EFFECTS OF ALCOHOL ON THE FETAL BRAIN. A BRIEF REVIEW OF PATHOPHYSIOLOGIC MECHANISMS AND STRUCTURAL LESIONS

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Abstract

Alcohol is commonly used among people in social occasions and in everyday life but alcohol use disorders are a major health concern with multiple negative effects. Among these, is the toxic effect of alcohol on the developing fetus, even though it has not yet received the proper attention. Ethanol is a well-known teratogen that crosses the placenta and causes diffuse structural and functional lesions in the developing brain. The spectrum of disorders that can be caused by prenatal exposure to alcohol is known as fetal alcohol spectrum disorders.

Ethanol administration to pregnant women, in order to examine its teratogenic effects, cannot be justified by the rules of medical ethics and law. Thus, experimental models are of great importance in order to gain understanding into these effects. The use of experimental models though presents some difficulties concerning the application of their results in human embryos, such as the time duration of embryologic developmental stages and the different rate of alcohol metabolism. Nevertheless, well-designed experimental models may provide useful information including the quantity of alcohol that can induce damage to the fetus, the critical neurodevelopmental periods of greater vulnerability for the brain as well as the underlying pathogenetic mechanisms.

Alcohol has been shown to affect practically the whole brain: its actions are relevant to neural crest formation, central nervous system differentiation, neuronal migration, myelination, synaptogenesis and gliogenesis. Among the anatomic structures, corpus callosum, hippocampus and cerebellum seem to be most vulnerable to the toxic effects of alcohol.

We initially describe the pathophysiologic and biochemical mechanisms underlying the toxic effects of alcohol in the fetal brain. Then, we describe the structural changes of the brain resulting from these toxic effects, based on results from experimental animal models.

Key words: alcohol, brain, experimental models, fetal alcohol syndrome

ΕΠΙΔΡΑΣΕΙΣ ΤΟΥ ΑΛΚΟΟΛ ΣΤΟΝ ΕΜΒΡΥΙΚΟ ΕΓΚΕΦΑΛΟ. ΒΡΑΧΕΙΑ ΑΝΑΣΚΟΠΗΣΗ ΠΑΘΟΦΥΣΙΟΛΟΓΙΚΩΝ ΜΗΧΑΝΙΣΜΩΝ ΚΑΙ ΑΝΑΤΟΜΙΚΩΝ ΒΛΑΒΩΝ

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Περίληψη

Το αλκοόλ είναι ουσία με ευρεία διάδοση και οι διαταραχές της χρήσης του αποτελούν σημαντικό πρόβλημα υγείας με πολλαπλές αρνητικές επιδράσεις. Μία από αυτές, που δεν έχει τύχει της ανάλογης προσοχής, είναι η τοξική του δράση στο αναπτυσσόμενο έμβρυο. Η αιθανόλη είναι γνωστό τερατογόνο που διαπερ-

νά τον πλακούντα και προκαλεί διάχυτες δομικές και λειτουργικές βλάβες στον αναπτυσσόμενο εγκέφαλο. Το φάσμα των διαταραχών που μπορεί να προκαλέσει η προγεννητική έκθεση στο αλκοόλ είναι γνωστό ως φάσμα των διαταραχών του εμβρυικού αλκοολικού συνδρόμου.

Καθώς η χορήγηση αιθανόλης σε εγκύους, προκειμένου να διαπιστωθεί η τερατογενετική της δράση, αντιβαίνει τους κανόνες ιατρικής ηθικής και δεοντολογίας, τα πειραματικά μοντέλα είναι πολύ χρήσιμα στην κατανόηση αυτής της δράσης. Η χρήση των ζωικών μοντέλων παρουσιάζει κάποια προβλήματα όσον αφορά τη συσχέτιση με τις αντίστοιχες βλάβες στους ανθρώπους (π.χ., χρονική διάρκεια των σταδίων εμβρυολογικής ανάπτυξης και διαφορετικός ρυθμός μεταβολισμού του αλκοόλ), αλλά καλά σχεδιασμένα ζωικά μοντέλα μπορούν να παρέχουν χρήσιμες πληροφορίες όπως, ποια ποσότητα αλκοόλ είναι επιβλαβής, ποιες είναι οι νευροαναπτυξιακές περίοδοι που ο εγκέφαλος είναι περισσότερο ευάλωτος και ποιοι είναι οι υποκείμενοι μηχανισμοί των βλαβών.

Έχει βρεθεί ότι το αλκοόλ μπορεί να επηρεάσει πρακτικά όλο τον εγκέφαλο: οι δράσεις του σχετίζονται με τον σχηματισμό της νευρικής ακρολοφίας, τη διαφοροποίηση στο κεντρικό νευρικό σύστημα και τη νευρωνική μετανάστευση, τη μυελίνωση, τη συναπτογένεση και τη γλοιογένεση. Από τις ανατομικές δομές φαίνεται να επηρεάζονται ιδιαίτερα το μεσολόβιο, ο ιππόκαμπος και η παρεγκεφαλίδα.

Περιγράφουμε αρχικά τους παθοφυσιολογικούς και βιοχημικούς μηχανισμούς διά των οποίων το αλκοόλ ασκεί τις τοξικές του δράσεις στον εμβρυικό εγκέφαλο. Κατόπιν, αναφερόμαστε στις δομικές μεταβολές που επέρχονται στον εγκέφαλο συνεπεία αυτών των τοξικών δράσεων, έτσι όπως έχουν παρατηρηθεί σε πειραματικά μοντέλα.

Λέξεις κλειδιά: αλκοόλ, εγκέφαλος, εμβρυικό αλκοολικό σύνδρομο, πειραματικά μοντέλα

Introduction

Alcohol use during pregnancy, especially if it is heavy and continuous, can lead to fetal growth restriction and concurrent cognitive defects and behavioral problems. Except for the permanent defects in cognitive, functional, anatomic, neurobiological, and neurobehavioral level, the negative consequences of maternal alcohol use on the child extend beyond the in utero effects, affecting also the maternal care [1]. The neurodevelopmental effects of in utero exposure to alcohol are long-lasting and are the main reason of concern about prenatal exposure to alcohol [2].

Knowledge about the negative effects of alcohol use during pregnancy on the fetal development has a long history. In fact, experimental data concerning these effects have been known since the beginnings of the 20th century [3]. The spectrum of the major effects that alcohol may exert to the developing fetus is known as fetal alcohol spectrum disorders [3]. The consequences of maternal alcohol use during pregnancy in the neurobehavioral development of the fetus depend upon various factors including the pattern of alcohol use, the period of pregnancy during which alcohol is used, and, perhaps the most important, the quantity of alcohol used [2]. Nevertheless, it is probable that even non continuous pattern of alcohol use, such as binge drinking, may cause more serious defects [2].

The usefulness of animal models for the understanding of the harmful effects of alcohol is well documented. Since the neuroanatomical, molecular and cellular effects of alcohol use during pregnancy cannot be studied in vivo, well-designed animal models can provide very useful information [4] answering questions such as which quantity of alcohol is harmful, which are the neurodevelopmental periods of greatest vulnerability for the brain and which are the underlying mechanisms of the harmful effects [2].

The first studies using experimental animals confirmed the relationship between prenatal alcohol exposure and fetal alcohol syndrome in observational studies in humans [5]. The better mechanistic understanding of the effects induced by alcohol in the developing brain, in experimental studies, can prove useful for two reasons. First, it can help the development of effective therapeutic strategies. Second, it may make possible the discovery of biological markers which could aid to diagnosis [6].

Of course, using animal models presents some difficulties as far as it concerns associating findings in animals with similar problems in humans. One difficulty has to do with the duration of various stages of embryological development and a second one with the different rate of metabolism of alcohol in humans versus experimental models [6]. The critical periods for the harmful effects and the teratogenic action of alcohol are embryological time periods during pregnancy when specific developmental changes take place. These changes vary between different species [5].

Mechanisms of fetal alcohol toxicity

Alcohol is a well-known teratogen and the developing central nervous system (CNS) is very vulnerable to its effects [2]. It is a CNS depressant that crosses

Table 1. Mechanisms of fetal alcohol toxicity

Hypoxia	
Oxidative stress	
Placenta	Decreased blood flow in the umbilical artery Vasoconstriction Disrupted architecture Decreased size Decreased ability to transport nutrients
Disordered absorption of nutrients from the gut	
Effects on hormonal levels	
Decreased protein synthesis	
Disrupted signaling of growth factor	
Disordered microvascular development	
Transient cortical astrogliosis	
Effects on the activity of ion channels, receptors and enzymes	
Neuronal cells	Effects on neuronal development, synaptic function, neuronal plasticity Cellular death Depressed migration of neural crest cells Disruption of normal aggregation and adhesion of neuronal cells

the placenta, is rapidly distributed to the fetus [7] and affects the fetal brain through various mechanisms, such as hypoxia, decreased blood flow in the umbilical artery [8], oxidative stress, which increases through the depression of oxidative phosphorylation and NADH addition by ethanol oxidation [9], as well as negative effects in neuronal development, synaptic function and neuronal plasticity [10] (Table 1).

Alcohol affects the fetal brain through neuronal anomalies or cellular death [11], decreases protein synthesis and affects hormonal levels [12], disrupts signaling of growth factor [13] and depresses migration of neural crest cells resulting to the migration of fewer cells from prosencephalon, mesencephalon and rhombencephalon. Furthermore, this migration takes place in a distorted way in higher concentrations of ethanol and causes cellular death in neural crest at a great degree [10, 14]. It can cause disorders in microvascular development and transient cortical astrogliosis through increased expression of glial fibrillary acidic protein [15]. This protein has been a focus of research in various studies concerning fetal alcohol syndrome and is frequently used as a biomarker for astrocytes, their up-regulation being an indication of reactive astrogliosis [15].

Contrary to adults, the fetus is more vulnerable to the effects of ethanol. Its fetal concentrations are estimated to be at about 2/3 of those in maternal blood. Furthermore, ethanol is found in amniotic fluid and the fetal liver does not metabolize it sufficiently, thus the fetus depends on the mother for its catabolism [16]. The rate of ethanol removal from the fetus is estimated roughly at 3-4% of maternal rate of removal, thus it accumulates in amniotic fluid resulting to greater exposure of the fetus to ethanol: the fetal renal system removes xenobiotics in amniotic fluid which are consequently reassumed by the fetus with its swallowing movements [17]. It should be also bore in mind that both alcohol and its metabolite, acetalheyde, are teratogenic, irrespectively of the presence of dietary deficiencies or other environmental factors [18]. The effect of alcohol on the fetus is long-lasting due to its accumulation in amniotic fluid, decreased concentration of metabolic enzymes in fetal liver and decreased removal [19].

Alcohol affects absorption of nutrients from the gut which, in turn, leads to decreased availability of nutrients for the fetus despite the fact that it has higher metabolic rate compared to the mother [20]. Concurrently, both the size of the placenta and its ability to transport nutrients are decreased while its architecture is disrupted [21]. Necessary factors for the fetal development (biotin and vitamin B6) may not be properly transported to the fetus owing to mechanisms such as ethanol-induced vasoconstriction in the placenta, oxidative stress leading to decrease of nitrous oxide, which has vascular dilatation properties, and the disruption of balance between thromboxane, which causes vasoconstriction, and prostacyclin, which causes vascular dilatation [22].

Ethanol affects the activity of ion channels, receptors and enzymes. It increases glutamate levels and

decreases NMDA receptors [23], blocking their activity, increases the GABAergic activity, thus decreasing cortical and subcortical activity [24]. Blockade of glutamate receptors causes their up-regulation leading possibly to increased vulnerability in cases of increased excitability and to epileptic seizures during alcohol withdrawal [25]. Lack of neuronal inhibition by GABA receptors [26] and changes to voltage-gated calcium channels [27] also lead to increased neuronal excitability during alcohol withdrawal.

Free radicals can cause brain cellular damage through uncontrollable apoptosis, as has been shown in experimental models [28]. Thus, the characteristic facial morphology in fetal alcohol syndrome may relate to apoptosis of cells of neural crest in cranium, while the neuropsychiatric symptoms may relate to apoptosis in serotonergic neurons [29]. Ethanol disrupts the normal aggregation and adhesion of neuronal cells by increasing molecules that play an important role in the normal brain development: laminin-1 (α and β), β-integrins 3 and 5, sarcoglycan-ε and phosphoprotein-1 [30].

The placenta is very vulnerable to the effects of alcohol. Experiments in animals have shown that prenatal exposure to alcohol has wide, multiple effects both on its morphology and function [31]. The relevant mechanisms include disordered transport of nutrients [32], vasoconstriction in the placenta and the umbilical cord [33], effects on signaling of insulin and its growth factor leading to problems in the mobility of trophoblast [34], disorders in the natural change of maternal spiral arteries and inhibition of cellular mobility of trophoblast in a dose-dependent manner [34, 35], disruption of placental angiogenesis, decreased efficacy of nutrient-waste exchange, increased oxidative stress [36], DNA damage, lipid peroxidation, and mitochondrial dysfunction [35], decreased survival of trophoblast and disordered gene expression [37]. Yet, the effects of alcohol on brain development are not confined to processes, such as partition, increase, and migration of neurons [18].

Alcohol and structural lesions in the fetal brain of experimental animals

Even though, as a rule of thumb, studies about fetal alcohol syndrome focus on the maternal use of alcohol during pregnancy, there are indications concerning the role of paternal alcohol use, even in the absence of alcohol use by the pregnant mother. This has been shown in studies in rodents over a century ago [38].

Many animal models have been developed and tested in order to understand better the effects of alcohol on the developing brain. The majority of the studies though are done in mice and rats [15]. For decades, rodents have been used as models to examine the teratogenic effects of alcohol which seem to include developmental delay, congenital defects of the CNS, intellectual disability, and craniofacial anomalies [39]. Animal models show that prenatal alcohol use affects multiple neurodevelopmental processes. The importance of the effects varies from person to person and depends on factors, such as the pattern, the dose and the duration of alcohol use, genetic, environmental and nutritional effects [40].

Animal models of prenatal alcohol exposure have revealed increased teratogenic effects and congenital defects, including serious neurological injuries. The effects of alcohol are devastating for the developing brain during all the stages of pregnancy [15] with structural anomalies being similar to those observed in human embryos: hypoplasia or aplasia of the corpus callosum [41], microcephaly [42], disorders of the neocortex [14], the hippocampus [43], with disruption in organization and allocation of hippocampal nervous fibers, decreased number of neural cells and dendritic size [44], the cerebellum and the basal ganglia [45], decreased myelination in major tracts of the white matter of brain's midline [46], decreased production and size of brain and spinal motor neurons, increased neuronal cell death, disordered cellular migration, problems in the integration of receptors of cytoplasmic membrane [47] (Table 2).

The anatomical lesions may include decreased volume of the brain and the brain cells in specific regions, spoiling of neuronal cells, molecular, biochemical and cellular events that contribute to the formation of the brain, such as defects in cellular development, differentiation and migration [48]. Short-term exposure to substances that block NMDA receptors or act synergistically to $GABA_A$ receptors (both are properties that alcohol has) causes diffuse apoptotic neurodegeneration of the developing brain, the period of brain development being the one of greatest vulnerability [49].

Experimental animals of young age, exposed to alcohol in utero, manifested disorders of neuroendocrine and immunological nature, that is changes in hypothalamic-pituitary-adrenal axis resulting to increased response to stress and immunological disorders resulting to increased vulnerability to infections [50]. In rodents of very young age, even only one high dose of alcohol is sufficient enough to cause diffuse apoptosis of neurons in the forebrain, the mecencephalon, the cerebellum, the spinal cord and the retina [51]. Phenotypes similar to the fetal alcohol syndrome in these models have been related to changes in gene expression in the developing brain [52].

Exposure of experimental animals to alcohol during the developmental period, which is equivalent to the 1st trimester of pregnancy in humans, may cause facial morphological disorders similar to those observed in fetal alcohol syndrome. Furthermore,

Table 2. Alcohol-induced structural lesions in the fetal brain of experimental animals

Diffuse apoptotic neurodegeneration	Forebrain Mecencephalon Cerebellum Spinal cord Retina
Decreased production and size	Brain Spinal motor neurons
Defects	Cellular development, differentiation, migration Neural crest
Decreased	Myelin Neural tissue
Disorders	Neuroendocrine Immunological Integration of receptors of cytoplasmic membrane Cellular migration Neuronal development Neural circuits
Inhibition	Catabolism of brain proteins Dendritic spreading
Increased	Neuronal cell death Neuronal loss Cellular disorganization Gliosis
White matter	Reduced volume Complete absence of formation and decreased myelination of important white matter tracts Hypoplasia or aplasia of the corpus callosum
Cerebellum	Reduced number of granular and stellate cells Basket and Purkinje cells less affected Partial dendritic hypertrophy
Hippocampus	Decreased volume, number and density of cells Disorders in synaptic morphology, mechanisms of intracellular signaling and plasticity

defects of neural crest and disorders of neuronal development are observed [18, 42]. Exposure during the period that is equivalent to the 2nd trimester in humans may cause loss of neurons, disorders of neuronal differentiation and migration with resultant decrease of neural tissue, cellular disorganization and developmental delay. During the 3rd trimester, it can lead to disorders in neural circuits, extended gliosis and serious neural loss. In fact, this last period is a period during which the brain is extremely vulnerable to its insult from alcohol [18, 53].

During the 3rd week after fertilization, alcohol-specific defects begin to appear in the developing fetus. Between weeks 3 and 6, the cells of cranial neural crest become vulnerable to the toxic effects of alcohol, while from the 3rd week until the 3rd trimester of pregnancy morphological anomalies of the CNS and decrease of the white matter of the brain may appear. During weeks 6 and 7 after fertilization, the corpus callosum is very vulnerable to the effects of alcohol and the same is true for the cerebellum during week 8 [54].

Alcohol is a teratogen that affects the whole brain, nevertheless, cerebellum, hippocampus, corpus striatum and frontal cortex are more vulnerable [55]. Experimental models reveal that hippocampus is subjected to structural damage, including decrease of its volume, the number and the density of its cells, as well as disorders in synaptic morphology, the mechanisms of intracellular signaling and its plasticity [56]. Hippocampus, which plays an important role in memory, knowledge and cognitive functions, is especially vulnerable to the neurotoxic effects of ethanol [57]. Exposure to alcohol may lead to serious decrease (up to 40%) of pyramidal cells of the ventral hippocampus, which are considered more vulnerable contrary to those of dorsal hippocampus [58], of granular cells of dentate gyrus (which are

considered less sensitive to the effects of ethanol compared to the previous ones) [59], and of the cells of relay neuronal circuits in these areas [60]. The loss of the above cells probably comes as a result, mainly, during cessation than exposure to ethanol, or beyond the exposure period continuing even to withdrawal period [61].

The cerebellum is also vulnerable to the effects of prenatal alcohol exposure. Cerebellar lesions may, at least in part, probably be reversible. Slowing of the naturally occurring thinning of the outer granular layer or even delay in the decrease of the outer granular layer in rats is observed. These processes may be indicative of a delay of inward migration of granular cells [62]. Alcohol reduces the number of granular and stellate cells of cerebellum while the basket and Purkinje cells seem to be less affected, and the Golgi cells are probably not affected at all [63]. Cerebellar neurons of rats, which survive ethanol exposure for 12 months, show partial dendritic hypertrophy [64].

Prenatal exposure to alcohol inhibits dendritic spreading in various brain regions, which could finally lead to disordered neuronal interconnection [44]. Furthermore, delay in neuronal myelination may be observed [65]. Chronic administration of ethanol leads to decrease of myelin [66], inhibition of catabolism of brain proteins [67] and gliosis [68]; it is speculated that glial cells possibly play an active role in ethanol metabolism in the brain [69].

Alcohol can induce activation of microglia, and even cellular death. This activation may take place through Bcl-2-associated X protein, as has been shown in mice [70]. Alcohol increases the release of inflammatory cytokines from microglia and reduces intracellular cyclic AMP and brain-derived neurotrophic factor [71], increases the vulnerability of microglia [70], which can lead to its long-term sensitization resulting in persistent inflammatory signaling in the brain after an insult [72].

The white matter is a target of alcohol's harmful effects on the developing CNS (reduced volume and/or complete absence of formation of important white matter tracts in the brain). These effects on the oligodendrocytes and the microscopic structure of myelin have been well documented [73]. In rats, clear changes in the expression of oligodendrocytes, delay and reduction in the expression of basal protein of myelin in cerebellum, disorders of the myelination of optic nerve with reduced thickness and fewer myelin sheaths with microscopic lesions, have been shown [54, 74]. Studies in experimental animals also reveal that genetic factors play an important role in the risk and the importance of alcohol-induced damage in the developing brain [5, 75].

Conclusions

Alcohol's toxic effects in the fetal brain are well documented and have been shown both in experimental models and human embryos. These effects are caused through various mechanisms and depend on various factors, such as the dose and the duration of alcohol use. Since experimental studies in pregnant women are not ethical, the lowest dose of alcohol that can be safely used by the pregnant woman without causing harm to the fetus has not been yet documented with safety. For this reason, total abstinence from alcohol should be proposed during pregnancy and this suggestion should be part of a systematic effort of preventive medicine in pregnant women.

All studies converge to the fact that alcohol affects practically the whole brain. This is evident in infancy, adolescence and adult life, but the most important is that the effects may last throughout the whole life. Experimental models have been of great value in trying to understand the effects of alcohol on the CNS. Ethanol readily crosses the placenta and reaches the embryo, whose brain is a major target of its toxic action. This action has many sides and various clinical manifestations depending on the developmental stage of the embryo.

Ethanol induces massive neuroapoptosis in the developing brain. Studies in experimental models have shown the toxic effects of ethanol through various mechanisms causing major damage in critical neurodevelopmental periods [76] leading to changes in the volume of the brain, to anatomic lesions in areas such as the cerebellum, the corpus callosum, the basal ganglia and the optic nerve. Furthermore, morphological defects and disorganization in microcellular level in areas such as the frontal cortex, the corpus striatum and mecencephalon may be observed.

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