Vitamin D3 Versus Gliadin: A Battle to the Last Tight Junction

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Celiac disease (CD), the most common chronic autoimmune enteropathy present in Western populations, is triggered by gluten ingestion in genetically susceptible individuals carrying the HLA DQ2 and/or DQ8 loci [1]. Gluten, a high molecular weight protein present in the endosperm of grass-related grains, including wheat, barley, and rye, is stored within seeds in order to ensure a stable nutrient supply supporting the germination and development of young plants. Gluten-containing cereals, the most important crop in the world, are used to make food products such as pasta, bread, other baked and pastry products. The viscoelastic and stabilizing properties of gluten have fostered its use as an additive in the baking industry; furthermore, it gives food greater palatability due to the creation of disulfide bonds that in combination with atmospheric oxygen and nitrogen alter the properties of the dough as a function of the sulfhydryl and disulfide content [1]. Gluten is a composite of two classes of protein, glutenins and prolamins (gliadin, secalin, and hordein), which can be further fractionated to produce peptides. Pepsin–trypsin-resistant gliadin (PT-G) is an undigested gliadin fragment that substantially contributes to the pathogenesis of CD by altering intercellular tight junctions (TJs) [2].

In CD, a genetic predisposition and exposure to environmental triggers cooperate in the loss of the intestinal barrier function. Gluten peptides and gliadins permeabilize the gut, which provokes an immunomodulatory cytotoxic effect and opens TJs. Subsequently, the deregulated traffic of macromolecules, due to the “leaky gut,” severely damages the intestine, thus fueling the chronic inflammatory process.

The TJ, a dynamic protein complex, is the primary determinant of the paracellular flux of fluid and solutes in a healthy epithelium. The integrity of the barrier is important for the separation of the two compartments: the luminal and the submucosal sides [3]. TJs govern the permeability of the intestinal mucosal barrier; even minimal alterations in TJ function allow the passage of potentially toxic macromolecules through the intercellular spaces in the subcellular matrix, a condition called “leaky gut syndrome” that is common in many inflammatory diseases of the small bowel [4–6].

The three-dimensional structure of many cells is governed by a network of polymeric proteins including actin filaments termed the cytoskeleton, the organization of which is implicated in the pathogenesis of CD. Gliadin causes the disarrangement and disappearance of epithelial cellular organization in CD patients [7]. TJs are demonstrably associated with the actin filaments in epithelial cells assembling in a fusion structure called “kissing points.”

The inflammation and autoimmunity that occurs secondary to the ingestion of gluten in predisposed subjects disconnects innate and adaptive immunity. Zonulin is the only known modulator of intercellular TJs, regulating intestinal permeability [8]. During the acute phase of CD, the zonulin pathway is upregulated. For all the aforementioned reasons, investigation is extremely active into drugs

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and other bioactive molecules that restore intestinal barrier function and suppress the inflammatory mechanism.

Vitamin D, a fat-soluble vitamin naturally present in a variety of animal and plant foods and also produced endogenously as a result of sun exposure, maintains and supports the health, the bones, the immune system, and the nervous system. Vitamin D also influences the expression of genes implicated in cancer development and preserves lung function and cardiovascular health. Serum vitamin D concentrations depend on the balance between intake and absorption; vitamin D deficiency can cause muscle weakness, an increase in fracture risk, and may intensify bone loss, requiring vitamin D supplementation for the management of osteopenia or osteoporosis. The suggested dosage of vitamin D depends on the severity of its deficiency. The two common forms of the vitamin supplements are ergocalciferol and cholecalciferol, the latter being the more active and efficient form.

In this issue of *Digestive Disease and Sciences*, Dong et al. published an innovative paper that demonstrated the beneficial effects of the vitamin D metabolite 1,25-dihydroxycholecalciferol (VD3) or calcitriol, a health-promoting molecule [9]. VD3 is thought to be the most active metabolite of vitamin D in humans, a modulator of calcium homeostasis and regulator of electrolytes and blood pressure. Their manuscript reports interesting results about the effects of VD3 on PT-G-induced TJ injuries in experimental systems in vitro and in vivo. The strongest effect of VD3 in reducing the monolayer barrier function was measured at 10^{-8} M, also the optimal concentration for upregulating TJ protein expression. 10^{-8} M VD3 has the most powerful effect in suppressing the release of zonulin and, moreover, the same concentration inhibits MyD88 expression, a factor inducing zonulin release. These results have been also confirmed in an in vivo model of gluten-sensitized mice. Thus, VD3 can protect the intestinal mucosal barrier from PT-G in a dose-dependent fashion in both model systems.

Several studies have debated the positive effects of VD3 on a range of gastrointestinal diseases, but, in particular, have described its protective action on TJ injury induced by chemical compounds and by PT-G. Other studies have helped define the mechanism by which VD3 inhibits intestinal MyD88 expression [10]: Specifically VD3 interrupts the MyD88-dependent zonulin release signaling cascade, a key pathway that should serve as the basis of effective new treatments for CD. Indeed, zonulin is the major TJ regulator, associated with deregulation and damage of TJs, of particular interest according to available studies of zonulin inhibitors such as larazotide that protect the small bowel mucosa and reduce the passage of toxic peptides and molecules into the subcellular matrix [11].

In view of the low toxicity of VD3 and its considerable clinical experience, the positive findings of Dong et al. are encouraging for the future research on CD treatment. In particular, VD3 is potentially an attractive therapy for administration to CD patients following an on-demand strategy in the case of accidental or suspected occult gluten ingestion. Nevertheless, the bioavailability of VD3 and the form of vitamin D that is most efficient in humans warrants further study. VD3 is a fat-soluble vitamin that is absorbed by a pathway similar to that followed by long-chain lipids that may need to be optimized in malabsorbing patients, along with research into newer more active forms of vitamin D.

The results of the present study go beyond the potential use of VD3 in CD; indeed, the proven benefits of VD3 can be widely applied to the other gluten-related disorders such as non-celiac gluten sensitivity (NCGS) [12], thus giving the patients an alternative to the gluten-free diet for reducing their symptoms. The pathogenesis of NCGS differs from the autoimmune pathomechanism of CD and its associated diseases or complications [13], although increased bowel permeability is present in NCGS, as described by Camilleri et al. [14]. Due to the benign nature of NCGS and its usually mild or moderate clinical picture, the therapy based on a strictly and sometimes chronic gluten-free diet may be considered excessive, considering its impact on the quality of life of patients. The intake of VD3 is potentially helpful to attenuate the TJ injuries directly induced by PT-G and improve the condition of the “leaky gut” syndrome that persists in these patients. Furthermore, vitamin D intake is potentially perceived as positive due to its “natural” as opposed to synthetic origin.

In conclusion, there is a strong need to translate the experimental studies conducted in vitro and in the gluten-sensitized mouse model into clinical trials—both randomized and double-blind—in order to detect the effects of vitamin D on humans. On the basis of this interesting study, new therapeutic strategies are needed to re-establish intestinal barrier function, inhibiting the zonulin pathway, and discovering new approaches for the management of these chronic diseases.

Key Messages

- The pathogenesis of celiac disease is complex and multifactorial; however, a zonulin-driven tight-junction alteration seems to be an early step in the inflammatory cascade.
- Tight-junction damage and leaky gut syndrome facilitate the passage of gliadin peptides into the submucosal layer, where the autoimmune process starts and is fueled.

- Vitamin D is a health-promoting molecule involved in different biological processes and potentially preserving the tight-junction structure through a MyD88-related downregulation of zonulin.
- The study by Dong et al. demonstrates that the vitamin D metabolite calcitriol prevents the tight-junction damage induced directly by gliadin in vitro and in an animal model.
- The positive effect of vitamin D on tight junctions can be used in celiac disease therapy to prevent the passage of peptides into the lamina propria that enhances the inflammatory process.
- The findings related to intestinal barrier damage and the benefit from vitamin D use suggest its application to also other gluten-related disorders (such as non-celiac gluten sensitivity) where the presence of altered intestinal permeability is proven.

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