

Research Article

Sex differences in alcohol use disorders: the role of steroid hormones

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Abstract

Clinical and preclinical studies suggest a close and mutual relationship between sex hormones and the development of alcohol use disorders. Even drinking behavior may be affected by certain hormones as testosterone and allopregnanolone that reinforce alcohol desire and reward. Sex hormones mostly affect women where it was seen, by the way, a causal link between alcohol and severe illness such as estrogen-mediated breast cancer even when alcohol intake is so low that it does not affect other tissues. Gender studies about the role of hormones in the development of alcohol related disease are yet poor and the determination of hormones as possible biomarkers of alcohol use disorders is still to be explored. Furthermore, more reliable analytical tools and better standardized experimental condition are required to obtain comparable results and to improve efficacy of the clinical treatment and the preventive actions. In the light of current knowledge all women are recommended to limit alcohol use and, in the case of women at risk of breast cancer, to consume even less or to completely abstain from drinking.

Introduction

Epidemiologic studies show that alcohol abuse and alcohol related problems that in the past concerned mainly men, are more and more involving female population so that the gender gap in the prevalence of alcohol related risk is narrowing mostly among young people [1]. On the consequence, alcohol clinical studies not including female patients present only half of the story and are not suitable enough for improving scientific knowledge and for developing effective intervention strategies. Recent clinical and preclinical studies show that gender matters a lot in the development of alcohol use disorders and that women are more vulnerable than men to alcohol impairment because of anatomical and physiological differences [2]. Results from alcohol research and clinical studies highlight that gender differences in alcohol impairment may be due also to the action of sex hormones since sex steroid hormones especially estrogen can modulate alcohol effects and alcohol itself may modulate hormonal status [3]. The reciprocal influence between alcohol and sex hormones seems to concur significantly to the gender differences in alcohol impairment and neurobehavioral consequences and seems to have a key role in mediating alcohol disorders [4]. This hypothesis is supported by some evidences such that the fact that dopamine system, that is strongly implicated in reinforcement effect of alcohol, is sexually dimorphic and it is modulated by sex hormones mostly estrogens [5]. Human sex hormones include androgens, estrogens and progestins whose secretion is regulated by a hierarchy of hypothalamic and pituitary hormones. Estrone and estradiol control sexual and reproductive functions in men and women. Progesterone, produced in ovaries and placenta when a woman gets pregnant and in adrenal glands, regulates the monthly menstrual cycle, prepares body for conception and it is essential for maintaining pregnancy. The gonadotropin releasing hormone (GnRH) is synthesized and released by neurons in the hypothalamus and induces the secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the anterior pituitary gland. These two hormones regulate the levels of sex hormones derived from cholesterol and produced in the male testes, women

ovaries and, in small amounts, in the adrenal glands. Testosterone is the principal male sex hormone with anabolic properties such as the growth of muscle tissue, bones, and body hair. In men, approximately 5% of testosterone undergoes 5 α -reduction to form the more potent androgen, dihydrotestosterone (DHT) that governs development of male external genitalia during embryogenesis and in the adult acts as the primary androgen in prostate and hair follicle [6]. Alcohol intake can interfere with sex hormones function even by the aromatization of androgens in the bioprocess that leads to estrogen synthesis and so it can impair androgen/estrogen ratio [7]. The link between alcohol and hormones is so close that male sex is considered itself an important risk factor for the onset of alcohol dependence because psychoactive effects of testosterone favor tendency to alcohol drinking [8-10]. Experimental studies show that the interaction alcohol/hormones is related not only to the development of alcohol-related illnesses, such as many type of cancer including breast cancer, but also to the neurobehavioral impairment because some hormones exert an important role in the reinforcement of alcohol reward and so they favor drinking behavior. It was demonstrated that acute alcohol intoxication was associated with an increase of plasma levels of progesterone and allopregnanolone in human male and female adolescents [11]. Allopregnanolone (ALLO), a metabolite of progesterone, is an endogenous neurosteroid and a strong modulator of gamma-aminobutyric acid (GABA)_A receptors. ALLO exerts anxiolytic and anticonvulsant effects and recent studies ascribed to it an important role in the reinforcement of alcohol reward mainly for women.

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The present manuscript summarizes and discusses updates about the reciprocal relation alcohol-hormones and, from this point of view, highlights sex differences in the development of alcohol-related clinical and behavioral disorders. In the last section of the paper is taken into consideration the status of the art about analytical tools suitable for gender-tailored studies in clinical practice even in order to plan prevention and intervention strategies..

Clinical issue

During fetal development, the gonadal hormones organize permanent differentiation of the nerve centers that control specific behaviors of each sex (*organizational effect*), while in the adult age gonadal hormones activate specific sexual behavior (*activation effect*). Some studies suggest that early exposure to sex hormones triggers neuro-structural adjustments (organizational) that influence cellular responses to hormones and sexual behavior of the adult [12]. These neural adaptations seem to sensitize the reward system of the brain and to reinforce alcohol power in modulating behavior and abuse. So prenatal exposure to alcohol not only could be responsible of a number of fetal alcohol disorders including neuro-behavioral damages but could also increase the risk for the child of developing alcohol dependence later in life [14,15]. As reported below, relationship between sex hormones and alcohol is mutual since hormone levels influence willingness to alcohol intake and alcohol-related effects, whilst alcohol intake influences hormonal status. In studies about healthy male college students, higher testosterone levels were associated with increased alcohol consumption, more tendency to get drunk and to practice “binge drinking”, and more risk to develop alcohol dependence in respect to males with lower testosterone levels [16-18]. Women are more susceptible to alcohol-related diseases including those of endocrine interest such as Cushing’s syndrome. Female alcohol drinking induces delay of menarche, menstrual irregularities, reduced fertility, amenorrhea, premature menopause, altered bone metabolism (osteoporosis), and the onset of hormone-dependent breast cancer [19,20]. The results of the studies about relationship hormones/ alcohol are often inconsistent even because the clinical research is affected by a quantity of variables that may influence the results both in man and woman. Surely, the deep hormonal changes that occur throughout the entire lifespan of a woman make these studies more and more complex for female sex but also external factors, such as simultaneous exposure to other chemicals, lifestyle, type of alcohol drink and pattern of use, could significantly affect the hormonal status and the effects of alcohol intake. As far as we know, even a moderate alcohol use (one drink/ day) in woman can be associated with increased risk of spontaneous abortions and breast cancer, and it seems to raise estrogens of in pre- and post-menopausal women and to decrease progesterone in premenopausal women [21]. Some authors justify these effects by the hypothesis of an increased rate of aromatization of testosterone or a decreased rate of oxidation of estradiol to estrone. Studies by Mendelson and co-workers reported increased levels of plasma estradiol associated with alcohol intake in premenopausal women [22] and the same association was found in postmenopausal women who receiving estrogen replacement therapy. It was observed that the plasma estradiol elevation occurred with a decreasing plasma estrone levels [23]. The authors suggested that alcohol-related increase in estrogen levels is a consequence of the alcohol metabolism by alcohol dehydrogenase that leads to an accumulation of reduced NAD (NADH) in the liver. So the breakdown of estradiol to estrone is limited and estradiol accumulates [24]. Even other authors found a positive association between a moderate alcohol

consumption and plasma estradiol and dehydroepiandrosterone sulphate levels in premenopausal women [25]. Sarkola et al. observed an acute elevation in plasma estradiol in premenopausal women using oral contraceptives and alcohol intake was also associated with an acute decline in progesterone among women using oral contraceptives as well as among non-users. Furthermore, alcohol use seems to decrease luteinizing hormone (LH) mainly in women using oral contraceptives [26]. These evidences may be due to the action of the 17 β -hydroxysteroid dehydrogenase type 2 enzyme, which is induced by synthetic progestins contained within some oral contraceptives. This isoenzyme, found in the endometrium and in the liver, mainly catalyzes the catabolic conversion of estradiol to estrone and testosterone to androstenedione and the oxidation of 20 α -dihydroprogesterone to progesterone, using the same cofactor (NAD) of alcohol dehydrogenase. A decreased oxidation and/or increased reduction of these steroids in the liver is due to the alcohol-related oxidative stress. Teoh et al. also showed that alcohol may compromise the survival of the blastocyst (increased abortion rate) by decreasing progesterone production [27]. In a recent manuscript alcohol consumption was positively associated with concentrations of luteal estradiol, luteal estrone and sex hormone binding globulin and was inversely associated with concentrations of free testosterone. Authors did not observe significant association between alcohol and testosterone, progesterone, dehydroepiandrosterone and dehydroepiandrosterone sulphate, but highlighted the differences of sex steroid concentrations according to the type of alcohol drinks (beer, wine and liquors). Significant positive associations were observed with beer intake, but not other alcohol types, for Dehydroepiandrosterone (DHEA) (Pinteraction = 0.003) and androstenedione (Pinteraction = 0.006). However Alcohol consumption was positively associated with plasma luteal estrogen concentrations, but not with androgen levels, nor estrone or estradiol measured in the follicular phase. Differences in premenopausal estrogen levels may contribute to the association between alcohol and breast cancer [28]. In postmenopausal women without hormone replacement therapy (HRT) no significant changes in plasma estrogens were found; on the contrary, in women receiving HRT (either as a patch or orally) acute alcohol exposure increased estradiol [23,29]. The increase was related to the route used for HRT delivery since the increase in blood estradiol was almost the 22% by transdermal patch, but it achieved the 300 % by oral administration [21]. Ginsburg et al. did not find any significant increase in plasma estrogen after a single dose of alcohol, however these results are not consistent with those reported by Gavalier and Love who reported significant increase of plasma estradiol in postmenopausal women who drink moderately [23,30]. In pre- and postmenopausal women. Rinaldi et al. 2006 reported that a daily alcohol use was associated with higher concentration of serum androgens, both of adrenal (dehydroepiandrosterone sulphate, androstenedione, and testosterone) and ovarian origin (androstenedione and testosterone) suggesting a direct action of alcohol on the steroidogenesis in both types of glands. A strong association between estrone and alcohol intake was observed. The concentration of the sex hormone binding globulin was inversely associated to alcohol intake only in postmenopausal women, while no relationship of estradiol with alcohol intake was observed in pre/postmenopausal women [31]. The mechanisms by which alcohol increases sex steroids in humans remain still not elucidated. It’s postulated a stimulation of ovarian theca cells to produce androgens through increased LH secretion, a direct action on adrenal androgen productions, induction of a different catabolism of androgens in the liver or an increased aromatase activity in the liver leading to higher conversion of androgens into estrogens. Unfortunately results of the

studies both in premenopausal and postmenopausal women are poorly consistent [32-35]. Mendelson et al suggested that increased estradiol levels reduce FSH secretion to levels which in turn, by suppressing folliculogenesis, lead to anovulation [36]. Evidences of unsuccessful conceptions related to alcohol use have been also reported and the alcohol related impairment of ovulation not only may decrease fertility but also reduces the production of cardioprotective hormone in the second (luteal) phase of the menstrual cycle [37]. The long-term loss of these hormones in young women implicates heavy health damages. Hartman et al in 2016 found that alcohol intake was associated with higher estradiol levels and wine use was positively associated with a number of estrogen metabolites measures including estradiol [38]. Experimental results strengthen the evidence that alcohol consumption play a key role in the development of breast cancer and other estrogen-related conditions and that alcohol consumption is a consistent risk factor for breast cancer. Further studies are required to clarify the association between specific types and patterns of alcohol drinking and modulation of estrogen metabolites in blood, urine, and tissue.

Neurobehavioral issue

Even as regards neuro disorders, clinical and preclinical studies have shown that alcohol effects on the central nervous system are different between males and females and to consider the factor "gender" in neurobiological studies is essential. Inadequate alcohol intake is associated with a wide range of neuro behavioral damages including impairment of perceptual-motor skills, of visual spatial function, of learning-memory, of problem solving. Some neurological damages are indirectly due to alcohol drinking by alcohol-related nutritional deficit. This is the case of thiamine (vitamin B1) deficiency that can result in Wernicke's Encephalopathy (WE), a serious neurologic disorder, and Korsakoff amnesic syndrome that is a late neuropsychiatric manifestation of WE with memory loss and confabulation. This condition is generally referred to as Wernicke-Korsakoff syndrome (WKS). In the Western countries, thiamine deficiency is characteristically associated with chronic alcoholism because alcohol affects thiamine uptake and utilization. WKS is more prevalent than commonly supposed and it is frequently unrecognized so that Thiamine Deficiency is diagnosed only post- mortem above all in alcoholics affected by strong neurological impairment [39]. Alcohol has even a direct toxicological effects as happens, for example, when maternal alcohol consumption during pregnancy induces impairment of the development of Central Nervous System of offspring. Fetus cannot metabolize alcohol and prenatal alcohol exposure affects children by many and different grade of physical and neurological impairment (FASD Fetal Alcohol Spectrum Disorders) up to the most heavy birth illness named Fetal Alcohol Syndrome (FAS). Not all children prenatally exposed to alcohol present morphological changes typical of FAS but they can suffer for many mental dysfunction such as impaired cognitive and motor skills, reduction in intellectual function deficits in verbal learning, spatial memory and reasoning [13]. The basal ganglia appear to be sensitive to prenatal alcohol exposure since they are smaller in the children with FAS. In particular corpus striatum is the critical structure involved in motor, perceptual and cognitive skills and its innervated by excitatory glutamergic and inhibitory GABAergic neurons. The function of these nerve terminals is modulated by neurotransmitters and neurotrophic factors acting on their specific receptors. Alcohol influences the function of many neurotransmitters systems with the interaction at gamma-aminobutyric acid A (GABA A) receptors being integral for ethanol's reinforcing and several withdrawal -related effects. An interesting

group of molecules called neurosteroids may act as modulators of ion channel-coupled receptors and thereby also mediate neuronal response. Neuroactive steroids are steroid hormones derivatives that act primarily on GABA system, share some of the neurobiological effects and may contribute to alcohol effects. Recent research shows that brain concentration of allopregnanolone a potent positive modulator of GABA_A, is affected by ethanol administration both in rats [40] and in female and male humans [11,40,41]. The neurosteroid Allopregnanolone, (3 α -hydroxy-5 α -pregnan-20-one or 3 α ,5 α -tetrahydropregesterone) is a steroid metabolite of progesterone and it is the most potent endogenous GABAergic steroid identified that exerts its action on alcohol reward with significant sex differences [3]. Clinical studies found that alcohol increases the level of the Allopregnanolone that has effects similar to those of other potentiators of the GABA_A receptor such as the benzodiazepines and alcohol, including anxiolytic, sedative, and anticonvulsant activity [42]. Despite a lack of evidence showing hormone cycle effects on alcohol reinforcement, studies using controlled progestin manipulation techniques suggest a strong relationship alcohol- progestins and it was observed a direct correlation between alcohol intoxication, Allopregnanolone increase and reinforcement of alcohol rewarding effects and alcohol seeking. By animal experimentation, during ethanol withdrawal were shown decreased level of Allopregnanolone and it was observed that the severity of withdrawal symptoms enhanced when allopregnanolone was decreased. On the contrary, the experimental increase of allo levels alleviated ethanol-induced withdrawal effects [43]. Preclinical studies in female rats suggest that the rewarding effects of alcohol are stronger in females than in males and, although the behavioral effects of alcohol seem not to vary across the estrous cycle, it's recognized that the neurochemical effects of alcohol may fluctuate across the estrous cycle under the influence of hormones on the rewarding effects. Several studies are consistent in highlighting the importance of ovarian hormones as mediators of the rewarding effects of alcohol in females and in proving the major vulnerability of female sex to the neurological effect [44].

Analytical issue

As previous reported, relatively little is known about the mood-altering and behavioral effects of psychoactive substances in women since the most of studies were based on male volunteer's population. In particular, little is known about the possible variation across the menstrual cycle even for the burden of managing all possible variables such as interaction between drugs and circulating ovarian hormones, variations in non-ovarian hormones and individual pharmacokinetics/ pharmacodynamic factors. So, up to today the differences in research protocols and methodologies, including the dose and the way of alcohol administration, make poorly consistent the results of studies about the neurobiological interactions between alcohol and hormones. Some human studies rely on self-reports data about alcohol intake and dates of menstruation, others identify the day of ovulation by the measure of body temperature but these procedure depend on the compliance of the examined subject. The best strategy to characterize the phases of the cycle should be the quantification of plasma or salivary levels of hormones in order to rule out anovulatory cycles and to recognize unanticipated variability on the length of the cycle. This requires the availability of rigorous clinical protocols and analytical tools so reliable that are able to show even small variations and to produce comparable results [45]. It is important to consider that, in order to actually evaluate the alcohol effect, the blood alcohol concentration must be quantified by a reliable analytical method at the same time of the hormones determination [46].

The analytical methods used for the detection of sex hormones in biological fluids are based on screening techniques such as immunometric assays: Immunoassay (IA) Radioimmunoassay (RIA), Enzyme Immunoassay (EIA) and enzyme-linked immunosorbent assay (ELISA). The advantages of these methods are due to their practicability, i.e. high analytical efficiency, suitability for high sample throughput and good sensitivity and specificity. However, these screening test need to be confirmed by chromatographic procedures that provide full or complementary information enabling the substance to be unequivocally identified. If needed, the chromatographic procedures let us quantify hormones at the level of interest to avoid false positive or false negative results and to eliminate matrix interferences. To date there are several immunoassay kits available on the market but there are yet significant differences intra- and inter laboratories and even between kits and batches. So more standardization studies are required in order to ensure the reliability and comparability of the results and their suitability for clinical researches [47]. Furthermore, immunometric tests are able to highlight pathologies that implicate a large deviation from the reference values for healthy people. Instead, when the relationship between alcohol and hormones is studied, we need to highlight small fluctuations that may be within the reference values. So alcohol research requires analytical sensitivity and specificity higher than that usually provided by the most of commercial kits and the analytical cut-off needs to be adjusted according to the diagnostic thresholds of concern by the development of more rigorous analytical tools and more sensitive and specific tests. Technology is always improving and now there are available more sensitive instrumentation able to detect differences such that were not previously detectable. In particular Liquid chromatography-mass spectrometry-based methodology has evolved to the point that very accurate analyses of trace levels of estrogens and androgens in serum and plasma can be accomplished with high precision and accuracy [48].

Discussion

Alcohol use disorders include a wide spectrum of diseases and depend both on individual endogenous peculiarities such as age, race, gender, nutritional status, alcohol exposure during pregnancy and on exogenous factors such as the worsening of environmental condition. So health problems of today are different from those of some decades ago because of deep changes in family values, social education, influence of media and even environmental and work exposure to new toxics, including endocrine disruptors, that may affect our physical and mental health by combined exposure to multiple chemicals. Biological ("sex-related") and psycho-socio-cultural ("gender-related") factors influence the developing of alcohol-related diseases and neurobehavioral impairment with sex differences that go to a significant disadvantage for women. The review of scientific literature highlights that differences between men and women involve the biological response to alcohol intake, the progression to alcohol related diseases, and the neuro behavioral damages which may be due to both sociocultural factors and innate biological differences. The crucial role played by steroid hormones (oestrogens and progesterone) has been documented by human and animal model studies. Epidemiological data on how particular psycho-biological and physiological characteristics in females influence vulnerability to alcohol addiction and toxicological consequences are still at the beginning. In this paper the reviewed data show that there is a close relationship between alcohol and hormones and that this interaction with sex hormones can result in a lot of problems concerning different fields from neuroscience to oncology, mostly in women. In particular, evidence based data suggest that

premenopausal plasma hormones are associated with breast cancer and even global health problems, such as infertility, need to be evaluated also in the light of the possible role of alcohol intake that today has little or no attention. The frequent lack of consistency between experimental data shows the actual difficulty to harmonize analytical methods and experimental protocols, but also the presence of a lot of critical issues such as type of drink (beer, wine, liquors), patterns of use (moderate, heavy, regular or by binge drinking, at short and long term, acute, chronic), differences in the experimental protocols including the time interval between alcohol intake, sample collection and hormone assays, that affect alcohol studies. About that, in some studies where no association between alcohol use and estrogen levels was detected, it is possible to think that estrogen levels increased and then returned to normal levels before blood samples were collected. Another critical issue is how the quantity of alcohol intake was estimated because some studies consider only the self-report consume obtained by questionnaire. Furthermore, the assessment of alcohol use on the basis of the average weekly intake is strongly questionable because the effects of more drinks consumed only in a day are very different in respect to the same number of drinks consumed during several days. Too many time it happens that the actual blood concentration is not reported and to overcome this problem, the analytical determination of blood alcohol concentration (BAC) must be performed at the same time of hormones determination. All this considered, it is difficult to predict how individual endocrine profiles will influence drinking behavior, propensity to initiate and sustain alcohol use, outcome of any treatment in case of abuse and likelihood of relapse. Surely it was established a causal link between alcohol and breast cancer and evidence-based data show that carcinogenesis of breast tissue is favored by drinking even when alcohol intake is so low that it does not affect other tissues. This effect is tissue-specific and is mediated by estrogens since even a low increase of blood alcohol concentration results in a marked increase of circulating estrogens so that the hormonal modulation by alcohol may justify the sex differences in the onset of alcohol harm. Great attention should be given to the concomitant use of contraceptives, hormonal replacement therapy and alcohol because of synergic negative effects are possible and women should be warned of this risk. As regards neuro behavioral impairment, sex differences in the modulation of GABAergic neurosteroids seem to be important to clarify physiological mechanisms and to ameliorate therapeutic interventions in alcoholics affected by neuro-impairment. It is never emphasized enough the deleterious effects of alcohol drinking during pregnancy since in the western countries maternal drinking during pregnancy is the first preventable cause of mental retard in the child. Today a large percentage of young women are accustomed to drink since adolescence and do not have enough perception of the possible harm to themselves and to the fetus during pregnancy. For all we know, alcohol harm cannot be correlated to the dose therefore it is essential that doctors inform their patients about the need to abstain from alcohol during pregnancy and, even better, since when they are planning pregnancy. Significant gaps remain in our knowledge, and new multidisciplinary studies are required to better understand the influence of sex hormones in the processes that mediate enhanced female vulnerability to alcohol effects and to clarify the mechanisms underlying biological (sex-related) and psycho-socio-cultural (gender-related) differences in alcohol use and related disorders. In order to develop strategies suitable for prevention and treatment of alcohol use disorders in men and women, available data suggest the need of improving clinical research and the opportunity of beginning breast cancer prevention early in life. A good strategy of intervention should be to sensitize health operators in order

to give great attention to alcohol problems that, mainly by women, are often hidden. In every case, it is to be recommended the need for adult healthy women to restrict alcohol intake to no more than one unit / day. The teenagers as well as the pregnant women should not drink at all, and women at risk of breast cancer for familiarity or other physical/ environmental condition should consume less or completely abstain from drinking.

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