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Vitamin D toxicity redefined: Vitamin K and the molecular mechanism

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Summary The dose of vitamin D that some researchers recommend as optimally therapeutic exceeds that officially recognized as safe by a factor of two; it is therefore important to determine the precise mechanism by which excessive doses of vitamin D exert toxicity so that physicians and other health care practitioners may understand how to use optimally therapeutic doses of this vitamin without the risk of adverse effects. Although the toxicity of vitamin D has conventionally been attributed to its induction of hypercalcemia, animal studies show that the toxic endpoints observed in response to hypervitaminosis D such as anorexia, lethargy, growth retardation, bone resorption, soft tissue calcification, and death can be dissociated from the hypercalcemia that usually accompanies them, demanding that an alternative explanation for the mechanism of vitamin D toxicity be developed. The hypothesis presented in this paper proposes the novel understanding that vitamin D exerts toxicity by inducing a deficiency of vitamin K. According to this model, vitamin D increases the expression of proteins whose activation depends on vitamin K-mediated carboxylation; as the demand for carboxylation increases, the pool of vitamin K is depleted. Since vitamin K is essential to the nervous system and plays important roles in protecting against bone loss and calcification of the peripheral soft tissues, its deficiency results in the symptoms associated with hypervitaminosis D. This hypothesis is circumstantially supported by the observation that animals deficient in vitamin K or vitamin K-dependent proteins exhibit remarkable similarities to animals fed toxic doses of vitamin D, and the observation that vitamin D and the vitamin K-inhibitor Warfarin have similar toxicity profiles and exert toxicity synergistically when combined. The hypothesis further proposes that vitamin A protects against the toxicity of vitamin D by decreasing the expression of vitamin K-dependent proteins and thereby exerting a vitamin K-sparing effect. If animal experiments can confirm this hypothesis, the models by which the maximum safe dose is determined would need to be revised. Physicians and other health care practitioners would be able to treat patients with doses of vitamin D that possess greater therapeutic value than those currently being used while avoiding the risk of adverse effects by administering vitamin D together with vitamins A and K.

Introduction

Although vitamin D was initially discovered in the early 20th century as the factor that prevented and treated rickets, more recent research has suggested numerous roles for vitamin D beyond those which have been classically recognized, ranging from the prevention of heart disease and cancer to the regulation of blood sugar and support of neuromuscular function. Recommendations regarding the requirement for and safety of vitamin D vary

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widely. The US Institute of Medicine recommends only 200 IU per day for adults under the age of 50 [1], while some individual researchers recommend as much as 4000 IU per day [2,3]. Whereas Dr. Vieth of the University of Toronto asserts that the latter amount can be safely consumed in addition to 4000 IU per day obtained from sunlight [2], it exceeds the tolerable upper limit set by the Institute of Medicine by a factor of two [1].

Because the dose that some researchers consider optimally therapeutic exceeds the maximum dose officially recognized as safe, many physicians may be discouraged from employing vitamin D to treat conditions for which its use may be appropriate. It is therefore important to elucidate the precise mechanism of vitamin D's toxicity so that we can better understand the interacting factors that affect its safety profile and more clearly comprehend how to reap its benefits without the risk of adverse effects.

The Institute of Medicine derived the tolerable upper limit from a study purporting to show that 3800 IU per day of vitamin D resulted in an increased incidence of hypercalcemia [1]. Although the rigor of this study's experimental protocol has been called into question [4], the fundamental premise that vitamin D exerts its toxic effects primarily through an elevated level of calcium in the blood has remained largely unchallenged.

There are nevertheless several lines of evidence suggesting that hypercalcemia is of secondary importance to vitamin D toxicity. First, both vitamin A [5,6] and the bone resorption inhibitor ibandronate [7] reduce or eliminate soft tissue calcification, anorexia, weight loss, lethargy and death induced in animals by toxic doses of vitamin D without reducing the concomitant hypercalcemia. Second, Warfarin, a coumadin derivative that inhibits the recycling of vitamin K, produces a toxicity profile almost identical to that of vitamin D but does not increase serum calcium levels [8]. Third, vitamin D can induce renal calcification in chickens [9] and possibly bone resorption in humans [10] at doses that do not result in hypercalcemia. These observations show that vitamin D can induce hypercalcemia in the absence of toxicity and can exert toxicity in the absence of hypercalcemia, and therefore demand an alternative explanation for the mechanism of this toxicity.

Presentation of the hypothesis

I propose that vitamin D exerts its toxic actions primarily by inducing a deficiency of vitamin K. According to this model, vitamin D upregulates the expression of certain proteins that must be activated by the vitamin K-dependent process of carboxylation; when the level of these proteins exceeds that which the pool of available vitamin K has the capacity to carboxylate, this pool becomes depleted. Because of this depletion, vitamin K-dependent processes that support the nervous system, retain minerals in the bone matrix and protect the soft tissues from calcification cannot be performed. Additionally, the excess of inactive, undercarboxylated proteins overwhelms the limited supply of active, carboxylated proteins, and the inactive proteins themselves may facilitate the release of calcium from the bone matrix and the deposition of calcium into the soft tissues.

I further propose that the more than 70-year-old observation that vitamin A protects against the toxicity of vitamin D can be explained by vitamin A's ability to downregulate matrix Gla protein, a vitamin K-dependent protein found in soft tissues. This reduction of the demand for carboxylation exerts a vitamin K-sparing effect that counteracts the depletion of vitamin K induced by vitamin D.

While official recommendations consider the toxicity of vitamin D to be a function of the absolute amount of vitamin D, this hypothesis presents a model in which the toxicity of vitamin D is instead a function of the balance between vitamins A, D and K. If it is correct, it requires a revision of the models by which the safe oral dose and serum level of vitamin D are determined. Proper consideration of the synergistic and protective context of other fat-soluble vitamins could allow vitamin D to be administered in doses of greater therapeutic value yet simultaneously of lower risk.

Evaluation of the hypothesis

The ''avitaminosis A'' hypothesis: an historical predecessor

In 1935, the German researcher Thoenes put forward the hypothesis that vitamin A is essential to the functioning of vitamin D and that high doses of vitamin D cause toxicity by producing a state of ''relative avitamonosis A'' [11]. This hypothesis gathered circumstantial evidence from a number of experiments showing that high doses of vitamin A substantially protected against the growth retardation, soft tissue calcification and bone resorption induced in rats by dietary vitamin D_3 concentrated from fish oils [12] and dietary vitamin D_2 synthesized by irradiating ergosterol [6,12,13], and that vitamin A completely protected against renal calcification induced by dietary vitamin D_3 in turkeys [14].

In 1951, French researchers showed that the same protective effect was observed when the vitamins were administered to rats through intramuscular injection [15], demonstrating that the interaction was not a result of competitive intestinal absorption. More recently, Aburto and Britton showed that vitamin D depletes blood levels and liver stores of vitamin A in chickens, whether the vitamin D is provided through diet or by ultraviolet light [16]. Recent research has further shown that 9-*cis*-retinoic acid, a derivative of vitamin A, is required for the full functioning of the vitamin D receptor [17].

These findings are all consistent with Thoenes' hypothesis that vitamin D exerts its toxic effects by depleting the body's pool of vitamin A through cooperative actions between the two vitamins. However, while vitamin A exerts a strong protective effect against vitamin D toxicity, its protection is not complete and vitamin D toxicity is not an exact mirror of vitamin A deficiency. Experiments showed vitamin A to be much more effective at reducing vitamin D-induced growth retardation, bone loss and renal calcification than it was at reducing vitamin D-induced calcification of the lungs and heart [12]. Traditional measures of vitamin A deficiency such as xerophthalmia and hyperkeratosis were not reported in response to toxic amounts of vitamin D, and vitamin A deficiency was never observed to result in death via widespread and massive calcification of tissues as occurs during hypervitaminosis D. The hypothesis that vitamin D exerts its toxicity by inducing a relative deficiency of vitamin A, then, has substantial support but is insufficient to account for the full range of observations.

The vitamin K hypothesis: circumstantial evidence

Vitamin K activates a select group of vitamin K-dependent proteins by adding carboxyl groups to, or ''carboxylating,'' these proteins. Although vitamin K is best known for its role in activating blood clotting factors, it is also essential to the activation of osteocalcin, a protein involved in bone mineralization, and matrix Gla protein (MGP), a protein that protects a wide variety of soft tissues from calcification. Vitamin K_1, or phylloquinone, is preferentially utilized by the liver to carboxylate clotting factors while vitamin K_2, or menaquinone, is preferentially used by the extrahepatic tissues for the carboxylation of the other vitamin K-dependent proteins. Vitamin K_2 is therefore believed to be important for the prevention and treatment of osteoporosis and calcification-mediated cardiovascular disease [18]. Vitamin K_2 is also found in high concentrations in the brain, where it contributes to the production of myelin and other important compounds [19]. My hypothesis concerns those physiological effects preferentially attributable to vitamin K_2; I will therefore use the general term vitamin K in this paper to mean vitamin K_2.

Animals fed toxic doses of vitamin D bear a striking resemblance to animals deficient in vitamin K or the vitamin K-dependent proteins. Like rats fed toxic doses of vitamin D, MGP-null mice suffer from bone demineralization, growth retardation, extensive calcification of the aorta and its branches, the arteries, the trachea and the lungs, and calcification-mediated death. Although the mechanism by which vitamin D causes growth retardation has never been clarified, the same effect in MGP-null mice results from extensive calcification of the cartilaginous growth zones of long bones [20]. Vitamin K-dependent proteins also protect the kidneys and urogenital tract from calcification [21].

Warfarin, a coumadin derivative that induces a functional vitamin K deficiency by inhibiting the recycling of the vitamin, produces extensive hypervitaminosis D-like calcification of the soft tissues and exerts toxicity synergistically with vitamin D when the two are combined [8]. Both Warfarin and vitamin K-deficient diets induce hypoactivity and reduce exploratory behavior in rats [22], which may result from the same or a similar phenomenon reported as lethargy and anorexia by other researchers observing hypervitaminosis D. By an unknown mechanism, the bone resorption inhibitor ibandronate fully protects against all toxic endpoints induced by both vitamin D [7] and Warfarin [23] that have been measured, strengthening the hypothesis that vitamin D and Warfarin exert toxicity through a common mechanism. In the case of vitamin D, these endpoints included anorexia, weight loss, lethargy, calcification of the aorta, arteries, trachea, lungs and kidneys, and death. Vitamin K is itself sufficient to fully reverse the calcification induced by Warfarin [24], confirming that the drug's vitamin K-inhibiting property is directly responsible for its induction of calcification, and strongly suggesting that the same or a similar mechanism underlies the toxicity of vitamin D.

The vitamin K hypothesis: a mechanism

Although carboxylated MGP is known to protect the arterial lining and other soft tissues from calcification, undercarboxylated MGP is found abundantly in calcified arterial plaque. Undercarboxylated

MGP has generally been assumed to be an innocent bystander and its presence in calcified plaque has been assumed to reflect a reactive attempt by the local tissue to protect itself from calcification; this attempt, according to the conventional understanding, has in turn been rendered futile by an insufficient capacity for carboyxlation due to the limited supply of available vitamin K [18].

Price et al. showed that toxic amounts of vitamin D increase the expression of MGP between 6-fold (cartilage) and 100-fold (lung) depending on the tissue, and that the expression of MGP correlated with the degree of calcification [7]. Although the increase in MGP expression occurred simultaneously with hypercalcemia, ibandronate restored normal levels of MGP without reducing serum calcium levels, suggesting that the elevated level of MGP was not a reaction to elevated serum calcium *per se*. While Price et al. argued that MGP was likely to be produced locally in order to retard the process of soft tissue calcification, the hypothesis presented in this paper proposes that the overexpression of MGP contributes directly to the development of both bone resorption and soft tissue calcification.

In rat vascular smooth muscle cell culture, vitamin D directly increases the expression of MGP while retinoic acid, a derivative of vitamin A, directly decreases its expression [25]. When provided at physiological doses, vitamins A and D may therefore contribute to the maintenance of healthy levels of MGP; when the pool of available vitamin K is sufficient to carboxylate the MGP, the combined effect of these three vitamins would be expected to protect against soft tissue calcification.

When vitamin D is provided at doses that greatly exceed this level, however, the excessive stimulation of MGP and other vitamin K-dependent proteins could causally contribute to the toxicity observed. Overexpression of MGP could contribute to toxicity in one or both of two ways. If the demand for carboxylation exceeds the capacity of the limited supply of vitamin K, a *relative* deficiency of this vitamin would result in the undercarboxylation of most vitamin K-dependent proteins. A great excess of MGP could also rapidly deplete the reserves of vitamin K and thereby induce an *absolute* deficiency of the vitamin.

In the first case, a great excess of undercarboxylated MGP could facilitate soft tissue calcification by interfering with the functioning of carboxylated MGP through a crowding out mechanism or could even bind calcium salts directly and deposit them into the soft tissue matrix. In the second, overexpression of MGP could induce one of two distinct types of *absolute* vitamin K deficiencies. If excretion or degradation of vitamin K is dependent on its rate of carboxylation activity, the increased demand for carboxylation could result in a more rapid excretion or degradation of the vitamin, resulting in a decreased tissue concentration of vitamin K. Alternately, since vitamin K must be enzymatically recycled after it carboxylates a protein, the increased demand for carboxylation could exceed the maximum rate of enzymatic recycling, resulting in a decrease of the ratio of unrecycled to recycled vitamin K rather than a decrease of the total concentration of vitamin K in the tissues.

The undercarboxylation of osteocalcin is likely to contribute to the bone loss observed in animals suffering from hypervitaminosis D. In human osteoblast cell culture, vitamin D stimulates the production of osteocalcin, but this osteocalcin accumulates in both the extracellular matrix and the culture medium indiscriminately. When vitamin D is combined with vitamin K, the vitamin K carboxylates this osteocalcin, induces its accumulation in the extracellular matrix and inhibits its release into the culture medium in a dose-dependent manner. By contrast, Warfarin reduces the accumulation of osteocalcin in the extracellular matrix and increases its release into the culture medium. In each case, mineralization of the extracellular matrix occurs in parallel to the accumulation of osteocalcin [26]. This shows that vitamin D can only support bone mineralization insofar as a sufficiently large pool of vitamin K is available to carboxylate the osteocalcin whose production the vitamin D stimulates. The fact that vitamin D and Warfarin both induce the release of osteocalcin into the culture medium suggests that this osteocalcin would be released into the blood *in vivo*. This may represent the mechanism by which toxic doses of vitamin D and Wafarin induce the loss of bone mineralization.

Taken together, these observations suggest that vitamin D and Warfarin exhibit similar toxicity profiles because they both induce a deficiency of vitamin K, even though the mechanism by which they contribute to this deficiency is different: while Warfarin directly inhibits the recycling of vitamin K, vitamin D increases the demand for vitamin K beyond that which can be fulfilled.

According to this model, the induced vitamin K deficiency results in bone loss mediated by the release of osteocalcin into the blood; soft tissue calcification mediated by a deficiency of carboxylated MGP, possibly aggravated directly by an excess of undercarboxylated MGP; growth retardation mediated by the calcification of cartilaginous growth zones; and neurological symptoms including

lethargy and anorexia mediated by a reduction of the vitamin K available to the nervous system.

The vitamin K hypothesis: a critical evaluation

This model of vitamin D toxicity faces several criticisms: first, Warfarin enhances vitamin D toxicity even though it reduces MGP expression; second, because ibandronate is known as a bone resorption inhibitor, its ability to reduce vitamin D-induced MGP expression seems to suggest that the elevation of MGP may be a reaction to rather than a contributor to bone resorption; third, the fact that vitamin A upregulates osteoblast expression of osteocalcin presents a challenge to the interpretation that its protection against vitamin D toxicity results from its downregulation of vitamin K-dependent proteins. Each of these observations, however, can be assimilated into the proposed model.

The fact that Warfarin enhances vitamin D-induced soft tissue calcification despite reducing MGP levels [8] could be interpreted as evidence that undercarboxylated MGP does not itself facilitate soft tissue calcification. It is also possible, however, that the effect of Warfarin's reduction of the protective carboxylated MGP exceeds the effect of its reduction of the potentially harmful undercarboxylated MGP. Moreover, the observation that Warfarin reduces MGP while enhancing vitamin D-induced soft tissue calcification does not present any challenge to the proposal that excessive MGP expression depletes vitamin K reserves. This model would predict that Warfarin would aggravate this depletion regardless of its effect on MGP expression because Warfarin induces a functional vitamin K deficiency by an independent mechanism. This prediction is observed.

Although the ability of the bone resorption inhibitor ibandronate to restore normal expression of MGP in hypervitaminosis D [7] may superficially suggest that the dramatic increase in MGP expression observed in this state of toxicity is due entirely to bone resorption, this conclusion would be a mistake. The efficacy of ibandronate has been attributed to its inhibition of the mevalonate pathway in osteoclasts [27]. This mechanism cannot account for its ability to counteract vitamin D's direct transcriptional regulation of MGP or Warfarin's inhibition of vitamin K-mediated retention of osteocalcin within the extracellular matrix of osteoblasts, nor can it provide a satisfactory explanation for why ibandronate inhibits the neurological symptoms associated with vitamin D toxicity. The best interpretation of these observations is that ibandronate acts through multiple mechanisms, some of which have not yet been elucidated.

A final challenge to this model is presented by the synergistic effect of vitamins A and D on osteocalcin expression. In human osteoblast cell culture, incubation with either all-*trans*-retinoic acid, a metabolite of vitamin A, or calcitriol, a metabolite of vitamin D, increases osteocalcin expression only slightly. When the cells are incubated with the two vitamin metabolites simultaneously, however, osteocalcin expression is increased dramatically [28]. This suggests that vitamin A increases rather than decreases the demand for vitamin K in osteoblasts; one could argue that the proposed model predicts from this that vitamin A would aggravate vitamin D toxicity, despite the clear observation that vitamin A ameliorates vitamin D toxicity. Nevertheless, vitamin A downregulates the expression of MGP in rat [25] and human [29] cells. Since MGP is expressed broadly whereas osteocalcin is expressed only in osteoblasts, vitamin A is likely to exert a substantial net vitamin K-sparing effect, and would thus be predicted by the proposed model to ameliorate vitamin D toxicity, which is observed.

Testing the hypothesis

This hypothesis is composed of several dissociable parts, each of which makes testable predictions. These parts include the following: first, that vitamin D exerts toxicity by inducing a deficiency of vitamin K; second, that vitamin D induces this deficiency primarily through the excessive upregulation of vitamin K-dependent proteins; third, that the undercarboxylated forms of these proteins themselves contribute to the observed toxicity; and fourth, that vitamin A protects against vitamin D toxicity by exerting a vitamin K-sparing effect through the downregulation of the expression of vitamin K-dependent proteins. As in the previous section, the term vitamin K as used in this section refers specifically to vitamin K_2.

Vitamin D exerts toxicity by inducing a deficiency of vitamin K

If vitamin D exerts toxicity by inducing a vitamin K deficiency, administration of vitamin K to animals fed toxic doses of vitamin D should protect against all hypervitaminosis D-related endpoints in a dose-dependent manner. This observation would be consistent with the hypothesis but would not confirm

that a vitamin K deficiency had been induced. If vitamin K administration was only able to protect against some endpoints and not others, or if the maximally effective dose was unable to completely eliminate all manifestations of toxicity, it would suggest that the hypothesis has only partial power to explain the mechanism of vitamin D toxicity. If vitamin K administration were not able to protect against any hypervitaminosis D-related endpoints, the entire hypothesis would be falsified.

If vitamin D increases the excretion or degradation of vitamin K, administration of toxic doses of vitamin D to test animals should reduce the vitamin K levels of tissues in a dose-dependent manner. If vitamin D induces a functional deficiency of vitamin K by increasing the demand for carboxylation more quickly than vitamin K can be recycled, administration of toxic doses of vitamin D to test animals should increase the ratio of unrecycled to recycled vitamin K in a dose-dependent manner. Each observation would confirm the mechanism with which I have associated it; if, however, administration of vitamin D were able to affect neither the vitamin K level nor the unrecycled-to-recycled vitamin K ratio of tissues, the portion of the hypothesis proposing that vitamin D induces an absolute deficiency of vitamin K would be falsified; such observations would not, however, rule out the possibility that vitamin D induces a *relative* vitamin K deficiency, wherein the undercarboxylated proteins themselves directly contribute to bone loss and soft tissue calcification.

Depletion of vitamin K by the upregulation of vitamin K-dependent proteins

If the aforementioned experiments confirm that vitamin D exerts toxicity by inducing a deficiency of vitamin K, the mechanism by which vitamin D induces this deficiency would need to be clarified. This hypothesis proposes that vitamin D induces such a deficiency through excessive upregulation of vitamin K-dependent proteins, primarily of MGP. The fact that vitamin D increases the expression of these proteins is already confirmed, but the relationship of this expression to vitamin K deficiency has not been clarified.

The present hypothesis predicts that the induction of any sharp increase in the tissue level of MGP by any mechanism that does not involve the administration of an agent that can also carboxylate MGP should lead to an absolute deficiency of vitamin K. There are two such mechanisms by which MGP levels could be increased: test animals could be genetically altered using a hyperactive promoter of MGP expression or could be directly injected with uncarboxylated MGP. In each case, the increase in tissue levels of MGP achieved must be similar to that seen in hypervitaminosis D: between 6-fold and 100-fold. If increasing MGP levels by either of these mechanisms were to induce either a reduction of tissue vitamin K levels or of the unrecycled-to-recycled vitamin K ratio, it would confirm that vitamin D induces a vitamin K deficiency through the excessive upregulation of MGP.

On the other hand, if increasing MGP levels by either mechanism produced neither kind of vitamin K deficiency, it would falsify the specific portion of the hypothesis that explains the mechanism by which vitamin D induces a vitamin K deficiency. It would not, however, negate the finding that toxic doses of vitamin D deplete the tissues of vitamin K; instead, it would require that a new molecular explanation for this observation be developed.

The induction of a relative deficiency of vitamin K

In the event that both vitamin D and the independent overexpression of MGP are shown not to result in an absolute deficiency of vitamin K, experiments using a hyperactive promoter of MGP expression or direct injection of uncarboxylated MGP could also be used to test the hypothesis that a *relative* deficiency of vitamin K could result in soft tissue calcification. In this model, the pool of available vitamin K is not sufficiently large to fulfill the demand for carboxylation; undercarboxylated MGP then enhances soft tissue calcification by crowding out carboxylated MGP or by physically depositing calcium salts into the soft tissues. The observation that the overexpression of MGP does not result in a decrease in the vitamin K concentration or an increase in the unrecycled-to-recycled vitamin K ratio of the tissues but nevertheless enhances soft tissue calcification would support this hypothesis.

Although overexpression of MGP could lead to bone demineralization by depleting the vitamin K needed to carboxylate osteocalcin, it is also possible that overexpression of osteocalcin itself could play a role in facilitating bone loss. The latter possibility is a second example of a relative deficiency of vitamin K. Before testing whether excessive expression of osteocalcin can lead to bone loss, however, osteocalcin expression should be measured in animals to which toxic doses of vitamin D have been administered. The observation that

hypervitaminosis D is accompanied by a dramatic increase in the expression of osteocalcin would provide a basis for testing the effect of excessive osteocalcin expression on bone loss.

Genetic alteration of test animals using a hyperactive promoter of osteocalcin expression or direct injection of test animals with uncarboxylated osteocalcin could confirm that an excess of this protein contributes to bone loss. If bone loss increases when either of these methods is used to increase osteocalcin levels to those seen in hypervitaminosis D, this would confirm that excessive osteocalcin expression forms part of the mechanism by which hypervitaminosis D results in bone loss. Since osteocalcin is expressed exclusively in osteoblasts, osteocalcin circulating in the serum may not exert the same effects on bone matrix that would be exerted by osteocalcin produced endogenously by osteoblasts; this portion of the hypothesis could therefore only be reliably falsified if bone loss does not increase in response to genetic alteration; the observation that bone loss does not increase in response to direct injection of osteocalcin would be insufficient to falsify it.

In the event that initial experiments show overexpression of MGP to induce an absolute deficiency of vitamin K, it would be difficult to distinguish an independent effect of a relative deficiency. Since the two possibilities are not mutually exclusive, however, the latter could not be ruled out.

The protective role of vitamin A

If vitamin A protects against vitamin D toxicity by exerting a vitamin K-sparing effect through the downregulation of MGP, we should expect to be able to make several observations: first, the protective effect of vitamin A should be proportionate to the degree to which it reduces tissue levels of MGP; second, vitamin A should protect against toxicity induced by the genetic overexpression of MGP in the same way that it protects against vitamin D toxicity; third, in both cases vitamin A should exert a dose-dependent restoration either of the normal tissue levels of vitamin K or of the normal unrecycled-to-recycled vitamin K ratio. If each of these predictions is observed, it would confirm the proposed mechanism by which vitamin A exerts its protective effect, but it would not rule out the possibility that vitamin A exerts a protective effect through additional mechanisms. The strongest evidence for this latter possibility would be the observation that the combination of vitamins A and K exerts a more complete protective effect than can be achieved by using a higher dose of vitamin K alone.

Applications of the hypothesis

Confirmation of this hypothesis would demand that vitamin D toxicity no longer be seen as a function of the absolute amount of vitamin D, but instead as a function of the balance between vitamins A, D and K. This would in turn require substantial revisions to the way in which vitamin D is studied as well as the way in which it is used therapeutically.

The potential utility of these revisions can be illustrated by using several examples from the literature: in the first example, a case-control study of a South Indian population published in 2001 showed that men with 25(OH)D levels (the serum marker used to measure vitamin D nutritional status) above 223 nmol/L had three times the risk of heart disease than those with 25(OH)D levels below 223 nmol/L [30]; in the second example, a clinical trial testing the ability of 400 IU of vitamin D and 1000 mg of calcium to lower the risk of hip fracture in postmenopausal American women published in 2006 found that those using the supplements had a 17% increased risk of kidney stones [31]; in the third and final example, an epidemiological study of blacks, whites and Mexican Americans residing in the United States published in 2004 showed remarkably different effects of 25(OH)D levels on bone mineral density between racial and age groups with no apparent explanation for the difference [32].

In the last example, the Mexican Americans over the age of 50 with the highest bone mineral density had 25(OH)D levels between 110 and 140 nmol/L. By contrast, the bone mineral density of whites of the same ages began declining after 25(OH)D levels reached 98 nmol/L and that of blacks of the same ages began declining at 90 nmol/L. While Mexican Americans with a 25(OH)D level of 140 nmol/L had excellent bone mineral density, blacks with the same 25(OH)D level had as poor a bone mineral density as those with the borderline deficient level of only 40 nmol/L. For whites under the age of 50, by contrast, bone mineral density continued to increase past 170 nmol/L. There was no explanation for why the bone mineral densities of these different racial and age groups would exhibit such disparate responses to vitamin D levels.

In each example, neither the intakes of vitamins A and K nor the use of coumadin derivatives were assessed. If the hypothesis presented in this

paper were confirmed and accepted, however, arterial calcification, renal calcification and bone mineralization would be seen as functions of the interaction between vitamins A, D and K. The hypothesis therefore has the potential not only to explain why vitamin D may contribute to these endpoints at given doses, but why the responses of these endpoints to a given dose or serum level of vitamin D may vary widely between different groups that may have differential intakes of vitamins A and K or differential use of coumadin derivatives. Future studies of vitamin D toxicity and efficacy would need to evaluate these interacting factors together in order to generate valuable conclusions.

If the mechanism of vitamin D toxicity presented in this paper is correct, official recommendations regarding the safety of vitamin D will have to be revised to take into account the effects of vitamins A and K, while physicians and health care professionals would be able to treat patients with doses of vitamin D that have higher therapeutic values than those currently used but do not carry the risk of toxicity by administering this vitamin together with vitamins A and K.

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