

Fetal alcohol spectrum disorders: can alcohol-related oxidative stress concur to the prenatal damage?

R Mancinelli^{1*}

Abstract

Introduction

Alcohol consumed during pregnancy freely crosses the placental barrier and constitutes a significant risk for many adverse outcomes globally defined Fetal Alcohol Spectrum Disorders (FASD) up to the Fetal Alcohol Syndrome (FAS). Many teratogenic mechanisms have been proposed but, to date, one of the most studied and understood is the alcohol-related Oxidative Stress (OS). Alcohol-related OS can affect lipids and proteins and can produce DNA alteration and epigenetic modifications since DNA is very sensitive to the OS effect. Recent animal studies show that alcohol-related OS can affect mainly the brain that is physiologically more vulnerable than other organs as liver or kidney.

Experimental evidence show that alcohol-related OS significantly concur to produce the Fetal Alcohol Spectrum Disorders (FASD) and the Fetal Alcohol Syndrome (FAS). Animal experimentation show that OS is involved in developing FASD and that the individual vulnerability observed in different rat strain may reside, at least in part, in the regulation of REDOX equilibrium. The association between FASD and oxidative stress is confirmed by the evidence that alcohol effects are mitigated when antioxidants are administered. Clinical studies performed by OS biomarkers as plasmatic Malondialdehyde (MDA), Free Oxygen Radicals Testing (FORT) and Free Oxygen Radicals Defense (FORD) in the blood of heavy drinking subjects evidence their OS status. Gender difference are significant since OS level in drinking women is higher than that in drinking men.

Conclusion

Animal studies show that OS condition have a significant role in impairing pregnancy and in developing the neuro-behavioral symptoms of FASD. Evidence from clinical studies suggest that to include the study of OS biomarkers in obstetric and neonatal routine diagnostic procedures could be a good strategy to early detect at-risk situation and to improve treatment. The high reliability and practicability of current available tests for determination of OS biomarkers make easier to reach this goal.

*Corresponding author
Email: rosanna.mancinelli@iss.it

¹ Istituto Superiore di Sanità, Roma, Italy

Introduction

To date because of the widespread habit of drinking in women, the prenatal alcohol exposure should be considered a major public health concern. Alcohol consumed during pregnancy freely crosses the placental barrier and constitutes a significant risk for many adverse outcomes globally defined Fetal Alcohol Spectrum Disorders (FASD) up to the Fetal Alcohol Syndrome (FAS)¹. Many teratogenic mechanisms have been proposed but, to date, one of the most studied and understood is the alcohol-related Oxidative Stress. Redox regulation plays a pivotal role for the life and the health of the cell since redox equilibrium between production of the Reactive Oxygen Species (ROS) and the activity of antioxidant defenses is essential for cellular functioning and many of the protein involved in the signal intracellular transductional chains (receptors, kinases, phosphatases and transcriptional factors) depend on it. There are a growing number of studies that show the influence of redox modulation in cellular signaling and that demonstrate how even slight redox modifications serve to modulate cell functions¹. Intracellular ROS are produced in mitochondria by means of the electron transport chain that is the main source of free radicals, and in the cytoplasm via xanthine oxidase and reduced nicotinamide adenosine dinucleotide phosphate (NADPH) and NADPH oxidase (NOX) pathways. NOX has a dedicated function of generating reactive oxygen species and experimental evidence suggests that NOX has an important role in signal transduction in cellular stress responses². Oxidation reactions are crucial for life but they can also be damaging; so there is a complex system including various types of antioxidants, such as glutathione, vitamin C, vitamin A, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases, that acts as a defense to counteract the production of ROS. When the redox homeostasis is altered by endogenous or exogenous factors such as exposure to chemicals or to ionizing radiation, it is produced the so-called oxidative stress (OS) that is the redox imbalance due to the increase of ROS and the decrease of endogenous antioxidant defenses^{3,4}. When OS condition occurs for a long time, heavy cellular damages such as apoptosis, necrosis and epigenetic modification can be produced. Alcohol drinking can produce redox imbalance both directly by its own metabolism that generates ROS, both indirectly by impairing antioxidant power of the cells⁵. Alcohol-related OS can affect lipids and proteins and can produce DNA alteration and epigenetic modifications since DNA is very sensitive to the OS effect⁶. Alcohol is a teratogen and onset and severity of alcohol-

related teratogenic effects are the result of endogenous and exogenous factors. Fetal damages were put into relation with mother blood alcohol concentration (BAC) and with enzymatic activity of alcohol dehydrogenase but what determines the individual vulnerability to alcohol damage is yet far from being completely explored. Experimental evidence show that alcohol-related OS significantly concur to produce the Fetal Alcohol Spectrum Disorders (FASD) and the Fetal Alcohol Syndrome (FAS). The present paper shows a review about recent studies about the role of oxidative stress in the development of alcohol teratogenic effects and proposes a critical debate based on the evidence of experimental data.

Alcohol Metabolism and alcohol-related oxidative stress

Alcohol metabolism depends both on genetic polymorphism of the individual enzyme pattern and on metabolic induction consequent to environmental exposure to xenobiotics⁷. The enzymes involved in ethanol metabolism present significant differences in activities due to racial peculiarities, age and sex⁸. The major pathway for the degradation of ethanol is its oxidation to hydrogen and acetaldehyde to which many of the toxic effects of ethanol can be attributed. Variations in alcohol and acetaldehyde metabolism are genetically determined by polymorphisms in alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH), and seem to play an important role in individual and racial differences, in acute and chronic reactions to alcohol, in alcohol drinking habits, as well as in the vulnerability to organ damage after chronic alcohol abuse and in the development of teratogenic effects^{9,10,11}.

The metabolic pathways of metabolism are:

1. Alcohol-dehydrogenase (ADH) that is the main enzyme involved in biotransformation of ethanol. It is a cytosolic protein encoded by a poligenic family (seven genes) in chromosome 4. In humans the variability in alcohol metabolism is due mainly to the polymorphism of allele ADH2 and some studies hypothesize that the genotype ADH2, both of mother and of fetus, may be related to individual susceptibility to the onset and severity of FASD¹².

2. Cytochrome P450 2E1 (CYP2E1), that is a member of the cytochrome P450 mixed-function oxidase system, and it is involved in the metabolism of xenobiotics in the body. CYP2E1, as well as alcohol dehydrogenase and aldehyde dehydrogenase, is an important enzyme for the conversion of ethanol to acetaldehyde and to acetate in humans even if its affinity for ethanol as substrate is significantly lower than ADH. In case of moderate alcohol consume CYP2E1 is responsible of oxidation of ethanol to acetaldehyde for about the 10% but, in case of alcohol abuse, ADH pathways is saturated and CYP2E1 activity increases. CYP2E1 activity is induced by ethanol so that it was found increased from 4 to 10 times in liver biopsies from heavy drinkers^{13,14}. In addition, CYP2E1- dependent ethanol oxidation may occur

in other tissues such as the brain, where ADH activity is low. CYP2E1 produces ROS, including hydroxyethyl, superoxide anion, and hydroxyl radicals, which increase the risk of tissue damage. CYP2E1 enhances the clearance of ethanol and contributes to the onset of metabolic tolerance in parallel to the tolerance of the central nervous system.

3. Catalase that is located in the cell bodies called peroxisomes and is the enzyme capable of oxidizing ethanol in vitro in the presence of a hydrogen peroxide (H₂O₂)-generating system, such as the enzyme complex NADPH oxidase or the enzyme xanthine oxidase. Quantitatively, however, this is considered a minor pathway of alcohol oxidation¹⁵. Chronic alcohol consumption by rats has been shown to result in increased H₂O₂ production in pericentral regions of the liver and increased catalase activity. The role of CYP2E1 and catalase is important mainly for alcohol metabolism in the brain^{16,17}.

Alcohol toxicity is due both to alcohol per se and to alcohol metabolism products such as acetaldehyde and reactive oxygen species (ROS) that compromise redox regulation of the cell and may interfere with lipids, proteins and DNA. DNA is very sensitive to oxidative stress that causes DNA hydroxylation, ring opening or fragmentation, besides of epigenetic alteration such as DNA methylation. Furthermore OS is due also to indirect alcohol effect that reduces the cellular antioxidant capacity. Ethanol is mainly toxic for mitochondria reducing its number and functioning. Typically, hepatocytes of alcoholic patient exhibit morphological and biochemical signs of mitochondrial damage. Mitochondria participate to alcohol metabolism with the conversion of acetaldehyde into acetate and the generation of increased amounts of NADH. Mitochondrial impairment seems to be responsible for ethanol disease including the reduced muscle strength that frequently occurs in alcoholics and impairment of central nervous system^{18,19}. Recent studies demonstrate the direct participation of mitochondria as a potential target of compounds that can be used to treat alcohol abusers²⁰.

Alcohol-related oxidative stress and teratogenic effects

Alcohol exposure generates oxidative stress in fetus developing organs and affects mainly the brain causing the development of fetal neuro-disorders^{21,22,23}. Even a short exposure to ethanol during pregnancy can produce an imbalance in the brain's intracellular redox status so that even a moderate alcohol use may produce fetal damage. The brain is more susceptible to OS since it has the highest oxygen metabolic rate because brain cells utilize the 20% of the oxygen consumed by body and generate a high quantity of ROS during oxidative phosphorylation. Furthermore brain is rich in unsaturated fatty acids that can serve as substrates for ROS, has a high content of iron that can promote generation of ROS, and several neurotransmitters can spontaneously react with oxygen. On the other hand, the antioxidant defense system of brain is less effective than that of other organs such as liver or

kidney and fetal cells themselves are more vulnerable to OS than adults because their antioxidant system is less effective. Animal experimentation suggest different mechanisms that may explain the relation between alcohol-related OS and FASD. As mentioned above, ethanol acts directly stimulating mitochondrial respiratory chain, the xanthine oxidase and the NOX pathways with consequent increase in the production of superoxide, hydrogen peroxide and hydroxyl radical into the cells. Furthermore, ethanol impairs endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) and glutathione reductase (GR) besides of the intracellular levels of reduced glutathione (GSH) so decreasing antioxidant defenses. It's seems likely that alcohol-related oxidative impairment occurred during fetus development may have long lasting effects by rendering the antioxidant defense system less effective in the life span. All available data obtained by animal experimentation show that OS is involved in developing FASD and also that individual vulnerability observed in different rat strain may reside, at least in part, in the regulation of REDOX equilibrium. The association between FASD and oxidative stress is confirmed by the evidence that alcohol effects are mitigated when antioxidants are administered^{24,25}.

Alcohol-related Oxidative stress and placenta

Placenta is the organ that establishes the connection mother/fetus and permits the passage of nutrients from the maternal blood to the fetus and so it's essential that the exchange across placenta be adequate to guarantee a normal fetus metabolism and growth. Epigenetic regulation of the placenta evolves during the preimplantation developmental period and further gestation. Imprinting is regulated by epigenetic mechanisms under the control of environmental factors and nutrients: this may provide a linkage between maternal nutrition and fetal placental growth. Development of placenta is a highly regulated process and is therefore quite susceptible to perturbation. Placenta itself generates ROS and may contribute to the oxidative stress observed even in normal pregnancy. ROS from placenta increase in such cases as preeclampsia, intrauterine growth retardation and pre-gestational diabetes.. Exposure of developing placenta to environmental agents such as alcohol or acetaldehyde can produce anatomical anomalies leading to in utero death or structural birth defects. It was demonstrated that ethanol exposure induces OS in the human placental villi so affecting placental blood flow regulation and generating the restriction of growth observed in ethanol-exposed fetus²⁶. Gundogan et al in 2010 found placental apoptosis/necrosis in rats chronically exposed to ethanol during pregnancy and showed that the alcohol related OS impairs the placental trophoblast function and could account for pregnancy loss²⁷. So the indirect ethanol effects on fetal development were related to placental pathology especially within the rat placental barrier. Ethanol exposed

placentas show ischemia, infarction, reduced thickness due to increased cellular necrosis. On the consequence, the delivery of nutrients to fetus is impaired and intrauterine growth retardation is generated. A further placental abnormality observed in case of ethanol exposure, is the impairment of the physiological conversion of uterine vessels required for expansion of maternal circulation into placenta. This impairs the production of the vascular system essential for ensuring adequate blood and nutrient delivery to fetal growth^{28,29}.

Oxidative stress biomarkers

Biomarkers have an important role in clinical and research to understand the relationship between exposure to xenobiotics, development of diseases and identification of subgroups that are at increased risk for disease. Alcohol biomarkers include trait markers and state markers. Trait markers are used to reveal the risks of abusing alcohol and the dependence of alcohol in a person and are connected to genetics (for example, polymorphisms of ADH and CYP2E1). State markers indicate recent drinking, reveal if there are biochemical damages consequent to alcohol use, measure the progress of alcohol diseases and monitor the treatment. Example of state markers are: the presence of Ethylglucuronide that is a minor metabolite of ethanol used to determine ethanol up to 80 hours consumption; the increase of Glutamyl Transferase (GGT), Carbohydrate-Deficient Transferrin (CDT) and Mean Corpuscular Volume (MCV) that require several weeks or months of sustained alcohol consumption to be significantly elevated; the Blood Alcohol Concentration, Acetaldehyde, 5-hydroxytryptophol – that are able to demonstrated in short-term alcohol use and are measurable soon or only few days after consumption; the Vitamine B1 deficiency that is considered not only a biomarker of alcohol abuse but also a prognostic index of alcohol related brain disease³⁰. Oxidative stress biomarkers are considered state markers useful to measure the progress of alcohol diseases. Lipids are the class of molecules most involved in oxidative stress and lipid peroxidation is used as an indicator of oxidative stress. Malondialdehyde (MDA) derives from the polyunsaturated fatty acid peroxidation and it is considered an effective biomarker of OS; so the determination of plasmatic MDA is one of the most significant tool to evaluate oxidative stress. Recently, ISS researchers carried out a clinical study about OS in a population of alcoholics using different biomarkers. Besides of plasmatic Malondialdehyde (MDA) by a HPLC procedure, the Free Oxygen Radicals Testing (FORT) and the Free Oxygen Radicals Defense (FORD) by Callegari systems (Italy) were performed and used to determine free radicals and antioxidant defense in the blood of alcoholic patients. FORT It is a colourimetric test based on the ability of transition metals, such as iron, to catalyze free radicals that are formed in the breakdown of hydroperoxides (ROOH). The free radicals are subsequently trapped by chromogenic substance (CrNH₂) which is an amine derivative. Free Oxygen Radicals Defense (FORD) is a

colorimetric method to quantify the ability of antioxidants present in plasma to reduce a performed radical cation. The test measures antioxidant ability by the decrease of color intensity before and after adding sample: the decrease of color intensity is due to various antioxidants such as proteins, glutathione, vitamins that form the antioxidant plasmatic defense. Data obtained by of MDA, FORD and FORT determination resulted consistent. OS of alcoholics was significantly higher than controls and there are gender differences; in fact in drinking women OS was significantly higher than that in drinking men⁸. This evidence justifies the warning against alcohol-related fetal damage and the recommendation to completely avoid alcohol drinking during pregnancy³¹.

Discussion

The Fetal Alcohol Spectrum Disorders and the Fetal Alcohol Syndrome represent a complex malformation condition of the infant and child due to maternal drinking during pregnancy. Teratogenic effects of alcohol are very different and include physical abnormalities and behavioral/neurocognitive deficits. The mechanisms underlying the development of so devastating effects remain yet uncertain but the wide variety of cellular/biochemical effects on fetal tissues strongly suggests that teratogenic responses to alcohol exposure is the result of a multifactorial setting including endogenous and exogenous factors. According to a modern point of view, in the case of the FASD studies we can apply the concept of exposoma, i.e. of the totality of human environmental (i.e. non-genetic) exposures from conception onwards, complementing the genome. In respect to 70's when the Fetal Alcohol Syndrome was first described, to date we have the help of innovative diagnostic tools that let us to study and evaluate the peculiarities of the disease in terms of cellular and organs impairment. For example, new diagnostic perspective have been opening by the recent development of genomics, proteomics and metabolomics that is one of the newest 'omics' sciences. As regards alcohol related oxidative stress, we can affirm that it is involved in the development of some diseases including FASD since experimental evidence prove that alcohol drinking directly and indirectly triggers intracellular redox imbalance. Animal studies let us enhance our knowledge about the effects of prenatal alcohol exposure but there are yet open problems. For example, results of animal studies are not so consistent and comparable as desirable in consequence of different experimental condition used to treat animals. Furthermore remains to be clarified what is the most vulnerable gestational age and if the pre- or perinatal antioxidant treatment can really avoid or mitigate some neuro behavioral effects. All this considered, it must be clearly recommended to women to avoid alcohol drinking when they are planning pregnancy or are pregnant.

Conclusion

Alcohol related OS is a condition that has a significant role in impairing pregnancy and in developing neuro-behavioral symptoms of FASD. Nowadays different biomarkers are available to reveal and to monitor OS condition and many of them have been successfully used for clinical alcohol studies. A good prevention strategy may be to include determination of OS biomarkers in the obstetric and neonatal panel of routine diagnostic analyses in order to early detect at-risk situation and to improve treatment. The reliability and practicability of current available tests for OS determination make easier to reach this goal.

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