Glucosamine and O-GlcNAcylation: a novel immunometabolic therapeutic target for OA and chronic, low-grade systemic inflammation?

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Osteoarthritis (OA) is a disease with a very long course and varied clinical expression in the initial stages, when it is really challenging to adequately measure disease outcomes both in clinical trials and in daily life.[1] It is also very difficult to accurately study the efficacy of symptomatic slow-acting drugs for OA and that of non-pharmacological treatments.[2] Although the efficacy of glucosamine (GlcN) in the treatment of OA is still a controversial issue[3 4], recent high-quality epidemiological studies confirm previous data showing that prolonged GlcN intake, regardless of its effect on OA progression, could decrease cardiovascular disease (CVD) events, and the incidence of CVD-associated diseases.[5–8] These data should be analysed keeping in mind that CVD is the main cause of death in patients with OA.[9] Different authors suggest that this protective effect may be associated with the anti-inflammatory properties of GlcN,[5] although the molecular basis has been only partially defined.

The imbalance between the mechanical loading and its absorption by the articular cartilage is the origin of joint tissue alteration in OA. While a severe overload can deteriorate any kind of cartilage, a certain load that is physiologically well tolerated by a robust cartilage could be the origin of pathological alterations for a weakened one.[9] Cartilage damage begins when the load prevails over the resistance, which in the midterm activates innate immune response in the different joint tissues, and is, at least partially, responsible for joint deterioration. Unbalanced mechanical forces and damage-associated molecular patterns turn on the innate immune system through the activation of the Toll-like receptors. Once this response is activated, a secondary wave of inflammatory mediators is released, with a robust increase in the concentration of cytokines and metalloproteases, the final effectors of cartilage destruction.[9] Certainly, these mediators can be also partially released into the circulation in small amounts but during extended periods of time, then contributing to what has been defined as chronic low-grade systemic inflammation (CSI).

Several factors increasing CVD in patients with OA revolve around CSI and lack of exercise and physical activity.[10 11] CSI is clinically silent; it is not associated with energy expenditure—unlike classic inflammation that consumes it in huge amount—and it mainly involves molecular signalling instead of cell proliferation, all accounting for its difficult therapeutic management.[10] Low-grade CSI is commonly triggered by overnutrition, that leads to obesity and/or diabetes, and hypercholesterolaemia. These metabolic alterations result in a prominent increase in the synthesis of adipokines and proinflammatory mediators by adipose tissue, and its infiltration by immune cells.[11]

It is important to highlight that these OA pathogenic factors: mechanical and nutrient overload, as well as adipokines and proinflammatory cytokines, converge in the same regulatory master nodes within the cell. The chronic stimulation of the innate immune system results in a robust activation of nuclear factor kappa-B (NF-κB), mitogen-activated protein kinase (MAPK) and phosphatidyl-inositol-3-kinase (PI3K)-dependent pathways.[9]

Therefore, mechanical stress, low-grade CSI and metabolic imbalance are the driving factors for OA onset and progression. The last two are very active in CVD. Overall, this is the immunometabolic framework where OA is developed.[11]

In turn, epidemiological data provided by several sound studies strongly suggested an association between GlcN use and lower risk of CVD, cancer and other diseases.[5–8] The current report by Li *et al*, involving 495 077 individuals, provides further evidence for the association between GlcN use and lower risk for all-cause mortality (15%) and cause-specific mortality, including CVD (18%), cancer (6%), respiratory (27%) and digestive (26%),[6] supporting previous studies.[5 12] However, these data should be evaluated with caution, as unexpected bias in the study design could exist. Population taking regular prescribed medication or supplementations, including GlcN, could constitute a subgroup of patients with a better health rating, self-care or other unmeasured lifestyle-related factors. Indeed, it is impossible to evaluate these features in studies of this nature, despite a careful adjustment for the measured confounders undertaken. In addition, a 5.5% of response rate observed in this cohort could also limit the extrapolation of the results to the general population. Besides, data collection on medications intake did not include detailed information on GlcN dosage, duration of the treatment or GlcN formulation. Lastly, although authors exclude participants who died within 2 years of follow-up, a potential reverse causation cannot be ignored.

Authors demonstrate that GlcN exerts a greater protective effect for all-cause mortality in smokers, which could be related to a greater degree of systemic inflammation observed in this group.[6] Intriguingly, an inverse relationship between smoking and OA has been described.[13] Prolonged GlcN intake has been previously associated with a decrease in serum C-reactive protein (CRP).[14] Furthermore, studies on type 2 diabetes (T2D) also indicate that patients on GlcN treatment showed a decrease in T2D incidence, after adjustment for different confounding factors.[8] Again, the decrease in T2D incidence was greater in patients with higher serum CRP. Overall, a relationship between the higher rate of CSI and the protective effect of GlcN intake is observed.

The analysis of the effect of GlcN should consider pharmacokinetic data. After a usual oral dose intake, 1500 mg, GlcN serum concentration increases and began to be detected within 30–45 min, reaching maximum values after 2–3 hours, with a range between 2 and 12 µM (0.34–2 µg/mL).[15] In healthy volunteers, several daily doses of 1500 mg GlcN were administered until a steady state was reached, when the maximum concentration was around 10 µM, 3 hours after product administration. These concentrations were up to 100 times higher than endogenous levels.[16] However, GlcN levels rapidly decreased, being almost undetectable after 5–8

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hours.[15 16] It is unknown the GlcN serum concentration to consider this drug as a pharmacologically active agent, or the concentration needed to modulate some specific mechanisms. In ageing mice, long-time GlcN intake induced an increase in its plasma concentration up to 2 µM, that was able to increase the life span in these animals.[17] Overall, conventional GlcN administration appears to cause daily peak and trough levels in serum and tissue. GlcN concentration fluctuates in such a way that it can be considered an intermittent administration, even for long-term treatments. This pharmacokinetics could have unexpected consequences in the regulation of different metabolic cell pathways.

Different studies have tried to decipher the mechanisms by which GlcN could decrease CVD. Although no preclinical models that mimic OA-induced atherosclerosis have been described, the results in an experimental model of chronic arthritis-aggravated atherosclerosis in rabbits with hypercholesterolaemia could shed some light. Severe chronic arthritis in rabbits with hypercholesterolaemia and balloon-induced vascular damage increased plaque instability in the injured artery, and leads to the appearance of spontaneous plaques in the thoracic aorta.[18] This model mimics the accelerated atherosclerosis observed in CSI diseases, in the presence of a metabolic imbalance. Oral high doses of GlcN, following the human therapeutic regimen, substantially prevented the development of atherosclerotic lesions, reduced serum inflammatory markers and inhibited NF-κB activation in circulating mononuclear cells.[18] Although the ability of GlcN to inhibit NF-κB activation has long been recognised,[19] the precise molecular mechanism is still under study.

Recent data have demonstrated that GlcN administration to septic mice decreases systemic inflammation and improves cardiovascular dysfunction, while an increase in the O-N-Acetyl-glycosylation (O-GlcNAc) of NF-κB p65 subunit was reported.[20] Interestingly, we have also observed that prolonged GlcN administration to OA rabbits decreases joint damage and cartilage inflammation in correlation with a profound modification in the amount of O-GlcNAc proteins.[21] Furthermore, GlcN induced a decrease in cartilage NF-κB activation, in parallel to an increase in p65 O-GlcNAcylation (Largo, unpublished data).

O-GlcNAc, or the attachment of O-N-Acetyl-glucosamine to a protein, is a particular post-translational modification (PTM) by which this single sugar adds to serine and threonine residues of nuclear and cytoplasmic proteins (figure 1). O-GlcNAcylation occurs in thousands of proteins. A single pair of enzymes—O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA)—controls the highly dynamic cycling of this protein modification.[22 23] OGT transfers this sugar from a unique donor substrate, uridine diphosphate GlcNAc (UDP-GlcNAc), that is the final product of nutrient flux through the hexosamine biosynthetic pathway (HBP), which integrates glucose, amino acid, fatty acid and nucleotide metabolism to synthesise the molecule (figure 1).

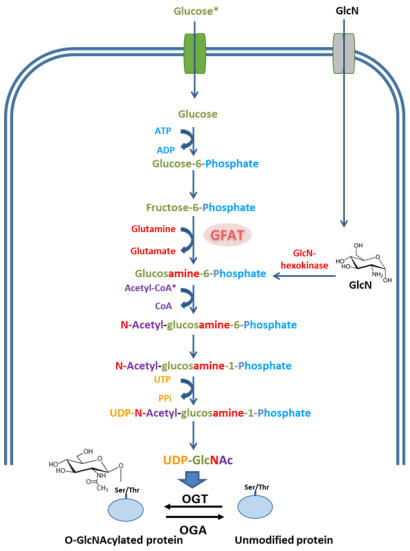

Figure 1 The hexosamine biosynthetic pathway (HBP) and O-GlcNAcylation process. Glucose enters the cell and is phosphorylated and then converted to fructose-6-phosphate. This product is then oxidised through glycolysis, and 3%–5% of it is converted to glucosamine-6-phosphate (GlcN-6P) by the limiting enzyme of this pathway, glutamine:fructose-6-phosphate-amidotransferase (GFAT), that transfers the amine group from the amino acid pool. Glucosamine-6P can also be obtained by the phosphorylation of GlcN. In this way, GlcN intake is able to increase HBP flux when the cell is exposed to pharmacological extracellular concentrations of this molecule, bypassing GFAT regulation. The corresponding transferase adds the acetyl group from Acetyl-CoA, a key metabolic node mainly coming from fatty acid metabolism. The sequential synthesis of uridine-diphosphate-N-Acetyl-glucosamine-1-P (UDP-NAcGlc-1-P) incorporates the residue that proceeds from the nucleotide metabolism, the uridine-triphosphate (UTP). The final molecule synthesised in this pathway, UDP-GlcNAc, is the unique donor for O-GlcNAc modification of nuclear and cytoplasmic proteins by O-GlcNAc transferase (OGT). In addition, O-GlcNAcase (OGA) catalyses the removal of O-GlcNAc. *The metabolic molecules whose excess has demonstrated to increase HBP flux, thus increasing the amount of O-GlcNAcylated proteins.

O-GlcNAcylation plays a key role in the regulation of both cellular homeostasis, in response to nutritional or hormonal cues, but also in response to stress or damage, such as that induced by inflammation or immune activation.[24–26] In fact, protein O-GlcNAc has been proposed as a nutrient sensor that regulates crucial cell responses to very different pathophysiological processes,[24–27] ranging from cell transcription to protein folding or degradation.

The large number of proteins and processes modified by O-GlcNAc by two such promiscuous enzymes gives an idea of the complex regulation of this process within the cell. The exposure to increased concentration of nutrients, particularly glucose and fatty acids in cultured cells, increases HBP flux, then enhancing the pull of O-GlcNAc proteins. In fact, the negative effects of chronic hyperglycaemia have been associated with the accumulation of O-GlcNAcylated proteins in different tissues.[28] However, changes in HBP flux and UDP-GlcNAc concentration are not the only factors driving this PTM. O-GlcNAc levels also vary on a substrate-specific basis, and depending on OGT and OGA and its adaptor proteins location and interaction with the substrate.[23] In addition, O-GlcNAc signalling is under tight temporal control. Cellular O-GlcNAcylation levels decrease in hours after glucose deprivation and increase at later time points. Adipocytes in response to insulin, or neurons following depolarisation, change the O-GlcNAc amount within minutes. Therefore, protein O-GlcNAc is highly changing and not only dependent on OGT and OGA presence and activity, but also on nutrient availability, which makes this type of PTM highly sensitive to very different forms of cellular stress.

Acute and chronic alterations in the amount of O-GlcNAcylated proteins have been associated with different human diseases. Experimental cardiac ischaemia/reperfusion models showed that acute increases in protein O-GlcNAcylation induced by GlcN or glutamine correlated with a better functional recovery, while similar conclusions were obtained with the more clinically applicable remote ischaemic preconditioning.[29] In contrast to the benefit in acute and transient elevations, chronic increments or decreases in the levels of O-GlcNAcylated proteins have been associated with the pathogenesis of degenerative diseases such as Alzheimer's and Parkinson's, cancer, diabetes and

its complications, as well as ageing and OA.[23 30]

Interestingly, the cellular O-GlcNAcylation has been described as a buffering system, finely tuned, designed to tolerate moderate and acute cell alterations and adapt cell response accordingly.[23] Moderate or acute alterations in nutrient availability will produce transient fluctuations in the pull of O-GlcNAc proteins. In turn, chronic alterations will overcome the system, resulting in cell and tissue damage.[23] It is conceivable that different interventions able to restore an exhausted buffering system, such as caloric restriction or low-carbohydrate diet, could in some way trigger the return to cell homeostasis.

Long-term GlcN intake would lead to daily intermittent changes in the concentration of this pharmacologically active agent, that would act as mild acute impacts on HBP flux, improving cell autoprotective systems as those induced after cardiac ischaemia/reperfusion. GlcN, as an HBP metabolite that bypasses the rate-limiting step of this pathway (figure 1), has demonstrated to significantly modulate O-GlcNAc. In different preclinical models, GlcN improved local and systemic inflammation and prevented tissue damage simultaneously with a significant increase in O-GlcNAc.[17 20 21] GlcN could restore the exhausted buffering capacity of the O-GlcNAcylation system, improving overnutrition-related chronic inflammation. However, the time, concentration and tissue-dependent specific effects of GlcN are mainly unexplored.

One of the most outstanding conclusions of these observations is to recognise that a modest pharmacological effect, if it is time extended, could render a reasonable benefit. Although a difficulty arises, as the measurement of the effect size is extremely difficult. The 20% improvement observed in CVD with GlcN; it is challenging to be detected in OA over years of disease progression, with the clinical and imaging methodology currently used in clinical trials. In any case, what is important is not the magnitude of the effect but the door opened in the search for new therapeutic targets.

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