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Dimercapto-1-propanesulfonic acid (DMPS) induces metaphase II mouse oocyte deterioration

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ABSTRACT

In light of the recent lead contamination of the water in Flint, Michigan and its potential adverse outcomes, much research and media attention has turned towards the safety profile of commonly used chelators. Dimercapto-1-propanesulfonic acid (DMPS) typically used in the treatment of lead, mercury and arsenic poisoning also displays a high affinity towards transition metals such as zinc and copper, essential for biological functioning. It is given in series of dosages (0.2–0.4 g/day) over a long period, and has the ability to enter cells. In this work, we investigated the mechanism through which increasing concentrations of DMPS alter oocyte quality as judged by changes in microtubule morphology (MT) and chromosomal alignment (CH) of metaphase II mice oocyte. The oocytes were directly exposed to increasing concentration of DMPS (10, 25, 50, 100 and 300 μM) for four hours (time of peak plasma concentration after administration) and reactive oxygen species (mainly hydroxyl radical and superoxide) and zinc content were measured. This data showed DMPS plays an important role in deterioration of oocyte quality through a mechanism involving zinc deficiency and enhancement of reactive oxygen species a major contributor to oocyte damage. Our current work, for the first time, demonstrates the possibility of DMPS to negatively impact fertility. This finding can not only help in counseling reproductive age patients undergoing such treatment but also in the development of potential therapies to alleviate oxidative damage and preserve fertility in people receiving heavy metal chelators.

1. Introduction

Given the recent lead poisoning crisis in Flint, MI, media attention and investigational research studies have focused on heavy metal toxicity, however little has been done on the safety of the heavy metal chelators and their effect on fertility [1–5]. Dimercapto-1-propanesulfonic acid (DMPS) acts as an effective chelator that is derived from British Anti-Lewisite (BAL), used for the treatment of acute and chronic heavy metal poisoning such as lead, arsenic, cadmium, and mercury [6]. The capacity of DMPS to chelate metal is due to the two free sulfhydryl groups (SH-) and appears to be more efficacious with sequential dosing rather than administration of large single-dose treatment [7]. It is water-soluble, displays a long half-life of 20 h, and has the ability to diffuse across cell membranes, though organic anion transporter 1(OAT1) [8,9]. In the plasma, the majority of DMPS (~ 60%) is bound by protein, primarily albumin, through a disulfide linkage [8,9]. It is also a reducing agent, which is metabolized rapidly and eliminated by the kidney and bile [10]. Based on these properties, optimal dosing appears to involve administration of DMPS at 0.2–0.4 g/day over a period of up to 15 days [11]. Importantly, the use of DMPS may lead to the elimination of biologically essential trace metals such as zinc, chromium, cobalt, copper, and iron, which may interfere with normal physiologic function [12]. The affinity of DMPS to zinc and copper is higher than its affinity to heavy metals such as mercury and arsenic [12].

Zinc is one of the most abundant transition metals and plays an essential part in cells including oocytes with multipurpose roles such as catalytic, structural, and regulatory functions [13]. Its intracellular accessibility is firmly controlled [14–16], as deviation from normal levels are potentially toxic to cells [17]. For mammalian oocytes, zinc is accumulated during oocyte growth and is thought to be stored in lipoproteins in preparation for later stages such as embryonic

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development [18,19]. It is a cofactor for multiple enzymes and transcription factors that are involved in biological functions including growth and reproduction [13] such as fetal formation during pregnancy and parturition [20]. Additionally, zinc plays a critical role in the completion of meiosis I in oocytes [21–23]. Most of the zinc transporters, superoxide dismutase (SOD), metallothioneins, and metal regulatory transcription factors are expressed in oocytes and not in cumulus cells [24]. In regard to female reproduction, zinc deficiency can cause ovulation failure as indicated by lacked corpora lutea, [25,26] it can also affect male fertility through altered sperm morphology and motility [27,28]. Despite these findings, intracellular mechanisms regulated by zinc, pertaining to female reproduction, more specifically ovarian function remains unclear. There is however, evidence that intracellular zinc depletion leads to apoptosis of animal cells [25,26]. Additionally, zinc has been suggested to play a role in oxidative defense systems as part of superoxide dismutase [29,30]. As a result, inadequate levels of zinc will consequently enhance the generation of reactive oxygen species [29,30]. This enhanced formation of free radicals results in tissue damage as evidenced in rats fed low zinc diets [31]. Extension of these findings to human subjects has also been made; increased oxidative stress and resulting damage has been documented with suboptimal zinc intake [32,33].

Reactive oxygen species is considered to be the major cause of the deterioration of oocyte quality and infertility [34]. Recently we have shown that reactive oxygen species such as superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\bullet}OH$), hypochlorous acid (HOCl), and peroxynitrite ($ONOO^-$) can alter the oocyte quality in a dose dependent manner, as manifested by hypergranulated cytoplasm, absence of the perivitelline space, and abnormal spindle dynamics [35]. Therefore, we hypothesize that DMPS can deteriorate oocyte quality through its ability to scavenge zinc, which leads to the generation of reactive oxygen species. We choose to study the metaphase II spindle structure and chromosome alignment as markers of oocyte quality, as these are sensitive to alterations in the oocyte microenvironment [31–35]. We also examined the mechanisms through which DMPS mediates oocyte deterioration by following the depletion of intracellular zinc content and the subsequent generation of reactive oxygen species.

2. Materials and methods

2.1. Materials

All materials used were of the highest grade of purity. No further purification was necessary. Human tubal fluid (HTF) medium, anti-α tubulin antibody, fluorescein isothiocyanate (FITC) conjugate anti-goat antibody, 4′,6′-diamino-2-phenylindole (DAPI), 0.1% Triton X- 100, and SYTO 64 red fluorescent nucleic acid stains were obtained from Sigma–Aldrich (MO, USA). Anti-fade agent was obtained from Biomedia (CA, USA) Cellular reactive oxygen species detection assay kit (Abcam, Cambridge, United Kingdom), DMPS and zinc detection kit (Zinquin ethyl ester) were obtained from Enzo Life Sciences, Inc. (NY, USA).

Metaphase II oocytes without cumulus cells from B6C3F1 mice crossed with B6D2F1 mice were obtained commercially (Embryotech Inc., MA, USA) in cryopreserved straws using ethylene glycol-based slow freeze cryopreservation protocol.

2.2. Methods

We chose to use frozen-thawed oocytes instead of fresh as we previously demonstrated that both fresh and frozen produce similar and reproducible results [36–40]. This was supported by other studies which showed that incubating thawed oocytes in the media for at least 60 min allows the spindles to repolymerize to normal architecture [41,42]. Institutional Review Board approval was not required, as the oocytes were obtained from Embryotech (MA, USA).

2.3. Effect of DMPS on MT and CH alignment of metaphase II mouse oocytes

Oocytes were thawed and transferred from straws to phosphate buffer saline (Dulbecco PBS) and washed for 3 min to remove cryoprotectant. Oocytes were then transferred to HTF media and incubated at 37 °C and 5% carbon dioxide (CO_2) for 60 min to allow spindle repolymerization and attainment of normal oocyte architecture. The oocytes were then screened for the presence of the polar body confirming their MII stage. Immature oocytes or those that displayed disrupted zona pellucida were discarded.

In triplicate experiments, oocytes without cumulus cells (n = 60 oocytes in each group) were exposed to 10, 25, 50, 100 and 300 μM DMPS for four hours where the effects on the oocyte MT and CH alignment were observed. The concentrations were selected based on peak plasma drug levels of patients treated with DMPS [43]. DMPS is typically given at doses of up to 1600 μmol/kg orally which is much higher than the concentrations we tested on our oocytes [44,45]. Untreated oocytes served as controls.

2.4. Immunostaining of the oocytes and confocal microscopy examination

Oocytes were fixed in a solution prepared from 2% formaldehyde and 0.2% Triton X-100 for 30 min then washed with PBS for 3 min. Subsequently, the oocytes were subjected to indirect immunostaining by incubation in mouse primary anti-alpha tubulin antibody against the MT for 60 min, and secondary FITC conjugated anti-goat antibody for 45 min. The chromosomes were stained using DAPI by incubating for 10 min. Stained oocytes were loaded into anti-fade agent on slides with two etched rings and cover slips placed using transparent nail varnish. Slides were stored at −20 °C and protected from light until they were evaluated for more details by confocal microscopy.

Slides were examined to obtain confocal images by Zeiss LSM 510 META NLO microscope using FITC (green) and DAPI (blue) fluorescent filters with excitation and emission wavelengths of 495 and 519 nm, and 358 and 461 nm, respectively. Oocytes were localized using a 10X magnification lens and spindle alterations assessed using 40X oil immersion lens. The MT was stained fluorescent green, which was distinct from the fluorescent blue staining of chromosomes. Fluorescence images were saved as graphic files in TIFF format.

2.5. Assessment of MT and CH alignment (Scoring System)

Treated and control oocytes in each experiment set were closely examined for spindle and chromosome configurations. The categorization of oocytes based on MT and CH configurations was performed by three independent observers who were blinded to treatment group assignment, and used comprehensive evaluation of the individual optical sections.

The alterations in the MT and CH alignment were scored (score1-4) based on a previously published scoring system and compared with controls. Scores 1 and 2 were combined for good and score 3 and 4 for poor outcomes [39,46]. Briefly, a good spindle configuration was coded for scores 1 and 2 where microtubules were organized in a barrel-shape, whereas, abnormal or poor configuration (scores 3–4) were for spindle length reduction, disorganization and complete absence of spindle. Chromosomal configuration was considered as good (score 1–2) when chromosomes are normally arranged at the equator of the spindle, while poor (score 3–4) when the chromosomes are dispersed or show aberrant or less condensed appearance [40,46–48] (Fig. 1).

2.6. Detection of intracellular zinc in oocytes after exposure to DMPS

In triplicate experiments, oocytes (n = 30 oocytes in each group)