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New insights in Foetal Alcohol Syndrome: A Literature Review

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Abstract
Foetal Alcohol Syndrome falls under the umbrella of Foetal Alcohol Spectrum Disorders which are caused by prenatal alcohol exposure. Foetal Alcohol Syndrome is characterised by craniofacial abnormalities, central nervous system abnormalities and growth deficiencies. Alcohol consumption during pregnancy is teratogenic causing issues in multiple aspects of neurological development in the foetus. It is a vital preventable cause of mental disability in the West.

The main craniofacial abnormalities that are present in Foetal Alcohol Syndrome include a thin vermillion border, short palpebral fissures and a smooth philtrum. Alcohol exposure can also cause various epigenetic changes in the developing foetus. This alters gene expression resulting in various abnormalities in different organs and may also affect future behaviour.

Prenatal alcohol exposure also affects brain morphology and biochemistry. Alcohol alters survival, migration and function of various cells in the brain. It also alters the Gamma-Aminobutyric Acid system, a vital neurotransmitter system in the brain. Brain neovascularisation is also altered with consequences on brain perfusion.

This literature review shall highlight various effects of alcohol on craniofacial development, epigenetics, glia, the gamma-aminobutyric system, neovascularisation, and cell death in the developing foetus.

Keywords
Foetal Alcohol Syndrome, Alcohol, Embryo, Prenatal

Introduction

All the disorders caused by prenatal alcohol exposure (PAE) can be grouped under the term Foetal alcohol spectrum disorders (FASD). Foetal Alcohol Syndrome (FAS) comprises the group of characteristics associated with PAE.(1) Other disorders within this spectrum include partial foetal alcohol syndrome (pFAS), a neurobehavioral disorder associated with PAE (ND-PAE), alcohol related birth defects (ARBD) and alcohol related neurodevelopmental disorder (ARND).(2) It is the primary cause of preventable mental disability in the West. (3)

Although any amount of PAE is teratogenic, the risk of a child being born with FAS was associated with timing and dose of alcohol.(4) A greater blood alcohol content (BAC) was found to increase the

risk of harming the foetus.(5) Ethanol diffuses through the placenta and takes much longer to be eliminated.(6) Therefore, the concentration in the amniotic fluid increases.(7) Since the liver is still developing in the foetus, the placenta has a major role in metabolism, particularly in the first trimester.(8) Cytochrome P450 2E1 (CYP2E1), as opposed to alcohol dehydrogenase (ADH), is the main enzyme which metabolises ethanol in the placenta due to ethanol's greater affinity for this enzyme in the placenta.(9)

Method

A MEDLINE search was carried out using the search terms "foetal alcohol syndrome"," effects of alcohol", "neurological development of a foetus" and "prenatal exposure to alcohol", from inception to December 2021

Craniofacial Abnormalities in Foetal Alcohol Syndrome

The three main facial abnormalities associated with Foetal Alcohol Syndrome (FAS) are: a thin vermillion border, short palpebral fissures, and a smooth philtrum. A flattened nasal bridge, a small jaw, a shorter epicanthal and interpupillary distance and epicanthal folds may also accompany these. (10) Changes in bone and tissue imply alcohol's negative effect on the development of neural crest cells (NCCs) which include expansion, apoptosis, migration, induction, and differentiation. (11) Other changes are likely to result from a decrease in brain growth. (12)

Cranial NCC migration was found to decrease, become asymmetrical and display a lack of direction at low ethanol concentrations.(13) Ethanol causes a morphological alteration in migrating NCCs (less filopodia and focal adhesions, rearrangement and reduced branching of actin bundles and a decrease in cell surface area and perimeter).(14) Studies show that cranial NCCs adopted a pyknotic appearance when exposed to ethanol.(15,16) Ethanol causes an increase in the calcium level intracellularly through the inositol triphosphate (IP3) pathway, however one third of the calcium comes from an extracellular source.(17) It also causes a reduction in oxidative phosphorylation and nicotinamide adenine dinucleotide (NADH) accumulates due to the metabolism of alcohol, resulting in oxidative stress.(18) NCCs also have naturally lesser levels of superoxide dismutase,(19) which makes them increasingly sensitive to reactive oxygen species (ROS).(20) This contributes to the apoptosis of NCCs. It was found that NCCs produced high ROS concentrations when subjected to alcohol. (21)

The facial features of FAS fit in the holoprosencephaly (HPE) spectrum.(22) In mouse models prenatal alcohol exposure (PAE) was found to hinder the development of the neuroectoderm by decreasing migration of the prechordal plate, apoptosis of the anterior prechordal mesoderm (PME) and causing a significant reduction of PME signalling, including sonic hedgehog (SHH).(23) Reduction in SHH signalling occurs due to a reduction in cholesterol ester pools, preventing protein membrane assembly, and due to the increase in protein kinase A, which causes signal suppression.(23,24)

The Effect of Alcohol on Epigenetics

Epigenetic mechanisms involve histone modification (Table 1), DNA methylation and small noncoding ribonucleic acids (RNAs). (25) Ethanol increases the messenger RNA (mRNA) formation of histone altering genes (26) and induces hypoacetylation which reduces gene expression. (27) (see Table 1, below).

Global hypomethylation has been shown in animal models.(39) Neural tube defects are induced due to methylation changes in the 7^{th}, 10^{th} and X chromosome.(40) Mouth swabs taken from children with foetal alcohol spectrum disorder (FASD) show that genes associated with neurodevelopmental and neurological diseases, such as anxiety, are hypermethylated.(41) Hypermethylation of the proopiomelanocortin (Pomc) gene promoter region was observed, causing a reduction in expression of this gene, which is associated with a reduction in formation of beta endorphin, which suppresses the stress axis.(42)

Table 1: Effects of alcohol on various histones. (28-38)

Histone	Effect of Alcohol	Location and Effect
H3K9/18	Acetylation	Apoptosis of Lung Tissue. Also seen in in vitro cardiac progenitor cells at H3K9, increasing dHAND and eHAND expression and impairing heart development and suggesting that alcohol may cause congenital heart disease.
H3K14	Acetylation	Rodent brains during the generation of synapses inducing mild neurodegeneration in the developing brain. This hyperacetylation is also seen developing foetal heart resulting in increased dHAND and eHAND expression and impairing heart development.
H3 and H4	Downregulation of histone acetyltransferase and CREB binding protein resulted in hypoacetylation	Rat cerebellum. This could imply an association between the decrease in CREB binding protein and the motor coordination deficits in FAS.
H3K9 and H3K23	Hypoacetylation and increased methylation causing decrease of CREB binding protein.	Foetal brain.
H3K4 and H3K27	Decrease in trimethylation	Cerebral cortical neuroepithelial stem cells. This alters the epigenetic programming of the brain and may affect development together with other factors.

H3K4me3 and H3k27me3	Low dose of ethanol caused elevation and high dose showed a reduction in these histone marks	Cerebral cortical neuroepithelial stem cells. This alters the epigenetic programming of the brain and may affect development together with other factors.
H3K4me2	Reduction of this histone	Rat arcuate nucleus.
H3K4me3	Reduction of this histone in arcuate nucleus in the hippocampus of neonatal rats and increased incidence of histone in the adult hippocampus after PAE.	Rat arcuate nucleus and adult hippocampus.
H3K9me2	Increased incidence of histone methylation. Depletion with a high ethanol concentration.	During synaptogenesis. Increased in rat arcuate nucleus. In the study on the rat arcuate nucleus (also referred to in the two rows above) suggests an alteration in an alteration in histone posttranslational modification and causes an increase in DNA methylation resulting in suppression of POMC gene.
H3	Alteration in phosphorylation at the 10th and 28th serine associated with gene expression and regulation.	Both altered in rat livers and serine 10 altered in hippocampus. Phosphorylation of these 2 molecules is associated with histone acetylation in epithelial growth factor stimulating cells.

High alcohol concentrations reduce the expression of miRNA-153, miRNA-21 and miRNA-335, which regulate genes which control the maturation and proliferation of neurons.(43) Studies show that PAE increases miRNA-9, miRNA-10a and miRNA-10b expression and reduces the expression of miRNA-200a, miRNA-496 and miRNA-296 in the brain. This causes learning impairment and congenital malformations.(44) Exposure during early gestation alters the expression of miRNA138-2 (dendritic spine density), miRNA290 (gene regulation) and miRNA16-2 in the hippocampus.(45,46)

Effects of Prenatal Alcohol Exposure on the Glia

Infants with FAS have a disruption in migration of neurons, neuroglial displacement, and microcephaly.(47) Neuronal plasticity is greatly affected by PAE. Studies show that dendritic branches and spine density in the hippocampus and pyramidal neurons greatly decrease with PAE.(48,49)

PAE damages neural progenitors causing decreased survival and inhibiting their differentiation into astrocytes.(50,51) Primary astrocytes treated with ethanol in culture also showed inhibited proliferation.(52) Factors released by astrocytes such as activity-dependent neuroprotective protein (growth of axons in the cerebellum) and serum response factor (neurite formation) have been implicated in the effects of alcohol.(53) PAE affects the ability of astrocytes to secrete substances required for neuritogenesis such as laminin and fibronectin which are important extracellular matrix (ECM) proteins.(54) Ethanol inhibits the increase of plasminogen activator inhibitor-1 (PAI-1) which prevents proteolysis of plasminogen to plasmin and therefore the breakdown of the ECM.(55) Laminin, fibronectin and PAI-1 are all upregulated through the stimulation of muscarinic receptors. Ethanol also upregulates tPA in astrocytes, through DNA hypomethylation, causing a reduction in laminin resulting in neuronal breakdown.(56,57) PAE causes the formation of chondroitin sulphate proteoglycan nuerocan, a neurite growth inhibitor, via the inhibition of arylsulfatase B.(53)

Several studies have shown that PAE impacts the programming of oligodendrocyte precursor cells.(58) Alcohol slows down myelination and alter the myelin structure.(59) Myelin malformation and abnormal oligodendrocyte morphology were observed. Myelin basic protein, an integral element of the myelin sheath, was found to be less expressed and delayed in the cerebellum of PND15 rats after alcohol exposure. (60) Acetaldehyde has been implicated to be highly toxic to oligodendrocytes.(61) These alterations in oligodendrocyte maturation and survival were associated with the impairment of neurocircuitry and conduction pathways.(62)

Microglia have multiple receptors that detect potentially threatening signals in order to mount a response. (62) Alcohol is able to activate TLR2 and TLR4 which stimulates phagocytosis and ROS and cytokine production.(63) Alcohol augments inflammatory cytokine release and diminishes intracellular cyclic adenosine monophosphate and brain derived neurotrophic factor (BDNF) in hypothalamic neurons and microglia in culture.(64)

Gamma-Aminobutyric Acid (GABA) System in Foetal Alcohol Syndrome

GABA binds to ionotropic ($GABA_A$ and $GABA_c$) and metabotropic ($GABA_B$) receptors. This neurotransmitter is then taken up by neurons to terminate its action. In the cytoplasm it is metabolised by GABA transaminases. When GABA receptors are activated, cell hyperpolarization occurs through the entry of chloride ions or the efflux of potassium ions, preventing cell activation.(65) It has been suggested that ethanol causes excessive inhibition through N-methyl-D-aspartic acid (NMDA) receptor inhibition and activation of $GABA_A$ receptors.(66) Ethanol is an antagonist of NMDA and mimics GABA.(67) $GABA_A$ receptor activation caused elevations in calcium which lead to apoptosis in developing neurons.(68) $GABA_B$ receptor mRNA expression has been found to be affected by PAE in the brains of rat embryos.(69)

Migrating neurons and radial glia express $GABA_A$ receptors in the early developmental stages and therefore can respond to GABA stimulation.(70) Depolarisation caused by GABA causes the opening

of calcium channels resulting in calcium ion influx. This causes the NMDA receptors to open.(71) This shows that GABA can influence cell movement and neurite development through calcium flux in migrating neurons.(72,73) Studies have shown that PAE reduced GABAergic cell density primarily in the developed rodent somatosensory cortex (74) and primates.(75) The latter study suggests that neurons that remain local, as opposed to neurons that project to other cortical layers, are more susceptible to ethanol induced apoptosis.

The GABAergic interneurons are also involved in developmental plasticity in the brain.(76) Therefore, long term potentiation and long-term differentiation were found to be affected by the effects of ethanol on GABAergic cells.(77,78) Increased GABA receptor activation, either through an increase in GABA itself or through $GABA_A$ activation by ethanol, possibly lead to GABAergic cells ending up in abnormal cortical layers or columns resulting in the formation of abnormal brain circuitry.(79) Several studies have shown that the effects of ethanol in early development could be due to the effect of PAE on various transcription factors.(80-82) SHH regulates GABAergic neuron maturation in the cortex.(83) Loss of this signalling resulted in interneuron loss and HPE.

$GABA_A$ receptors are important for interneuron migration across the corticostriatal junction into the cortex whereas $GABA_B$ receptors are involved in the final placement of interneurons in the cortical plate. Ethanol results in a faster migration rate of cells originating from the medial ganglionic eminence.(65, 79)

Neovascularisation and Prenatal Alcohol Exposure

After exposure to alcohol, 20 genes connected to angiogenesis are downregulated and 2 are upregulated. Alcohol also increases 19 proteins and decreases up to 30 in the endothelium. Genes associated with cell structure, protein synthesis, histone, calcium ion, NO and redox reactions are downregulated.(84)

CNS vasculature develops via angiogenesis.(85) Angiogenesis and neurogenesis are intertwined as microvessels provide necessary substances to neural cells.(86) Alcohol causes a dose-dependent decrease in the length and diameter of microvessels, as well as an increased vascular cell death rate.(87) Vascular endothelial growth factor A (VEGF) is a strong modulator of angiogenesis. VEGF functions are mediated by VEGF-R1, VEGF-R2 and VEGF-R3.(88)

In a study, 10 children with FAS showed a mild decrease in left hemisphere perfusion (89) and a reduction of blood flow to the cerebrum was found when a stress (hypoxia) was introduced in foetal and newborn sheep.(90) Pia mater vessels infiltrate the developing cortex and give rise to radial micro vessels and then cause the generation of collaterals.(91) Ethanol treatment results in disorganised vasculature showing random distribution of vessels. Radial organisation in human subjects was found to be heavily altered after 30 weeks of gestation. PAE results in a greater vascular cell death rate, which indicates that alcohol has an effect on vascular plasticity and survival.(92) It has been suggested that the formation of collaterals in the cortex is especially sensitive to PAE.(93)

PAE decreases the levels of platelet endothelial cell adhesion molecule mRNA in the cortex, which correlates with reduced vascular density. As opposed to a mouse model, human FAS patients do not exhibit a difference in cortical microvessel density, whereas cortical VEGF, R1 and R2 mRNA levels are also decreased. The R2 protein levels are decreased whereas R1 protein levels increased.(92) This decrease in R1 mRNA contrasting with an increase in protein could be attributed to post-translational modifications which contribute to protein stability.(94) Placental Growth Factor (PIGF) mRNA levels in mice placentas are found to be low after alcohol exposure.(95) PIGF activates VEGF-R1 which causes transphosphorylation of VEGF-R2 and an increase in angiogenesis modulated by this receptor.(96)

Prenatal Alcohol Exposure and Cell Death

Ethanol causes widespread apoptosis in the cerebral cortex, cerebellum and hippocampus during the brain growth spurt time in rat and mice models.(97) Binge-like ethanol treatment triggers apoptosis especially during synaptogenesis.(98) Pyramidal neurons in the 5th layer, which are the main output source from the cortex, are more susceptible to alcohol induced apoptosis.(99) It was suggested that ethanol may also delay maturing which prevents a physiological reduction of the neurons. Increase in parvalbumin neurons, due to alcohol consumption, contribute to a shift of the excitatory/inhibitory balance in favour of inhibition.(79, 100)

Other proapoptotic molecules, such as caspase-3, have been described as being upregulated or showing alterations of the timing of their development, post alcohol exposure.(101) Besides being proapoptotic, caspase-3 is also involved in dendritic spine remodelling and plasticity.(102) The neurotrophin signalling system was also altered by early alcohol exposure and has been linked to apoptosis and changes in developmental plasticity.(103) A decrease (104) or increase (105) of BDNF support has been shown after prenatal and postnatal alcohol exposure respectively. BDNF-TrkB is involved in apoptosis (106) and has a role in the formation of dendrites.(107) The latter function of BDNF-TrkB could be linked to the changes in dendritic branching which was seen in experimental FASD models.(108)

Molecules that modulate apoptosis are also important in neural plasticity. Therefore, the molecules responsible for cell death can affect neural plasticity and the neurocircuitry of the brain. There is an overexpression of p75 low-affinity neurotrophin receptor (p75-NTR) in the sensorimotor ethanol exposed rat cortices in the first week postnatally was found.(109) This molecule is also important in brain plasticity. Dendrite structure and neurite development are directed by p75 signalling.(110) The p75-NTR pathway modulates synaptic plasticity and formation in the hippocampus of mice.(111) p75-NTR was increased in ethanol treated neuroblastoma cells. The proapoptotic effects of ethanol can be counteracted by using RNA which targets p75-NTR.(112)

Limitations

Further studies are required to better understand the precise mechanisms by which alcohol alters development. Focus must be made on creating methods to identify women at risk of having a child with FAS and studies can be undertaken whereby treatments are given to possibly mitigate any damage done during gestation and the initial development of the offspring when born. Because FAS is the commonest preventable cause of mental retardation, it is vital for healthcare professionals to be aware of this syndrome and its consequences to the neonate and further on in development.

Conclusion

In conclusion, prenatal alcohol exposure results in multiple postnatal consequences including characteristic facies as well as mental retardation due to a deficiency in brain development through a variety of mechanisms. Alcohol results in deficient cell migration and oxidative stress of NCCs as well as decreased signalling in the neuroectoderm, contributing to the craniofacial abnormalities found in FAS.

Prenatal alcohol exposure also alters the expression of genes resulting in anatomical abnormalities in various parts of the body, as well as learning difficulties.

In the developing brain, alcohol results in alterations in cell development and structure, resulting in morphological and functional abnormalities. Alcohol may also cause cell death. These factors contribute to a decrease in neural plasticity. The GABA signalling system, which is a prominent neurotransmitter system in the brain, is also tampered with. The formation of vessels in the brain is also affected which has implications in postnatal brain perfusion.

Education and awareness of the dangers of alcohol in pregnancy in prospective mothers or women of childbearing age is essential in the prevention of FAS. Furthermore, understanding and further investigated the pathophysiology that underlies FAS may help healthcare professionals understand the underlying mechanisms of this disorder and highlight the phenotypic expression. A multidisciplinary approach to care of children with FAS is essential to ensure that these children are allowed to develop to their fullest potential.

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