Selenite suppresses hydrogen peroxide-induced cell apoptosis through inhibition of ASK1/JNK and activation of PI3-K/Akt pathways

SANG-OH YOON, MOON-MOO KIM, SOO-JIN PARK, DOHOON KIM, JONGKYEONG CHUNG, AND AN-SIK CHUNG

Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Taejon 305–701, South Korea

SPECIFIC AIMS

There are few reports on the relationship between the biological essential element selenium and signal transduction in cells. The main objective of our research is to study how selenite regulates signal transduction, especially induction of cell proliferation and maintaining survival.

PRINCIPAL FINDINGS

1. Selenite inhibits ASK1-JNK pathway

It was observed that the activities of ASK1 and JNK, one of the downstream molecules of the ASK1, were inhibited with selenite treatment in HT1080 cells (Fig. 1A). The amount of phosphorylated c-Jun was also decreased with selenite treatment (Fig. 1B). Furthermore, the inhibitory effect of selenite on ASK1 activity was sustained even for 48 h (Fig. 1C). However, protein levels of ASK1 were not changed much even for 24 h treatment with 2 μM selenite (Fig. 1D).

2. Selenite inhibits ASK1 activity directly and through PI3-K/Akt pathway

Selenite inhibited ASK1 activity not only directly by sulfhydryl modification of ASK1 but also by activation of PI3-K/Akt pathway. Treatment with both selenite and LY294002 did not restore Akt activity induced by selenite, suggesting that selenite activates Akt via the PI3-K-dependent pathway. Selenite increased Akt activity in a time- and dose-dependent manner (Fig. 2A, B, respectively), which was closely correlated to the inhibitory pattern of ASK1 by selenite; the amount of protein was not changed with selenite (Fig. 2C).

3. Physiological concentration of selenite increases mitochondrial membrane potential, bcl-2 expression, ATP generation, and glucose uptake through PI3-K/Akt-dependent pathway thereby increases cell proliferation

Cellular proliferation was stimulated by a physiological concentration of selenite below 3 μM. Selenite increased glucose uptake rate and ATP generation in HT1080 cells as well as 3T3-L1 rat adipocyte. Selenite also increased Bcl-2 expression and membrane potential. Treatment with LY294002 blocked an increase of the above parameters. These results indicate that selenite exerts the above parameters through PI3-K/Akt.

4. High concentration of H₂O₂ increases both apoptotic and antiapoptotic signals, eventually inducing apoptosis

H₂O₂ (500 μM) increased apoptotic (ASK1-dependent) and antiapoptotic pathways (Akt-dependent) simultaneously. However, the apoptotic signal was more sustained than the antiapoptotic signal, which resulted in Bcl-2 protein down-regulation, mitochondrial membrane potential disruption, and decreasing ATP and glucose uptake. Overexpression of Akt blocked apoptosis induced by H₂O₂.

5. Selenite inhibits H₂O₂-induced apoptosis through the inhibition of ASK1 activity and activation of PI3-K/Akt

H₂O₂ (500 μM) induced apoptosis of HT1080. Pretreatment with 2 μM selenite decreased significantly cell death induced by H₂O₂. Selenite also inhibited ASK1 activation and enhanced Akt activation by H₂O₂ treatment. Both 400 and 500 μM H₂O₂ treatment rapidly decreased glucose uptake, bcl-2 expression, mitochondrial membrane potential, and ATP generation. Pretreatment with selenite inhibited disruption of membrane potential by 400 μM H₂O₂, but not by 500 μM H₂O₂, and just delayed rapid disruption of membrane potential. Selenite almost blocked apoptosis induced by both concentration of H₂O₂. Treatment with H₂O₂ induced caspase-9 and -3 activation. Selenite was

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2 Correspondence: Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Taejon 305–701, South Korea. E-mail: aschung@mail.kaist.ac.kr

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able to effectively block caspase activation induced by 500 μM \( \text{H}_2\text{O}_2 \). These results indicate selenite blocks apoptosis through caspase inhibition even after mitochondrial disruption.

**CONCLUSIONS**

Selenium is known as an essential biological trace element involved in many physiological functions such as an antioxidant, modulation of immune system, and chemoprevention of cancer. There are few reports on what mechanisms are involved in the increased cell proliferation by a low level of selenite. In our studies, physiological concentration of selenite (1–3 μM) increased cell proliferation by increasing glucose uptake and ATP generation through activation of glycolysis, Bcl-2 up-regulation, and maintaining mitochondrial membrane potential. All these phenomena are known to be regulated by Akt activities, and selenite increased activities of PI3-K/Akt pathways (Fig. 3). The above parameters are also important for cell survival against stress.

Next, it is shown that selenite regulates signal molecules, especially apoptotic and antiapoptotic signals. Physiological levels of selenite inhibited ASK1-JNK pathway not only through PI3-K pathway, but also by direct interaction with ASK1 through modification of

![Figure 1](image1.png)

**Figure 1.** Inhibitory effects of selenite on ASK1 and JNK activities. A) HT1080 cells were incubated with selenite for 24 h and lysed. 200 μg of lysate was used for kinase assay. B) To detect the amounts of phosphorylated c-Jun, selenite-treated cells were lysed and subjected to Western blot using anti-phospho-c-Jun and anti-c-Jun antibody. C) HT1080 cells were incubated with 2 μM selenite for the periods indicated, lysed, and ASK1 activity (C) and protein amount (D) were measured. The results shown are representative of three independent experiments.

![Figure 2](image2.png)

**Figure 2.** Stimulatory effect of selenite on Akt activity. A) HT1080 cells were incubated with 2 μM selenite for the indicated periods, lysed, and Akt activity was measured. B) HT1080 cells were incubated with the indicated concentrations of selenite for 24 h, lysed, and Akt activity was measured. C) HT1080 cells were incubated with 2 μM selenite for the indicated time periods, lysed, and the amount of Akt protein was measured by Western blotting. Results are representative of three independent experiments.

![Figure 3](image3.png)

**Figure 3.** Schematic diagram of the effects of \( \text{H}_2\text{O}_2 \) and selenite on cell death and survival. Physiological level of selenite increases cell proliferation and survival by blocking apoptosis induced by \( \text{H}_2\text{O}_2 \) through inhibition of ASK1/JNK and activation of PI3-K/Akt pathways.
its sulfhydryl groups. Moreover, the duration of the inhibition of ASK1 and activation of PI3-K/Akt pathways by selenite were maintained for at least for 48 h. This duration period is much longer than that of growth factors and other stress factors.

Apoptosis by oxidative stress has been implicated in several biological and pathological processes like aging, inflammation, carcinogenesis, and diseases including AIDS, Parkinson’s, Huntington’s, and cataract formation in the eye. However, the mechanisms of cell death by oxidative stress, especially by hydrogen peroxide, are not clarified. One possible mechanism of H₂O₂-induced apoptosis is through the activation of the ASK1-JNK mitochondrial dysfunction/caspase activation pathway (Fig. 3). In reality, 500 μM of H₂O₂ increased ASK1 activity but decreased glucose uptake, cellular ATP level, Bcl-2 expression, and mitochondrial membrane potential, finally inducing cell death. H₂O₂ also activated the antiapoptotic pathway PI3-K/Akt, but the duration was much shorter than that of ASK1. It is likely that the amount and duration of apoptotic stress and antiapoptotic factors can determine cell survival or death. Selenite inhibited apoptosis induced by H₂O₂ through the PI3-K/Akt and ASK1 regulation.

Selenite contributes to cell survival and proliferation against external stress through inhibition of apoptotic signals and activation of the antiapoptotic signal. These can provide a good defense system for cells, so that selenium would be beneficial in protecting cells from oxidative stress factors, AIDS, and neurodegenerative disease. This is the first demonstration that selenite is closely related to cell survival signals such as the relationship between reduction of ASK1 and induction of Akt activities. This study will elucidate many biological and physiological functions of selenium compounds and will lead to new directions in selenium research.