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Vitamin D: Controversy Cancer and Beyond

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Vitamin D: Controversy Cancer and Beyond

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Abstract

Vitamins are an essential part to wellbeing. This was not something always known however, as the Germ theory was the accepted thesis of the 18th century. It was found that certain accessory factors helped mitigate and even cure these diseases such as beriberi, scurvy, and rickets. Accessory factors, later coined vitamins by Casimir Funk, are an essential constituent of the human diet. Vitamin D is technically not a vitamin but functions as a steroid hormone whose most well-known purpose is calcification of the human skeleton. This helps prevent osteomalacia in adults and rickets, a serious problem in children due to their developmental stages. But besides its well-known role, vitamin D also has other important non-calcification purposes, including being a possible anticancer agent. Calcitriol (1,25 dihydroxycholecalciferol, vitamin D3) has found to treat certain cancers in various ways: from the VDR-RXR complex to analogs being able to decrease tumor growth, lowering expression of stem cell marker genes, and inhibition of the Wnt pathway. Although there is promise in its effectiveness based on studies of squamous cell carcinoma, prostate, breast, colon, and ovarian cancer; hypercalcemia, lack of consistent data, and insufficient clinical trials are serious issues that constantly get in the way of progression of vitamin D's legitimacy as a solution.

Keywords: Vitamin D, Calcitriol, Disease, Cancer, Nutrient, Human health

Abbreviations: CSC: Cancer Stem Cell; CYP: Cytochrome P; PTH: Parathyroid Hormone; SCC: Squamous Cell Carcinoma; UV: Ultraviolet; VDR: Vitamin D Receptor

Introduction

At the beginning of the 18th century, scientists believed that a human was perfectly capable of being healthy based on only 4 necessary nutritional factors: proteins, carbohydrates, fats, and minerals. However, some diseases would severely affect human population at the time. Diseases such as beriberi, scurvy, pellagra, and rickets would baffle researchers as there weren't any abnormal microorganisms found in the body at the time. The reason why these diseases were so difficult to understand was due to the accepted hypothesis of diseases at the time known as Germ theory. It states that any disease is due to some action or presence of a microorganism within the body [1]. Scientists later found out that these diseases were due to a lack of a specific component in the body [1]. According to Frederick Gowland Hopkins, states "no animal can live upon a mixture of pure protein, fat, and carbohydrate, even if necessary inorganic materials are supplied, an animal still can't flourish" [2].

Historical background

During the Franco-Prussian war, the Siege of Paris occurred between 1870 and 1871 where several supplies for the French were cut off. Infant mortality rate was absurdly high during this period of time as there was a lack of milk. Experiments with rats conducted by Wilhelm

Stepp found that milk gave them normal growth; however, a milk substitute synthesized by opportunists (people who wanted to take advantage of the situation) failed to provide sustenance to the infants [3][4]. This lead to the conclusion that a lipid substance was found in milk which is essential to mice growth [5]. These accessory factors were found in very small amounts. These accessory factors, coined by Hopkins, were later termed vitamins by Casimir Funk in 1912, and were linked to solving vitamin deficient diseases [6][7]. Beriberi due to a lack of Vitamin B-1 (thiamin), Pellagra insufficient B-3 (niacin), scurvy insufficient Vitamin C, and rickets insufficient Vitamin D. Sir Edward Mellanby (1884-1955) was a researcher at King's College for Women in London [8]. It was at this place in 1918-1919 that he discovered an accessory factor that played an essential part in preventing rickets. He fed puppies a diet that was found to produce rickets by giving them two different diets (Table 1). Milk was limited to less than 200 mL, if not, a rachitic diet would not be produced [9].

Table 1: Rachitic diets for the puppies.

Rachitic Diets	
Diet 1	Diet 2
Milk	Milk
Rice	Bread
Oatmeal	
NaCl	

Other puppies were given this nutrition with added foods into the diet, this was done to observe if some factor found in said foods could inhibit rickets (Table 2).

Table 2: Diets for two different groups.

Group I (Prevented)	Group II (Did not prevent)
Meat	Protein of meat
Watery extract of meat	Casein
Malt extract	Linseed oil
Commercial yeast extract	10g of yeast a day
500 c.c of milk everyday	
Butter	
Margarine	
Cod liver oil	

It was concluded as there were some sort of accessory factors that prevents rickets and is fat-soluble as these were found in the extract of protein and fat [10]. This also showed that fats, proteins, and carbohydrates had no influence on the production of rickets, merely that rickets are a deficiency of a certain accessory factor. Later research my Mellanby and McCollum found out that the factor that prevents rickets and even cures them was a new vitamin termed Vitamin D [10].

Discussion

Vitamin D

Vitamin D (taken together) is technically not a vitamin. This is due to the fact that vitamins are substances that can't be synthesized by our body and must be obtained by the foods we eat. Vitamin D, although it must be obtained from the environment, is mostly gathered from how long we are exposed to the sun as a typical diet won't give sufficient amounts because of how little sources come from animals (the most common source being fatty fish such as tuna, salmon, or cod liver oil). Our geographic location and atmospheric pressure also explains why some regions of the world have people more susceptible to vitamin D deficiency diseases [11]. It's important to note one can be Vitamin D deficient, but not be able to over consume Vitamin D from being exposed to UV radiation [12]. The only way an overdose could happen is due to excessive amounts of Vitamin D supplementation, which could be just as fatal as under consumption [12]. Vitamin D, however, is actually considered to be a steroid hormone. The active form of Vitamin D, known as Calcitriol (Figure 1), is a steroid hormone because it can regulate levels of calcium and phosphorus in our body. Calcitriol is capable of being synthesized within our body; this is done within our kidney and liver. Cholecalciferol, the inactive form of Vitamin D (Figure 1), is generated in our skin when UV radiation is absorbed by the precursor molecule, 7-dehydrocholesterol. Cholecalciferol is the supplement that can be found in stores where overconsumption can be toxic. This makes Vitamin D technically not a vitamin because they are unable to be synthesized in our body [12].

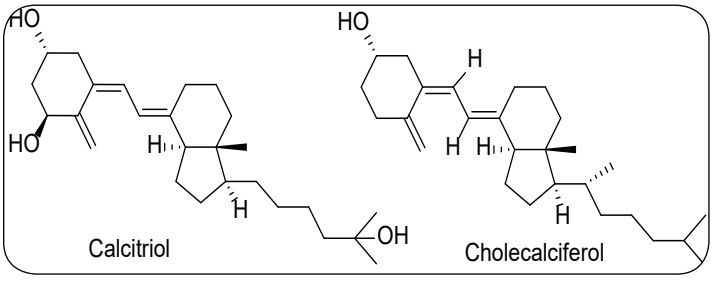

Figure 1: Structures of Calcitriol and Cholecalciferol.

Pharmacokinetics

Human skin contains a sterol derivative known as 7-dehydrocholesterol. Once this compound is exposed

to UV radiation it converts to the inactive form known as cholecalciferol (typically known as Pre-Vitamin D_3) [12]. This cis-cis conformer is thermodynamically unfavorable and is energetic but is the only form that isomerizes to Vitamin D_3. Vitamin D_3 (or D_2 if obtained from plants known as ergo sterol) is not biologically active however they still need to be converted to its physiologically active form, calcitriol [12]. First, cholecalciferol is hydroxylated (introduced a hydroxyl group) inside the liver where it becomes 25-hydroxy derivative of Vitamin D. This is done under the influence of the enzyme 25-hydroxylase. Next, it must be transported to the kidney where it again is hydroxylated, acting as a substrate for 1-alpha hydroxylase, where it is converted to 1,25-dihydroxycholecalciferol, the active form (Figure 2). It travels throughout the body *via* blood with its carrier protein, DBP (vitamin D-binding protein) [12]. Also, it's worthy to mention that since it is difficult to lead a vitamin D fortified diet, it is possible for food companies to irradiate Vitamin D into our food, fortifying it [13].

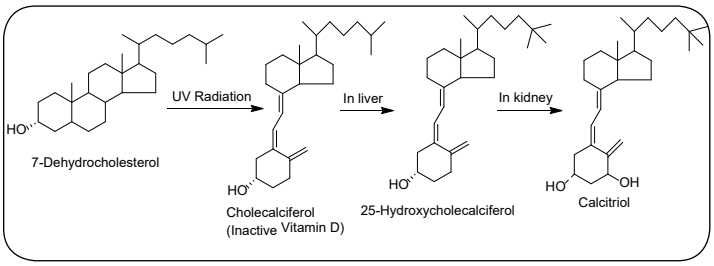

Figure 2: Formation of Calcitriol from 7-Dehydrocholesterol.

Calcification

Activated Vitamin D cannot play any 'active' role in human body without the Vitamin D receptor, a member of the nuclear receptor family whose purpose is allowing Calcitriol's gene expression [14]. The most well-known function of Vitamin D is regulation of calcium in human body. This is monitored by the serum calcium levels in our blood, kidneys, and parathyroid hormone. If human serum calcium levels are found in low amounts, the parathyroid gland secretes a hormone, which responds to these levels (as well as phosphate) and secretes PTH (parathyroid hormone). This hormone then signals the kidney to begin producing activated Vitamin D and pulling calcium from our bones and into our circulatory system. This is done until the calcium levels have become balanced. This is all an example of our bodies' excellent practice of homeostasis [15]. By this relatively simple function, it prevents disastrous effect that might occur in the body, including osteomalacia: a softening of the bones. There are a number of reasons why osteomalacia could occur, obviously from lacking Vitamin D; this could be due to not going out to get direct UV radiation or lacking from the diet. Although it was quite difficult to get Vitamin D from our diet, it is possible to irradiate many of our foods with Vitamin D and commonplace groceries, such as milk, have become Vitamin D fortified. There is debate however, about the effectiveness from fortified Vitamin D_2 found in most foods as compared to D_3 obtained from the sun. Another reason for osteomalacia occurring could be due to kidney or liver problems, this can halt the mechanistic formation of Vitamin D in our body, and even if we do have a Vitamin D rich diet and are exposed daily to the sun, we will not be getting anything out of it since our body isn't able to synthesize the active form. Osteomalacia in children is known as rickets and is considerably more severe as children's bones are still forming. An example of the severity of rickets is bowed legs that are curved outward as well as other skeletal deformities [16]. Osteoporosis can also occur as a consequence of years of insufficient vitamin D thinning the bones and making them brittle. An insufficient calcium intake can prove dangerous for the elderly as a fall could prove a trip to the hospital over a broken hip [17]. Despite Vitamin D being most-well known for its role in bone formation, recent studies have shown that it also displays excellent noncalcemic functions. The reason is due to its Vitamin D receptor and analogs.

Vitamin D receptor (VDR)

The Vitamin D receptor regulates many roles of vitamin D in human body due to its versatility. Calcitriol executes its functions only through this receptor. VDR has several binding domains for several occupants. The C-domain for binding to the DNA, E-domain for ligand-binding, and an activating F-domain [18]. This means that VDR standardizes all the functions Vitamin D performs. This is excellent, however may create complications in preparation of its analogs and their functions. Regardless, this 427 amino acids' constituted receptor works with Vitamin D-responsive elements (VDREs) found at the starting site of the specified gene. These VDREs' 5' arm attaches to the RXR, and its 3' arm to the VDR [18]. This in turn heterodimerizes as well as acquiring an activator [18].

VDR possesses remarkable versatility. Interestingly, it's found in many places including enterocytes, osteoblasts, and more recently discovered in parathyroid gland cells, keratinocytes, colon cells, and more [19]. Because VDR is present in these locations strongly suggests its functions are far beyond the scope of just bone formation. For

example, pioneering research by Suda et al. (2015) found that calcitriol can help terminally differentiate between promyelocytes and monocytes, precursors of the bone dissolving and absorption cell: osteoclasts. This demonstrates the importance in production of osteoclasts by use of Vitamin D [20]. As mentioned before, VDR is found in parathyroid gland cells, this has been found to be therapeutically useful in the treatment of renal osteodystrophy: a disease of the bones occurring due to kidneys being unable to regulate calcium and phosphorus levels in the blood. Treatments so far were mainly through the use of phosphorus retention, which in turn inhibited hyperphosphatemia [21]. This allowed serum calcium concentrations to return to regular levels [21]. Studies have also found that calcitriol (and analogs) also function to prevent parathyroid gland cells from rapidly multiplying. When the kidneys aren't working as intended (renal failure), can affect circulation of calcium in the body, causing hyperproliferation of the parathyroid gland, secreting large amounts of parathyroid hormone, resulting in secondary hyperparathyroidism [22]. Regulation through calcitriol maintains healthy parathyroid glands in patients undergoing dialysis [22]. Vitamin D also has shown to have some therapeutic effect in treatment of certain autoimmune diseases such as multiple sclerosis and regulation of type 1 diabetes mellitus by increasing lag time, resulting in more time before diabetes actually occur [23][24]. Further studies are required to make firm conclusion.

Cancer and Vitamin D

It is well-known that calcitriol and VDR have increased utility and influence on the human body. It's also worthy to restate that it can regulate gene expression, allowing influence to several signaling pathways involved in such processes like apoptosis and proliferation. This is especially seen when discussing Vitamin D's impact on the following cytochrome P450 enzymes.

CYP27B1 and CYP24A1

These renal enzymes are regulated by three hormones: calcitriol, fibroblast growth factor 23, and parathyroid hormone [25][26]. CYP27B1 is found in several extrarenal areas of interest. It's also found in cancer cells, allowing it to impose anticancer influence [27]. Although CYP27B1 is regulated by the hormones mentioned above, it's only regulated as renal CYP27B1, their influence on extra renal CYP27B1 is nonexistent and cannot regulate its effects [27][28]. Synthesis of this enzyme depends on calcidiol substrate concentration. This indicates that Vitamin D could be a possible anticancer agent due to the inactive form easily being converted to calcitriol inside cancer cells [29][30]. This also means that since it is not under the influence of the 3 hormones, it can exert high levels of calcitriol concentration [31], but there is no question of hypercalcemia being a danger as of right now [27][28][32]. CYP24A1 is also found within cancer cells, yet serves no anticancer therapeutic effects, actually it suppresses calcitriol's influence on them [33][34][35] classifying it as an oncogene (gene that may cause growth of cancer cells) [36]. Luckily, there are ways around this gene by use of cytochrome P450 inhibitors such as genistein [37], ketoconazole [38], and liarozole [39]. This in turn allows calcitriol's influence to be prevalent once again. Unfortunately the use of these inhibitors enhances the calcemic effects of calcitriol, introducing hypercalcemia as a risk once more [40]. Caution must be taken when using these compounds in tandem. Furthermore, expression of CYP27B1 depends on the tumor, its progression, and targeted organ [29][30][41][42][43]. Regardless, this cytochrome metabolizing enzyme shows potential in being able to influence cancer-related pathways for treatment of it (information was obtained by performing studies on patients with breast, prostate, and other cancers) [44]. In a nutshell, calcitriol influences the activity of certain cytochrome metabolizing enzymes and subsequent interactions with various cancer cells. An outlined discussion is presented below considering the types of cancer based on organs and its promise as a future therapeutic agent.

Leukemia

An abnormality of the body's bone marrow forming cancerous white blood cells, Leukemia is a cancer that affects less than 200,000 people in the United States per year [45]. Depending on its aggression, treatment may vary [46]. Calcitriol can regulate mineral metabolism in the body and can impose a differentiation inducing factor on this leukemic cell line (HL60), converting them into end cells (this means that it can transform a cancerous cell into a completely different cell) [47]. *In vitro* and *in vivo* tests were performed on patients who had preleukemia. In vitro study, calcitriol had a differentiating factor induced on the leukemic cells, but in vivo the results were not as well. Therapeutically speaking, it was virtually absent as it didn't prevent 7 of the 18 patients from developing leukemia and 9 others developed hypercalcemia [47]. An analog that does not impose calcemic effects would be needed in order to make this therapy effective in patients suffering leukemia [47].

Squamous cell carcinoma (SCC)

Often regarded as one of the most common skin cancers, squamous cell carcinoma usually affects the epidermal layers of the body. The reason as to why it's much more common than leukemia (1 million diagnosed annually) could be due to too much exposure to UV radiation. Being exposed to the sun year-round and being a participant in tanning beds could proliferate abnormal cell growth to the exposed areas [48]. Calcitriol has the ability to change the expression of proteins that regulate the cell cycle in cancer cells resulting in the dephosphorylation of a retinoblastoma protein (function to suppress tumors and regulate cell cycle, doesn't function in different cancers). Calcitriol also is known to down modulate a possible oncogene known as p21 (WAF1/CIP1), an inhibitor thought to suppress tumors [49], but studies have shown that it prevents apoptosis and encourages rapid cancer cell growth. Finally, calcitriol also increases expression of p27Kip1, another cyclin-dependent kinase inhibitor whose function is to slow down or even stop the cell cycle from proceeding. Calcitriol, along with help from dexamethasone, have shown to prevent SCC proliferation, and this is done by preventing cell cycle progression rather than programmed cell death [50] through apoptosis. This is therapeutically useful as studies were shown to be effective both *in vitro* and *in vivo*.

Prostate and Colon cancers

Prostate cancer is a very frequent cancer (males) worldwide. As many as 3 million men a year are diagnosed with prostate cancer although in most of the cases the rate of proliferation in prostate cancer is relatively slower than many other types of cancer. Treatment is simple if detected early and sometimes not necessary, however when it becomes metastatic, its aggression and advancement can be deadly [51]. Calcitriol demonstrated huge potential to fight against prostate cancer as found in vitro, however, with frequent uptake, the chance of hypercalcemia raises. EB1089, a synthetic analogue of Vitamin D (Figure 3), however might resolve this issue. It has the same anticancer effects that calcitriol would have on prostate cancer cells but does not induce a calcemic effect of any kind [52]. EB1089 and calcitriol were injected in various doses to 4 groups of male rats. Each group had 10 rats treated with MAT-Lylu cells (a cell line that induced proliferation of prostate tumor cells in male rats). There was also an extra group that served as the control, totaling 50 rats in 5 groups overall. The results were as expected: the control group had increased amount of tumor progression as compared to any of the 4 experimental groups. The rats treated with calcitriol had tumor progression slowed, and with a higher dosage it also lowered the progression further, but with marked weight loss and development of hypercalcemia. On the other hand, rats with EB1089 showed tumor progression similar to that of calcitriol, without marked weight loss or hypercalcemia on either of the two groups [52]. There is much promise to be shown here and if possible how EB1089 could be incorporated to other cancers. This is truly the first successful synthetic analog that can be used for anticancer therapy. It is also worthy to note that EB1089 was able to successfully and effectively induce apoptosis of two carcinoma cell lines: SW620 and PC/JW [53]. This indicates that EB1089, along with possibly other synthetic analogues produced, could offer an alternative solution to colorectal cancer.

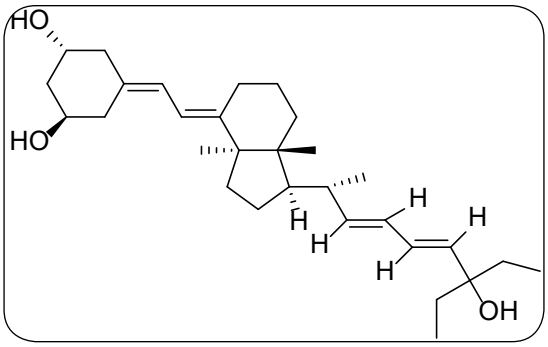

Figure 3: Structure of EB 1089.

Breast cancer

Breast cancer is known as the most commonly diagnosed cancer, being the 2nd most lethal type of cancer found in women. It initiates by the use of tumor-initiating cells (also known as cancer stem cells) that creates cancer cells and continues to do so repeatedly at the site of the original tumor mass. The significance of this study was not necessarily if calcitriol or vitamin D as a dietary supplement could treat breast cancer, but how it does so. A mouse mammary tumor virus was induced in rats and later treated with calcitriol injections or a diet focused on vitamin D supplementation [54]. The results were not very exciting, but progressive toward support its push to therapeutic legitimacy. What was surprising was how effective calcitriol was when combined with ionizing radiation therapy, results far better than what either one could've done alone, creating a synergistic effect. The way it halted tumor progression was hypothesized to have been located at the Wnt/β-Catenin pathway [55]. This pathway is very important as it regulates stem cells that are considered pluripotent, meaning that it can create any kind of cell needed in the human body.

Ovarian cancer

Ovarian Cancer is a perplexing cancer for females. It's undetectable in early stages, and symptoms usually don't show up until it's become advanced. There has been no real information on how to prevent ovarian cancer [56] although there are many instances that ovarian cancer functions the same way that breast cancer does *via* cancer stem cells. Interestingly, xenograft models of mice produced with ovarian cells from human, was shown that calcitriol therapy greatly slowed down tumor growth [57]. This was done by disrupting its cancer stem cells through the Wnt signaling pathway. The capacity to create its form under cultured conditions, the frequency of CSCs, was done in vivo by limited dilution analysis [57]. Much promise by seeing repeated paths or similar means of action by calcitriol indicates some form of consistency in its method of approach.

Conclusion

In conclusion, vitamin D and its analogs demonstrated key role in regulating various diseases in human including cancer. The mechanism of action is not completely understood yet. Accordingly, further research is required to explore its therapeutic potential more appropriately for human wellbeing.

Conflicting interests

The authors have declared that no conflicts of interests exist.

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