Toxicology 357–358 (2016) 44–51

Contents lists available at ScienceDirect

Toxicology

journal homepage: www.elsevier.com/locate/toxicol

Ethanol impedes embryo transport and impairs oviduct epithelium

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ARTICLE INFO

Article history:
Received 16 May 2016
Accepted 1 June 2016
Available online 3 June 2016

Keywords:
Alcohol
Embryo transport
Ectopic pregnancy
Fertility
Nitric oxide

ABSTRACT

Most studies have demonstrated that alcohol consumption is associated with decreased fertility. The aim of this study was to investigate the effects of alcohol on pre-implantation embryo transport and/or early embryo development in the oviduct. We reported here that ethanol concentration-dependently suppressed the spontaneous motility of isolated human oviduct strips (EC50 50 ± 6 mM), which was largely attenuated in the present of L-NAME, a classical nitric oxide synthase(NOS) competitive inhibitor. Notably, either acute or chronic alcohol intake delayed egg transport and retarded early development of the embryo in the mouse oviduct, which was largely rescued by co-administration of L-NAME in a acute alcohol intake group but not in chronic alcohol intake group. It is worth mentioning that the oviductal epithelium destruction was verified by scanning electron microscope (SEM) observations in chronic alcohol intake group. In conclusion, alcohol intake delayed egg transport and retarded early development of the embryo in the oviduct by suppressing the spontaneous motility of oviduct and/or impairing oviductal epithelium. These findings suggested that alcohol abuse increases the incident of ectopic pregnancy.

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1. Introduction

Studies have shown that the importance of lifestyle factors is often disregarded by couples attending infertility clinics (Olatunbosun et al., 1997). Approximately 10% of all couples in western countries suffer from undesired infertility (Soule and Jacobs 1995), and less than half of all couples with these problems seek medical care (Olsen et al., 1996). Tubal factor infertility (TFI) is probably the most important factor in infertility (Cates et al., 1985; Sciarra 1994). Most studies of the relationship between alcohol intake and fertility have analyzed the issue from a short term or long term perspective. The results suggest that high consumption is associated with decreased fertility (Grodstein et al., 1994; Hakim et al., 1998; Jensen et al., 1998). In a short-term, prospective study by Jensen et al. (Jensen et al., 1998) found that conception was delayed proportionally with increased alcohol intake.A similar studyshows a dose-related, negative effect of alcohol on fertility (Hakim et al., 1998). High alcohol consumption is associated with decreased fecundity (Olsen et al., 1997). Alcohol intakes during the week of conception increased the risk of spontaneous abortion (Henriksen et al., 2004; Windham et al., 1997). These results suggest a negative influence of alcohol on fertility. However, the data show no evidence of any of the effects of alcohol intake during preimplantation. We propose that female alcohol intake during early pregnancy affects the tubal transport of embryos. The purposes of this study were to determine if ethanol affects the contraction of human fallopian tube strips, and if alcohol intake by mice at doses equivalent to those received by human affects in vivo functioning of the oviduct. Specifically, we examined the effects of acute and chronic alcohol intake on the preimplantation embryo transport and oviduct epithelium in mice.

2. Methods

2.1. Ethic statement

All experimental procedures were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals of

Abbreviations: TFI, tubal factor infertility; NO, nitric oxide; NOS, nitric oxide synthase; L-NAME, L-N^{G}nitroarginine methyl ester; SEM, scanning electron microscope; AUC, area under the curve.

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http://dx.doi.org/10.1016/j.tox.2016.06.001
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Shandong University, and the study was approved by the Medical Ethics Committee for Experimental Animals, Shandong University, China (number ECAESDUSM 2012029). Informed consent was obtained from each patient and approval granted from the Medical Ethics Committees of Shandong University (number MECSDUMS 2010032).

2.2. Tension recording

The human fallopian tubes were obtained from salpingectomy for ectopic pregnancy and hysterectomy or postpartum sterilization samples collected from women aged 23–48 years who had not undergone exogenous hormonal treatment for at least 3 months prior to surgery. The strips made from human oviduct were placed in isolated organ baths filled with Krebs solution, which contained 95% O_2 and 5%CO_2, incubated and connected with tension transducers. The Krebs solution contains: 5.9 mM KCl, 1.2 mM NaH_2PO_4, 1.2 mM $MgCl_2$, 120.6 mM NaCl, 15.4 mM $NaHCO_3$, 11.5 mM glucose and 2.5 mM $CaCl_2$. The strips were stretched and reached a resting tension of 1 g. Until tension was recorded the strips needed to equilibrate for 30 min, as necessary, bathing solution was periodically changed. Subsequently, different concentrations of ethanol were added, and the respective contraction curve was recorded. Whenever the reagents were replaced, the tissues were washed three times. Data were analyzed for average changes in basal tension, frequency, amplitude and area under the curve(AUC)for 3 min by a software programme (Chart 5, ADInstrument, Sydney, Australia).

2.3. Measurement of nitric oxide (NO) levels in incubated rat oviduct or human fallopian tube

The rat oviduct or human fallopian tube was carefully removed and placed in isolated organ baths filled with Krebs solution for 15 min, which contained 95% O_2 and 5% CO_2. Then they were treated with different drugs for 15 min before it was prepared for the measurement of NO content and Western blots. The samples were thawed, weighed and homogenized in 1:9 w:v in 0.9% saline. The homogenates were then centrifuged at 1000 r/min for 5 min at 4 °C, the supernatant was taken for NO assay and total protein determination. NO was assayed spectrophotometrically by measuring total nitrate plus nitrite (NO_3^- plus NO_2^-) and the stable end products of NO metabolism. The procedure that nitrate was enzymatically converted into nitrite by the enzyme nitrate reductase was followed by quantization of nitrite using Griess reagent at the absorbance of 550 nm. The level of NO was expressed as μmol/g protein.

2.4. Western blots

After centrifugation of homogenates at 12,000 rpm for 20 min at 4 °C, the supernatant was added the same amount of sample buffer boiled 10 min at 100 °C. 10–30 μg of the denatured proteins were separated on 10% SDS polyacrylamide gels and transferred to a PVDF membrane. Membranes were blocked in a blocking buffer (contains 5% nonfat dry milk and Tween-Tris-buffered saline) for 1 h at room temperature,then washed in Tween-Tris-buffered saline (0.1% Tween 20, 50 Mm Tris,and 150 mM NaCl), followed by overnight incubation at 4 °Cwith rabbit polyclonal nitric oxide synthase(NOS) (Abcam) antibody (1:2000). Membranes were washed in Tween-Trisbuffered saline and incubated with anti-horseradish-peroxidase-conjugated secondary antibody (1:5000). Membranes were washed again and exposed to ECL.

2.5. Acute alcohol exposure experiments

Female C57BL/6 mice (6–8 weeks of age) were mated randomly, while vaginal plug-positive mice were used in the experiment. Pregnant mice were divided into three groups and were given different drugs as follows: vehicle (normal saline, 0.1 ml), ethanol (4 g/kg/day), ethanol and L-N^G nitroarginine methyl ester(L-NAME; 5 mg/kg/d, Sigma). Vehicle or ethanol was administered via gavage after the vaginal plug 12 h, 24 h, 36 h, 48 h, 60 h and 72 h, respectively. L-NAME was intraperitoneally injected before ethanol administration. The mice were sacrificed by cervical dislocation at 74 h after vaginal plug, and oviduct and uterus were removed to count embryos.

2.6. Chronic alcohol intake experiments

Female C57BL/6 mice (6–8 weeks of age) were randomly divided into three groups and were given different drugs daily for 30 days as follows: vehicle (normal saline,0.1 ml), ethanol (4 g/kg/day), ethanol and L-NAME (0.5 mg/kg/d, Sigma). Vehicle or ethanol was administered twice via gavage. L-NAME was intraperitoneally injected before ethanol administration. Thirty days after the administration, some mice (non-pregnant) were sacrificed by cervical dislocation and the oviducts were removed to Western blots, HE staining and SEM studies. Other mice were mated and sacrificed by cervical dislocation at 74 h after vaginal plug to count the number of embryos in oviducts and uterus.

2.7. Scanning electron microscope (SEM) studies

Mouse oviducts were fixed in 2.5% (v/v) glutaraldehyde and stored at 4 °C in fixative until processing. After a wash in buffer (PBS pH 7.4) the ducts were post fixed for 1 h in 2% (w/v) osmium tetroxide. The isthmus and ampulla region were cut along the longitudinal axis with a small scalpel, rinsed in distilled water, dehydrated through graded ethanol (10 min each concentration), then critical-point dried in liquid carbon dioxide using a Polaron E3000 drier. The dried segments were mounted with the luminal surfaces uppermost and coated with approximately 20 nm gold/palladium to facilitate examination in a JEOL JSM6610LV field SEM (resolution 4 nm).

2.8. Statistical analysis

Statistical analyses were performed with Sigmaplot software (SPSS). Data are expressed as the mean ± s.e.m. and were examined with two-tailed Student's *t*-tests or one-way ANOVA where appropriate. A *P* value of less than 0.05 or 0.01 was considered statistically significant or very significant, respectively.

3. Results

3.1. Ethanol inhibited the spontaneous motility of isolated human oviduct in concentration-dependent manner

Preparations from all patients showed spontaneous activity comprised of slow phasic contractions with amplitude of 0.37 ± 0.12 g and frequency of 3–8 cycles perminute (Fig. 1A). Ethanol (17, 34, 50, 67, 85 mM) evoked a concentration-dependent suppression of spontaneous contractions of either the isolated fallopian tube ampulla or isthmus strips from human (Fig. 1B). Moreover, this phenomenon is reversible (data not shown).

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