

Novel Molecular Targets for the Prevention of Fetal Alcohol Syndrome

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Abstract: Alcohol abuse produces damaging effects on the CNS that leads to several types of disorders. When consumed during pregnancy, alcohol may cause craniofacial malformations, growth retardation and brain damage in offspring. These symptoms are grouped by the term fetal alcohol syndrome (FAS). FAS is the most common cause of non-genetic mental retardation in the western world. Substantial efforts to elucidate the molecular basis of these impairments are currently in progress. Whereas FAS is totally preventable by avoiding alcohol intake during pregnancy, efficient therapies to prevent or mitigate the effects of prenatal alcohol exposure are still not available but many pharmacological treatments have been developed to avoid alcohol intake and dependence in adults. The present article reviews the most relevant mechanisms of alcohol injury in developing brain and the strategies and patents that are currently available and in progress to prevent therapy for FAS.

Keywords: Fetal alcohol syndrome, fetal alcohol spectrum disorders, ethanol, patents, antioxidants, retinol, lysophosphatidic acid, glia, neuron, central nervous system.

INTRODUCTION

Children who are exposed to alcohol (ethanol) during gestation can exhibit a spectrum of abnormalities, which in the most severe cases lead to the Fetal Alcohol Syndrome (FAS). The diagnosis of FAS includes anomalies at three distinct levels: prenatal and/or postnatal growth retardation, characteristic facial pattern (e.g. short palpebral fissures, thin upper lip vermillion, smooth philtrum) and neurocognitive deficits [1-3]. However, there are children who have been adversely affected by prenatal alcohol exposure but who do not fit the current FAS diagnostic criteria. Gestational ethanol exposure leads to a continuous scope of disabilities and malformations that varies and depends on several exposure factors (Table 1): (i) dose and pattern of alcohol exposure (e.g. regular drinking, binge drinking, or just one-off drinking); (ii) timing of alcohol exposure; and (iii) genetic background and nutritional factors of the mother. Thus, individuals exposed to similar amounts of alcohol during gestation do not have the same outcomes. Nowadays, Fetal Alcohol Spectrum Disorders (FASD) is the term used to define the full range of prenatal alcohol damage varying from mild to severe symptomatology [4, 5]. These effects may include physical, mental, behavioral and/or learning disabilities. FASD, although it is not a clinically used term, comprises other terms with current diagnostic criteria such as FAS, partial fetal alcohol syndrome (pFAS), alcohol related birth defects (ARBD) and alcohol related neurodevelopmental disorder (ARND) [3, 5-7].

Table 1. Risk factors Associated with FASD

Pattern of alcohol consumption: binge <i>versus</i> chronic drinking, frequency of consumption
Levels of alcohol intake
Critical periods of exposure: periods of brain development specially vulnerable to ethanol effects
Genetic background: e.g. genetic polymorphisms in <i>ADH2</i> gene
Sociocultural status and high risk populations
Exacerbating effects by co-drug administration: pharmacological agents and abuse drugs
Nutritional status of the mother: e.g. vitamin deficiency.
Paternal drinking

The existence of a wider terminology to include the overall alcohol related pathologies dramatically increases the impact of this drug on public health, which places alcohol in a prevalent position as a cause of mental disorder [6,8]. At present, FASD is considered the leading known preventable cause of mental retardation and birth defects. Given the magnitude of the syndrome, further preventive therapies to avoid alcohol intake during pregnancy and strategies to mitigate the deleterious effects of prenatal alcohol exposure on fetus are required. Over the years, a considerable effort has been focused on understanding the molecular mechanisms underlying FASD. Several lines of research in different animal and cellular models are being pursued in

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order to contribute to the successful development of treatments for FASD.

Mechanisms underlying the actions of ethanol in the central nervous system (CNS) have been extensively studied and pharmacological agents have been developed in the treatment of alcohol dependence, some of which are patented and currently in use [9]. The present article reviews some of the multiple molecular mechanisms caused by alcohol ingestion during pregnancy that have been implicated in CNS impairments, which represent the most serious consequences of prenatal alcohol exposure. The review explores the putative role that potential therapeutic strategies (some of which have been patented) can play in the prevention of FASD.

MOLECULAR MECHANISMS ASSOCIATED TO ALCOHOL-INDUCED DEVELOPING BRAIN DAMAGE

Alcohol exerts pleiotropic deleterious effects in the developing nervous system. Extensive experimental evidence supports that alcohol affects a variety of cellular processes by different molecular mechanisms (Fig. 1). These processes include neurogenesis, gliogenesis, synaptogenesis, myelination, proliferation, migration, differentiation and survival of cells [10-18]. In order to target these cell-specific processes, alcohol may engage simultaneous or sequentially

different molecular mechanisms such as acetaldehyde formation [19-21], oxidative stress [22-27], cell-cell interaction and cell-adhesion disassembly [28-30], deregulation of neurotransmission systems [15,31-34], growth-factor and trophic support alteration [35-40], aberrant glycosylation and glucose uptake [41-47] and the interference with several cell-signalling pathways (Ca²⁺-dependent, cyclic-nucleotide dependent [18,48], and other signaling pathways [49-52]). The severity of alcohol deleterious effects will mainly depend on how specific CNS areas and cell types are exposed during susceptible stage of development.

THERAPEUTICAL STRATEGIES FOR PREVENTION OF FASD

Nowadays, there is no treatment for FASD and the classical disjunctive between prevention and cure prevails. The former is the big goal for its specificity, whereas the latter is characteristic and widely used for mental retardation and for associated disorders. Next, we review the most promising therapeutic approaches, paying a particular attention to those with a preventive strategy.

Antioxidant Supplements

Oxidative stress is a postulated key mechanism that contributes to ethanol-induced cell damage, cell death and nervous system dysfunction observed in FASD [22, 23-27,

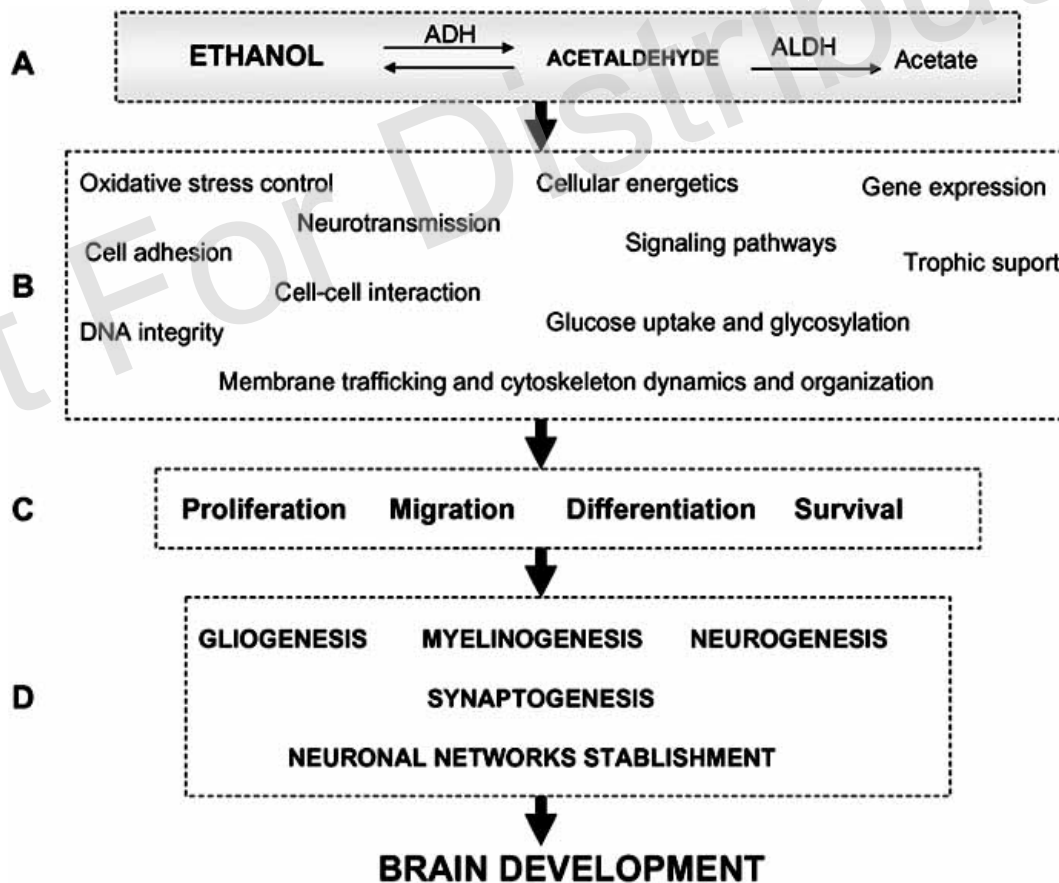


Fig. (1). Ethanol directly and/or through its metabolism within the cell, (A) interferes with a set of different molecular and biochemical processes (B). The alteration of these processes affects a large variety of fundamental cellular events (C and D), which lead to the impairment of critical stages of CNS development. ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase.

53]. The cellular response to an excess of free radicals and the consequences of this response generate a state in the cell commonly referred to as oxidative stress. Free radicals are highly reactive molecules normally generated during various biochemical reactions [54]. Since many of these free radicals contain oxygen, they are usually called reactive oxygen species (ROS). Typically, levels of ROS are controlled by cellular antioxidant activities, which are provided by enzymes and by non-enzymatic factors. Well characterized antioxidant enzymes are catalase, superoxide dismutase (SOD) and glutathione peroxidase. Major non-enzymatic antioxidants are vitamin C (ascorbic acid), vitamin E (tocopherol) and β -carotene [27,54]. Once formed, ROS may interact with carbohydrates, proteins, lipids (lipid peroxidation), and nucleic acids to form intermediates that can produce severe cell damage and death [54]. ROS can also inactivate electron-transport chain complexes, which decrease the production of mitochondrial energy and eventually activate apoptotic pathways [55,56]. Thus, when mitochondria become dysfunctional, cytochrome C is released into the cytoplasm, which in turn leads to caspase cascade activation and apoptosis [55,57].

Several studies have shown that alcohol exposure induces oxidative stress in developing the brain basically through two mechanisms: (1) induction of ROS generation [23,25, 56,58-60] and/or (2) decreasing the amounts or efficiency of endogenous-antioxidant systems [27,61-63]. Strikingly, embryos have a reduced intrinsic antioxidant capability [62], which represents a risk factor for deleterious abnormalities when embryos are exposed during specific stages of development to even moderate levels of ROS. Increasing evidence strongly suggests that antioxidant supplementation can prevent oxidative stress and alcohol-induced damage in developing brain in experimental models of FASD. Next, we provide some relevant examples.

Vitamin C is a potent scavenger of ROS and maintains intracellular levels of glutathione. *Xenopus laevis* embryo

has been recently patented as a useful experimental method for screening agents with protective or therapeutically effects for FASD (Table 2) [63,64]. In *Xenopus* embryos in which alcohol exposure produced several developmental defects analogous to those observed in FASD, vitamin C offered protection against alcohol-induced growth retardation and microcephaly. Vitamin C seems to exert its neuro-protective effects by acting as a scavenger of ROS, reducing oxidative stress and lipid peroxidation and by restoring the expression of neural marker genes such as Pax6, Sox2, Sox3, and/or NCAM, which all are downregulated by alcohol treatment [63, 64].

Vitamin E supplementation has also been shown to prevent alcohol-induced cell loss [65-68]. In a recent study, a modified form of vitamin E, chemically-engineered to target mitochondria, inhibited intracellular oxidant accumulation, mitigated alcohol-mediated suppression of antioxidant systems, and promoted cell survival at much lower concentrations than natural vitamin E [68]. Therefore, this modified vitamin E provides significant neuroprotection against alcohol concentrations as high as 1600 mg/dL. Bioavailability studies of this orally-administered mitochondrial-targeted vitamin E show that it crosses both placenta and blood-brain barriers. Therefore, this modified vitamin E can promisingly be used in the future as a therapeutic strategy against some of the severe damages caused by prenatal alcohol exposure. In another study, the direct ethanol exposure of fertile chicken eggs decreased cerebral long-chain polyunsaturated fatty acids [69]. Lipid peroxidation, the oxidation of long-chain fatty acids, which are essential components of cell membranes, has been considered a process that eventually leads to a reduction in the brain mass. When α -tocopherol or γ -tocopherol (vitamin E derivatives) was injected concomitantly with ethanol, no reduction in the brain mass was observed and lipid peroxidation levels did not increase [69].

Table 2. Patents currently available to prevent or mitigate FASD

Name	Mechanism of action	Patent number and references
<i>Xenopus laevis</i> embryo model	Animal model for study the effects of alcohol on fetal development. The invention provides a method for treating FAS by administering vitamin C or other agents	US20060037085A1 [64]
1-Octanol	Methodology and compounds (e.g. 1-octanol) for antagonizing inhibition of alcohol on cell adhesion	US6359015 [111] US20040077731A1 [112] US6977272 [113]
ADNF polypeptides	Prevention of FASD and neuronal cell death by antagonizing the inhibition of alcohol on cell adhesion	US6933277 [121]
Ipsapirone Buspirone	5-HT _A receptor subtype agonists, which are patented for treating a patient suffering from disorder of CNS associated with 5-HT _{1A} receptor subtype	US7053092 [212]
Cholinesterase inhibitors	Treatment of cognitive impairments and other brain disorders by blocking the cholinesterase activity	WO04034963A2 [196] US20060018839A1 [195] DE102004029325A1 [194]

Addition of SOD decreased oxidative stress and ethanol toxicity on both cell and whole embryo cultures [24-26]. However, only partial prevention was observed because of the accumulation of hydrogen peroxide, a product of superoxide dismutation by SOD [70]. A plausible explanation could be insufficient levels of catalase or peroxidase, which are the enzymes responsible for the elimination of hydrogen peroxide. More recently, EUK-134 (superoxide dismutase/catalase mimetic) has proven to be more efficient than SOD treatment [71]. EUK-134 administered concurrently with alcohol in C57BL/6J mice, significantly reduced oxidative stress and decreased forelimb malformations and the percentage of resorbed fetuses [71,72]. EUK-134 strongly destroys superoxide anion and hydrogen peroxide, mimicking both SOD and catalase activities. The therapeutic potential of EUK-134 as an antioxidant agent has previously been observed in the prevention of brain damage caused by ischemia [73], -amyloid peptide [74] and aging [75].

Flavonoids are also potent antioxidants. Silymarin-Phytosome, a mixture of several flavonoids, has been shown to reduce the levels of -glutamyl transpeptidase (GGT), a classical marker of alcohol-induced tissue damage, in the brains of both ethanol-fed rats and their fetuses [76]. In addition, Siliphos, a mixture of silybin, prevented the elevation of fetal brain GGT activity and improved the mortality rate of pups [77]. It has been suggested that the mechanism of action of flavonoids is the prevention of ethanol-induced lipid peroxidation. Other studies on atherosclerosis models [78] support this hypothesis. Interestingly, the administration of these compounds seemed to improve the spatial cognitive and social learning deficits induced by prenatal exposure of ethanol [79], although the knowledge of molecular mechanisms underlying their action need further investigation.

Pycnogenol®, a combination of bioflavonoids extracted from the bark of French maritime pine (*Pinus maritima*) [80], inhibits apoptosis of developing neurons acutely exposed to ethanol. In ethanol exposed cerebellar granule cells, Pycnogenol reduced ROS, counteracted suppressed SOD and glutathione peroxidase/reductase system activities, upregulated SOD protein expression, mitigated ethanol-mediated exacerbation of catalase activity and inhibited specific binding and activation of caspase-3 [67]. These results suggest that Pycnogenol antagonizes ethanol-induced effects by preventing oxidative stress and apoptosis.

Collectively, although antioxidants may appear to be protective in some *in vitro* and *in vivo* models, there are still many uncertainties as to whether antioxidant supplements may serve as effective potential interventions. However, all these studies suggest the possibility that nutritional therapies incorporating antioxidants provide protection against the deleterious effects of alcohol on brain development. An application of antioxidant treatment in pregnancy is not a new concept, since oxidative stress also plays a role in the pathogenesis of pre-eclampsia [81]. Treatments with vitamin C or E significantly reduced the occurrence of pre-eclampsia whereas no adverse effects to the fetus were noted [82], which suggested that dietary supplementation with antioxidants in therapeutic doses, would not cause adverse

effects to both mother and fetus. In addition, treatment of alcoholic pregnant women with antioxidants and vitamins supplements is appealing, since they also contribute to reverse the nutritional deficits detected in this population [83]

Vitamin A Supplementation

Vitamin A (retinol) is the precursor of retinoic acid (RA), which is a potent mediator in embryogenesis and differentiation that plays a central role in the development of the limbs and of the CNS [84,85]. *In vitro* and *in vivo* studies demonstrate that ethanol interferes with RA-dependent signaling pathways [86,87]. Furthermore, ethanol ingestion alters vitamin A metabolism [88]. Strikingly, there are numerous phenotypic similarities between malformations associated to FAS and those induced by vitamin A deficiency (VAD) [89,90].

Retinol and ethanol are alcohols that are metabolized by the same enzymatic systems. Thus, ethanol and retinol are respectively converted to acetaldehyde and retinaldehyde by cytosolic alcohol dehydrogenase (ADH) enzymes [91-95] (Fig. 2). Moreover, the oxidation of these two aldehydes to acetic acid and RA respectively, is catalyzed by the same members of the aldehyde dehydrogenase (ALDH) family [86] (Fig. 2). Since certain isoenzymes of ADH and ALDH are critical for synthesis of RA, the competitive inhibition of retinol oxidation by ethanol during critical periods of embryonic development is considered as a mechanism of teratogenesis [49, 86, 96-98].

Interventions designed to prevent the suppression of RA synthesis due to alcohol's competitive inhibition would be difficult because RA homeostasis is carefully regulated, since either transient excesses or depletions can be both teratogenic. Vitamin A supplementation in heavy drinkers may be indicated, but it could become complicated by the intrinsic hepatotoxicity of large amounts of vitamin A, which in turn is highly potentiated by concomitant alcohol intake [99]. β -Carotene is a precursor and a nontoxic substitute for retinol but alcohol interferes with its conversion to vitamin A and even moderate alcohol intake can result in increased concentrations of β -carotene. Although vitamin A and β -carotene supplementation could show secondary effects, β -carotene has the potential advantage of acting more efficiently as antioxidant than retinol and due to its inhibitory effect of the free radical-induced lipid peroxidation, it is one of the most efficient quenchers of ROS [100].

Recently, Yelin *et al.* [101] showed that *Xenopus laevis* embryos could be used as a suitable model for examining FASD, since when treated with ethanol, the embryos recapitulate many aspects of human FASD. In this interesting experimental system, the embryonic period that goes from late blastula to early gastrula is the most critical developmental window of highest sensitivity to alcohol. Embryonic phenotype and gene expression pattern analysis show that development is affected by ethanol exerting effects opposite to RA. They provide evidence that support the competition model (Fig. 2), in which ethanol and its product acetaldehyde both compete with retinol and retinal for their oxidation by ADH and ALDH respectively [86, 87]. Such competition must result in a transient reduction of RA levels,

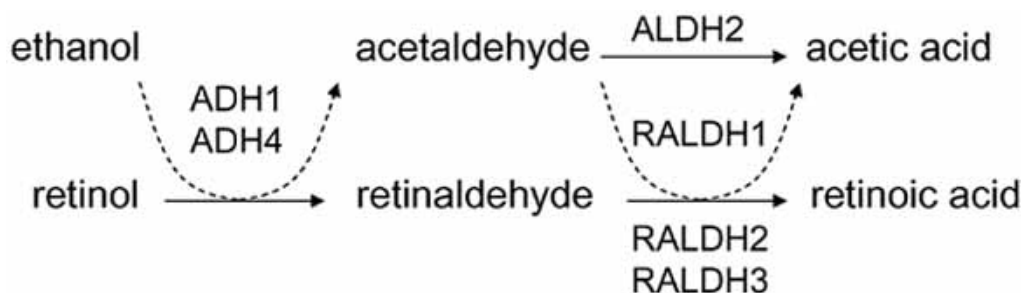


Fig. (2). Hypothetical model of competition produced by ethanol on the physiological metabolic pathway of retinoic acid synthesis. ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; RA, retinoic acid; RALDH, retinaldehyde dehydrogenase.

which are necessary for normal embryonic development through the control of regulatory genes such as *Hox* genes [102]. The expression pattern of these genes showed opposite responses to ethanol and RA [101], which is in agreement with the postulated competition model (Fig. 2). In addition, the reduction of RA levels by XCYP26 (a RA hydroxylase) overexpression [101,103] or depletion of retinaldehyde dehydrogenase-2 gene (*Raldh2*) [104] results in the shortening of the anterior-posterior axis, facial dysmorphogenesis and CNS defects extensively overlapping with the VAD and FASD associated phenotypic defects [1-3, 89,90]. The phenotypes of *Xenopus* embryos obtained by the reduction of endogenous RA levels (as a consequence of citral treatment or XCYP26 overexpression) are indistinguishable from those observed in ethanol treatments. These results strongly support the idea that ethanol causes a transient reduction in RA levels in developing embryos and that embryonic malformations induced by ethanol in *Xenopus* embryos can be phenocopied by inhibiting the biosynthesis of RA. The addition of RA, retinal or retinol could then perform a significant rescue of ethanol-induced impairments.

Antagonistic Action of 1-Octanol

Another mechanism by which alcohol seems to interfere with brain development is by reducing cell adhesion or disrupting cell-cell interactions. Several cell adhesion molecules (CAM) assist different aspects during CNS development: cell adhesion, cell-cell interaction, neurite outgrowth and migration [105,106]. Since some of the neurodevelopmental defects found in children with mutations in L1 CAM gene resemble those of children with FAS, it has been suggested that alcohol may disrupt L1 actions during fetal development [29,30,107]. In accordance to this hypothesis, several studies have shown that indeed alcohol disrupts L1-mediated cell-cell adhesion [29,94,108]. Importantly, only short chain alcohols altered L1-mediated cell adhesion, which is consistent with a ligand-receptor interaction [109]. In contrast, alcohols containing five or more carbons in their chains exhibited opposite properties. Specifically, 1-octanol was not only inefficient disrupting L1-mediated adhesion but remarkably antagonized ethanol effects in cell-adhesion by a non-competitive and reversible mechanism [109, 110]. These results have generated several patents [111-113] (Table 2). Additionally, low concentrations of 1-octanol (3 μ M) significantly prevented alcohol-induced teratogenesis in embryonic mouse model [114].

Neuroprotective Action of SAL/NAP Peptides

NAPVSIPQ (NAP) and SALLRSIPA (SAL) are small peptide fragments of the glial-derived activity-dependent neuroprotective protein (ADNP) and activity-dependent neurotrophic factor (ADNF) respectively [115,116]. These two peptides provide protection in C57B/6J mouse strain, a mammalian model of FAS [117]. In addition to antioxidant and neuroprotective effects [117], NAP and SAL have been recently shown to antagonize alcohol inhibition of L1-mediated cell adhesion [118-120]. These results have generated a patent [121] (Table 2). Interestingly, experiments with series of NAP derivatives in which alanine has been substituted revealed distinct structure-activity relationships for NAP neuroprotection and for NAP antagonism of ethanol-induced inhibition of L1 adhesion [119]. NAP derivatives that retained alcohol-antagonist capacity in cell-adhesion assays also preserved the protective capability in front of the alcohol-induced embryonic defects [120,122]. These findings support the hypothesis that these neuroprotective peptides prevent teratogenic effects of alcohol during early embryonic development by avoiding the disruption of L1-mediated adhesion induced by ethanol. Variants of NAP or SAL containing D- instead of L-amino acids (D-NAP, D-SAL) were equipotent as alcohol antagonists [120]. Note that peptides containing D-amino acids are resistant to endogenous proteases, which make them particularly useful as therapeutic agents.

Neural crest cells are especially sensitive to ethanol-induced cell death, which makes them a valuable model for *in vitro* studies, in which SAL and NAP peptides have been revealed as potential therapeutic agent diminishing the oxidative stress induced by ethanol [25, 109, 120].

The Role of Neurotrophic and Growth Factors

Ethanol also interferes with signaling pathways related to several neurotrophic and growth factors that control important cell processes such as proliferation, differentiation and cell death during brain development (Fig. 1) [40]. Insulin-stimulated CNS neuronal survival mechanisms have been recently shown to be impaired by chronic gestational exposure to ethanol [123,124]. Notably, the generated abnormalities persist in the early postnatal period, which is critical for brain development. Therefore, the effect of ethanol on insulin-dependent signaling pathways is another important point to take into account in ethanol teratogenesis. In the developing CNS, insulin and insulin-like growth factor type 1 (IGF-1) receptors are abundantly expressed

[125] and the corresponding growth factor-stimulated responses are critical mediators of neuronal growth, viability, energy metabolism, and synapse formation. Insulin and IGF-1 signalling pathways are important targets of ethanol neurotoxicity in immature nervous system [36,50,126,127] and therefore neuronal loss associated with microencephaly in ethanol-exposed fetuses may be caused, in part, by ethanol inhibition of insulin/IGF-1 stimulated survival mechanisms. Using a rat model of chronic gestational exposure to ethanol, ethanol-exposed pups showed cerebellar hypoplasia, reduced mitochondrial function and increased neuronal apoptosis [123]. Phosphatidylinositol 3-kinase (PI3 kinase)-mediated signalling pathway has been proposed as the underlying biochemical mechanism of the ethanol deleterious effects on insulin-stimulated neuronal viability and mitochondrial function in cerebellum. Interestingly, treatment with IGF-I reduced ethanol neurotoxic effect on neuronal survival when it was given subsequently to the ethanol intake [128]. Moreover, overexpression of IGF-1 diminished ethanol susceptibility in transgenic mice [129].

Other factors neuroprotective against ethanol include the nerve growth factor (NGF), the basic fibroblast growth factor (bFGF) and estrogens [130-132, 128]. Recently, it has been shown that treatment with Heparin-binding EGF-like growth factor (HB-EGF) prevented apoptosis and completely ameliorated cell death at the gastrulation stage in mouse embryos cultured in medium with a high concentration of ethanol [133]. HB-EGF promotes cell survival during normal development and is highly expressed during early embryonic development [134-136].

The novel peptide activity-dependent neurotrophic factor-12 (ADNF-12) has been recently shown to prevent alcohol-induced fetal death and developmental and learning abnormalities in C57B16/J mice [137]. C57B16/J mice on gestational day 8 were treated with ADNF-12, 30 min prior to alcohol treatment. Fetal death assessed on gestational day 18 and neonatal and adult behavior tests demonstrated that a single treatment with the peptide was efficient enough for the prevention of alcohol-induced damage.

In these studies, both neurotrophic and growth factors were administered at concentrations being within the pharmacological range [138,139]. In addition, NGF has been used at various concentrations in human beings for Alzheimer's disease [139]. Overall, these findings suggest that the delivery neurotrophic factors or other effective survival factors to the fetus or stimulation of their endogenous production, might reduce some of the adverse effects of prenatal alcohol exposure.

Lysophosphatidic Acid as a Cytoprotectant

Some of the harmful effects of prenatal alcohol exposure may also be directly associated with the reported alteration in the glucose uptake. Glucose is a crucial source of energy and its availability for neurons is highly regulated by neighbor glial (in particular astrocytes) and endothelial cells. The effect of alcohol on glucose uptake and on glucose transporters (GLUTs) has been widely studied in several cell types, although with disparate results [44,43]. The varying results could be attributable to differences in cell lines used and to the variety of experimental designs such as alcohol

treatments (chronic or acute). Results from our laboratory have demonstrated that alcohol induces an altered monosaccharide uptake and increases the levels of the glucose transporter GLUT1 in primary cultures of rat astrocytes [45,46]. The GLUT1 increase after alcohol exposure has also been observed in rat [43] and chick brain [140]. Taking into account the large functional relevance of astrocytes in glucose metabolism and in brain energy availability, even slight alteration in the regulation of glucose uptake and/or transport by glial cells would significantly compromise neuronal function and brain development [141,142]. Unfortunately, the molecular mechanisms by which ethanol produces these changes in astrocytes remain unclear, but as glucose uptake by GLUT proteins are in some extent dependent on actin [143], we have postulated that ethanol could primarily alter the organization and dynamics of actin cytoskeleton. In this respect, we and others have reported that ethanol induces an alteration in the actin cytoskeleton organization [45,46,144] and microtubules dynamics [46] in rat astrocytes. Strikingly, the treatment with lysophosphatidic acid (LPA) not only prevented the ethanol-induced defects in actin and microtubule cytoskeleton organization but also normalized monosaccharide uptake [46]. LPA is a simple phospholipid and a potent intracellular lipid mediator present in serum that elicits a broad spectrum of responses in a variety of cell types including CNS cells [145-151]. In addition, LPA induces actin cytoskeleton rearrangements and stabilizes microtubules through the stimulation of the Rho signaling pathway [152-154]. However, LPA cannot cross the blood-brain barrier, but the use of derivatives that could overcome it and/or pharmacological agents that stimulate the endogenous production of LPA by astrocytes [146] and endothelial cells [155], could be of potential interest for preventing cellular injuries produced by ethanol ingestion. In this content, experiments are in progress in our laboratory.

Treatment with 5-HT_{1A} Agonists

Developing neurons containing serotonin (5-hydroxytryptamine, 5-HT) are highly sensitive to damaging effects of ethanol in both *in vivo* and *in vitro* models [15,17,156-161]. Serotonergic neurons are particularly vulnerable to alcohol exposure during the early stages of brain development; thereby their decrease could be crucial to lead to the widespread cascade of abnormalities in other brain systems. 5-HT, has two very important roles in brain: (i) as a neurotransmitter and (ii) as a neurotrophic factor regulating neuronal differentiation and maturation in developing brain [163, 164]. 5-HT functions are mediated by 5HT_{1A} receptors both in neurons [165] and astrocytes [166]. 5-HT stimulates nearby astrocytes to release S100 β , which provides important feed-forward trophic support for the survival and growth of 5-HT neurons. In fact, alcohol ingestion induces a decrease of S100 levels [159,167]. The mechanism by which ethanol impairs the development of the 5-HT system seems to involve the induction of apoptosis on 5-HT neurons and an inhibitory effect of the trophic interactions between 5-HT neurons and astrocytes [157,159,161,168,169].

5-HT receptor has been targeted to prevent ethanol-induced damage by the development of 5-HT_{1A} agonists such as bupirone and ipsapirone, which in turn avoid ethanol-

induced reduction of 5-HT neurons [157,161,168]. The administration of buspirone and ipsapirone to pregnant mice prevents the loss of fetal 5-HT neurons, maintains normal levels of 5-HT and facilitates neurotrophic effects of 5-HT [156,158,170]. The mechanism by which 5-HT_{1A} agonists neuroprotect is unknown, but it has been suggested that the activation of pro-survival/anti-apoptotic pathways, which involve the activation of either p42/p44 mitogen-activated protein kinase (MAPK) or phosphatidylinositol 3-kinase (PI-3K) [171,172] plays a prominent role.

S100 prevents ethanol-induced apoptosis and reduction of 5-HT neurons [161]. On healthy astrocytes, the stimulation of 5-HT_{1A} receptors [173] triggers the release of S100 [160,166,174], which appears to contribute to the anti-apoptotic effects produced by 5-HT_{1A} agonists [174]. In addition, S100 stimulates the development of 5-HT neurons and promotes differentiation and neurite outgrowth of 5-HT neurons [167, 175].

Treatment with Nicotinamide

There is another hypothesis that links alcohol-induced brain damage to the interference onto specific neurotransmitter systems. Ethanol selectively and strongly inhibits the function of N-methyl-D-aspartate (NMDA) glutamate receptors [176,177] and overstimulates GABA_A receptors [178]. Chronic exposure to ethanol causes adaptive upregulation of NMDA receptors, which can result in an increased vulnerability for glutamate-induced cytotoxic response (excitotoxicity) [177,178]. This 'sensitization' of neuronal cells to excitotoxic insults is one of the most important factors in the mechanism underlying ethanol-induced brain damage during adulthood [179]. However, no evidence of this excitotoxic cell death has been found during brain development [31,180,181]. Glutamate has been implicated in neuronal proliferation and migration during early development [182] and it is thought to critically regulate neuronal survival in very specific periods of brain development (e.g. growth spurt). There are experimental evidences supporting that ethanol, like ketamine and nitrous oxide, acts as a NMDA antagonist [31,32,176,177] and triggers a neurodegenerative apoptotic response in developing brain. Importantly, the window of vulnerability to ethanol-induced apoptosis coincides with the period of synaptogenesis (brain growth spurt), which in human extends from the sixth month of gestation to several years after birth [183]. In addition, ethanol, like benzodiazepines and barbiturics, mimics or potentiates the action of γ -amino butyric acid (GABA) at GABA_A receptors triggering apoptotic neurodegeneration in the developing brain [31, 32]. Therefore, ethanol acting by a dual mechanism, NMDA antagonist and GABAergic agent, triggers a robust and widespread apoptotic-induced neurodegeneration in developing rat brain [31, 32, 184]. As previously mentioned, this vulnerability is coincidental with the period of synaptogenesis, in which transient ethanol exposure can delete a large amount of neurons and glial cells. This phenomenon could easily explain the reduced brain mass and life-long neurobehavioral disturbances associated with human FASD [180]. Interestingly, it was demonstrated that the minimum condition for trigger neurodegeneration was a blood ethanol concentration at or above 200 mg/dL for a brief period of 4

hours (ethanol dose equivalent to a binge-like drinking by the human mother [185]). Higher concentrations and longer periods of exposure lead to more severe and widespread neurodegenerative response.

In another interesting study, Iraci and Herrera [186] showed that subcutaneous injection of ethanol triggered neurodegeneration in seven-day-old mice (P7). Mice P4–10 stages were equivalent to the third trimester of human pregnancy, coincident to the brain growth spurt period (synaptogenesis). They reported that one single ethanol injection raised blood ethanol levels up to 200 mg/dL, which was sufficient to induce the release of mitochondrial cytochrome C and caspase-3 activation. The majority of brain damage occurred in the anterior cingulate cortex, the hippocampus and the thalamus, all of them being regions particularly sensitive to ethanol at this stage of development. Interestingly, these ethanol-treated mice, when were tested at adult age, showed hyperactivity, a decrease in fear and severe difficulties in learning and memory. An important aspect of this work is that it showed a correlation between ethanol-induced brain damage during development and neurocognitive deficits in adulthood. Furthermore, when nicotinamide was injected after ethanol exposure, it completely prevented alcohol-induced neuronal death in these specific brain regions and long-term behavioral disturbances. Nicotinamide, a precursor for coenzyme - nicotinamide adenine dinucleotide (NAD⁺), acted as a neuroprotector by preventing oxidative stress and NAD⁺ depletion thus maintaining mitochondrial functionality and energy supply [187, 188]. The action of nicotinamide as against the ethanol effects at the mitochondrial level could be the result of re-balancing cellular energy metabolism [189], although the precise mechanisms underlying this neuroprotection require further investigation. Nicotinamide and other forms of vitamin B3 have been used as dietary supplements to treat and prevent pellagra, a vitamin deficiency disease [190]. Large oral doses of nicotinamide have also been used in clinical trials to treat type I diabetes and bullous pemphigoid (a chronic, autoimmune skin-blistering disease) [191,192]. A very important advantage of using nicotinamide is that it causes neither birth defects nor side effects [193].

CURRENT & FUTURE DEVELOPMENTS

The simplest method for the prevention of FASD is avoiding any alcohol intake during pregnancy. However, given the widespread and apparently increasing incidence of FASD [3, 6, 8], it is obvious that the risk that implies alcohol consumption during pregnancy is not fully envisioned as a serious public health problem. Whereas a great effort should be done to avoid ethanol consumption, several pharmacological approaches for the prevention of FASD are currently under active research and some of them have already generated patents.

As previously discussed, one promising set of preventive candidates would initially comprise NAP and SAL peptides, 5-HT agonists, neurotrophic factors, antioxidants and vitamins. Currently, all these therapeutic approaches still remain in a pre-clinical stage and effectiveness and non-teratogenicity in pregnant women must be confirmed by further studies. A complex spatio-temporal pattern of action

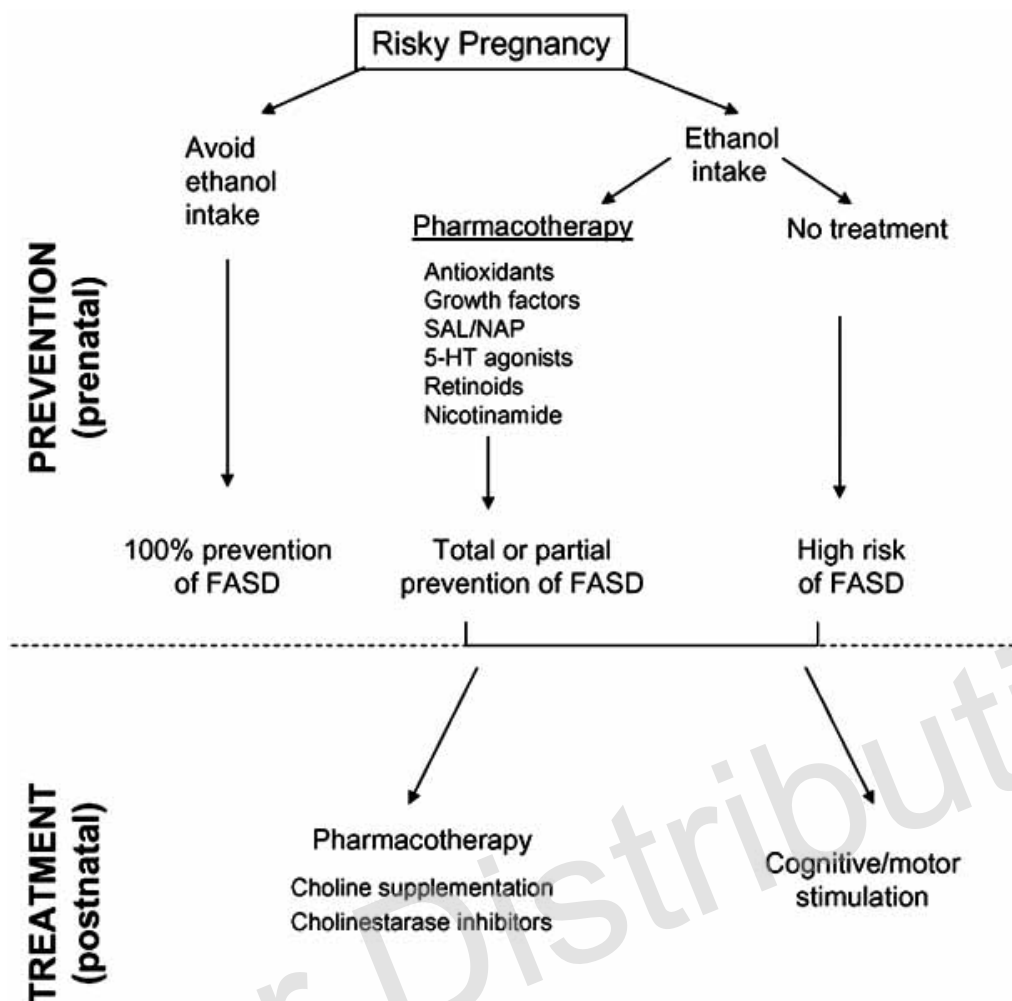


Fig. (3). Current strategies for the prevention and treatment of FASD.

on distinct molecular pathways underlies ethanol-induced brain damage, generating multiple pleiotropic effects. This complexity is reflected in the heterogeneity of the pharmacological targets and in the limited period of action of potential treatments, which imposes an intrinsic caveat to reach clinical stages. In this respect, it is illustrative the promising work done with nicotinamide by Iraci and Herrera [186], in which they provide molecular, cellular and behavioral data of treatments with this compound. Their findings suggest the possibility of prevention of the alcohol generated damage in the fetus by the administration of nicotinamide to the mother soon after alcohol consumption. Whereas the beneficial effects observed were most pronounced when nicotinamide was given at the same time or shortly after alcohol intake, the study suggests that there is a window of a few hours in which the treatment seems to be effective. The reversal effect of nicotinamide on alcohol damage disappears upon the expiration of this time period and consequently newborns exhibit FASD.

A global view of the impact of alcohol abuse in our societies makes unlikely a total success in the prevention of FASD. At this stage, pharmacotherapy and/or behavioral interventions have to be considered to improve outcomes in children with FASD (Fig. 3). Among the applicable

pharmacotherapy at this stage, those based on the control of the neurotransmitter acetylcholine should receive our attention because cholinesterase inhibitors (ChEIs) have recently been patented for the treatment of FASD (Table 2) [194-196]. The authors provide strategies for treating and preventing cognitive impairments and/or dementia caused by FASD by the administration of therapeutically effective amounts of at least one or a combination of different ChEIs (such as donepezil, galantamine, rivastigmine). ChEIs block the catabolism of acetylcholine (ACh) by acetylcholinesterase (AChE), which leads to larger pool of available ACh to interact with postsynaptic ACh receptors and induce more lasting effects of this neurotransmitter during postnatal brain development. Alcohol exposure alters cholinergic development and increases AChE and decreases acetylcholine transferase (AChT), diminishing the levels of ACh in cholinergic neurons [197-201]. Cognition is further compromised by the loss of cholinergic neurons [199]. Thus, it is reasonable to think that the expected augmentation of ACh available in the remaining cholinergic neurons, as a consequence of ChEIs treatment, may improve FASD symptoms. ChEIs have been extensively used for the treatment of Alzheimer, a disease also associated with a loss of cholinergic neurons in brain areas related to memory and learning [202,203]. In the same direction, postnatal

supplementation with choline reduces the severity of ethanol-related hyperactivity and learning deficits in adult rats exposed to ethanol during gestation [204,205]. Choline is an essential nutrient that is readily transported across the blood-brain barrier in the neonate [206]. Importantly, these data provide evidence that ChEIs and choline supplementation may serve as a treatment for children with alcohol-related neurodevelopmental disorders.

There is another important type of intervention designed to ameliorate the symptoms and problems of the children already affected by FASD. This treatment is focused on the stimulation of neuroplasticity mechanisms involved in the behavioral deficits, such as motor deficits, balance problems, and gait anomalies commonly associated with FASD. Several investigators have provided evidence that complex motor training, behavioral and environmental interventions may successfully reduce the severity of fetal alcohol effects [207-210].

Efficient intervention in FASD children requires an early identification and the subsequent treatment after birth. Therefore, increasing efforts are needed to identify offsprings with alcohol-related neurodevelopmental disorder but without the appearance of characteristic facial malformations crucially considered for FAS diagnosis [211]. Recent reports define alcohol-related effects on offsprings which might provide new parameters for a more accurate FASD diagnosis [3-5,7]. Eventually, the effectiveness of FASD diagnosis will rely on the integration and cross-talk of clinical and educational systems. This is an accurate assessment of alcohol consumption of pregnant women and detection of the signs and symptoms of the children affected by FASD.

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