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Perspectives

The mechanisms of vitamin D toxicity

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Vitamin D intoxication is a hypercalcemic and hypercalciuric state which is suggestive of excess $1,25(\text{OH})_2\text{D}$ -like activity. Major aspects of vitamin D toxicity have been reviewed by Coburn and Barbour [1] and by Stern and Bell [2]. Among the factors that may predispose individuals to vitamin D intoxication are increased calcium intake, decreased renal function, diminished estrogen levels, the existence of sarcoidosis [1] or other vitamin D-hypersensitivity syndromes associated with overproduction of $1,25(\text{OH})_2\text{D}$ [2]. The key issue that has not been resolved is the question of just what it is about the vitamin D endocrine system itself that makes excess vitamin D toxic even in normal individuals.

The role of $1,25(\text{OH})_2\text{D}$ in vitamin D intoxication was discounted once it was shown that $1,25(\text{OH})_2\text{D}$ levels were relatively unchanged in affected subjects. Instead, toxicity has been attributed to the high circulating concentrations of $25(\text{OH})\text{D}$ [3]. This view was based on in vitro studies showing that high concentrations of $25(\text{OH})\text{D}$ stimulated resorption of cultured fetal rat bone [4]. However, it is by no means clear that excessive $25(\text{OH})\text{D}$ or some unspecified agonist will act at the $1,25(\text{OH})_2\text{D}$ receptor in vivo. This article discusses three of the newer aspects of the vitamin D system which can differ among individuals who do not have overt disease and which should influence the susceptibility to vitamin D toxicity: the concentration of free vitamin D metabolites, the activity of 1α -hydroxylase and degradative metabolism. The hypothesis presented here is that $1,25(\text{OH})_2\text{D}$ is the agent causing toxicity.

Free metabolite

Like other fat-soluble compounds, very little of any vitamin D metabolite is freely dissolved in plasma. Vitamin D binding protein (DBP) and, to a much lesser degree, albumin, bind the metabolites in plasma. Recent evidence indicates that the

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free vitamin D metabolites, and not the bound ones, are functional *in vivo* [5] and in cultured cells [6,7]. If the free hormone hypothesis applies to the vitamin D system, then the high-affinity binding to DBP is the most important factor in determining the concentration of metabolite accessible to cells [8]. The metabolite bound with low affinity to albumin is virtually free because it will dissociate rapidly during passage through the tissue microvasculature [8].

The concentration of DBP in human plasma is 5-6, $\mu\text{mol/l}$ [9]; in rat plasma it is 3.7, $\mu\text{mol/l}$ [10]. The DBP concentration is substantially higher during pregnancy or when estrogens or proestrogens are taken [5] and lower in patients with liver disease [11]. Normally, it can be assumed that the level of DBP inversely determines the free fraction of each vitamin D metabolite in the circulation. However, once the total concentration of all vitamin D metabolites in circulation is no longer negligible in relation to the DBP concentration, this assumption no longer applies and the free fraction of every vitamin D metabolite will increase dramatically.

With this in mind, it is worthwhile to re-evaluate results reported a decade ago by Sheppard and DeLuca [3]. These investigators measured concentrations of vitamin D₃ metabolites in rats after daily treatment with increasing doses of vitamin D₃. At the lowest dose that caused hypercalcemia, the total concentration of vitamin D₃, 25(OH)D₃ and other metabolites in plasma was equal to 5.3, $\mu\text{mol/l}$ (2120 ng/ml), which exceeds the reported capacity of rat DBP [10]. The concentration of 1,25(OH)₂D₃ in the intoxicated rats was slightly lower than in those given lower doses. The principles of binding equilibrium, however, indicate that the fraction of this hormone in the free form was greatly increased. It has been suggested that a

Table 1
Comparisons of affinities and potencies of vitamin D₃ metabolites

Biological feature	Metabolite tested			Unit	Ref.
	25(OH)D ₃	24,25(OH) ₂ D ₃	1,25(OH) ₂ D ₃		
Affinity for human DBP	1-2 x 10 ⁶	1-2 x 10 ⁻⁵	1 x 10 ⁻⁷	M-1	12
Metabolite extracted from 720 ng/ml DBP by perfused dog tibia	2a	2a	28	%	14
Metabolite extraction from 5 g/dl bovine albumin by perfused rat brain	2.2		5.2	%	15
Binding to chick intestinal cytosolic receptor	1	0.5	1000	relative affinity	13
Stimulation of ⁴⁵ Ca release from fetal-rat bone in culture	1	0.5	1000	relative potency	4,13

^a The value was not significantly different from zero.

role for DBP is to provide a 'buffering' capacity to protect against vitamin D intoxication [8]. The evidence in the rat supports this view.

The affinity of DBP for $1,25(\text{OH})_2\text{D}$ is low compared to that of most of the other vitamin D metabolites [12]. Therefore, the free or unbound fraction of $1,25(\text{OH})_2\text{D}$ in circulation should increase by more than that of other vitamin D metabolites as the occupancy of DBP increases. In contrast, intracellular receptors have far greater affinity for $1,25(\text{OH})_2\text{D}$ [13]. The net effect of the relative affinities of plasma DBP and the intracellular $1,25(\text{OH})_2\text{D}$ receptor for $1,25(\text{OH})_2\text{D}$ is that micromolar levels of extracellular $25(\text{OH})\text{D}$ and $24,25(\text{OH})_2\text{D}$ will promote, not inhibit, the entry of $1,25(\text{OH})_2\text{D}$ into target cells. This phenomenon has been demonstrated with intestinal epithelial cells in the presence of calf serum [6].

Table 1 summarizes some of the features that influence the cellular entry and action of $1,25(\text{OH})_2\text{D}$ and $25(\text{OH})\text{D}$. If one multiplies the ten-fold difference in DBP affinity between $25(\text{OH})\text{D}$ and $1,25(\text{OH})_2\text{D}$, times the two-fold difference in metabolite availability from albumin and the 1000-fold greater affinity of the intracellular receptor, then it appears that on a molar basis, $1,25(\text{OH})_2\text{D}$ is 20 000 times more potent than $25(\text{OH})\text{D}$ in vivo. This value does not include the effect of other vitamin D metabolites at promoting the cellular entry of $1,25(\text{OH})_2\text{D}$ during vitamin D intoxication. One cannot rule out entirely the possibility that $25(\text{OH})\text{D}$ binds to some degree to the $1,25(\text{OH})_2\text{D}$ receptor in vivo. However, most of the activity at target tissues would appear to be due to the presence of excess $1,25(\text{OH})_2\text{D}$.

Residual 1 α -hydroxylase

When enough vitamin D is provided, then tissues other than the kidney can synthesize $1,25(\text{OH})_2\text{D}$ in anephric humans [16] and pigs [17]. Furthermore, in subjects with normal kidneys it appears that 1 α -hydroxylase cannot be switched off completely. Existence of a residual level of 1 α -hydroxylase somewhere in the body is indicated by the fact that $1,25(\text{OH})_2\text{D}$ concentrations are only slightly suppressed [3,18] or increased [19,20] in reported cases of vitamin D intoxication.

If residual 1 α -hydroxylase were regulated properly in people intoxicated with vitamin D, then $1,25(\text{OH})_2\text{D}$ levels would be either very low or undetectable because of the suppressed PTH levels and severe hypercalcemia. An important feature of 1 α -hydroxylase in vivo is that the synthesis of $1,25(\text{OH})_2\text{D}$ is determined by the availability of $25(\text{OH})\text{D}$ through a 'mass action' effect [20-22]. In essence, both 1 α -hydroxylase and 24-hydroxylase present in vivo behave as if they are below their K_m [22]. The inability to regulate residual 1 α -hydroxylase will result in inappropriate synthesis of $1,25(\text{OH})_2\text{D}$ during vitamin D intoxication.

Another feature that supports the concept that $25(\text{OH})\text{D}$ levels drive $1,25(\text{OH})_2\text{D}$ production during vitamin D intoxication is the increased metabolic clearance of $1,25(\text{OH})_2\text{D}$ that most vitamin D metabolites induce [22-24]. To sustain the normal or increased $1,25(\text{OH})_2\text{D}$ levels seen during vitamin D intoxication, there must be a substantial increase in the production rate of this hormone.

Degradative metabolism and hepatic excretion

Vitamin D intoxication will eventually develop if the input of vitamin D or its metabolites exceeds the adaptive capacity of the body to eliminate them. Inducible mechanisms for clearance of vitamin D metabolites involve oxidation and cleavage of the sidechain beyond carbon 23 of the secosteroid molecule [25] as well as metabolism and excretion of vitamin D metabolites by the liver into the bile [26]. At present it appears that both 25(OH)D and 1,25(OH)₂D are subject to the same clearance mechanisms.

Younger children with idiopathic hypercalcemia tend to have elevated 1,25(OH)₂D concentrations [27]. Young rats have been shown to be more susceptible to the toxic effects of 1,25(OH)₂D₃ doses than older rats [28]. In both cases, the authors proposed that diminished degradative metabolism of 1,25(OH)₂D could explain the hypercalcemia. However, there is still no direct evidence that clearance of 1,25(OH)₂D is diminished with immaturity. In adults, there does not appear to be an effect of aging per se on the production or metabolic clearance rates of 1,25(OH)₂D [29]. Another mechanism for the susceptibility of young children to vitamin D intoxication may be that their 1,25(OH)₂D production is 'loosely regulated', with 1,25(OH)₂D levels that appear to be proportional to those of 25(OH)D [30].

Conclusions

There are at least three features of the vitamin D system per se which together impose a limit on the safe intake of vitamin D: (a) the capacity of DBP, (b) the level of residual 1 α -hydroxylase, the activity of which is driven by 25(OH)D, and (c) the capacity to clear vitamin D metabolites from the body. Because free, not total, 1,25(OH)₂D is functional in vivo, a probable mechanism for the toxicity of vitamin D is that high 25(OH)D concentrations will cause both excessive synthesis of 1,25(OH)₂D and, together with vitamin D and its other metabolites, cause displacement of the hormone from DBP thereby increasing the amount of free 1,25(OH)₂D that is accessible to target cells. To understand what is going on in individual cases, vitamin D intoxication should be characterized by measuring the total concentration of all vitamin D metabolites and the concentrations of 1,25(OH)₂D and DBP.

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