Letter to the Editor

Effects of Selenium on *Fusarium* Growth and Associated Fermentation Products and the Relationship with Chondrocyte Viability*

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This study determined the effects of selenium on the growth of *Fusarium* strains and the effects of products extracted from the fungal cultures on relevant indicators of chondrocytes injury. The results showed that selenium supplementation resulted in differential effects on the mycelial growth of the strains. Levels of the chondrocyte injury indicators, including cell viability, proteoglycan and type II collagen contents and their mRNA expressions, were all reduced to varying degrees when the chondrocytes were incubated with fermentation extracts, the inhibitory effect varied depending on selenium content supplemented to fungal culture media. The results indicated that certain chain relations existed between the content of selenium in the environment, the production of some metabolites by fungi, and the occurrence of chondrocyte damage. The extent of this relationship and the role it plays in Kaschin-Beck disease pathogenesis merit further study.

Kashin-Beck disease (KBD) is a serious endemic osteoarthritis that predominantly affects the growth and development of children. Although environmental factors are recognized as important determinants regarding the incidence and development of KBD, the pathogenesis of the disease is not yet fully understood[1]. In relation to the currently accepted pathogenesis model, much evidence suggests that low selenium (Se) content in the environment and the presence of mycotoxins lead to KBD. However, the specific pathogenic mechanism that underpins KBD remains unclear. Hence, this research used endophytic *Fusarium* strains, which are dominant in many KBD-prevalent areas, to study the effects of different selenium concentrations on *Fusarium* growth and the effects of *Fusarium* fermentation products on *in vitro* rabbit chondrocytes damage-related indicators, to help understanding the possible role of selenium and mycotoxin during chondrocyte damage.

The experimental endophytic *Fusarium* strains were isolated from wheat in a KBD-prevalent region in Qinghai province and numbered 6-5-1 (*F. tricinctum*) and 1-8-B (*Fusarium* spp.). To avoid the impact of content variation in the natural ingredients of fungal culture medium, this study used synthetic medium[2], to which different concentrations of sodium selenite were added for fungal culture. The experimental animals were four-week old New Zealand white rabbits purchased from Medical Rabbit Breeding Base, Shaanxi, China.

Fungal culture and measurement of associated growth curves, extraction of fermentation products from fungal cultures were performed in accordance with the methods described in Zhang et al.[3]. Rabbit chondrocytes were isolated according to the methods described in Wu et al.[4] and cultured in Dulbecco's modified Eagle's medium (DMEM) containing 15% fetal bovine serum. The chondrocytes at passage 1 were used for the subsequent tests. The detection of chondrocyte injury indicators, including chondrocyte viability, proteoglycan and type II collagen contents and their mRNA expressions, were carried out by tetrazolium (MTT) assay, toluidine blue staining, immunohistochemistry, and RT-PCR methods, according to the reference[3], respectively. All data were analyzed by the One-way Analysis of Variance using SPSS 20.0 statistical software (SPSS, Chicago,

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IL,USA). The experiment was approved by the Ethics Committee of Northwest University, Xi'an, China.

Figure 1 shows the growth curves of both *Fusarium* strains in culture medium containing different concentrations of selenium, the legends in the Figure 1 and Figure 2 show the selenium contents added in the fungal culture medium (mg/L). The study demonstrated that selenium supplementation in the range of experimental concentrations promoted mycelial growth in the 6-5-1 strain, and the optimal concentration of selenium ranged from 0.10-1.00 mg/L (Figure 1A). In contrast, selenium supplementation was unfavorable for mycelial growth in the 1-8-B strain (Figure 1B), and the inhibitory effect was enhanced with increasing selenium concentrations.

As shown in Figure 2, incubation with fermentation products extracted from the two strains had different degrees of inhibition on the viability of rabbit chondrocytes. Although fermentation products derived from the 6-5-1 strain reduced the viability of rabbit chondrocytes, the inhibitory effect of the fermentation products in the presence of 0.05-0.10 mg/L selenium was milder than that observed with fermentation products generated using other selenium concentrations (Figure 2A). However, except for the inhibitory effect on chondrocyte viability caused by 1-8-B fungal cultures generated using higher concentrations of selenium (0.50-1.00 mg/L), the impact of the 1-8-B fungal cultures incubated with lower selenium concentrations (0.05-0.10 mg/L or no selenium) on chondrocyte viability remained the similar (Figure 2B).

As shown in Figure 3, treatments with extracts from both *Fusarium* strains demonstrated inhibitory effects on the synthesis of proteoglycan and type II collagen and the expression of their mRNA in chondrocytes. Compared to the treatment with fermentation products from non-selenium treated *Fusarium* cultures, the inhibitory effects on proteoglycan and type II collagen synthesis were comparatively lower in chondrocytes treated with fermentation products from cultures that were incubated with 0.05-0.10 mg/L selenium (Figure 3A and 3B).

The inhibitory effects on proteoglycan and type II collagen mRNA expression in chondrocytes treated with

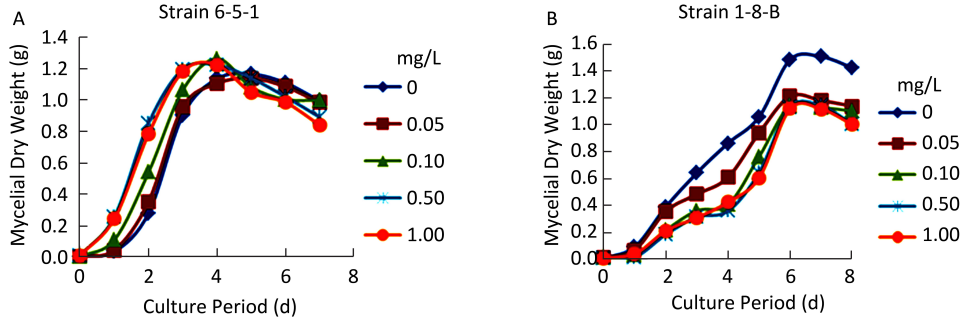

Figure 1. Mycelium growth curves of the two *Fusarium* strains treated with different doses of selenium in the medium.

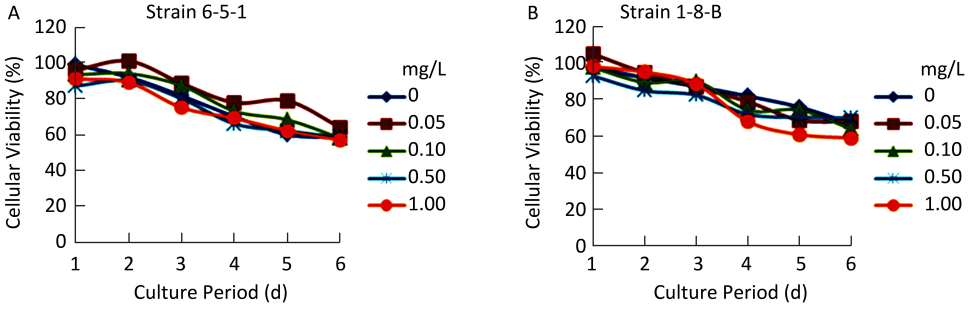

Figure 2. Impact of fermentation extracts obtained from the two *Fusarium* strains treated with different doses of selenium on the viability of rabbit chondrocytes (n = 6).

fermentation products derived from the 6-5-1 fungal cultures treated with 0.05-0.50 mg/L selenium were relatively reduced (Figure 3C). Proteoglycan and type II collagen mRNA expression in chondrocytes treated with fermentation products derived from the 1-8-B fungal cultures incubated with any tested concentration of selenium was higher than that observed in the absence of selenium treatment (Figure 3D).

Selenium exhibits important biological roles in many living organisms. The mineral is also a key determinant on the growth and apoptosis of *in vitro* human articular chondrocytes[5]. However, the relationship between selenium and fungal activity has rarely been reported. A previous study showed that *Fusarium* growth resulted in significant consumption of selenium[6]. The strains used in this study, as well as a *F. poae*s train used in our previous report[3], belong to the same *Fusarium* genus. However, the effect of selenium on mycelial growth varied for the different strains. Apparently, different *Fusarium* strains had different requirements for selenium. Therefore, we speculate that although different areas might have similar *Fusarium* species, different selenium concentrations in the endemic areas might lead to deviations in mycelial growth.

Cell viability is an important marker for cell growth, cell division, and cell proliferation and for the detection of chondrocyte growth. In this study, the fermentation products reduced chondrocyte viability to varying degrees. This demonstrated that the fermentation products extracted from these *Fusarium* strains contained substances that inhibited chondrocyte survival. However, fermentation products from fungal cultures supplemented with different selenium concentrations produced different effects on chondrocytes. Fermentation products extracted from the 6-5-1 strain that had been incubated with appropriate selenium concentrations resulted in higher chondrocyte viability than that from the culture without selenium treatment. These findings were similar to our previous report where fermentation products isolated from the *F. poae* strain were analyzed[3]. This suggested that appropriate selenium concentration treatments had inhibitory effects on the activity or synthesis of some substances, which were unfavorable to chondrocyte survival. However, the fermentation products extracted from the 1-8-B fungal cultures that had been treated with different selenium concentrations demonstrated different inhibitory effects on chondrocyte viability from that extracted from the strain 6-5-1. These results suggested that 1-8-B-generated substances might be

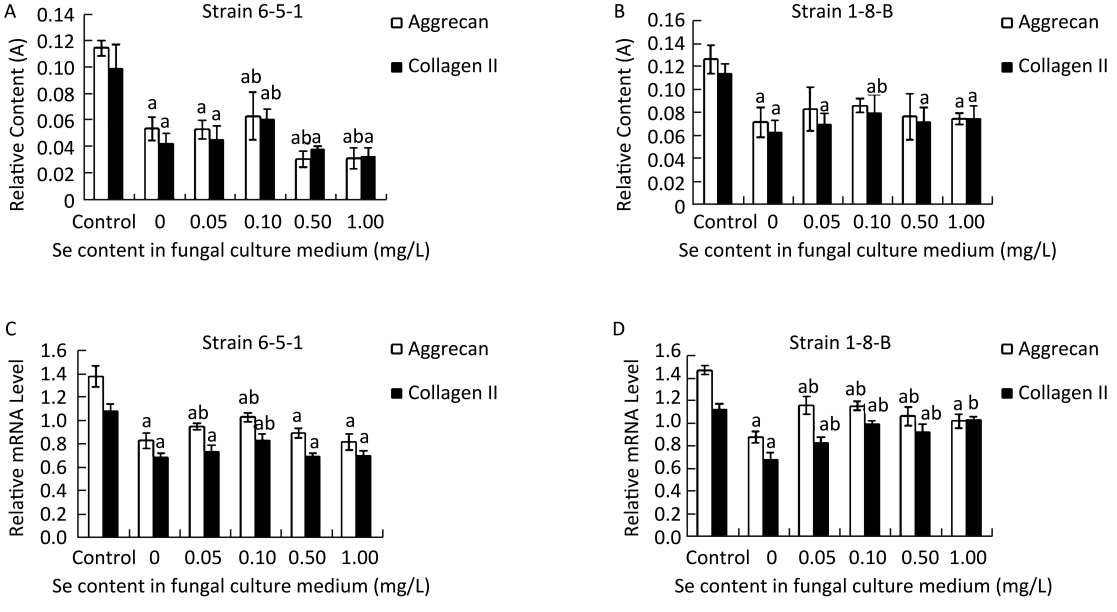

Figure 3. Impact of fermentation products obtained from two *Fusarium* strains treated with different doses of selenium on the expression of proteoglycan and type II collagen (A and B) and their mRNA (C and D)**.** [a], Comparison with control without the addition of fermentation products, $P < 0.05$; [b], Comparison with the treatment of fermentation products from fungi cultured without the addition of selenium, $P < 0.05$, $n = 6$.

different from 6-5-1-generated substances, thereby causing different effects on chondrocyte viability. Hence, different selenium concentrations might regulate changes in the quality or quantity of different substances, which cause chondrocyte damage, thereby altering chondrocyte viability to varying degrees.

Since the 1980s, numerous studies have shown that the incidence of KBD is associated with abnormal metabolism of extracellular matrix proteins in chondrocytes. Type II collagen and proteoglycan are constituents of the matrix of chondrocytes and play critical roles in maintaining cartilage matrix structure and functional integrity. Under normal conditions, decomposition and synthesis of type II collagen and proteoglycan are in a dynamic equilibrium, while expression of them is reduced upon occurrence of cartilage disease. Expression of type II collagen and proteoglycan is suppressed following interference with external factors, thereby resulting in partial functional loss of chondrocytes, ultimately leading to chondrocyte apoptosis[3,7]. Therefore, measurement of cell viability together with the detection of type II collagen and proteoglycan expression are key indicators of damage in chondrocytes.

Our results showed that fermentation products of the two *Fusarium* strains reduced proteoglycan and type II collagen protein expression in rabbit chondrocytes, indicating that both strains could produce substances that inhibited their synthesis. When the *Fusarium* strains were cultured in conjunction with specific selenium concentrations, the inhibitory effect on proteoglycan and type II collagen protein levels was significantly reduced. These findings were similar to those observed in our previous report where different *Fusarium* strain were analyzed[3], suggesting that appropriate selenium concentrations might inhibit some *Fusarium* strain-generated mycotoxicities, which might damage chondrocytes. Following comparison of treatment results of fermentation products from both *Fusarium* strains, a significant difference in inhibitory effects associated with selenium supplementation on proteoglycan and type II collagen was observed. This observation might be caused by the generation of different substances capable of promoting chondrocyte damage by the two *Fusarium* strains. Combining our findings with observations from other studies, we speculate that different *Fusarium* strains might generate different substances, which cause differential cellular damage[8]. It is likely that production of these substances is regulated to some extent by selenium levels in the fungal culture medium.

Proteoglycan and type II collagen mRNA exhibited similar expression patterns as the corresponding proteins. Rabbit chondrocytes treated with fermentation products extracted from the two *Fusarium* strains incubated with different selenium concentrations showed varying degrees of proteoglycan and type II collagen mRNA expression suppression, suggesting that the inhibitory effects of selenium on the chondrocyte damaging substance might occur in both mRNA and protein synthesis levels. So, selenium played a crucial role in both protein and mRNA expression of proteoglycan and type II collagen. These effects should be mediated through regulation of fungal metabolism, and be responsible for the occurrence of chondrocyte damage.

Different fungi populate different regions and environments. The effects of selenium on the growth of different fungal species and associated metabolism (including mycotoxin production) also vary significantly thereby differentially affecting human populations that have consumed grain crops infected with fungi. Our findings demonstrated that appropriate concentrations of selenium reduced the mycotoxin generating capacities of some fungal strains, and effected T-2 toxin production in other study[9]. Fermentation products of fungi contained many types of substances, some of which can cause chondrocyte damage. Therefore, KBD, which is closely related to cartilage-injury, might be a consequence of combined mycotoxicities[8]. In addition, this study showed that selenium produced different effects in relation to the production of mycotoxins in different *Fusarium* strains. This might partly explain why KBD occurs in endemic areas associated with different selenium levels.

Previous studies demonstrated that fungal strains of the same classification status produced different metabolites, while fungal strains with different classification statuses might produce the same or similar secondary metabolites. Some secondary metabolites were only produced by some fungal strains under certain conditions[10]. Therefore, although similar fungal strains might occur in different regions, variations in fungal growth and metabolite production might occur due to different environments (including differences in selenium availability). In contrast, different dominant fungal species might occur in some regions. However, it is

likely that these dominant fungal species produce some metabolites, either similar or different to those produced by fungi resident in other regions, which vary with respect to the capacity to damage cartilage even when selenium is present at similar levels in the environment. We speculate that this is one of the reasons why KBD is prominent in regions with different selenium concentrations and different types of fungal contamination.

To summarize, this study demonstrated that selenium affected both fungal growth and mycotoxicity. The significance of these effects varied for different *Fusarium* strains and concentrations of selenium. Fermentation products extracted from the experimental fungal strains incubated with different selenium concentrations altered rabbit chondrocyte viability, chondrocyte proteoglycan and type II collagen protein and their mRNA expression to varying degrees. These results suggest the existence of a relationship between the content of selenium in the environment, alterations in the production of fungal metabolites and the occurrence of chondrocyte damage. The extent of this relationship and the role that it plays in KBD pathogenesis merit further study.

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