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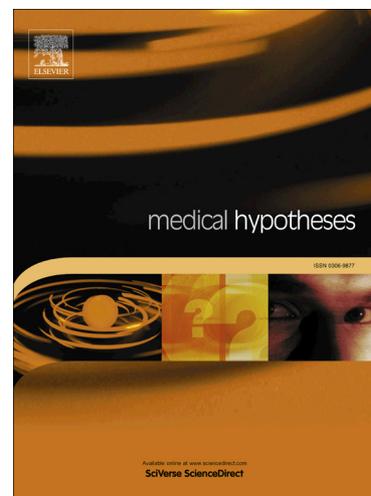
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Randomized clinical trials of oral vitamin D supplementation in need of a paradigm change: The vitamin D autacoid paradigm.

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Abstract

Epidemiological studies highlight the negative correlation between vitamin D levels and the incidence of many non-skeletal diseases including inflammatory diseases, cancer, and metabolic and neurological disorders. However, most randomized controlled trials (RCTs) with oral vitamin D supplementation give mixed results or are inconclusive. It has been said that *“discovery commences with the awareness of anomaly”*. The *“anomaly”* between our preclinical and clinical data provides the opportunity to propose an alternative paradigm to the vitamin D endocrine system: the vitamin D autacoid paradigm. In the vitamin D autacoid paradigm, the extra-skeletal effects of vitamin D depend on the tissue reserves of vitamin D metabolites. These vitamin D autacoid systems are inducible oscillatory ecosystems in which

1,25D is produced, acts and is inactivated locally. In the vitamin D autacoid paradigm, attaining adequacy of vitamin D in the systemic circulation is necessary but not sufficient; we must also ensure the repletion of the tissue stores. The co-existence of two different vitamin D systems, endocrine and autacoid, with different functions and regulations leads to “*significant shifts in the criteria determining the legitimacy both of problems and of proposed solutions*”. With respect to our clinical trials of vitamin D supplementation for unconventional effects, the proposed solution is administering and quantifying vitamin D metabolites directly to the target tissue.

Key words: *vitamin D; autacoid; paracrine; brain; paradigm shift.*

Introduction

Epidemiological studies highlight the negative correlation between vitamin D levels and the incidence of many non-skeletal diseases including inflammatory diseases, cancer, and metabolic and neurological disorders [1]. Even if the association does not imply causation, a large number of laboratory experiments further support these extra-skeletal functions of vitamin D. Nevertheless, most randomized controlled trials (RCTs) with oral vitamin D supplementation on these such so-called unconventional health effects give mixed results or are inconclusive [2–14]. The possible reasons why the results of randomized controlled trials do not meet our expectancy have been already extensively discussed. Co-variation, reverse causality, long-term outcomes, population heterogeneity with individual differences in

response to supplementation and the issue of co-nutrient status are some reasons proposed (see for example [15–18]). Another more drastic possibility is that the paradigm in which we conduct our trials, namely, the vitamin D endocrine system, is not adequate to handle the non-skeletal effects. *“Discovery commences with the awareness of anomaly”*[19]. The discrepancy between our preclinical and clinical data should be considered as an opportune worth investigating anomaly.

The vitamin D endocrine system.

The vitamin D endocrine system paradigm states that vitamin D, either produced in the skin upon UVB exposure or provided by the diet, circulates in the blood and is hydroxylated in the liver to form 25-hydroxyvitamin D (25D), and thereafter in the kidney to generate the most active vitamin D metabolite, namely, 1,25 dihydroxyvitamin D (1,25D). The blood concentration of 1,25D is strictly regulated and does not significantly change with vitamin D intake, except in the case of extreme vitamin D deficiency or vitamin D overload [20]. On the other hand, the blood level of 25D varies depending on vitamin D synthesis by the skin, dietary intake and vitamin D supplementation [20]. For example, 25D levels vary with sun exposure due to vitamin D skin synthesis, while circulating 1,25D levels remain nearly constant throughout the year [21,22]. Similarly, in adults, the daily ingestion of 1000 IU vitamin D₃ increases the serum concentration of 25D, but the level of 1,25D does not change [23]. This mechanism is why our vitamin D clinical trials are based on 25D status and not 1,25D status. Note, however, that a meta-analysis of randomized controlled trials concluded that vitamin D supplementation could increase circulating 1,25D concentrations, but did not sufficiently affect calcium homeostasis [24]. Hence, even if vitamin D supplementation might enhance circulating 1,25D levels during some clinical trials, it is still not sufficient to affect the reference marker of the vitamin D endocrine system, which is calcium homeostasis. The physiology of

the vitamin D endocrine system in which 1,25D blood levels are strictly regulated generates the paradox of our ongoing clinical trials of oral vitamin D supplementation; our oral vitamin D supplementation effectively increases circulating 25D, but the results provide no evidence of a functional effect on the reference markers that are serum 1,25D and calcium levels. In the absence of any positive control for the functional efficiency of vitamin D supplementation, these trials cannot be conclusive. In the endocrine paradigm, and in the absence of vitamin D deficiency, blood 1,25D is so tightly regulated that increasing its concentration by oral vitamin D supplementation to achieve unconventional effects without causing hypercalcaemia in trial participants is similar to trying to square the circle.

In other words, we cannot handle the unconventional effects of vitamin D supplementation through the endocrine paradigm. For extra skeletal diseases, attaining vitamin D adequacy in the systemic circulation is necessary but not sufficient: we need a paradigm shift.

The vitamin D autacoid paradigm.

The word "autacoid" comes from the Greek *autos* (self) and *akos* (remedy). In autacoid systems, molecules are produced on demand and act locally in the same cells or tissues through autocrine and paracrine signalling [25]. Some examples of autacoids are histamine, serotonin, bradykinin and several lipids involved in the modulation of inflammation, including members of the Specialized Pro-resolving Mediators (SPM) family[25,26]. Recently it has become evident that extra-liver and extra-renal metabolism of vitamin D does occur [27]. For example, cutaneous [28,29] , fat [30], immune and nervous tissues [31,32], among others, can express the genes encoding the enzymes that activate vitamin D or 25D in 1,25D. The expression in these tissues of these enzymes together with the presence of the Vitamin D receptor (VDR) represents vitamin D autacoid systems [33–35]. Hence, when investigating the

unconventional effects of vitamin D, we must address two frameworks: the endocrine and the autacoid paradigms.

Several critical points differentiate the vitamin D autacoid systems from the endocrine system. They are inducible, for example by inflammatory stimuli, and the local increase in 1,25D is transient and resolves itself by the induction of *CYP24A1*, which encodes the enzyme that inactivates 1,25D into 1,24,25D. This mechanism means that 1,25D is produced, acts and is inactivated locally. Autacoid systems are oscillating ecosystems that do not affect serum 1,25D. A corollary of the existence of such inducible vitamin D autacoid systems is that they are not constitutively turned on by the circulating levels of 1,25D. This mechanism makes sense if we consider, for example, that the immunomodulatory function of 1,25D must be limited both in time and in space at the foci of inflammation. In the autacoid framework, the regulation is achieved by inducing locally the production of 1,25D and the expression of the VDR.

Another distinctive point is that the local synthesis of 1,25D requires that its precursors, namely, vitamin D and 25D, have sufficient local bioavailability. It is commonly accepted that circulating 25D makes the bioavailable precursor pool for 1,25D. However, what is correct in the vitamin D endocrine framework may prove to be incomplete in the autacoid paradigm. In the vitamin D autacoid paradigm, not only the circulating 25D but also the tissue reserves of vitamin D metabolites are important for the non-conventional effects of vitamin D [36]. Hence, in the autacoid framework, it is necessary but not sufficient to attain adequacy of vitamin D in the systemic circulation; we also need vitamin D adequacy in the sites of storage [36]. Adipose, skin and muscle tissues are the main body stores [30,37]. In the vitamin D autacoid framework, these reserves are used to produce 1,25D in the microenvironment thereby controlling, for example, the resolution of inflammation in a restricted location.

In addition to adipose, skin and muscle tissues, other sites of storage likely exist. For example, in the human brain, the highest levels of 25 D are found in the corpus callosum that does not contain detectable 1,25 D [38]. This observation suggests that 25D might be stored in the myelin sheath (Fig. 1A). If this hypothesis is correct, the degradation of the myelin sheath by microglial cells during demyelinating or neurodegenerative processes [39] makes possible the synthesis by microglial cells of 1,25D from the myelin 25D stores [40]. This would in turn trigger the immunomodulatory and neuroprotective response mediated by 1,25D (Fig. 1B). Note that the local levels of 25D and 1,25D would be much higher in the inflammatory microenvironment than in the circulating blood or cerebrospinal fluid. In humans, 86% of the final volume of myelin is observed at 5 years, and reaches its maximum at 17 years [41]. This raises the question to know if an oral vitamin D supplementation in the adulthood is sufficient to replete the 25D stores outside of these critical periods and how long will it take? Myelination is also dynamic in adults and increases in response to external stimuli such as a learning stimulation. The possibility that the 25D storage capacity and therefore the response to oral vitamin D supplementation also depends on adult myelin plasticity warrants further investigation.

Another point raised by the existence of autacoid vitamin D systems is the question of whether the local delivery of vitamin D or 25D directly at the tissue level would be a better option than the oral supplementation to replete the tissues stores and to achieve the unconventional effects of 1,25D without causing hypercalcaemia [36]. For example, could the transcutaneous delivery of vitamin D or 25D in the breast adipose tissue be more effective than oral supplementation on breast cancer incidence, progression or therapeutic response? Note that covering the female breast is specific to humans, recent in evolution and prevents the synthesis of vitamin D by the breast skin. We should perhaps evaluate whether some of the

vitamin D and 25D that should have been locally produced by the skin naturally exposed to the sun is intended to be directly stored in the underlying adipose tissue as part of an evolutionary preventive mechanism against breast cancer.

Concluding remarks and perspectives.

The unconventional effects of vitamin D cannot be fully accounted for by means of the endocrine paradigm used to address calcium homeostasis and rickets. Nearly a century after the discovery that rickets is an endocrine disease associated with low 25D blood levels, we are discovering that autacid diseases associated with low vitamin D or 25D tissue levels also exist. However, this issue cannot be adequately handled within the framework of the vitamin D endocrine paradigm. The goal to attain adequacy of vitamin D in the systemic circulation is necessary for endocrine functions, but is insufficient for autacid systems that also depend of the local reserve of vitamin D metabolites at the tissue level. Assuming the co-existence of two different vitamin D systems, endocrine and autacid, with different functions and regulations leads to *“significant shifts in the criteria determining the legitimacy both of problems and of proposed solutions”*[19].

With respect to our clinical trials of vitamin D supplementation for unconventional effects, the proposed solution is administering and quantifying vitamin D metabolites directly to the target tissue.

Conflict of interest statement:

The authors declare no conflict of interest.

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Legend to Figure.

In the human brain, the highest level of 25D is found in the myelin-rich corpus callosum. The storage of 25D in the myelin sheath makes sense from physicochemical reasons; 25D is lipophilic (A). Microglial cells actively participate in the clearance of myelin debris produced

either in the course of myelin turnover or during neuroinflammatory or neurodegenerative diseases [41]. Activated microglial cells also metabolizes 25D into 1,25D. In the proposed hypothetical autacoid model (B), the 25D myelin reserves are made available at the site of neurodegenerative processes because of myelin destruction. In the presence of myelin debris microglial cells metabolize 25D to 1,25D in the immediate extracellular space that would in turn trigger the immunomodulatory and neuroprotective function of 1,25D [31,42–44]. Note that when the autacoid vitamin D system is turned on, the level of 1,25D only increases in the microenvironment where it is much higher than its physiological blood or cerebrospinal fluid concentration. In autacoid systems, 1,25D is produced from the local tissue reserves, and it acts and is degraded locally. All these processes are independent of the vitamin D endocrine system [36].