Protective effects of selenium on Bisphenol A-induced oxidative stress in mouse testicular mitochondria and sperm motility

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ABSTRACT

Objective: This study aimed to explore the impact of selenium (SE) on Bisphenol-A (BPA)-exposed sperm and isolated testicular mitochondria of mice.

Methods: Mouse sperm and isolated mitochondria were exposed to BPA (0.8 mM) and different concentrations of SE (50, 100, and 200 µM) for four hours. The viability of sperm and isolated mitochondria as well as the mitochondrial membrane potential (MMP) were evaluated. SOD (superoxide dismutase), GSH (glutathione), MDA (malondialdehyde), and ROS (reactive oxygen species) levels in testicular mitochondria were also examined.

Results: BPA concentration-dependently enhanced ROS and MDA levels in isolated mitochondria, while MMP and activity of GSH and SOD significantly reduced. BPA also considerably impaired spermatozoa survival and motility. SE concentration-dependently reduced mitochondrial oxidative stress, MMP, sperm survival, and total sperm motility.

Conclusions: Our findings collectively suggested that SE concentration-dependently reversed BPA-caused mitochondrial toxicity and reduced sperm motility by suppressing oxidative stress.

Keywords: selenium, sperm motility, oxidative stress, Bisphenol A

INTRODUCTION

Bisphenol A (BPA), an industrial chemical, is used to produce polycarbonate (a clear and hard, plastic) and epoxy resins (lining on the inside of beverage cans and metal-based foods (Mikolajewska et al., 2015). BPA is also used in the manufacturing of toys, eyeglasses, lenses, compact discs, thermal paper, and dental sealants (Löfroth et al., 2019). BPA penetrates the body through ingestion, dermal contact or inhalation (Konieczna et al., 2015). The exposure of humans to BPA has been related to biological systems, environmental BPA, and food intake (Engel et al., 2014). BPA has been found in amniotic fluid, semen, plasma, urine, and breast milk (Ikezuki et al., 2002).

Evidence indicates that BPA has deleterious effects on the reproductive system (Anjum et al., 2011; Ullah et al., 2018). BPA diminishes the weight of the epididymis and testicles and impairs sperm quality of rodents (Chitra et al., 2003; Tyl et al., 2008; Knez et al., 2014). Moreover, BPA impairs mitochondrial function by diminishing ATP, reducing mitochondria mass, and disrupting the mitochondrial membrane potential (Kaur et al., 2018). Mitochondrial dysfunction affects sperm production and spermatozoa motility (Kamali Sangani et al., 2017). Besides, BPA diminishes antioxidant levels and stimulates ROS generation in testicular tissue (Chitra et al., 2003).

Selenium (2-aminoethanesulfonic acid, SE), an essential trace element with antioxidant properties, is involved in successful male reproduction due to its role in testosterone synthesis and development of spermatozoa (Khurana et al., 2019). SE plays a key role in spermatogenesis and sperm motility (Das & Ghosh, 2010). Sperm SE content has been positively correlated with the volume of mitochondria in humans and several animal species, such as boars, horses, bulls, and rams (Saarinen et al., 1989). SE exists in the outer membrane of the sperm mitochondria as selenoproteins (Calvin et al., 1981). Thus, SE deficiency impairs sperm motility and morphology and leads to infertility (Ursini et al., 1985). SE improves sperm numbers and motility by selenoproteins (Kaur & Bansal, 2015).

This study looked into the effects of SE on BPA-induced mitochondrial toxicity and impaired mouse sperm motility.

MATERIALS AND METHODS

Experimental design (Figure 1)

Samples of sperm and isolated testicular mitochondria were collected from 48 NMRI mice aged 8-10 weeks. The Ethics Committee on Animal Research approved this study (certificate: ABHC.REC.1397-079). Spermatozoa were isolated from the epididymis of the mice and divided into six groups, as follows (5×10⁴ sperm/ml were used in each group):

- Group I. (Control): treated with medium only
- Group II. (BPA): treated with 0.8 mM BPA for two hours
- Groups III-V: exposed to 50, 100, and 200 µM SE, respectively, for two hours before exposure to BPA.
- Group VI (SE): exposed to 200 µM SE for four hours

The untreated spermatozoa died after four hours. Thus, a total incubation time of four hours was set for exposure of sperms to SE or BPA. BPA (Sigma) or SE (Sigma) was dissolved in dimethyl sulfoxide (DMSO, 0.1%) and diluted in Ham’s F-10 media (Invitrogen). BPA concentrations were based on the results of the MTT test (Table 1). Isolated mitochondria and sperm were exposed to DMSO (1%) for four hours to assess the safety of DMSO by MTT test. DMSO had no toxic effect on isolated mitochondria or mouse sperm (Table 2).

Mitochondria isolation

The testicular tissue of euthanatized mice was dissected and minced in medium containing fat free bovine serum albumin (0.1%), sucrose (250 mM), EGTA (0.2 mM), EDTA (0.1 mM), and HEPES-KOH (5 mM). The minced testicles were homogenized and centrifuged at 3,000-g (10 minutes at 4°C). The supernatant was centrifuged for 7 minutes at 10,000-g. The prepared mitochondrial fractions were centrifuged for 6 minutes at 10,000-g (twice). The amount of protein was determined using Bradford reagent (Invitrogen). Isolated mitochondria (0.5 mg protein/mL) was treated with BPA or SE.
Figure 1. Schematic illustration detailing the experimental design of the study.

Table 1. The effect of different concentrations of BPA on spermatozoa viability

| Concentrations | 1 hour | 2 hours |
|----------------|--------|---------|
| 0 (control)    | 100.00±0.00 | 100.00±0.00 |
| 0.1 µM         | 98.4±2.15  | 95.7±3.23 |
| 0.2 µM         | 92.1±5.41  | 86.3±4.45 |
| 0.4 µM         | 76.5±5.36  | 65.9±4.58 |
| 0.8 µM         | 63.9±4.12* | 49.8±3.81** |
| 1 µM           | 56.7±3.72* | 35.1±3.09** |

Values are expressed as mean ± SD (n=6). *p<0.05, **p<0.01; * comparison against controls.

MTT assay
Spermatozoa or mitochondrial fractions were placed in 96-well plates and exposed to SE or BPA. After treatment, 5 mg/mL MTT (Sigma, USA) was added to the wells and kept for one hour at 37°C. Then the medium was removed, and DMSO (100 µL) added to each well. A micro-plate reader was used to read absorbance at 570 nm.

Determining MDA content, ROS formation, and antioxidant levels
The treated mitochondria was poured into the micro-tubes and 10 µM DCFH-DA (Sigma) plus Hank’s buffered salt solution (100 µL) was added and kept at 37°C for 40 minutes. A spectrofluorometer (LS50B, USA; Em: 535 nm; Exc: 490 nm) was used to examine ROS levels. Protein content of the treated mitochondrial fraction was measured using Bradford reagent (Invitrogen). Malondialdehyde (MDA), superoxide dismutase (SOD), and GSH (glutathione) activities were evaluated based on the kit’s guidelines (ZellBio Company).

Mitochondrial membrane potential (MMP) evaluation
The treated mitochondria (0.4 mg protein/mL) was incubated in 10 µM Rhodamine 123 for 15 minutes. A spectrophotometer (LS50B, USA; emission: 535 nm; excitation: 490 nm) was used to measure fluorescence.

Sperm motility
Sperm motility was evaluated according to WHO guidelines. Sperm suspensions (10 µL) were poured in the semen analysis chamber. Six fields were examined to rate the motility of at least 200 spermatozoa for each specimen. Percent sperm movement was evaluated to estimate the proportions of fast progressive (A), slow progressive (B), no progressive (C), and immotile sperm (D).

Statistical Analysis
One-way analysis of variance was performed in SPSS (version 22.0) followed by post-hoc pairwise comparison; p-values<0.05 were deemed significant.

RESULTS

MTT assay
Following exposure to BPA, percent viability decreased considerably in spermatozoa and testicular mitochondrial fractions (p<0.01). Survival rates grew considerably in the SE-treated mitochondria and spermatozoa (p<0.01). SE concentration-dependently elevated survival of the BPA-treated spermatozoa and mitochondrial fractions (Figure 2).

MDA content, ROS formation, and antioxidant levels
Following exposure to BPA, ROS production and MDA levels increased considerably in mitochondrial fractions (p<0.01). MDA level and ROS production of the
Table 2. Effects of DMSO on testicular mitochondria and spermatozoa

| Parameters                        | Control  | DMSO     |
|----------------------------------|----------|----------|
| Viability of sperms (%)          | 100±0.00 | 100.01±1.4 |
| Viability of mitochondria (%)    | 100±0.00 | 99.5±1.2  |
| MMP (% of control)               | 100±0.00 | 100.03±1.1 |
| ROS formation (% of control)     | 100±0.00 | 98.9±2.3  |
| MDA of mitochondria (nM/ mg protein) | 17.6±5.6 | 17.4±3.6  |
| GSH of mitochondria (pM/ mg protein) | 48.9±2.7 | 49.1±3.9  |
| SOD of mitochondria (U/ mg protein) | 8.8±2.2  | 8.92±2.4  |
| Total sperm motility (%)         | 70.2±5.8 | 70.9±6.5  |

Values are expressed as mean ± SD (n=6).

Figure 2. Viability of mitochondrial fractions and spermatozoa (mean ± SD; n=6).*p<0.05, **p<0.01, †p<0.05, ††p<0.01; * and † indicate comparisons against untreated control and BPA-treated groups, respectively.

Mitochondria decreased in the group treated with SE compared with controls. SE concentration-dependently diminished ROS formation in the BPA-exposed mitochondrial fractions (Figure 3). GSH and SOD activity decreased after exposure to BPA (p<0.01). In the SE-treated mitochondria, SOD and GSH activity was higher than the in the untreated (control) group. SE concentration-dependently reversed the BPA-decreased antioxidant level of mitochondrial fractions (Figure 3).

MMP Assay
SE significantly increased the MMP of the isolated mitochondria (p<0.05). In the BPA-exposed mitochondria, MMP decreased considerably compared with controls (p<0.01). SE concentration-dependently elevated the MMP of BPA-exposed mitochondrial fractions (Figure 4).

Sperm motility
SE considerably increased total sperm motility in comparison with controls. Following exposure to BPA, the proportion of fast progressive spermatozoa (p<0.05) and total sperm motility (p<0.01) diminished considerably, while immotile sperm percentages significantly increased (p<0.01). SE concentration-dependently diminished the proportions of immotile and fast progressive sperm, and increased total sperm motility (Table 3 and Figure 5).

DISCUSSION
The present study demonstrated that SE concentration-dependently reversed the motility and survival of BPA-treated spermatozoa. Consistent with our results, BPA diminished sperm quality in humans and some animal species (Kotwicka et al., 2016; Knez et al., 2014; Rahman et al., 2017).

Rezaeian et al. (2016) showed that SE increases the quality of sperm submitted to freezing and thawing procedures. Beneficial effects of SE on Monosodium glutamate-induced spermatogenesis damages have also been reported (Hamza & Diab, 2020).

We observed that SE concentration-dependently enhanced viability, total motility, and fast progressive movement of BPA-exposed mouse sperm cells. In line with our findings, SE provided protection against reproductive system damages induced by zearalenone in male mice (Long et al., 2016). SE dramatically altered the changes induced by ethanol on sperm survival and motility to near-normal levels (Swathy et al., 2006). SE reportedly reversed the decreased number of spermatogonia and sperm cells induced by radiation (Bagheri et al., 2020). Beneficial impacts of SE on the quality of boar sperm have also been documented (Marin-Guzman et al., 1997; Bano et al., 2018).

As revealed in the results, SE effectively improved the motility of BPA-exposed spermatozoa. Improved motility of spermatozoa might be due to SE effects on mitochondrial mass or function (Shi et al., 2010).

This study did not intend to explain the effects SE produced on sperm survival. There is a possibility that SE improves sperm viability by suppressing cell death signaling. Ma et al. (2017) reported that SE prevents...
glutamate-induced cell death in HT22 hippocampal cells. Abu-El-Zahab et al. (2019) showed that SE inhibits apoptosis induced by cadmium in mouse hepatic tissue. SE prevented apoptosis induced by lead in chicken nervous tissues (Zhu et al., 2017). SE has an important role in the maintenance of follicles and suppression of apoptosis in ovaries (Yang et al., 2019). SE inhibits mitochondrial dysfunction and apoptosis in cadmium-induced ROS production within LLC-PK(1) cells (Zhou et al., 2009). Kahya et al. (2014) showed that mobile phone radiation (900 MHz) induced apoptosis in breast cancer cells through increased oxidative stress. The authors reported that SE attenuated increased oxidative stress and apoptosis.

As mentioned in our results, BPA elevated MDA and ROS levels in testicular mitochondria. In line with our results, BPA increased ROS formation and MDA levels in sperm in previous studies (Rahman et al., 2019; Kaur et al., 2018). According to our results, SE reversed MMP, ROS production, MDA levels, and antioxidant biomarkers in the BPA-treated mitochondria. Therefore, SE may protect testicular mitochondria by decreasing oxidative stress. It has been shown that SE has a protective effect against mitochondrial oxidative disorders in different pathological cases. These results are in direct correlation with available evidence that indicates the beneficial impacts of SE on mitochondrial activity (Yeo & Kang, 2007; Yoon et al., 2002). Neuroprotection provided by SE through reducing ROS production, preventing DNA oxidation, preserving MMP, and mitochondrial function have been reported by Mehta et al. (2012).

Ghafarizadeh et al. (2018) showed that MMP, sperm survival, total motility, and fast progressive sperm movement of infertile patients were considerably enhanced in SE-exposed samples after four hours of incubation. They concluded that SE protected spermatozoa from mitochondrial damage due to its antioxidant properties.

As shown in our results, BPA decreased the MMP of isolated mitochondria and SE concentration-dependently reversed the effects of BPA on MMP. Barbonetti et al. (2016) indicated that BPA diminished MMP and increased human spermatozoa loss. MMP correlates positively with progressive sperm motility and total sperm numbers (Zhang et al., 2016). The decrease in MMP caused by BPA was accompanied by increased oxidative stress in testicular isolated mitochondria, reduced sperm motility, and attenuated sperm viability. BPA can cause mitochondrial oxidative damage of testicles via increasing lipid peroxidation (del Hoyo et al., 2010), and lead to disruption of spermatozoa function (Catalá, 2009).
Table 3. Velocity distribution of sperm cells in various groups

| Groups          | Fast progressive | Slow progressive | No progressive | Immotile |
|-----------------|------------------|------------------|----------------|----------|
| Control         | 39.9±4.2         | 30.3±3.9         | 16.8±2.5       | 12.8±2.7 |
| BPA             | 18.8±2.27**      | 19.9±2.14*       | 28.5±3.25*     | 32.2±2.7** |
| SE50 + BPA      | 21.5±3.3**       | 23.4±2.2         | 28.1±2.7*      | 26.6±3.2** |
| SE100 + BPA     | 27.8±3.9**†      | 25.1±3.1         | 24.9±3.4       | 15.7±2.9† |
| SE200 + BPA     | 38.7±4.8††       | 22.9±3.8         | 23.8±3.2       | 13.9±1.7†† |
| SE200           | 50.3±5.3††       | 30.2±3.6         | 14.3±2.7       | 6.2±1.2†† |

The mean ± standard deviations are shown (n=6). *p<0.05, **p<0.01, †p<0.05, ††p<0.01, †††p<0.001; *and †show comparisons against control and BPA-treated groups, respectively.

Figure 5. Total mouse sperm motility in the various groups (mean ± SD, n=6). * and † indicate comparisons against control and BPA-treated groups, respectively.

SE reduces the glutamate-caused mitochondrial damage and ROS generation in HT22 neuronal cells (Kumari et al., 2012). SE has a crucial role in the enzymatic process for the elimination of ROS and helps to preserve membrane integrity. Treulen et al. (2016) showed that mitochondrial outer membrane permeabilization induction enhances intracellular ROS and reduces the mean velocity of human sperm cells.

CONCLUSIONS
Overall, SE concentration-dependently improved MMP and reduced mitochondrial oxidative stress. SE also effectively improved the survival and motility of mouse spermatozoa. It is suggested that SE might ameliorate BPA-caused mitochondrial damage and mouse sperm quality impairment by preventing oxidative stress.

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CONFLICT OF INTEREST
The authors have no conflict of interest to declare.

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