 51-8.
6. Adams JS and Lee G. Gains in bone mineral density with resolution of vitamin D intoxication. *Ann Intern Med* 1997; 127(3): 203-6.
7. Perkin RM, Swift JD and Newton DA. *Pediatric Hospital Medicine: textbook of inpatient management.* 2nd edition. Philadelphia. Lippincott William & Wilkins. . 2007; P.94.
8. Tshibangu K, Oosterwijck K and Doumont-Meyvis M. Effects of massive doses of ergocalciferol plus cholesterol on pregnant rats and their offspring. *J Nutr* 1975; 105(6): 54-8.
9. Federica M, Pierluigi A and Claudio F, editors. Vitamin D into the skin: Therapeutic applications into the skin, In: Stolzt VD. *Vit D: New research*. Nova Science Publisher 2006; p.103.
10. Saji F, Shigematsu T, Sakaguchi T, Ohya M, Orita H, Maeda Y *et al*. Fibroblast growth factor-23 production in bone is directly regulated by 1 (alpha) 25–dihydroxyvit D, not PTH. *Am J Physical Renal Physiol* 2010; 299 (5): F122-7.
11. Yamamoto R, Minamizaki T, Yashiko Y, Yashidaa H, Tanne K, Auvin JE *et al.* 1 alpha, 25-hydroxyvitamin D_3 acts

predominantly in mature osteoblasts under conditions of high extracellular phosphate to increase fibroblast growth factor 23 production in vitro. *J Endocrinol* 2010; 206(3): 279-86.

12. Fernandez-Cacio M, Andaluz P, Toran N, Esteban C, Carrascosa A and Audi L. Vitamin D stimulates growth hormone-insulin-like growth factor (GH-IGF) gene axis expression and potentiates GH effect to reverse the inhibition produced by glucocorticoids in human growth plate chondrocytes. *Horm Res* 2007; 67(supp 1):204-5.

13. Tryfonidou MA, Steven-Hagen JJ, Van den Bemd GT, Oosterlaken-Dikksterhuis MA, Deluca HF, Mol JA *et al*. Moderate cholecalciferol supplementation depresses intestinal calcium absorption in growing dogs. *J Nutr* 2002; 132(9):2644-50.

14. Tryfonidou MA, Holl MS, Steven-Hagen JJ, Buurman CJ, Deluca HF, Oosterlaken-Dikksterhuis MA *et al*. Dietary 135-fold cholecalciferol supplementation severely disturbs endochondral ossification in growing dogs. *Domest Anim Endocrinol* 2003; 24(4):265-85.