2-METHOXYETHANOL EXPOSURE AFFECTS PROTEIN PROFILE OF TESTICULAR SPERM MEMBRANE IN MICE (*Mus musculus*)

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ABSTRACT

The 2-Methoxyethanol (2-ME) is a one of the toxic agents which affect on male reproduction from phthalate ester. The 2-ME is used for making resins, lacquers and paint in industrial solvent and may enter in the body through skin, by inhalation or per oral. The aim of this study was to determine the effect of 2-ME in varied doses toward sperm membrane protein of mice testis. This study used male BALB/C strain mice (Mus musculus), aged 4-5 weeks with 24-30 g weight. Based on the types of treatment, the subjects were divided into 3 groups. One control group was injected with physiological saline solution and 2 treatment groups were injected with 2-ME dose respectively 50 and 100 mg/kg. All groups were injected daily for 12 days by subcutaneous. Spermatozoa membrane protein was taken from testicular spermatozoa. The protein profiles of spermatozoa membrane were analyzed by sodium dodecyl sulphate-polyacrilamide gel electrophoresis (SDS-PAGE 15%) techniques. Results of this research showed the existence of differences between profile of spermatozoa membrane protein in control group and treatment group. In control and treatment group receiving a dose of 50 mg/kg body weight there was around 15 protein bands with variation of the molecular weight between 93.3-7.4 kDa. Whereas, in treatment group receiving dose of 100 mg/kg body weight there was 14 protein bands with 60 kDa protein not expression. Conclusion of this research was that the exposure of 2-ME can affect the protein profile.

Keywords: *2-Methoxyethanol, protein profile, testicular spermatozoa*

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INTRODUCTION

Spermatozoa are the male gamete cells that have a plasma membrane that surrounds the outside of cells from head to tail. In its life cycle, plasma membrane functions in the process of maturation, motility, acrosome reaction, and interaction of spermatozoa with the oocyte (Reisse et al. 2001). Structurally, the sperm membrane is composed by glycolipid, lipids that are dominated by unsaturated fatty acids, but it also contained membrane proteins that control the function of spermatozoa in fertilization. Membrane proteins are synthesized during the process of sperm development (spermatogenesis) that occurred in the testis. This process stops after spermatozoa leave the testicular seminiferous tubules (Barrios et al. 2000). Compounds of protein and lipid membrane that constitutes the spermatozoa are very sensitive to changes in the microenvironment of them are free radicals. Free radicals are largely derived from micro-environmental changes, including pH, microbial infections and toxic substances (phthalate ester group of compounds) in the reproductive tract, so it can affect sperm function and activity (Gaudreault et al. 2001)

One of the most hazardous phthalate ester compounds is 2-methoxyethanol (2-ME). 2-ME compound is one of the most toxic group of compounds of phthalic acid esters that are widely used as a plasticizer in plastics manufacturing (Johanson 2000). 2-ME compound enters the body through food or drink that has been contaminated and consumed, through breathing or can be directly absorbed by the skin during a contact. In addition to be teratogenic, it is also toxic for reproductive organs, especially in males, with testes as its main target (Butterworth et al. 1995). The exposure of 200 mg/kg 2-ME in rats can reduce the quality of spermatozoa and inhibit protein expression in epididimal sperm membrane, the 60 kDa protein (Bio 2007).

Information on spermatogenic cell damage and changes in membrane protein profile of mice due to exposure to epididimal toxic materials have been discussed, but the changes in testicular sperm membrane protein profile and exposure to toxic substances (2-ME) doses of less than 200 mg/kg was still unknown. Therefore, it is very interesting to do further research to determine the variation of testicular sperm membrane protein profiles after exposure of 2-ME.

MATERIALS AND METHODS

This study used 27 adult male BALB/C strain mice (*Mus musculus*) who had never mated, aged 4-5 weeks with 24-30 g body weight, and divided into 3 groups (control, treatment 1 and 2, respectively). Each group comprised nine mice. Treatment of experimental animals was done on day 8, after acclimatization in the cage for 7 days. The control group (C) was injected with physiological saline solution and treatment groups 1 and 2 (P1 and P2) were injected with a solution of 2-ME doses respectively of 50 and 100 mg/kg body weight. The injection was done subcutaneously in the dorsal neck of the mice every other day for 12 days.

Isolation of the sperm membrane referred to the isolation of mice sperm membrane by Vernet et al. (2001) and Chitra et al. (2001). Spermatozoa collected from all testes from one group (9 mice) were placed in one tube, then made a suspension of spermatozoa in 6 ml of physiological saline solution. In this way, we obtained three tubes of sperm suspension derived from 3 groups (K, P1, and P2). This is done to get the required sperm plasma membrane proteins in large quantities.

To separate spermatozoa from other cells, such as epithelial cells, fat, and leukocytes, spermatozoa suspension was centrifuged at 500xg (1.100 rpm) for 5 minutes at 4°C. Pellets was resuspended in hypoosmotic state in 10 mM PPB pH 7.4 at 4°C. Then it wascentrifuged again at 3000 rpm for 15 minutes at 4°C. The supernatant containing spermatozoa were collected (S1). Pellets were resuspended again in 10 mM PPB, and centrifuged 3000 rpm for 15 minutes at 4°C, and the supernatant was collected (S2). S1 and S2 supernatant contained sperm cells only. Furthermore, S1 and S2 supernatant were mixed with 0.2 M NaCl and 2 mM MgSO4. The mixture was centrifuged at 3000 rpm for 30 minutes at 4°C to separate mitochondria from the plasma membrane fraction. Pellets containing mitochondria were stored in -20°C. Plasma membrane-containing supernatant was centrifuged again at 40,000 rpm for 90 minutes at 4°C. Supernatant containing cytosolic sperm cells was stored in -20°C and the pellet containing the plasma membrane of spermatozoa was ready to isolate plasma membrane proteins.

In the isolation of sperm membrane proteins, the transmembrane protein of the pellets containing plasma membrane were separated from lipid membrane by means of extraction with 1% octyl-beta-D-thioglucopyranoside (OSGP) (w: v) 10% glycerol (v: v) 20 mM Tris, pH 8.6, and containing protease inhibitors (Complete Mini, Boehringer Mannheim, Germany). Then it was homogenized with a vortex for 30 minutes at 4 degree C. Fraction of protein solution is ready to set the concentration of proteins (Vernet et al. 2001, Chitra et al. 2001). Measurement of the concentration of sperm membrane proteins was performed by using UV spectrophotometer in a wavelength of 595 nm to obtain absorbance values (OD) (Vernet et al. 2001). Electrophoresis was conducted to determine the sperm plasma membrane protein profiles. It was electrophoresis was done by using sodium dodecyl sulphate-poliacrilamide gel electrophoresis (SDS-PAGE).

Determination of protein molecular weight (MW) was done by measuring the distance of proteins band movement or silver staining movement from the start (well holes in the stacking gel) up to the final limit of diresolving running gel on electrophoresis gel, and then compared with the measurement of protein molecular weight marker (protein marker). Furthermore, BM protein samples can be determined by looking for the algorithmic values molecular weight and Rf of the protein marker, then determined the Rf by dividing the migration distance of proteins after staining with a length of acrylamide after staining.

Measurement of the thickness of the protein band was performed using a densitometer. This phase is conducted to determine the thickness of the ribbon protein electrophoresis results through extensive measurements of each protein band that formed in the gel electrophoresis. Measurements were taken at all tested protein (control group, 2-ME treatment groups, and the marker protein) with wavelength of 560 nm (visible light), linear scanning, and transmission absorption (Marques et al. 2000). Data were analyzed descriptively. The comparison of protein band profiles of the 3 groups (control, treatment of 2-ME in doses of 50 and 100 mg/kg) are shown in gel electrophoresis.

RESULTS

The observation of protein profiles separated by molecular weight showed that 2-ME exposure affects sperm membrane protein profiles. This can be seen in Figure 1. There are eleven types of standard proteins used as marker proteins, i.e proteins with molecular weight 150 120 100, 85, 70, 60, 50, 40 20 15, and 10 kDa.

Sperm membrane protein band profiles of control and treatment group 2-ME receiving dose of 50 mg/kg looked similar. In the group there was 15 different protein bands with molecular weight 93.3, 79.5, 70, 60; 54; 44.6, 41.6, 35.4, 22.3, 19.4, 18; 16.7 , 11.4, 10.2, and 7.4 kDa. In treatment group 2-ME receiving dose of 100 mg/kg there was different protein band profile

compared with the two groups mentioned above. In this group there were 14 types of bands with molecular weight 93.3, 79.5, 70, 54; 44.6, 41.6, 35.4, 22.3, 19.4 and 18, 17.2, 11, 4; 10.2, and 7.4 kDa. Proteins with molecular weight of 60 kDa was visible in the control group and the treatment receiving dose of 50 mg/kg, but the treatment group receiving dose of 100 mg/kg showed no expression. This indicates that the toxic material (2-ME) in a certain dose (100 mg/kg) can affect and inhibit the synthesis of a protein, including proteins 60 kDa.

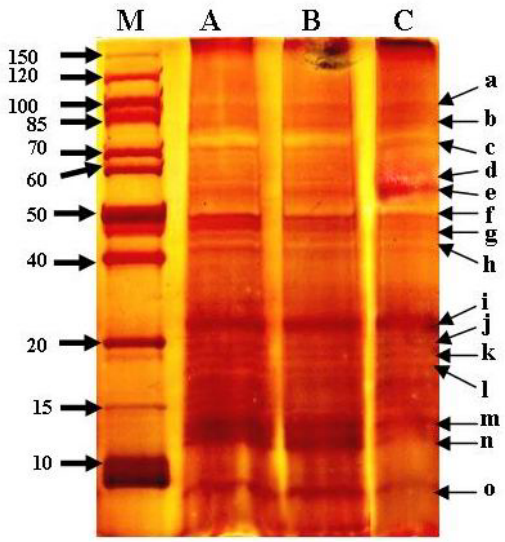

Figure 1. Testicular sperm membrane protein profile in mice. M = marker (protein molecular weight markers), A = control group, B and C = 2-ME treatment group in doses of 50 and 100 mg/kg.

DISCUSSION

Protein synthesis in spermatogenic cells occurs in the testicular seminiferous tubules. In addition, somatic cells (Sertoli cells) are also formed. Protein synthesis in spermatogenic cells begins during spermatogenesis and the synthesis ends after the formation of spermatid tail, while protein synthesized in Sertoli cells is used to support the process of spermatogenesis (Braun 2000). During the process of spermatogenesis the process of transcription and translation occurs, resulting in new proteins. Newly formed proteins are required in the preparation of the components of new spermatogenic cells, to produce adult spermatogenic cells or spermatids.

Protein constituent of sperm membrane has a variety of functions, among which are proteins with a weight of 60 kDa. 60 kDa protein plays a role in fertilization, when there is interaction between the membrane of spermatozoa with oocytes zona pellucida(Seaton, et al. 2000). These proteins are synthesized during elongation process of spermatids cell to form the tail and synthesis ends when the spermatozoa leave the testis.

In the exposure of 2-ME to treatment groups receiving a dose of 50 mg/kg there was visible decrease in the thickness of the 60 kDa protein band, whereas in the group treated with a dose of 100 mg/kg of 60 kDa protein does not appear (disappear). This shows that exposure of 2-ME can inhibit the expression of 60 kDa protein that causes decreased function of spermatozoa in fertilization. Other proteins found in this study were proteins with molecular weight of 70 kDa. Proteins are also found in the outer membrane of the acrosome and principal piece. These proteins are synthesized during germ cell to differentiate into spermatozoa (Wakle et al. 2005). According to Naz and Vanek (1998), 70 kDa proteins function as transcriptional control during spermatogenesis. A decrease in thickness (concentration) of 70 kDa protein band in control group (11638.04 mv.mm), the treatment dose of 50 mg/kg (11063.23 mv.mm) and followed by the treatment dose of 100 mg/kg (8910, mv.mm 82), showed that 2-ME exposure compounds suspected to inhibit the process of transcription in spermatozoa.

The mechanism of decrease in the thickness of the sperm membrane protein band BM 70 mg/kg bw was suspected that 2-ME compound entering the body is metabolized into methoxyacetic acid (MAA). MAA compounds in the body can cause oxidative stress. Oxidative stress can increase levels of free radicals in cells, thus causing oxidation of proteins. Oxidation of proteins begins with the reaction of protein molecules with radicals OH*, O2*, or both can change the structure of the protein to become abnormal. Changes in the structure are through the inhibition on the transcription and translation in protein synthesis (occupation et al. 2000). In addition, exposure of 2-ME also affected the thickness of the protein bands of protein 41.6. There was a decrease in the thickness of the protein bands after exposure to 50 and 100 mg/kg 2-ME, which was 9361.28 and 5702.14 mv.mm. This shows that exposure of 2-ME could be expected to result in infertility due to the non-expression of protease protein.

The function of other testicular sperm membrane proteins has already been known by some researchers. One of which is the proteins with molecular weight 32-34 kDa that function enzymatically in the merging process of sperm cells with oocytes (Hao et al. 2002). Proteins with 6.2 to 13 kDa molecular weight are

believed to be the sperm membrane protein constituent of mice sperm tail (Zhao 2001). 17-24 kDa proteins are found on the membrane of rat sperm tail (Zanich et al. 2003), whereas the 26 kDa protein is found in cell membranes of hamster spermatozoa tail (Gaudreault et al. 2001).

CONCLUSION

The 2-ME exposure affects testicular sperm membrane protein profile and the exposure of 100 mg/kg 2-ME for 12 days cause no expression of 60 kDa protein.

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