Effect of Smoking and Alcohol Consumption on Seminal Quality in Young Mice

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ABSTRACT

The purpose of this study is to evaluate the effects of alcohol or cigarette smoking on seminal parameters in a large group of mice model. Nine groups (n=20/group) of mice were treated intensive noxious materials that abdominal injection of 21% (v/v) of ethanol, cigarette smoke (10, 20, 30 minutes/day), and combination of ethanol and 30 minutes of smoking. In addition, vitamin C and selenium were also treated to mice exposed to combination of alcohol and smoking to identify the recovering effect. Sperm viability and motility were significantly decreased in either alcohol consumption or smoking exposed group, and combination of both materials have additive detrimental effects on seminal parameters. Mice groups that exposed to alcohol and smoking showed statistically significant decrease in motility and increase of static spermatozoa. Moreover, combination of both treatments showed cumulative effect in increase of static spermatozoa. Treatments of either vitamin C or selenium dramatically recovered detrimental effects of alcohol and smoking on seminal quality, although combination of both anti-oxidant molecules did not show any additive effect. In conclusion, detrimental effects of alcohol and cigarette consumption on sperm quality and motility were identified in mice model, and these detrimental effects can be compensated to uptake of anti-oxidant molecules.

(Key words : Vitamin C, Selenium, Smoking, Alcohol, Seminal prameter)

INTRODUCTION

The detrimental effects of smoking and alcohol consumption on semen quality, sperm motility, percentage of morphologically normal spermatozoa, and relation to male infertility have been intensively studied on animal and patient model (Anderson *et al*., 1983; Kucheria *et al*., 1985; Stillman *et al*., 1986; Anderson *et al*., 1987; Dunphy *et al*., 1991; Sofikitis *et al*., 1995; Vine *et al*., 1996; Zavos *et al*., 1998; Rubes *et al*., 1998; Donnelly *et al*., 1999; Dare *et al*., 2002; Kapawa *et al*., 2004; Marinelli *et al*., 2004). Smoking has been correlated with poor sperm function in sperm penetration assay (Close *et al*., 1990; Sofikitis *et al*., 1995). In addition, paternal cigarette smoking has been associated with a significant increase of production of DNA damaged spermatozoa (Fraga *et al*., 1996; Sofikitis *et al*., 1995; Shen *et al*., 1997; Potts *et al*., 1999), a decrease of early embryonic development (Kapawa *et al*., 2004), and a higher risk of birth defects and childhood cancers in the offspring (Zhang *et al*., 1992; Sorahan *et al*., 1995; Sorahan *et al*., 1997;). Although, the detrimental effect of alcohol consumption has been proposed, moderate alcohol drinking has shown an apparent protective effect on sperm parameters, probably due to the antioxidant effect of some alcoholic beverages (Marinelli *et al*., 2004).

A number of antioxidants have proven beneficial in treating male infertility, such as vitamin C, vitamin E, selenium, glutathione, and coenzyme Q10 (Sinclair, 2000). Antioxidants can protect against the damaging effect of leukocyte-derived reactive oxygen species (ROS) on sperm movement, and overproduction of ROS can be detrimental to sperm, being associated with male infertility (Akiyama, 1999). Among the number of antioxidants, L-ascorbic acid or vitamin C has shown an improvement of sperm viability, motility, and total mature sperm count (Dawson *et al*., 1992). Decrease of body levels of vitamin C is extremely sensitive to te-

* This research was supported by the research grant of Konkuk University.

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stes and seminal plasma (Chinoy *et al*., 1986). In addition, ascorbate has been considered as an essential biochemical molecule in the reproductive process and as a potentially significant factor in human fertility (Luck *et al*., 1995). Selenium has been considered as a significant substance which has a role in sperm cell development (Brown and Burk, 1973; Calvin, 1979) and sperm motility (Segerson *et al*., 1977). Inadequate dietary selenium fed to boars results in reduced sperm motility and higher structural abnormalities in the sperm (Marin-Guzman *et al*., 1997). In addition, sperm mitochondrial alterations and an abnormal plasma membrane attachment to the tail midpiece have been reported in selenium deficient mice (Calvin *et al*., 1981), suggesting that selenium has an important role in sperm function.

In the present study, detrimental effect of smoking, alcohol consumption, and both smoking and alcohol consumption at the same time have been examined on sperm parameters in mice. More frequently, smokers have other habits, such as alcohol consumption, however the effect of both harmful habits at the same time on sperm motility has not been reported so far. Present study also has been examined of the recovery effect of antioxidant molecules, vitamin C and selenium, on damaged sperm functions by smoking and alcohol consumptions. Therefore, the aims of this study were to evaluate the detrimental effect of smoking and alcohol consumption at the same time, and to identify the role of antioxidant molecules on recovering sperm quality.

MATERIALS AND METHODS

Animals and Treatments

The ICR mice used in present study were 3-week-old males (Dai-Han biolink, Chung-buk, Korea). The experimental groups were divided in nine: control group (group A; n=10), alcohol injection for 8 weeks (group B; n=20; abdominal injection of 0.7 ml of Korean liquor "So-ju", containing 21% of ethanol, equivalent to one bottle to human consumption), 10 minute exposed to smoking for 8 weeks (group C; n=20; tar 9 mg and nicotine 0.6 mg), 20 minute exposed to smoking for 8 weeks (group D; n=20; tar 9 mg and nicotine 0.6 mg), 30 minute exposed to smoking for 8 weeks (group E; n=20; tar 9 mg and nicotine 0.6 mg), 30 minute exposed to combination of smoking and alcohol injection for 8 weeks (group F; n=20; abdominal injection of 0.7 ml of Korean liquor "So-ju", containing 21% of ethanol, and tar 9 mg and nicotine 0.6 mg), abdominal injection of selenium for 8 weeks to group F at the same time (group G; n=10; 0.005 μg of selenium per 1 g of mice body weight), abdominal injection of vitamin C for 8 weeks to group F at the same time (group H; n=10; 0.05 mg of vitamin C per 1 g of mice body weight), abdominal injection of both selenium and vitamin C for 8 weeks to group F at the same time(group I; n=10; 0.05 mg of vitamin C and 0.005 μg of selenium per 1 g of mice body weight). For the abdominal injection of alcohol was conducted three times a week, and smoking exposure was carried out 30 minutes for everyday.

Sperm Preparation and Motility Analysis

All treated mouse were subjected to CO_2 gas chamber for euthanasia, and sperm in the epididymis were extracted by surgical procedure. Collected sperms were washed three times with phosphate buffered saline, pH 7.4 and preserved in M2 medium (Sigma, USA). Ten microlitre aliquot from the control and treated spermatozoa were placed into a pre-warmed Makler chamber and analysed by computer assisted semen analysis (CASA, Hamilton Thorne Research, USA) system. The parameters recorded for each sample were as follows: percentage of motile sperm, and movement characteristics such as percentage of static sperm, track speed, average path velocity (VAP).

Statistical Analysis

Statistical analyses were performed by SPSS (ver. 14.0) statistical software to determine differences of mean value between control and experimental group through independent *t*-test. Results were considered significant when $p<0.05$.

RESULTS

Effects of Detrimental Treatments and Antioxidant Treatment on Spermatozoa Motility

All spermatozoa from treated mouse identified no phenomena of abnormal morphology with help of light microscope of CASA. Treatment of smoking and direct alcohol showed a significant decrease of spermatozoa ability (Fig. 1). Mouse exposed to smoking for 10 to 20 min had about 60 % motility, and 30 min exposed mouse had about 52 % of motility in spermatozoa. Consumption of alcohol exhibited also significant decrease in spermatozoa motility about 54 %. Mouse exposed both alcohol and smoking showed the cumulative decrease of spermatozoa motility less than 50 %. However, treatments group of anti-oxidative stress molecules with both alcohol and smoking did not show any diminish of the motilities of spermatozoa, they showed the similar sperm motility showed in control group (Fig. 1).

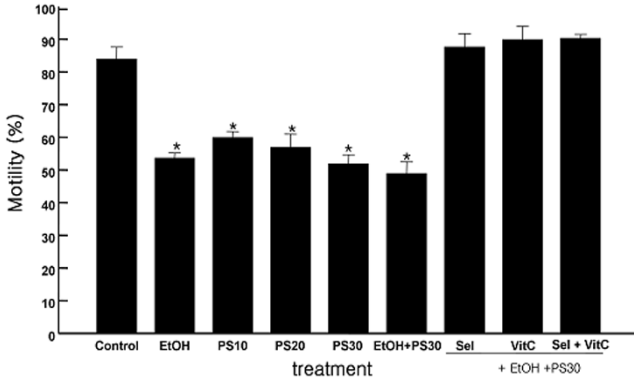
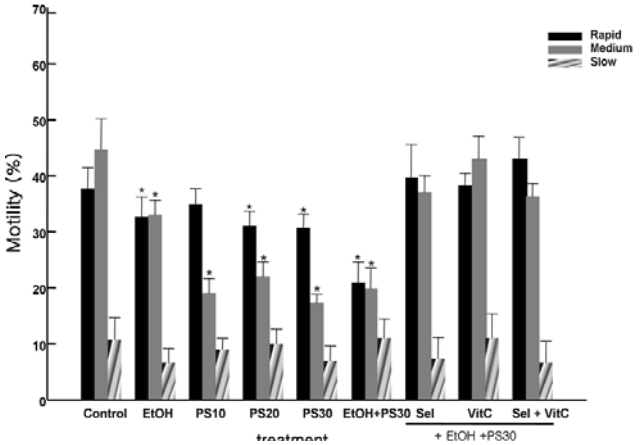
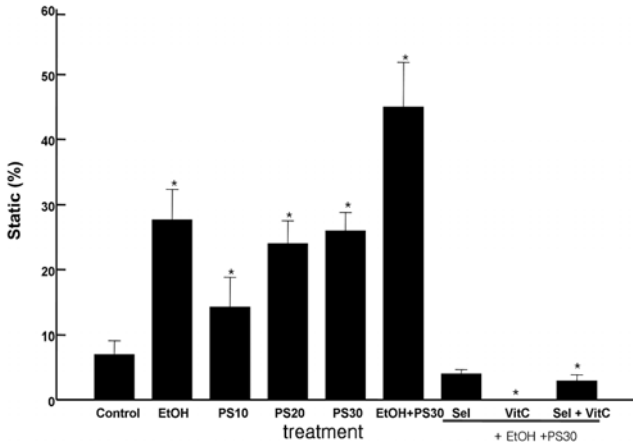


Fig. 1. Effects of alcohol consumption or cigarette smoking on viability of epididymal spermatozoa. 20 mice were used in each treated group and the results are expressed as mean±SD. All treatment are conducted for 8 weeks and treatments of each group are as follow; Control - normal mice, EtOH - 21% ethanol contained 'Soju', PS - smoking, Sel - selenium, and VitC - vitamin C. $p<0.05$.

Sperm motilities were characterized in both motile and static groups, motile groups were separated to three identical groups that are rapid, medium, and slow. Although 10 minute cigarette exposed group showed a decrease of medium motile spermatozoa, direct alcohol consumption, 20 minute, 30 minute, and alcohol-smoking exposed groups showed the decrease of rapid and medium motile spermatozoa (Fig. 2A). However, the ratio of slow spermatozoa did not changed by detrimental treatments (Fig. 2A). Interestingly, anti-oxidant molecules treatment at the same time with smoking and alcohol did not alter the percent ratio of motile spermatozoa compositions (Fig. 2A). Treatment of selenium and vitamin C showed a little improvement of rapid spermatozoa, however its statistical significant could not find. Among the detrimental molecules treated groups, both smoking and alcohol treated at the same time showed the significant decrease of rapid motile spermatozoa (Fig. 2A). Alcohol consumption and smoking were resulted the dramatic increase of the percent ratio of static spermatozoa, alcohol only and smoking only groups showed about ~ 2 fold increase of the static spermatozoa compare to control, non-treated, mice group (Fig. 2B). Moreover, treatment of both alcohol and smoking group showed a ~ 4.5 fold increase of the static spermatozoa compare to control group, however treatments of vitamin C and both with alcohol and smoking showed a significant decrease of the ratio of the static spermatozoa (Fig. 2B).



(A)



(B)

Fig. 2. Effects of alcohol consumption or cigarette smoking on motility of epididymal spermatozoa. (A) Rapid, medium, and slowly moved motile sperm, and (B) mean percentages±SD of static sperm are analyzed. 20 mice were used in each treated group and the results are expressed as mean±SD. All treatment are conducted for 8 weeks and treatments of each group are as follow; Control - normal mice, EtOH - 21% ethanol contained 'Soju', PS - smoking, Sel - selenium, and VitC - vitamin C. $p<0.05$.

Effects of Detrimental Treatments and Antioxidant Treatment on Track Speed and Average Path Velocity

Besides the sperm viability and motility, other sperm parameters were also evaluated. As shown in Fig. 3, 30 minute of smoking exposed group and both alcohol-smoking co-treated group showed the reduction of track speed by 85.0±3.1 μm/s and 77.3±6.52 μm/s, respectively. This is comparable to track speed of spermatozoa from control group that was 104.23±3.89 μm/s. The other treatments did not show any significant changes in track speed, although there were minor variations (Fig. 3). Average path velocity (VAP) was increased to 66.87±2.46 μm/s in co-treatment of alcohol, smoking, selenium, and vitamin C (Fig. 4). The other treated groups showed a little changes in VAP, however the statistical significances were not found.

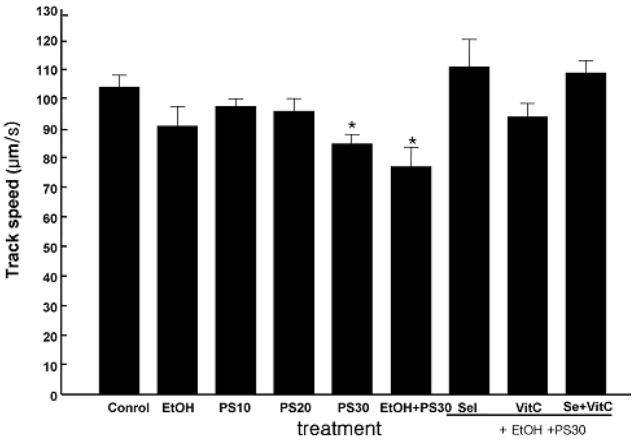


Fig. 3. Effects of alcohol consumption or cigarette smoking on track speed of epididymal spermatozoa. 20 mice were used in each treated group and the results are expressed as mean±SD. All treatment are conducted for 8 weeks and treatments of each group are as follow; Control - normal mice, EtOH - 21% ethanol contained 'Soju', PS - smoking, Sel - selenium, and VitC - vitamin C. $p<0.05$.

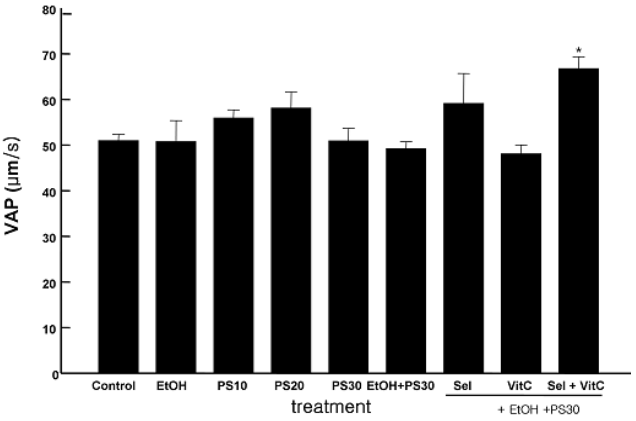


Fig. 4. Effects of alcohol consumption or cigarette smoking on VAP of epididymal spermatozoa. 20 mice were used in each treated group and the results are expressed as mean±SD. All treatment are conducted for 8 weeks and treatments of each group are as follow; Control - normal mice, EtOH - 21% ethanol contained 'Soju', PS - smoking, Sel - selenium, and VitC - vitamin C. $p<0.05$.

DISCUSSION

The effects of alcohol or tobacco consumption on male reproductive function are controversial. Several studies reported that tobacco negatively affects sperm concentration (Handelsman *et al*., 1984; Lewin *et al*., 1991), motility (Shaarawy and Mahmoud, 1982; Handelsman *et al*., 1984), and normal morphology (Evans *et al*., 1981; Shaarawy and Mahmoud, 1982). However, some studies could not find a relationship between smoking and alteration of sperm parameters (Dikshit *et al*., 1987; Trummer *et al*., 2002). The effects of alcohol on male reproductive function have been reported similar manners to smoking. A reduction in sperm concentration and in the percentage of normal morphological spermatozoa has been investigated in chronic alcohol consumers in human (Kucheria *et al*., 1985; Goverde *et al*., 1995). Other studies reported adverse effects of alcohol on male reproduction using animal models or ethanol induction (Klassen and Persaud, 1978; Anderson *et al*., 1983; Alvarez *et al*., 1988; Curtis *et al*., 1997).

Generally, smoking and taking alcohol habit could not be separated because most of the smokers have alcohol drinking habit moderate to high amounts, therefore investigation of both smoking and alcohol consumption at the same time on semen quality is significant. In agreement with our data, several studies also reported the detrimental effects of alcohol and cigarette smoking at the same time on male reproduction (Rubes *et al*., 1998; Wong *et al*., 2000; Kunzle *et al*., 2003). According to the others, our results also postulated the detrimental effect of the combination of smoking and alcohol on sperm motility without significant morphological changes. However, separate treatment of either alcohol or smoking caused a significant alteration in sperm motility in this study, this data is not accordance to other study that performed in human. In human, combination of both smoking and alcohol consumption showed a detrimental effect on seminal quality, but separate treatment did not (Martini *et al*., 2004). A recent study, Kapawa *et al*., evaluating the effect of paternal smoking on sperm quantitative and qualitative parameters, that rats exposed to cigarette smoke for 10 weeks had significantly smaller in sperm viability and motility. Moreover, this harmful habit affected to implantation of blastocyst. The different data obtained from the human and animal model on seminal parameter of detrimental effect of smoking may be caused by the intensity of exposure. In addition, mice used in this study are 3 weeks old male that are equivalent to youth age in human, suggesting that exposed to alcohol and smoking at earlier stage of growth may have severe defect in seminal quality.

The beneficial effects of ascorbic acid and selenium on spermatozoa development and seminal quality have been reported by many researchers (Brown and Burk, 1973; Segerson *et al*., 1977; Calvin, 1979; Chinoy *et al*., 1986; Dawson *et al*., 1992; Luck *et al*., 1995; Marin-Guzman *et al*., 1997). In agreement with other findings, our data identified significant association between anti-oxidant molecule and seminal quality. Although mice were exposed to smoking and alcohol consumption that caused the significant decrease in sperm viability and motility, absorption of ascorbic acid and selenium successfully recovered seminal quality. The

poor seminal quality caused by the noxious habit can be resulted in various chemical, hormonal, and physiological changes in body. Nevertheless of these changes, the smoking and alcohol consumption increased ROS, and overproduction of ROS has a detrimental effect on sperm and male fertility (Akiyama, 1999). In this regard, treatment of ascorbic acid and selenium to mice that exposed to smoking and alcohol might diminish the ROS in seminal fluid, and recovered the seminal quality.

The results of present study suggest that the either smoking or alcohol consumption and combination of both can be decreased seminal quality of sperm parameters and motility. Treatment with vitamin C and selenium can be considered a valid alternative to antioxidative stress of sperm in the treatment of smoking and alcohol consumption, and recover detrimental effects on seminal parameters.

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(Received: 15 October 2009 / Accepted: 15 December 2009)