The Effects of Smoking, Ozone Exposure and Alcohol on Pregnancy

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Abstract

Objective. *Our study evaluated the effects on gestation, in terms of oxidative stress(OS), of three risk behaviors (smoking, ozone exposure and alcohol consumption) with the purpose of applying the results in clinical practice.* ***Methods.*** *In this experimental study, we determined the oxidative stress markers: malondialdehyde(MDA) and carbonylated proteins(CP); and the antioxidants: the hydrogen donor capacity (DH) and the sulfhydryl groups(SH).* ***Results.*** *Smoking: significant increases of MDA and CP and a significant decrease of SH(multiparous animals). Ozone: significant increases of CP and decreases of antioxidants(primiparous animals); significant increases of MDA and decreases of DH(multiparous). Alcohol: significantly increases the OS markers and decreases the DH(primiparous animals).* ***Conclusions.*** *Smoking, ozone or alcohol associated with pregnancy (primiparous and multiparous) has a combined effect of increasing the oxidative stress and decreasing the antioxidant capacity. Because the results regarding O_3 exposure haven't been communicated by other authors, we suggest further research on this subject.*

Keywords: *pregnancy, oxidative stress, risk behaviors, alcohol, ozone, smoking*

Introduction

The pregnancy related complications benefit from a growing attention into the obstetrical practice, especially in the case of complications caused by risk behaviors. The pathogenic mechanisms that lead to these complications are still being studied and among them we can count the oxidative stress (OS). The oxygen is the main oxidant of the biomolecules found into the organic structures of the cells during the oxidation-reduction reactions. So, the oxygen continuously forms the reactive oxygen species (ROS) that react spontaneously and extremely quickly with lipids, glucides, proteins, nucleic acids etc. Also, the nitrogen forms the reactive nitrogen species (RNS). The imbalance between pro-oxidant and oxidant systems within intact cells, either by an excessive production of oxidants or by a significant decrease of the antioxidant capacity leads to oxidative stress.

Maintaining the homeostasis of the oxidants/antioxidants (O/AO) balance in physiologic limits is a characteristic of a healthy human organism. ROS and RNS are involved both in extreme reversible physiological conditions such as physical effort, pregnancy, ageing, living in hypo or hyperbaric conditions or pollution; and in pathologic conditions- the so-called "free radicals diseases" (over 100 types).

Objective

The presence of ROS and RNS into the female genital tract and the data concerning the modifications of the O/AO balance during a normal and pathologic pregnancy determined us to experimentally study the influence of the pregnancy on the pro-oxidants/antioxidants balance, such as the effects on pregnancy of the following factors: cigarette smoke, ozone exposure and alcohol consumption at primiparous and multiparous animals, with the purpose of applying the results in further clinical practice.

Materials and methods

The genetic resemblance between mice and humans and the reduced pregnancy duration (20-21 days) determined us to choose this species for experimental research on gestation.

For each aspect we have studied, we used three types of female rats of Wistar race (10 weeks un-pregnant, 12 weeks primiparous gestation, over 12 weeks multiparous gestation females), divided in 10 rats/group. From the blood we have sampled on the 21st-22nd gestation day, we have determined the OS markers: malondialdehyde (MDA) and carbonylated proteins (CP), but also the markers of the AO defense: the hydrogen donor capacity (HD) of the plasma and the sulfhydryl groups (SH).

Statistic interpretation

We used two-way Anova test to evaluate:

- the pregnancy (separately; we controlled each risk factor: cigarette smoke, alcohol, ozone)
- the risk factor (separately; we controlled the pregnancy)
- the combined effect of pregnancy and risks factors on O/AO markers

The analyze was made in SPSS, using General Linear Model and the statistic calculation with SPSS 13.0, Statistics 7.0 and Microsoft EXCEL

Cigarette smoke exposure

The animals were exposed to passive smoking - put into a covered cylindrical bowl, where we introduced, one by one, three lighted cigarettes (the product we used was composed of 0,9 mg nicotine, 10 mg CO, 10 mg tar/cigarette). The medium exposure duration was of 1 hour/day/ 28 days.

Ozone exposure

O_3 exposure was made with a ozonifier, a device that had the facility of regulating air ozone concentration from 50 mg/m^3 up to 500 mg/m^3. The air flux was of 5 litres/min (± 20%). The exposure duration to O_3 was of 10 min/day/28 days, at 0,5 ppm, according to international regulations approved in UE and USA.

Alcohol administration

The alcohol administration, in moderated doses, was made by tube. The quantity was of 0,5 ml ethyl alcohol 20%/ animal/day.

Results

Table 1 Effects of pregnancy, cigarette smoke, ozone and alcohol on MDA

	Cigarette smoke	Ozone	Alcohol
Primiparous pregnancy	Pregnancy decreases MDA (p<0.001) Cigarette smoke does not significantly affect(p=0.35) Pregnancy and cigarette smoke don't have a significant combined effect (p=0.20)	Pregnancy decreases MDA (p= 0.0008) Ozone does not have a significant influence (p=0.15) Pregnancy and ozone don't have a significant combined effect (p=0.40)	Pregnancy does not have a significant effect (p=0.14) Alcohol significantly decreases on MDA (p=0.004) Pregnancy and alcohol increase MDA(p=0.00002)
Multiparous pregnancy	Pregnancy decreases MDA (p<0.001) Cigarette smoke increases MDA (p=0.002) Pregnancy and cigarette smoke do not have a significant combined effect (p=0.95)	Pregnancy decreases MDA (p <0.001) Ozone has significant positive influence (p=0.04) Pregnancy and ozone do not have a significant combined effect (p=0.48)	Pregnancy significantly decreases MDA (p=0.000003). Alcohol significantly increases MDA (p=0.04) Pregnancy and alcohol have a significant effect on MDA (p=0.0001).

Table 2 Effects of pregnancy, cigarette smoke, ozone and alcohol on CP

	Cigarette smoke	Ozone	Alcohol
Primiparous pregnancy	Pregnancy decreases CP (p<0.001) Cigarette smoke decreases CP (p=0.01) Pregnancy and cigarette smoke have a significant combined effect on CP (decreases CP, but they cancel the decrease caused by cigarette smoke) (p=0.05)	Pregnancy does not affect CP (p=0.8949) Ozone increases CP (p=0.00001) Pregnancy and ozone significantly increase CP (p <0.001)	Pregnancy significantly increase CP (p=0.000001) Alcohol increases CP (p< 0.0001) Pregnancy and ozone significantly increase CP (p<0.001)
Multiparous pregnancy	Pregnancy decreases CP (p=0.002) Cigarette smoke increases CP (p=0.000003) compare to primiparous Pregnancy and cigarette smoke have a significant combined effect on CP (p=0.02)	Pregnancy decreases CP (p=0.001) Ozone does not significantly influence CP (p=0.12) Pregnancy and cigarette smoke have a significant combined effect on CP (p=0.04)	Pregnancy does not affect CP (p=0.49) Alcohol does not significantly influence CP (p=0.69) Pregnancy and alcohol increase excessively CP (p=0.00002)

Table 3 Effects of pregnancy, cigarette smoke, ozone and alcohol on SH

	Cigarette smoke	Ozone	Alcohol
Primiparous pregnancy	Pregnancy does not affect SH (p=0.22) Cigarette smoke does not significantly affect SH (p=0.32) Pregnancy and cigarette smoke don't have a significant combined effect on SH (p=0.66)	Pregnancy decreases SH (p=0.045) Ozone significantly decreases SH (p=0.0009) Pregnancy and ozone don't have a significant combined effect (p=0.23)	Pregnancy decreases SH (p=0.03) Alcohol decreases significantly SH (p=0.03) Pregnancy and alcohol significantly decrease SH (p=0.01)
Multiparous pregnancy	Pregnancy increases SH (p=0.01) Cigarette smoke decrease significantly SH (p=0.01) Pregnancy and cigarette smoke don't have a significant combined effect on SH (p=0.01)	Pregnancy has an important decrease on SH (p<0.001) Ozone significantly decreases SH (p=0.03) Pregnancy and ozone don't have a significant combined effect (p=0.78)	Pregnancy increases SH (p=0.0004) Alcohol significantly decreases SH (p=0.001) Pregnancy and alcohol don't have a significant combined effect on SH (p=0.33)

Table 4 Effects of pregnancy, cigarette smoke, ozone and alcohol on HD

	Cigarette smoke	Ozone	Alcohol
Primiparous pregnancy	Pregnancy increases HD (p=0.00001) Cigarette smoke does not significantly affect HD (p=0.37) Pregnancy and cigarette smoke don't have a significant combined effect on HD (p=0.27)	Pregnancy decreases HD (p=0.001) Ozone significantly decreases HD (p=0.001) Pregnancy and ozone have a significant combined effect (p=0.01)	Pregnancy decreases HD (p=0.03) Alcohol decreases significantly HD (p=0.002) Pregnancy and alcohol have a significant combined effect (multiplication) (p=0.0003)
Multiparous pregnancy	Pregnancy increases HD (p=0.00002) Cigarette smoke does not significantly affect HD (p=0.81) Pregnancy and cigarette smoke don't have a significant combined effect on HD (p=0.65)	Pregnancy doesn't significantly affect HD (p= 0.76) Ozone significantly decreases HD (p=0.00003) Pregnancy and ozone significantly increase HD (p=0.0001)	Pregnancy doesn't significantly affect HD (p=0.07) Alcohol significantly affect negatively HD (p=0.02) Pregnancy and alcohol significantly decrease HD (p=0.0003)

Discussions

During the normal pregnancy an oxidative and nitrosative stress is produced with benefic effects, but an excess leads to negative effects of SRO and SRN for the mother and for the embryonic/fetal development.

Smoking can initiate oxidative lesions through ROS and RNS. Cigarette smoke is a major exogenous pro-oxidant source; RNS are present both in gas phase and in particulate one[1].

Smoking during pregnancy represents one of the major challenges for the health of the mother and child. In 2002, World Health Organization published The Tobacco Atlas, a study about smoking incidence in different world regions. At that time, about 250 millions women in the world were smokers: approximately 22% of the occidental women and 9% of the women coming from developing countries. More than that, many Asian women chew tobacco. Smoking among occidental women is decreasing, but in Central, South and Eastern Europe this habit is increasing. The research warns about the fact that the tobacco industry promotes smoking among women by false images about emancipation, modernity and vitality[2].

Our research concerning the effects of cigarette smoke exposure shows that during gestation, it determines the increase of OS and the decrease of antioxidants in multiparous animals, just like the scientific literature that associates maternal smoking with a series of negative consequences that can have as pathogenesis the oxidative stress:

- **on pregnancy** (*abruptio placentae*, *placenta praevia*, hemorrhages, premature membrane ruptures or premature births)[3];
- **on fetus** (low birth weight)[3,4]; perinatal mortality (25-50% more increased in the case of smoking mothers)[3]; sudden death syndrome, before the child's first birthday. The mortality rate among babies with smoking mothers is

40% bigger than among those unexposed to cigarette smoke[5];
- diseases that affect the child later in life: tumors of the central nervous system, leukemia, lymphomas, respiratory diseases (asthma), neurological and behavioral disorders, obesity, diabetes, hypertension[5].

Other studies showed that smoking affects women's fertility the mechanism involved being the disturbance of the O/AO balance within the preovulator ovarian follicle, leading to intrafollicle OS and to the decreasing of OA defense. The intrafollicle content of lipid peroxides is associated with a local decrease in AO which pleads for the inducing of OS in Graaf follicle at passive and active smokers, as the nicotine level grows inside the follicle fluid, followed by the decrease of the oocyte number[6].

A study conducted on pregnant women passive and active smokers, discovered the presence of OS inside the fetal blood, the modifications being more pronounced in the case of active smokers who registered significant CAT, paraoxonaze 1 and AO decreases and increases of lipoperoxides, total oxidant status and OS index[7].

Another research made on smoking pregnant women (active and passive) discovered similar modifications in women and newborns, more pronounced in the case of active smokers: the alteration of the AO plasmatic status of the uric acid, vitamin C, the total AO capacity, the plasma capacity of reducing the Fe ferric to Fe ferrous. The research revealed increases of the uric acid and of the total AO capacity. The increase of the AO markers in the case of pregnant women exposed to cigarette smoking can be considered as an adaptive process to OS[8].

Ozone(O_3) is an exogenous pro-oxidant factor important in the formation of the OH radical in vivo. By oxidating the phospholipids, proteins and glucides of the cellular membranes, it causes oxidative lesions. Lately, the ozonotherapy (small doses O_3 treatment) is used as a revolutionary treatment with multiple benefits on human organism: the stimulation of the neutrophil production, the increase of the interferon and interleukins level, of the hematite's elasticity and consequently, it enhances the oxygenation of the tissues. In the same time, O_3 in increased doses is a pro-oxidant factor with adverse effects, generating the OS.

The effects of O_3 on human reproduction have not been the subject of many experimental studies. The O_3 exposure is used as a study model for OS, the respiratory airway being its way of penetrating the organism.

In the case of pre and/or postnatal exposure, O_3 can lead to:
- pulmonary ultra structural modifications: mitochondrial swelling, citoplasmatic vacuolization of the epithelial cells, structural damages caused by the OS, followed by exfoliations of the epithelial cells, mitochondrial edemas and lesions of their crypt[9];
- nervous and respiratory systems developing disorders (bronchia morphogenesis, cellular differentiation and proliferation, alveolisation and immune maturation of the vascular system)[10];
- increased respiratory necessities and the inflammation of airwaves at newborns, modifications that could cause the subsequent respiratory morbidity[11];
- the risk of oral malformations (hare lip, palatoschisis)[12];
- the reducing of the newborns' abdominal circumference in the case of exposure of their mothers between the 31st and the 60th days[13];
- significant risk of premature births in the case of exposure during the first trimester and during the first month of pregnancy[14].

Our research followed the effects of exposures during pregnancy in small doses, therapeutic and we determined modifications of the redox homeostasis in pregnant animals: the increase of OS markers and the decrease of the AO defense, data that hasn't been communicated by other authors yet. Our results bring experimental arguments in this direction and we do not recommend the ozonetherapy during pregnancy.

Moderate or low alcohol intake is associated with a lower risk of all-cause mortality and morbidity and with benefic effect on cardiovascular diseases, and our study showed a decrease of OS in non pregnant animals.

The scientific literature associates the **alcohol consumption** during pregnancy with a series of negative effects on the pregnancy and on the fetus: pregnancy loss, premature births, infantile mortality, neurologic disorders (Fetal Alcohol Syndrome, ADHD). The Fetal Alcohol Syndrome is associated with disorders such as: low birth weight (under 2500g), microcephaly, growing problems, congenital defects, cognitive and behavioral disorders[15,16]. Some of these abnormalities last until the adult life: camptodactyly, strabismus, hypoplasia of the nose, dental abnormalities (Autti-Ramo et al (2005), quoted by Seror et al. (2009)[16], a series of behavioral and social problems- low IQ, impulsivity, aggressiveness, social interaction problems[16], renal malformations[16], fetal alcohol habitude and the subsequent alcohol dependence, even in the case of moderate consumption[17].

The pathogenic mechanisms that relate alcohol consumption during pregnancy with the above mentioned disorders are not fully elucidated. It is well known that all kinds of stress on human organism (physical, psychological) have a common part: the oxidative stress and the results of our research showed that moderated alcohol doses during pregnancy determine significant oxidant increases and significant antioxidant decreases. The increasing of

oxidative stress in pregnant animals could be due to the OS induced by alcohol administration, which pleads in favor of the risk that can be produced by biochemical mechanisms on embryonary and fetal development and of the postnatal consequences revealed by other authors.

Conclusions

Our research brings experimental proof regarding the effect on redox homeostasis of some risk behaviors during gestation (smoking, ozone exposure and alcohol consumption) and it warns on the teratogenic consequences they could have on the conception product. Our study agrees with the literature and recommend the avoidance of these three factors in order to keep the mother and the baby safe.

If in the case of alcohol consumption and cigarette smoking there is an impressive number of studies that demonstrated their negative effects, we think that a continuation of the research on ozone exposure is necessary in order to determine more precisely its effects; this is because our study revealed the fact that ozonotherapy determines increases in OS and decreases in AO capacity, results that have not been communicated by other authors yet. ■

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