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Biochemical Alterations Accompanied to Zoledronic Acid Treatment after High Doses of Vitamin D in Experimental Model

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Abstract

Zoledronic acid is a long-acting compound of bisphosphonate class, and it has many health and medical benefits, among which are its effects in treatment of bone disorders and accordingly decrease the incidence of osteoporosis. Besides, vitamin D is recognized as one of the fat-soluble vitamins that affect the absorption of major minerals in the digestive system. The aim of this study was to verify the influence of vitamin D on the biochemical effect of zoledronic acid. To achieve the aim of the study, the experiment was conducted on ninety-six male Wistar rats that were divided into the following groups; control group, vitamin D treated groups and zoledronic acid treated groups. The results of the study found that, taking high doses of vitamin D before treatment with zoledronic acid results in significant changes ($P < 0.05$) in the levels of calcium, magnesium and phosphorus, as well as levels of thyroid hormone, cortisol and levels of immunoglobulin E in the blood. Which means that treatment with zoledronic acid in high levels of vitamin D promote worsening of such biochemical parameters.

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Major Minerals;
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Introduction

Vitamin D is a lipid soluble vitamin, which is a derivative of steroid compounds, so it has the ability to significantly influence the absorption of magnesium, phosphates, calcium and zinc into the digestive system.[1] It should be noted that there are two different types of vitamin D, namely vitamin D3 (cholecalciferol) and vitamin D2 (ergocalciferol).[2]

Regardless of the primary source of vitamin D from fish, cod liver oil, mushrooms, liver and eggs. The primary form of vitamin is inactive and must be

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converted to the other to obtain the primary function from it. Once this vitamin enters the bloodstream, it will be cleaned through the liver or storage tissue within a short period of time, not to exceed a few hours.[3]

The vitamin D activation process includes the addition of hydroxyl groups, and the first hydroxyl group is carried out in the liver and vitamin D is metabolized to 25 (OH) D with the help of an enzyme known as cytochrome P450. Next, 25 (OH) D binds to a special protein known as vitamin D binding protein (DBP), which passes into the bloodstream. But 25 (OH) D-DBP complex is excreted in the urine, and at the same time it is reabsorbed into the kidneys and later transformed into its active form 1,25-dihydroxyvitamin (OH) 2D with the help of an enzyme known as 25-hydroxyvitamin D-1α-hydroxylase enzyme.[4]

On the other hand, the levels of calcium in the bloodstream are maintained through calcitriol, and this is done by raising the levels of calcium to the normal rate gradually through three different mechanisms. In the first process, it does not require parathyroid hormone (PTH), but it depends on the role of calcitriol, which stimulates calcium absorption in the intestine.[5] As for the second process, calcitriol plays the main role in the mobilization of calcium from the bones, and this process requires the presence of PTH. With regard to the third and final process, calcitriol and PTH work to reabsorb calcium in the distal renal tubes, which in turn works to maintain the level of calcium in case of need for calcium.[2]

In this context, Vitamin D toxicity has various symptoms such as loss of appetite, weight loss, polyuria, and arrhythmia. Besides the possibility of raising calcium levels in the bloodstream, which will calcify the blood vessels and tissues. In addition to damage to the blood vessels, heart, and kidneys.[6] Many medical studies have proven that zoledronic acid is one of the most powerful bisphosphonates, which exists in a dose of 4-5 mg; its benefit is in preventing skeletal fractures, especially for patients with prostate cancer and multiple myeloma. In addition, it is used in the treatment of osteoporosis, as well as in the treatment of hypercalcemia of malignancy, and bone metastases.[7]

Therefore it must be clarified that zoledronic acid is nitrogen compound and classified as third generation of bisphosphonates, and the mechanism of its action lies in the inhibition of farnesyl pyrophosphate synthase (FPPS), which is an enzyme that works in a major regulatory way in the mevalonic acid pathway. This will prevent prenylation of guanosine triphosphate binding proteins, whose action is crucial because it will regulate the work of cellular activities of osteoclast cells, in particular, which will result in the programmed death of osteoclast cells.[8]

The aim of this study is to evaluate the influence of vitamin D on the biochemical effect of zoledronic acid through determination of calcium, magnesium, phosphorus, parathyroid hormone, immunoglobulin E and cortisol levels in the bloodstream in male Wistar rats.

Materials and Methods

In this experiment, the study sample was 96 male Wistar rats, they were 12 weeks old and weighed between 150 and 175 g.

Rats were obtained from the ophthalmology institutes in Egypt, and the research and procedures were supervised by a local ethical committee from Benha University for animal use and care. The recommendations were taken from the National Institutes of Health.

It is worth noting that animals were housed in metal cages, and fresh and clean drinking water was provided to them and fed at fixed intervals throughout their stay in the experiment, which would ensure them in a clean and healthy environment.

Chemicals and Drugs

Zoledronic Acid

which was purchased from Global Napi Pharmaceuticals Co. that is in Egypt. Metadronic vial is available at 0.15mg / kg, and was injected as intraperitoneal (I.P). That is, the amount of injection was 0.15 mg / kg body weight and given only once.

Vitamin D

which was purchased from Sigma Company in the United States of America. It is prepared in a form where it is ready for use, and it is available in

different capacities: 50 IU, 100 IU, 200 IU. In which the experimental rats are administered orally through the stomach tube.

Experimental Design

The study sample was 96 male Wistar rats, and they were divided as follow:

Control group

It consists of 12 rats; they fed on a regular diet and on drinking water.

Vit. D only Treated Groups

This group consists of 36 rats; they fed on a regular diet and receives drinking water, but it is divided into three sub-groups according to the dose taken from Vitamin D which was as follows:

- The first group of them, known as 2A subgroup, was given 50 IU intraperitoneal injection of vitamin D for one month
- The second group of them, known as 2B subgroup, was given 100 IU intraperitoneal injection of vitamin D for one month.
- The third group of them, known as 2C subgroup, was given 200 IU intraperitoneal injection of vitamin D for one month

Zoldronic acid Treated Groups

Which consists of 48 rats, feeding on a regular diet and receiving drinking water, and is divided into four sub-groups as follows:

- The first group, known as subgroup 3A, which received normal food and drink regular water for 30 days, after which they were given only one dose of zoldronic acid (0.15 mg / Kg), and kept for 15 days.
- The second group was known as subgroup3B, which was given vitamin D supplement in 50 IU dose daily for one month, after that they take only one dose of zoldronic (0.15mg / Kg), and kept for 15 days.
- The third group which is known as subgroup3C which was given 100 IU dose of vitamin D daily for one month, after that they take only one dose of zoldronic acid (0.15mg / Kg), and kept for 15 days.
- The fourth group, which is known as subgroup3D, which was given 200 IU dose of vitamin D daily for one month, after that they take only one dose of zoldronic acid (0.15mg / Kg), and kept for 15 days.

Sampling Collection

On the 45th day of the experiment, samples were collected from all groups. All blood samples were individually placed in clean, clear and spiral tubes, and then placed in the centrifuge at a rotational speed of 2500 rpm and for 20 minutes to separate the serum, and through a Pasteur pipette the serum sample is captured and placed in a sterile and dry sample tube. It is directly processed to enzymes determination, and then kept at -20°C, for use for subsequent biochemical analysis using available commercial kits.

It is worth noting that sera were analyzed to determine the following:

- The level of calcium in the blood according to 9.
- The serum phosphorous level in the blood according to 10.
- The level of vitamin D in the blood according to 11.
- The level of parathyroid hormone in the blood according to 12.
- The level of immunoglobulin Ig E in the blood according to 13.
- The level of cortisol in the blood according to 14.

Statistical Analyses

For statistical analysis, an SPSS / 11 software was used. Hypotheses were tested. One-way evidence (ANOVA) was analyzed to follow that least significant difference (LSD) test. P values of less than 0.05 were considered to indicate statistical significance.

Results

The results of the study showed that calcium levels were at high levels as a result of their association with different doses taken from vitamin D, except for one of the groups chosen in the experiment that received 200 IU dose which was compared with control.

In addition, there was a significant decrease in serum calcium levels in groups that received doses of zoldronic acid compared to other groups that took only vitamin D.

Besides, there is a significant increase in the levels of phosphorous in the blood in the groups that took only doses of vitamin D and the groups that took zoldronic acid, and those who were compared with the control groups.

However, it was not observed that there was a significant change in the levels of magnesium in the blood in groups that took only doses of vitamin D, although there was a significant increase in groups that received zoldronic acid compared to the control groups (Table 1).

On the other hand, the results showed that there was a noticeable increase in the levels of vitamin D in the blood in groups that took only doses of vitamin D compared to the control group, and the level of vitamin D was higher in the blood than the group treated with zoledronic acid.

In addition, there was a significant increase in parathyroid hormone, immunoglobulin E and cortisol levels in the blood of the group that took only vitamin D and the groups treated with zoldronic acid, compared to the control group (Table 1).

Table 1 : Levels of different biochemical parameters in control, vitamin D only and zoldronic acid groups.

Groups	Calcium	Phosphorus	Magnesium	Vitamin D	Parathyroid hormone (PTH)	Immuno-globulin Ig E	Cortisol
Control group	7.76 ± 1.3@	4.26 ± 0.25@	2.98 ±0.52@	1.89 ± 0.15@	5.27 ± 3.7@	14.95 ± 6.1@	7.31 ± 1.89@
Vitamin D only groups							
50 IU	10.43 ± 1.05@	4.52 ± 0.54 @	2.64 ± 0.53 @	45.93 ± 12.55 @	9.32 ± 2.0@	28. 79 ± 6.0 @	26.20 ± 10.2@
100 IU	11.38 ± 1.2@	5.71 ± 0.53@	2.08 ± 0.53@	75.48 ± 1.55@	8.29 ± 2.3@	36.69 ± 4.7@	52.18 ± 10.9@
200 IU	7.77 ± 1.3@	4.86 ± 0.54@	2.69 ± 0.52@	93.55 ± 3.54@	11.97 ± 0.9@	42.69 ± 4.7@	86.71 ± 11.2@
Zoldronic acid treated groups							
Control	9.23 ± 0.52@	4.07 ± 0.54@	3.43 ± 0.50@	8.79 ±1.3@	10.06 ± 0.8@	29.1 ± 3.3@	9.08 ± 2.11@
50 IU	9.43 ± 0.52@	6.09 ± 2.3@	3.52 ±0.51@	46.6 ± 2.54@	11.05 ± 1.2@	29.1 ± 3.3@	52.77 ±9.3@
100 IU	8.79 ±1.3@	7.51 ±1.3@	3.69 ±0.55@	67.77 ± 2.54@	8.66 ±0.45@	88.81 ±14.1@	91.4 ±6.1@
200 IU	7.58 ± 1.4@	7.47 ± 1.5@	3.85 ±0.54@	34.64 ± 4.53@	8.68 ± 0.55@	145.1 ± 13@	107.203 ± 21@

Data are expressed as means ± standard error (SE) for animals / group.

*: Insignificant change at P >0.01in comparison with control group.

@: Significant change at P < 0.05 in comparison with control group

Discussions

The mechanism by which vitamin D toxicity is reached includes increasing the concentration of vitamin D metabolites in order to reach the VDR in the desired and targeted cell nucleus, due to which many exaggerated genetic changes are made.[15]

Zoledronic acid is a powerful bone resorption inhibitor, it prevents the proliferation of bone cells and promotes apoptotic bone cells death.[16] The strength of which results from the high affinity of the metallic bones, especially for the sites where the high turnover of the bone occurs.[17]

This is consistent with the results of the current study, which confirms that zoledronic acid was specially developed with the aim of preventing the activity of bone cells, and therefore calcium remains in the bones for long periods of time. But it will lower the levels of calcium in the blood, whose symptoms appear as the presence of petechiae, paresthesias, tetany, laryngospasm and cardiac arrhythmias.[18]

Besides, zoledronic acid improves bone density, which confirms its reduction in accidents of fractures in the bones, and this has been shown in primary

and secondary prevention trials in patients with osteoporosis, especially among women who have had menopause.[19]

However, there is no evidence that bisphosphonates directly reduce PTH, and the inhibition of strong bone resorption is largely offset by the therapeutic effects of osteoporosis from a higher PTH. As for the pharmacokinetics, it can change secondary hyperparathyroidism, which requires frequent high doses even though a lower dose can be given.[20]

This confirms that, before starting treatment with zoledronic acid, both calcium and vitamin D levels must be high to prevent a deficiency of blood calcium, especially after the infusion of zoledronic acid. Although the decrease in calcium levels in the blood was less than that in other clinical trials in which bisphosphonate therapy was performed.[21]

This proves that zoldronic acid activates inflammatory vital signs, especially IL- 6, COX-2, and NF-κB. As for the pharmacokinetic aspect, zoledronic acid is gradually metabolized, after which different pharmacological effects are practiced, but with different doses.[22] Zoledronic acid has the ability to influence the large and repressive activity of Treg cells, and acts as an immunosuppressant by inhibiting several processes such as expansion, migration, immunosuppressive function and pro-metastatic ability of Treg cells.[23]

Conclusions

From obtained results, it could be concluded that, high doses of vitamin D lead to disturbance in the biochemical parameters, calcium, magnesium and phosphorus, as well as levels of thyroid hormone, cortisol and levels of immunoglobulin E. Also, treatment with zoledronic acid in high levels of vitamin D promote worsening of such biochemical parameters.

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Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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