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Shifting the focus of D-glucosamine from a dietary supplement for knee osteoarthritis to a potential anti-aging drug

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ABSTRACT

D-Glucosamine is a protective dietary supplement or medicine for osteoarthritis of the knee, a musculoskeletal disease that leads to a significant deterioration in daily activities and quality of life. As glucosamine can restore damaged cartilage worn down by joint disease, there was hope it could also improve symptoms. Data from several clinical studies on the efficacy of glucosamine on knee joint function conducted since the 1980s have been used in certain meta-analyses and epidemiological studies since 2010, yet the effect of glucosamine on the knee joints remains controversial. Concurrently, many drugs have been investigated for their anti-aging properties. Among these drugs, glucosamine has recently been discovered to be a potential substance with convincing evidence for increasing human lifespan. More interestingly, Zhi-Hao et al. have recently reported that the use of glucosamine was associated with a reduction in total mortality regardless of its effect on the knee (Annals of the Rheumatic Diseases 79(2020)829–836). Additionally, glucosamine prolongs the lifespan of the nematode *Caenorhabditis elegans*, possibly due to its calorie restriction-mimicking effect by improving energy metabolism and inducing autophagy. Thus, the recent large-scale epidemiological report on glucosamine intake and mortality, as well as our animal studies (Journal of Applied Glycoscience 65(2018)37–43), has become relevant. However, the potential significance of metabolism of glucosamine in anti-aging should be more clearly investigated in the future. This paper presents the novel concept of repositioning glucosamine from a dietary supplement or an OTC drug for osteoarthritis improvements to an anti-aging drug for healthy lifespan extension.

1. Introduction

Age-related diseases are becoming a social problem worldwide. Among them, the concept of "locomotive syndrome" has been proposed and attracted attention due to its association with a decrease in quality of life (QOL) [1]. Locomotive syndrome is a condition in which a person has difficulty walking due to impairment or deterioration of the motor organs. Consequently, there is an increased risk for a person to require nursing care. The causes of locomotive syndrome can be broadly divided into two categories: diseases of the musculoskeletal system and age-related musculoskeletal dysfunction. Diseases of the musculoskeletal system are associated with a variety of age-related musculoskeletal diseases, such as osteoarthritis (OA) and osteoporosis. In contrast, age-related musculoskeletal dysfunction includes muscle loss and muscle weakness [2,3]. Typical indicators of locomotive syndrome include loss of muscle strength and a decrease in walking speed. Walking speed decreases with age [4] and has been positively correlated with survival rates, which could be an indicator of vitality [5]. Thus, reducing locomotive syndrome may maintain QOL.

OA is a musculoskeletal disease that causes locomotive syndrome. Among OA and related diseases, knee OA (KOA) is the most common type and is associated with increased pain and difficulty in walking and a significant deterioration in QOL. Although current treatment options for KOA include surgical, pharmacological, and physical therapies, their effects are limited. Currently, treatment for KOA is dominated by pharmacotherapy to relive pain, including non-steroidal anti-inflammatory drugs (NSAIDs) and selective cyclooxygenase-2 (COX-2) inhibitors. However, NSAIDs carry a risk of causing gastrointestinal disorders, and COX-2 inhibitors can cause adverse cardiovascular effects. There are significant concerns regarding the side effects of these treatments [6]. Glucosamine (2-amino-2-deoxy-D-glucose) is a

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Abbreviations

AMP	adenosine monophosphate
AMPK	AMP-activated protein kinase
CI	confidence interval
CR	calorie restriction
CRMs	calorie restriction mimetics
COX-2	cyclooxygenase-2
GlcN	glucosamine
GlcNAc	N-acetyl glucosamine
HBP	hexosamine biosynthetic pathway
HR	hazard ratio
KOA	knee osteoarthritis
mTOR	mammallian target of rapamycin
Nrf-2	NF-E2-related factor 2
NSAIDs	non-steroidal anti-inflammatory drugs
OA	osteoarthritis
QOL	Quality of life
OTC	over the counter
SKN-1	transcription factor skinhead-1
UDP	uridine diphosphate

component of the cartilage worn out by joint diseases such as KOA and is used as a food supplement for the potential repair of damaged cartilage in patients with KOA based on the observation that symptoms improve as cartilage recovers. Clinical studies have been conducted to determine the efficacy of orally administered glucosamine on joint function since the 1980s [7].

Glucosamine is a constituent unit of chitosan, which exists in chitosan-based crustacean exoskeletons such as shrimp and crab (Fig. 1). It is industrially produced by hydrolysis of chitosan [8]. Unlike other anti-aging drugs, glucosamine is commonly used because it is inexpensive and has a low risk of side effects [9]. In the United States, glucosamine is classified as a dietary supplement and not for medical use. In most of Europe, glucosamine has been approved for use as a prescription drug and is sold in the form of glucosamine sulfate [10]. It is also distributed in the market as a dietary supplement and as a health food in most Asian countries. Thus, glucosamine has become very accessible worldwide, unlike other dietary supplements or drugs.

2. Meta-analysis of glucosamine

Glucosamine is usually taken in conjunction with chondroitin, but the individual effects of these substances remain unknown, thus resulting in confounding effects. A number of high-quality reviews and meta-analyses on glucosamine efficacy have been conducted since 2010. A meta-analysis including 10 large randomized, controlled trials of more than 200 patients with OA of the knee and hip were reviewed [11]. Glucosamine and chondroitin sulfate, taken alone or in combination, did not improve joint pain or the patient's condition. In another study in 2017, 6 of the 21 randomized, controlled trials were analyzed to evaluate the differences in the results of a total of 1663 people and found that the placebo had no effect on knee pain and joint function [12].

A meta-analysis in 2018 of 13 trials showed that glucosamine was not effective for alleviating pain, and no additional benefit was gained from its combination with chondroitin [13]. Another meta-analysis of various dietary supplements showed little significant difference between glucosamine and placebo, because glucosamine provided little moderate and clinically meaningful treatment effects on pain and function in patients with hand, hip or knee osteoarthritis [14]. On the other hand, a meta-analysis in 2018 showed improvement in 12 of 26 randomized, controlled trials of glucosamine [15]. Another 2018 meta-analysis of Japanese and Chinese data from randomized controlled trials showed that glucosamine slightly relieved knee pain [16], which differed from studies based on data from Europe and the United States [15] because this 2018 meta-analysis also incorporated data studies in Asian languages that focused on Asian populations [16]. Differences in effectiveness due to variations in race and dietary habits should also be examined to clarify the contrasts in the results of these studies. Thus, the effect of glucosamine on the knee joint remains controversial.

3. Effect of glucosamine on lifespan in epidemiological studies and animal studies, 2010–2020

Dietary restriction or calorie restriction (CR) is the most reliable method for healthy aging [17]. It reduces food intake without incurring malnutrition. Even if CR prolongs the human lifespan, it is difficult to enforce long-term CR in humans. Thus, it is preferable to develop a method or compound that reproduces the effect of CR without limiting the amount of food. The concept of CR mimetics (CRMs) was proposed by Lane et al. [18] in a study of 2-deoxy-glucose that showed anti-aging effects in animals. In addition, many drugs have been investigated for their anti-aging properties [19,20] and have included many CRMs, such as metformin, a diabetic drug [18,21]. While metformin has been considered the most promising CRM [19,20], there have been no long-term studies on healthy subjects. This is because metformin is a diabetic drug thus making it difficult to conduct large epidemiological studies in healthy subjects.

While the effects of glucosamine on the knee joint remain questionable, a large-scale epidemiological study of consumers of various dietary supplements showed that the use of glucosamine was associated with a reduction in total mortality regardless of the efficacy on the knee (HR: 0.83, 95%CI: 0.72 ~ 0.97) [22,23]. In addition, two animal experiments in 2014 and 2018 showed that the lifespan of mice and nematodes was extended with orally administered glucosamine [24,25]. In the 2014 mouse experiment, experimental data showed that the average lifespan of mice was extended by approximately 10% with improving energy metabolism. In our study using an aging animal model, the nematode *C. elegans*, life expectancy increased up to 30% at optimal conditions of administration dose (Fig. 2) and it was responsible for induction of autophagy. Additionally, a dose-dependent lifespan curve was observed, which is supported by previous epidemiological data showing lower mortality with higher doses of glucosamine [22]. Of further note is a large-scale prospective cohort study involving 490,000 subjects in 2020 reporting that glucosamine intake reduced the risk of cancer, heart disease, respiratory disease, digestive disease, and mortality [26]. This reduction in mortality (HR: 0.85, 95%CI: 0.82 ~ 0.89) may be due to a physiological effect that is quite different from the effect on the knee joint. There are reports of a possible explanation of

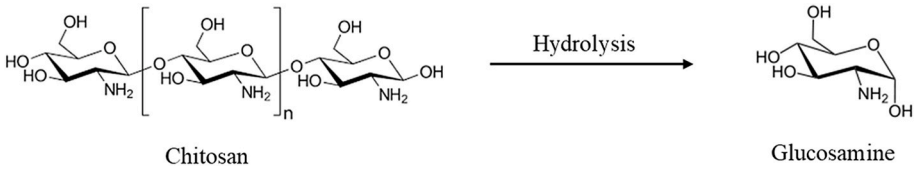

Fig. 1. Hydrolysis of chitosan.
Left molecular structures shows chitosan and right molecular structures shows glucosamine.Glucosamine is produced by hydrolysis of chitosan.

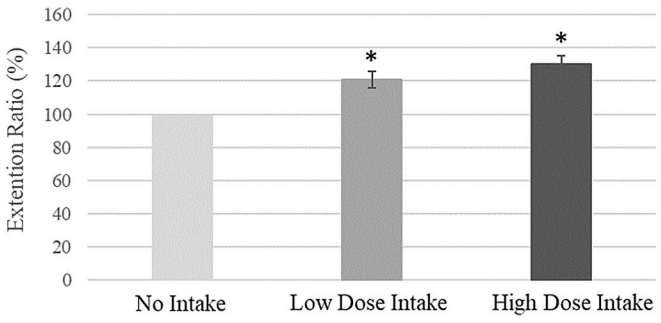

Fig. 2. Extension of lifespan by glucosamine in anematodes.
This graph is based on data from our reference [25]. Low dose refers to 10 mM and high dose refers to 25 mM as the glucosamine concentration in the nematode feed. The original data were statistically determined using the Kaplan-Meier method, and significant differences (vs control) were tested for significance using the log-rank test. Asterisk was considered significantly different when the *p*-value was lower than 0.001 vs control.

mechanism for this mortality reduction. In a clinical trial, oral administration of glucosamine modulates the intracellular redox state, thereby improving vascular endothelial function [27]. In addition, although this data has been reported in a preclinical study, the regulatory effect of glucosamine on carbohydrate metabolism and autophagy could play a role in its potential anti-aging effects, thus lowering mortality risk [28]. Therefore, based on the evidence that we could obtain, among the various CRMs and geroprotectors, glucosamine is one of the most likely to increase the healthy human lifespan.

4. Antiaging effects and mechanisms of glucosamine

Oral glucosamine is presumably absorbed from the intestinal tract, and much of it metabolized in the liver. It is conceivable that glucosamine metabolites in the liver or in other organs may have beneficial consequences such as improved metabolic regulation, or increased cellular stress resistance. There are two possible effects with respect to glucosamine metabolism. One involves implication in the synthetic pathway of UDP-GlcNAc, which is a raw material for glycoproteins, proteoglycans, glycolipids, and others termed the hexosamine biosynthetic pathway (HBP) [29]. Dietary glucosamine enters the cell via the glucose transporter, then becomes hexokinase-induced GlcN-6-P, and enters the HBP [30]. Thus, GlcN enhances HBP flux, ultimately reducing intracellular ATP [31]. A decrease in intracellular ATP levels causes autophagy [32]. The activation of HBP was recently reported to improve protein homeostasis through an integrated stress response [33].

Another is that an increase in HBP flux increases O-GlcNAcylated proteins in the nucleoplasm. O-GlcNAc is added to serine or threonine by an O-GlcNAc transferase even during phosphorylation. This is crucial for the regulation of intracellular signals, such as phosphorylation and dephosphorylation. O-GlcNAcylation of cardiac proteins has been recently shown to contribute to the regulation of transcription, metabolism, mitochondrial function, protein quality control and turnover, autophagy, and calcium processing [34]. In contrast, blockage of O-linked GlcNAcylation has recently been reported to induce AMPK (AMP-activated protein kinase)-dependent autophagy in mammalian cells [36]. In addition, O-GlcNAcylation of the transcription factor skinhead-1 (SKN-1), which is the ortholog of human NF-E2-related factor 2 (Nrf-2), has been reported to shorten lifespan in *C. elegans* [35]. Thus, the significance of O-GlcNAcylation of proteins in anti-aging should be more clearly investigated, because there are contradictory reports on the effects of O-GlcNAcylation on lifespan.

Interestingly, glucosamine is biosynthesized from two trophic factors, glucose and amino acids, which are thought to negatively regulate lifespan and autophagy, which is mainly modulated by glucose and amino acid sensor of mTOR (mammallian target of rapamycin) [37]. Although it may be involved in the regulation of sugar and amino acid metabolism as described above, the details of the anti-aging mechanisms of glucosamine are still unknown, with the exception of its efficacy in carbohydrate metabolism modulation [24] and autophagy induction [25,38].

Despite the recent reports on the effects of glucosamine on lowering mortality or extending lifespan, more studies are needed prior to reaching a consensus on considering glucosamine an anti-aging drug. These studies should aim to answer the following questions. Should everyone be advised to take glucosamine, at what age should they start, and which patient populations should be considered? How does the supplement compare to other interventions that lower mortality, such as a healthy diet, fasting, and exercise? What is the probability of side effects with glucosamine? What are the side effects and how severe are they? In addition, future studies on the use of glucosamine should determine its mechanism of action, as well as establish its safety, and efficacy. Finally, further attention should be paid to glucosamine's anti-aging effects, in addition to its use in treating KOA. Thus, in the future in addition to, or instead, of using glucosamine as a supplement for KOA, it could be used as an anti-aging drug.

Disclosure statement

The authors declare no conflict of interest. Tomoya Shintani is an employee of Matsutani Chemical Industry Co., Ltd. (Hyogo, Japan), whose company is manufacturing and selling sugar and carbohydrate products (not including glucosamine and the all-related products). However, the company provided no financial support for this study.

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