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Review

# Alcohol use disorders and current pharmacological therapies: the role of GABA_A receptors

Jing LIANG*, Richard W OLSEN

*Department of Molecular & Medical Pharmacology, David Geffen School of Medicine, University of California, Los Angeles, CA, USA*

Alcohol use disorders (AUD) are defined as alcohol abuse and alcohol dependence, which create large problems both for society and for the drinkers themselves.  To date, no therapeutic can effectively solve these problems.  Understanding the underlying mechanisms leading to AUD is critically important for developing effective and safe pharmacological therapies.  Benzodiazepines (BZs) are used to reduce the symptoms of alcohol withdrawal syndrome.  However, frequent use of BZs causes cross-tolerance, dependence, and cross-addiction to alcohol.  The FDA-approved naltrexone and acamprosate have shown mixed results in clinical trials.  Naltrexone is effective to treat alcohol dependence (decreased length and frequency of drinking bouts), but its severe side effects, including withdrawal symptoms, are difficult to overcome.  Acamprosate showed efficacy for treating alcohol dependence in European trials, but two large US trials have failed to confirm the efficacy.  Another FDA-approved medication, disulfiram, does not diminish craving, and it causes a peripheral neuropathy.  Kudzu is the only natural medication mentioned by the National Institute on Alcohol Abuse and Alcoholism, but its mechanisms of action are not yet established.  It has been recently shown that dihydromyricetin, a flavonoid purified from Hovenia, has unique effects on GABA_A receptors and blocks ethanol intoxication and withdrawal in alcoholic animal models.  In this article, we review the role of GABA_A receptors in the treatment of AUD and currently available and potentially novel pharmacological agents.

**Keywords:** alcohol use disorders; ethanol; GABA_A receptor; benzodiazepine; naltrexone; acamprosate; disulfiram; Kudzu; Hovenia

## Introduction

Alcohol use disorders (AUD) represent a substantial public health problem worldwide.  According to the World Health Organization (WHO) 2011 report, the harmful use of alcohol results in approximately 2.5 million deaths each year, with a net loss of life of 2.25 million, taking into account the estimated beneficial impact of low levels of alcohol use on some diseases in some population groups.  Also, alcohol abuse is the leading risk factor for death in males ages 15–59, primarily due to injuries, violence and cardiovascular diseases.  Globally, 6.2% of all male deaths are attributable to alcohol, compared to 1.1% of female deaths[1].  The Centers for Disease Control and Prevention (CDC) reported that excessive alcohol consumption is the third leading cause of preventable death in the United States[2].  Approximately 18 million Americans (8.5% of the population age 18 and older) suffer from AUD.  Only 7.1% of these individuals received any treatment for their AUD in 2006[3].  Prob-

lems related to the excessive consumption of alcohol cost the US society an estimated $185 billion annually[3].

AUD is defined as alcohol abuse and alcohol dependence.  Alcohol abuse is defined as a recurring pattern of high-risk drinking that creates problems for the drinker, for others, or for society.  Alcohol dependence, also called alcoholism (alcohol addiction), is a complex disease characterized by persistent and intense alcohol-seeking, which results in a loss of control over drinking, a preoccupation with drinking, compulsion to drink or inability to stop, and the development of tolerance and dependence[3].  The development of AUD involves repeated alcohol use leading to tolerance, alcohol withdrawal syndrome (AWS), and physical and psychological dependence, with the loss of ability to control excessive drinking.  Continued excessive alcohol consumption can lead to dependence that is always associated with AWS when alcohol consumption is ceased or substantially reduced[4].  Without a pharmacological adjunct to psychosocial therapy, the clinical outcome is poor, with up to 70% of patients resuming drinking within 1 year[5].  Clearly, in addition to external factors, the prevention and treatment of AUD must include stopping

repeated alcohol abuse and AWS. Currently, three oral medications (naltrexone, acamprosate, and disulfiram) and one injectable medication (extended-release injectable naltrexone) are approved for treating alcohol dependence by the US Food and Drug Administration (FDA). Topiramate, an oral medication used to treat epilepsy and migraines, has recently been shown to be effective in treating alcohol dependence, although it is not approved by the US FDA for this indication[6]. However, the efficacy of these medications is only approved for use in patients who are abstinent at the start of treatment. Although the clinical trials in the US have shown controversial, insignificant, and unsuccessful results[7, 8], the NIH has continually invested much effort and funding in clinical trials, which indicates some hope rather than no hope. However, the NIH nevertheless declared that there is an urgent need for the development of new and more effective medication (http://grants1.nih.gov/grants/guide/pa-files/PA-10-100.html). Therefore, understanding the mechanisms leading to AUD becomes critically important for finding the proper therapeutic.

The history of alcohol use can be traced back to 9000 years ago, when people discovered how to make fermented beverages[9]. Since then, people around the world have been drinking alcoholic beverages. The reasons for drinking alcoholic beverages vary, and include being part of a standard diet, for medical purposes, as a relaxant, for anxiolytic effects, for artistic inspiration, and for happiness. Clearly, alcohol consumption occurs to mark major life events from birth to death. Medical science noticed that drinking brings people happiness and relaxation but also adverse consequences. Short-term effects of alcohol consumption include intoxication and dehydration. Long-term effects of alcohol include changes in the metabolism of the liver and the brain. Like other food culture, once someone falls in love with alcohol, it is not easily stopped.

## Underlying mechanisms of AUD

The biological mechanisms leading to AUD are poorly understood. Alcohol consumption has profound effects on brain function and behavior[10]. Continued excessive alcohol consumption can develop a dependence on alcohol. Discontinuation or substantially reduced alcohol consumption triggers AWS[4]. To date, the mechanisms for how excess alcohol consumption leads to alterations in the human brain that produce alcohol dependence remain murky. The formation of AUD is a chronic and complex process. For example, a relapse in alcohol consumption may be spontaneous. A relapse may be due to internal stimuli of the body, such as mood changes, anxiety, or for reducing or stopping AWS; it also may be due to external stimuli, such as the observance of social drinking or bottles of the addict's preferred alcoholic beverage. Regardless of the perspective, the role of alcohol on the human brain cannot be ignored, given the many neuropharmacological and psychological actions of ethanol (EtOH), including its intoxicating, sedative, anxiolytic, reinforcing, and addictive properties[11, 12].

Alcohol affects brain function by interacting with multiple neurotransmitter systems. Alcohol can disrupt the delicate balance between γ-aminobutyric acid (GABA), the primary inhibitory neurotransmitter, and glutamate, the major excitatory neurotransmitter in the central nervous system (CNS)[13, 14]. Short-term alcohol exposure tilts this balance, while under long-term alcohol exposure, the brain attempts to compensate by bringing the balance back toward equilibrium. These neurological changes present as the development of tolerance to alcohol's sedative effects. When alcohol consumption is abruptly discontinued or reduced, these compensatory changes are no longer opposed by the presence of alcohol, thus leading to the excitation of neurotransmitter systems and the development of AWS[10]. Long-term, or chronic, alcohol consumption also induces changes in many neurotransmitter systems that ultimately lead to the development of tolerance and dependence[15]. Chronically relapsing alcohol consumption involves elements of both impulsivity and compulsivity that yield a composite addiction cycle composed of three stages: 'binge/intoxication', 'withdrawal/negative affect', and 'preoccupation/anticipation' (craving). Animal and human imaging studies have revealed discrete circuits that mediate the three stages of the addiction cycle with key roles of the ventral tegmental area (VTA), ventral striatum and amygdala. The transition to addiction involves neuroplasticity in all of these structures that may begin with changes in the mesolimbic dopamine system and a cascade of neuroadaptations from the ventral to dorsal striatum[16]. Furthermore, the study has shown that chronic drug experience increases the contrast, or 'signal to noise', of phasic dopamine release to basal dopamine levels in response to drug-related stimuli, which could result in aberrant associations between cues and reinforcers that contribute to the development of addiction[17, 18]. Since dopamine D2 receptors in the striatum are primarily localized in GABAergic neurons, these results provide evidence of GABAergic involvement in the dopaminergic abnormalities seen in alcoholics[19]. Therefore, the contribution of dopamine dynamics in the reward neurocircuitry, particularly the VTA, nucleus accumbens (NAcc), amygdala, and prefrontal cortex dopaminergic pathway, is an underlying mechanism leading to alcohol dependence[17, 18, 20–22].

GABA is the major inhibitory neurotransmitter in the mammalian brain. As a neurotransmitter, GABA is released into a synaptic cleft by its presynaptic nerve terminus when a GABAergic (GABA releasing) neuron fires an action potential. GABA$_A$Rs are a family of ligand-gated chloride anion (Cl$^-$) channels expressed throughout the CNS (ionotropic receptors)[23] and composed of five subunits, each of which has several isoforms, composed from a family of 19 related subunits (α1−6, β1−3, γ1−3, δ, ε, θ, π, ρ1−3)[23–27]. The neurotransmitter GABA binds to GABA$_A$Rs, changing their conformation state and then opening the pore to allow Cl$^-$ to pass down an electrochemical gradient. GABA$_A$Rs in the postsynaptic membrane mediate fast or phasic inhibition (through ionotropic GABA$_A$Rs) and slow synaptic inhibition (through metabo-

tropic GABA$_B$Rs); GABA$_A$Rs in the peri- and extrasynaptic membrane mediate tonic inhibition.

GABA$_A$Rs mediate several important effects of alcohol. Considerable evidence indicates that GABA$_A$Rs are the major target of EtOH in the CNS[28–32]. Some studies show that short-term alcohol exposure increases the inhibitory effect of GAB-A$_A$Rs; however, many factors determine whether GABA$_A$Rs respond to short-term alcohol exposure[33]. Alcohol can act as a depressant by increasing inhibitory neurotransmission, by decreasing excitatory neurotransmission, or through a combination of both[13, 34]. Commonly, alcohol consumption can induce decreases in attention, alterations in memory, reductions in executive decision-making, changes in mood, and drowsiness. Continuous alcohol consumption may result in lethargy, confusion, amnesia, loss of sensation, difficulty in breathing, and death[13]. GABA$_A$Rs mediate alcohol-induced sedation, anxiolysis, impairment of motor coordination, and withdrawal symptoms such as anxiety, hyperexcitability, insomnia, and seizures[35–44]. EtOH acts on certain subtypes of GABA$_A$Rs and induces rapid alteration of their subunit assembly, consequently altering the functional properties of these GABA$_A$Rs[38, 45]. As a result, GABA$_A$R-mediated behaviors are altered after alcohol exposure[43, 44, 46]. Clearly, GABA$_A$Rs play a critical role in the response to EtOH, modulating the altered balance between excitation and inhibition induced by EtOH, and contributing to withdrawal syndrome.

Human studies found that single nucleotide polymorphisms (SNPs) in the gene encoding the GABA α2 receptor subunit (GABRA2) are associated with complex behaviors considered to be part of alcohol dependence, suggesting that GABA$_A$Rs containing the α2 subunit contribute to the genetic risk for alcohol dependence[47]. The GABA$_A$R γ1 subunit gene GABRG1 and GABRA2 variants are associated with alcohol dependence in African Americans[48]. Indeed, a cluster of GABA$_A$R subunit genes encoding α2, α4, β1, and γ1 subunits on chromosome 4p has been associated with alcohol dependence[47, 49–52]. In human brain imaging studies, a persistent down-regulation of central GABA$_A$Rs in early abstinence was demonstrated[52–54]. One hundred and ten healthy social drinkers (53 men) in a drinking study showed that the GABRA2 gene is associated with subjective (pleasant or unpleasant sensations) effects of alcohol, suggesting that GABRA2 may play a role in the risk of developing alcohol use disorders by moderating the subjective effects of alcohol[55]. However, the neurobiological basis by which genetic variation and in which brain region translates into alcohol dependence is largely unknown.

GABA$_A$Rs have very unique characteristics. The large number of GABA$_A$R subunits generates the potential for various subunit compositions that may account for variable sensitivity to modulatory drugs such as EtOH, benzodiazepines (BZs), barbiturates, neurosteroids, and general anesthetics[31, 32, 50, 56, 57]. The change in numbers and subunit compositions of GAB-A$_A$Rs on cell surfaces has been demonstrated to be important in mediating inhibitory synaptic transmission[58, 59]. Neverthe-

less, GABA$_A$Rs can exist as either synaptic or extrasynaptic receptors that may facilitate rapid changes in inhibition. Most synaptic GABA$_A$Rs are composed of two α, two β, and one γ subunit, where the γ subunit is located between an α and β subunit[60, 61] and contributes to synaptically mediated (phasic) inhibition. In contrast, tonic inhibition is mediated by highly sensitive GABA$_A$Rs in peri- and extrasynaptic membrane where α4/α6-containing GABA$_A$R subunits are predominantly located and partner with the exclusively extrasynaptic δ subunits[27, 62, 63]. They are activated by ambient extracellular GABA or from 'spillover' of GABA from synaptic signaling, thought to be in the range of 100 nmol/L to 1 μmol/L[64–66]. Given this distinction, the study of synaptic *vs* extrasynaptic GABA$_A$Rs and EtOH effects on these receptors is essential to elucidate the mechanisms involved in the development of EtOH tolerance and dependence. On the other hand, synaptic and extrasynaptic GABA$_A$Rs are dynamic in response to EtOH. GABA$_A$Rs cycle in response to EtOH exposure between the surface of the synaptic membrane and intracellular sites, and traffic from intracellular pools to the surface of synaptic membranes is critically important in the postsynaptic and extrasynaptic control of neuronal excitability[63, 67, 68]. Due to the properties of GABA$_A$Rs, such as 1) various subunit compositions, 2) dynamic movement between extracellular and intracellular pools, and 3) postsynaptic and extrasynaptic membrane locations, studies of the interactions between GABA$_A$Rs and EtOH become very challenging.

## Effects of single alcohol consumption

Single or acute alcohol consumption is an alcohol intake that occurs over a short period of time. The effects of single alcohol consumption depend on alcohol concentration and the amount of intake. EtOH concentrations in the brain vary in a range from few millimolars to more than 100 millimolars. As a CNS depressant, EtOH in a concentration range of 5–10 mmol/L (less than 3 drinks) potentiates GABA$_A$Rs and decreases excitatory neurotransmission, leading to sedation accompanied by decreased attention, alterations in memory, mood changes, and lethargy[37].

A large number of animal experiments have shown EtOH effects on the brain. EtOH can produce an acute anxiolytic effect, which is related to the potentiation of GABAergic neurotransmission in the basolateral amygdala (BLA)[69]. Single-dose EtOH stimulates GABA-activated Cl$^-$ channels[70, 71]. In studies of acute EtOH effects on the kinetics of miniature inhibitory postsynaptic currents (mIPSCs), an EtOH (3 g/kg) intraperitoneal injection in rats produced a rapid down-regulation of extrasynaptic α4βδ–GABA$_A$Rs in hippocampus within 5–15 min. This change was accompanied by a decreasing surface expression of α4, β3, and δ–containing GABA$_A$Rs (internalized) and increasing phosphorylation of β3. In contrast, the down-regulation of postsynaptic α1β2 GABA$_A$Rs are observed, but only after several hours[68, 72]. Finally, there is an up-regulation of GABA$_A$Rs containing α4βγ2 after 1–2 d post-EtOH and increases in α2βγ1 GABA$_A$Rs[68]. These effects

of acute EtOH exposure on GABA$_A$Rs are transient and reversible, but altered GABA$_A$Rs will need two weeks to recover after acute alcohol intoxication. From the process of GABA$_A$Rs interacting with EtOH to recovery can provide valuable information for how alcohol dependence develops with long term exposure to EtOH.

## Effects of social drinking

Social drinking is casual drinking in social situations and only in moderate quantities, also referred to as moderate drinking. For men, it is no more than 4 drinks on any single day and no more than 14 drinks per week. For women, moderate drinking is no more than 3 drinks on any single day and no more than 7 drinks per week based on the NIH/NIAAA criterion. Social drinking can produce a low-to-moderate concentration of EtOH (≤30 mmol/L) in the brain. Animal studies have shown that a low dose of EtOH (0.01 g/kg, <10 mmol/L) applied to VTA could significantly increase GABA neuron firing rate and afferent-evoked synaptic responses[73]. Voluntary EtOH (6%, equal 1 g/kg, ~13 mmol/L) consumption induced an elevation of dopamine in the rat mesolimbic reward pathway[74]. Using viral-mediated RNA interference to transiently reduce α4-containing GABA$_A$Rs in the shell region of the NAc could reduce self-administration of EtOH intake[75]. Blockade of GABA$_A$Rs in the para-ventricular nucleus of the hypothalamus, which is the main integration site controlling the hypothalamic-pituitary-adrenal (HPA) neuroendocrine stress system, could reduce voluntary EtOH drinking[76]. Voluntary EtOH consumption (<10% EtOH) could induce an increase of GABA$_A$R α4/δ subunits in the hippocampus in socially isolated C57BL/6J mice and rats[77, 78]. These α4/δ-containing GABA$_A$Rs have been shown to exist on extrasynaptic membrane sites, which are sensitive to as low as 3 mmol/L EtOH (approximately 0.3% EtOH)[79]. Therefore, EtOH entering the brain by each episode of social drinking will target and stimulate GABA$_A$Rs, primarily extrasynaptic GABA$_A$Rs; these GABA$_A$Rs respond to the alcohol, then GABA$_A$R dynamic changes occur.

EtOH preference studies have shown that BZ receptor ligands modulate some of the reinforcing and/or aversive properties of alcohol in alcohol-nonpreferring (NP) rats, suggesting the potential importance of the GABA$_A$-BZ receptor complex in mediating palatability- (environmentally) induced EtOH drinking even in rats selectively bred for low alcohol preference[80]. Intrahippocampal infusions of an α5-containing GABA$_A$R subunit-selective BZ inverse agonist RY (RY, tert-butyl 8-(trimethylsilyl) acetylene-5,6-dihydro-5-methyl-6-oxo-4H-imidazo[1,5a][1,4]benzodiazepine-3-carboxylate), reduced EtOH-maintained responding in a dose-dependent manner, suggesting that the α5-containing GABA$_A$Rs in the hippocampus play an important role in regulating EtOH-seeking behaviors[81]. Mutations to the GABA$_A$R β1 subunit gene (GABRB1) increased alcohol consumption accompanied by spontaneous GABA$_A$R ion channel opening and increased NAcc tonic (extrasynaptic) current, providing an important link between

GABA$_A$R function and increased alcohol consumption that may underlie some forms of alcohol abuse[52]. The GABA$_A$R α5 subunit knockout mice showed reduced EtOH preference[31, 82, 83]. Additionally, GABA$_A$R δ-deficient mice have reduced EtOH preference[82]. Both studies suggest GABA$_A$R involvement in mediating drinking behavior. The PKCepsilon phosphorylation of γ2 regulates the response of GABA$_A$Rs to specific allosteric modulators, and in particular, PKCepsilon inhibition renders these receptors sensitive to low concentrations of EtOH[84]. Thus, alcohol consumption at a social level can target GABA$_A$R-mediated extrasynaptic inhibition; social drinking also appears to involve the mesolimbic dopamine system.

## Effects of chronic alcohol consumption

Chronic alcohol abuse refers to relapsing long-term alcohol consumption. In studies of animal models for chronic and repeated EtOH administration, rats receive multiple chronic intermittent EtOH (CIE) gavage administration with withdrawal (more than 2 d). CIE rats exhibit hyperexcitability in locomotion, rearing, and exploratory behavior. They are not altered in sensorimotor performance and exhibit no detectable brain or liver pathology[29]. In addition to the quantitative reduction in seizure threshold to pentylenetetrazol (PTZ), they exhibit increased anxiety, impaired hippocampal spatial memory, and perturbed sleep patterns. They also exhibit tolerance to the soporific effects of EtOH, BZs, neurosteroids, and several general sedative/hypnotic/anesthetics, including most commercial sleep aids. Significant tolerance to the soporific/anesthetic properties of diazepam remains long (>40 d) after the final EtOH dose in CIE rats, accompanied by decreased GABA$_A$R-mediated inhibition[43–46, 63, 85, 86]. Behaviorally, CIE rats show increased EtOH drinking[32]. Although no animal model can fully emulate the human AUD condition, the behavioral adaptations of human alcohol dependence/withdrawal are remarkably similar to those of CIE rats, particularly with respect to anxiety, increased seizure susceptibility, and tolerance to EtOH and cross tolerance to BZs[44, 46]. The mechanism studies have shown that the behavioral changes are primarily due to the plastic changes of GABA$_A$Rs that occur after chronic EtOH exposure, which include significantly reduced postsynaptic α1 and increased α4-containing GABA$_A$Rs[46, 63, 87, 88]. The subunit composition of GABA$_A$R subtypes is expected to determine their physiological properties and pharmacological profiles. An in-depth study of GABA$_A$R subunits using genetically engineered mice has shown that the α1 subunit is involved in sedation, anticonvulsant activity, and anterograde amnesia functions, *etc*[41], while the α4 subunit is involved in changes of mood and anxiety[89]. Thus, these GABA$_A$R subunit composition changes are a mechanism underlying the behavioral changes after chronic EtOH exposure.

Studies of psychological changes and alcohol consumption have determined that in young rats (postnatal days 28–42), binge drinking is related to anxiety-like behavior and leads to alcohol-dependence in adulthood[90]. Stress and withdrawal-

induced anxiety are correlated to increased voluntary EtOH drinking in alcohol-preferring P rats[91], and chronic psychosocial stressed male mice show increased voluntary EtOH drinking[92]. The data provide strong evidence that heavy drinking triggered by chronic stress and any type of induced anxiety are risk factors for developing alcohol dependence. Stopping or reducing alcohol consumption in turn aggravates stress or anxiety. The repeated psychological changes make it difficult to stop alcohol consumption.

Withdrawal occurs following the restriction of alcohol intake. The pathophysiology of alcohol withdrawal is complex because prolonged alcohol intoxication affects various circuits, each involving various neurotransmitter systems. After withdrawal from alcohol, the downregulation of the $GABA_ARs$ contributes to many of the symptoms of AWS. Prolonged intoxication also inhibits activity in the glutamate neurotransmitter system, the major excitatory neurotransmitter in the CNS, by acting on the ion-gated N-methyl-D-aspartate (NMDA) glutamate receptors. Abstinence from alcohol reverses the inhibition of the NMDA receptor, producing many of the signs and symptoms of AWS[93, 94]. Other mechanisms are also activated during alcohol withdrawal. Dysfunctional dopaminergic transmission[95] may be responsible for hallucinations. Signs and symptoms of alcohol withdrawal occur primarily in the central nervous system, including sleep disturbance and anxiety and negative emotional states, such as dysphoria. Chronic alcohol consumption leads to changes in brain neurotransmission, particularly in the GABAergic system, via induced $GABA_AR$ plasticity and DA release in the reward neurocircuitry. During acute alcohol withdrawal, changes also occur such as upregulation of α4-containing $GABA_ARs$ and downregulation of α1- and α3-containing $GABA_ARs$[29, 39, 44, 46, 63, 87]. $GABA_AR$ downregulation may contribute to the anxiety and seizures of withdrawal. During periods of withdrawal, rats show a significant decrease in DA and serotonin (5-HT) levels in the reward neurocircuitry[96–98], which is commonly associated with dysphoria, depression and anxiety disorders[98–100]. These psychological changes may contribute to EtOH-seeking behavior. Studies have shown that $GABA_ARs$ and opioid receptors within the central nucleus of the amygdala selectively regulate EtOH-maintained responding[101]. As in adults[68], CIE exposure during adolescence increased the EtOH sensitivity of tonic inhibition mediated by extrasynaptic $GABA_ARs$ and decreased the EtOH sensitivity of phasic, synaptic $GABA_AR$-mediated current in adult dentate gyrus cells, demonstrating long-lasting changes in the function and EtOH sensitivity of synaptic and extrasynaptic $GABA_ARs$ in DGCs[102]. GABAergic neurons in the VTA are a primary inhibitory regulator of DA neurons, generally recognized as having an important role in the development of addiction[103, 104]. In addition, a subset of VTA $GABA_ARs$ is implicated in the development of addictive behavior. In particular, the activation of central GABAergic neurotransmission is linked to mesolimbic dopaminergic neurotransmission during rewarding processes[73, 100, 105]. We suggest that preclinical and clinical studies should place more

emphasis on the GABAergic system as a pharmacotherapeutic target for the treatment of AUD.

## Cognition and alcohol dependence

Alcohol consumption is like other food culture; once an individual falls in love with alcohol, its consumption can be difficult to stop. In addition to the involvement of nervous system regulation, the changes in cognition caused by chronic alcohol consumption cannot be ignored. In mammals, alcohol acts on the CNS over time to enhance the excitatory NMDA signaling and discourage GABA signaling, inducing adaptation of $GABA_AR$-mediated inhibition and a hyperexcitable nervous system. This hyperactive nervous system is dependent on the presence of alcohol; otherwise, the hyperactive state can lead to over-excitatory consequences such as seizures[29]. Humans often drink too much because they find being drunk rewarding in some way, and/or they find abstinence difficult. Interestingly, in studies of alcohol recognition in *Drosophila*, flies exhibit voluntary consumption of EtOH. Kaun *et al*[106] developed a conditioned place preference paradigm for flies, and showed that flies perceive intoxicating levels of ethanol as rewarding. Flies were exposed to two odors, one in the presence of intoxicating levels of EtOH vapors, and the other without. After training, flies preferred the odor that had been paired with the high level of EtOH. Furthermore, trained larvae are able to learn, and develop cognitive dependence to EtOH[107]. A study of the human brain showed that that hippocampus is involved not only in learning but also in recognition ability[108]. Taken together, these studies may not provide direct evidence, but they suggest that EtOH-induced changes in multiple neurotransmissions likely involve EtOH-induced formation of cognition. These studies led us to consider that alcohol not only induces adaptation in the central nervous system but also forms cognition and memory of alcohol in the brain that promote the development of alcohol dependence. Understanding the effects of alcohol on the brain leading to AUD is essential to determine the direction for anti-AUD drug development.

## Current pharmacological therapies

One ideal property of therapeutic drugs for AUD is that the active ingredient acts on receptors directly targeted by alcohol to prevent interactions of these receptors with alcohol. To neutralize the effects of alcohol on $GABA_ARs$ and ameliorate the symptoms of AWS and/or to diminish cravings for alcohol is more likely to achieve success.

### Benzodiazepines

$GABA_ARs$ are down-regulated during chronic alcohol use. After abstinence from alcohol, the downregulation of $GABA_ARs$ contributes to many of the symptoms of AWS. BZs are classical medications for reducing the symptoms of AWS[109-111]. BZs, like EtOH, have a binding site on $GABA_ARs$[27, 112, 113]. Clinical studies suggest that BZs have efficacy in ameliorating symptoms and in decreasing the risk of sei-

zures. However, due to their addictive potential and lack of safety when combined with alcohol, BZs are usually not recommended for the maintenance of alcohol abstinence. BZs are usually not prescribed for more than 2 weeks or administered for more than 3 nights per week due to tolerance[114, 115] and other side effects. Two common side effects are that BZs may actually cause anxiety in patients and that they are potentially dangerous CNS depressants when used in combination with alcohol. Many pilot studies for improving BZs and/or related non-BZs acting at the same sites have failed. Furthermore, the frequent use of BZs can lead to dependence and cross-tolerance to alcohol[109, 116]. Together they are an even worse addiction problem and difficult to overcome. Therefore, BZs are not considered to be the proper choice for AWS[117, 118].

Other GABAergic medications represent potentially promising drugs useful for the treatment of AWS and for the maintenance of alcohol abstinence. Clomethiazole, gabapentin, and γ-hydroxybutyrate (GHB) present a similar efficacy as BZs in suppressing AWS. Current evidence also suggests that gabapentin and valproic acid may be beneficial in maintaining alcohol abstinence in alcoholics with psychiatric co-morbidity. Thus, given the importance of GABAergic mechanisms in the development and maintenance of alcohol dependence, and the interesting results that have currently been demonstrated, more research on GABAergic agents is warranted[119].

In addition to BZs, only three medications (oral naltrexone, acamprosate, and disulfiram) as well as extended-release injectable naltrexone are currently approved by the FDA for treating alcohol dependence[6].

### Naltrexone

Naltrexone is an opiate antagonist used primarily in the management of alcohol dependence and opioid dependence. In laboratory studies, naltrexone has been shown to reduce the number of drinks consumed[120, 121]. In clinical trials, naltrexone reduced the percentage of heavy drinking days[122]. Additionally, oral naltrexone reduces relapse to heavy drinking[123–125]. The standard dose is 50 mg daily, but a multisite study demonstrated that 100 mg daily was also effective when combined with medical management[7]. Naltrexone is less effective for the maintenance of abstinence[126, 127]. Naltrexone has some side effects, including the development of withdrawal symptoms, nausea, dysphoria, and fatigue[128–130]. Naltrexone also impairs thinking or reactions and induces anxiety[131–133].

### Acamprosate

Acamprosate (Campral) is thought to stabilize the chemical balance in the brain that would otherwise be disrupted by alcoholism, possibly by antagonizing glutamatergic *N*-methyl-*D*-aspartate receptors and agonizing $GABA_ARs$[134]. A study at the molecular and cellular level suggests that acamprosate attenuates hyper-glutamatergic states, which are thought to trigger relapse[135]. Two large US trials failed to confirm the efficacy of acamprosate, although secondary analyses in one of the studies suggested possible efficacy in patients who had a

baseline goal of abstinence[136, 137]. Recent studies have shown that acamprosate had a significantly larger effect size than naltrexone on the maintenance of abstinence, and naltrexone had a larger effect size than acamprosate on the reduction of heavy drinking and cravings, indicating that in treatment for alcohol use disorders, acamprosate is slightly more efficacious in promoting abstinence, and naltrexone is slightly more efficacious in reducing heavy drinking and cravings[138]. For best results, detoxification is needed before using acamprosate. The most common side effects reported for patients taking acamprosate in clinical trials included headache, diarrhea, flatulence, and nausea[139, 140].

### Disulfiram

Disulfiram (Antabuse) interferes with the degradation of alcohol, resulting in the accumulation of acetaldehyde which, in turn, produces a very unpleasant reaction including flushing, nausea, and palpitations if the patient drinks alcohol[136]. During normal metabolism, alcohol is broken down in the liver by the enzyme alcohol dehydrogenase to acetaldehyde, which is then converted by the enzyme acetaldehyde dehydrogenase to the harmless acetic acid. Disulfiram blocks this reaction at the intermediate stage by blocking the enzyme acetaldehyde dehydrogenase. After alcohol intake under the influence of disulfiram, the concentration of acetaldehyde in the blood may be 5 to 10 times higher than that found during metabolism of the same quantity of alcohol alone. Because acetaldehyde is one of the major causes of the symptoms of a "hangover", this produces a severe negative reaction to alcohol intake. Symptoms include flushing of the skin, accelerated heart rate, shortness of breath, *etc.*

The utility and effectiveness of disulfiram are considered limited because compliance is generally poor when it is given to patients to take at their own discretion[141]. Some patients, however, will respond to self-administered disulfiram, especially if they are highly motivated to abstain. Others may use it episodically for high-risk situations, such as social occasions where alcohol is present. It is also known that disulfiram may cause a peripheral neuropathy[142].

### Topiramate

Topiramate requires a very gradual dose escalation. The precise mechanism of action is unclear. The most common adverse events include cognitive dysfunction, abnormal sensations (*eg*, numbness and tingling), and anorexia and taste abnormalities. Additional rare but serious adverse events have been identified, such as metabolic acidosis, acute myopia, and secondary narrow-angle glaucoma. The optimal dose for alcohol dependence has yet to be established and may be lower than the target dose of 300 mg per day tested in previous research[143, 144].

### Traditional herbal medications and their effects on alcohol consumption and AWS

Historically, people in Asia have used herbal medicines and

dietary supplements for the treatment of excessive alcohol consumption and AWS. Although the precise chemical composition and mechanism are often unclear, many medicinal plants have been used to treat alcohol consumption and alcohol abuse for centuries, and some have shown efficacy. However, due to the lack of scientific evaluation of the properties of herbal medicines, the use of these herbal medicines has been greatly restricted. Recently, medical scientists re-evaluated the efficacy of some herbal medicines using modern scientific approaches.

Progress has been made toward research into the development of natural therapeutic agents for decreasing alcohol consumption and ameliorating AWS symptoms. Investigations of natural medicines have been focused on three aspects of alcohol intoxication: (1) decreasing alcohol consumption through decreasing the appetite for drinking and thereby suppressing alcohol intake; (2) inhibiting alcohol absorption in the gastrointestinal tract, and reducing alcohol concentration in the blood; and (3) enhancing liver metabolic functions to accelerate the elimination of alcohol and its metabolites and to alleviate injury to tissue and cells.

To date, several single herbal remedies have drawn attention for their anti-alcohol effects. These herbal remedies have a long history of use for the treatment of alcoholism in Asian countries such as China and Korea. For example, extracts of *Kudzu*, also known as *Pueraria lobata*, and *Hovenia* have been used as herbal medications in China since 200 BC, and both are noted in the Chinese pharmacopoeia of AD 600 as remedies for combating drunkenness. In addition to *Kudzu* and *Hovenia*, *Salvia miltiorrhiza* is another single herbal remedy for reducing cravings. Several compound formulas of herbal medicines are also commonly used to reduce the symptoms of excessive alcohol consumption, such as 'ge-hua-jie-xing-tang,' 'zhi-ge-yin,' and 'wu-ling-san.' These compound formulas consist of more than two single herbs. Even for medical scientists who specialized in herbal medicines, it is difficult or impossible to correctly distinguish which component is responsible for any given effect and if the medicine works.

A recent study showed that a complex containing *Kudzu*, bitter herbs (gentian, tangerine peel) and bupleurum reduced AUD identification test (AUDIT) scores in moderate to heavy drinkers. The underlying mechanisms may be through daidzin, which inhibits aldehyde dehydrogenase 2 (ALDH-2) and *Radix bupleuri* in the compound formula, which has some protective benefits not only in terms of ethanol-induced liver toxicity but also neurochemical effects involving endorphins, dopamine, and epinephrine[145]. Pilot studies provided useful evidence for using compound formulae to reduce heavy drinking. However, more time and effort are needed to understand the mechanisms and the safety/toxicity and to establish regulatory policies for using natural or other types of herbs.

### Kudzu

*Kudzu* is the only herbal medicine mentioned by the NIAAA[118]. *Kudzu* is popular as an agent for alcoholism and hangovers as noted in the Chinese Pharmacopeia.

Alcohol is removed from the body primarily through metabolism in the liver. Approximately 90% of the alcohol is metabolized in the liver by alcohol dehydrogenase (ADH) followed by aldehyde dehydrogenase (ALDH), while the gastrointestinal tract, lungs and kidneys play only a minor role[146]. Because alcohol-metabolizing enzymes such as ADH, ALDH and the microsomal alcohol oxidizing system (MEOS) contribute to the clearance of alcohol and toxic acetaldehyde, substances that stimulate these enzyme activities are expected to ameliorate alcohol toxicity. The development of an alcohol-metabolizing enzyme stimulant is one of the strategies for developing an alcoholism remedy. Attempts have been made to develop effective alcohol metabolic stimulants from natural dietary components and herbs; Kudzu is one of them.

Kudzu (*Pueraria lobata*) has two components. *Pueraria lobata* is the root-based herb, and *Puerariae flos* is the flower-based herb. Both of these herbal components have different claims and constituents. *Puerariae flos* enhances acetaldehyde removal and can be used as a hangover remedy. The extract of *Pueraria lobata* is a known inhibitor of mitochondrial ALDH2 and increases acetaldehyde[147]. A study of *Kudzu* effects on alcohol dependence/withdrawal shows that *Kudzu* root extract does not disturb sleep/wake cycles of moderate drinkers, indicating its utility as an adjunct treatment for alcohol dependence free of the potential side effects on sleep[148]. Furthermore, *Kudzu* root contains a number of useful isoflavones, including puerarin, daidzein and daidzin[149]. A study on the effects of *Pueraria lobata* on alcohol dependence showed that at times of high alcohol consumption, *Pueraria lobata* extract predisposed subjects to an increased risk of acetaldehyde-related neoplasm and pathology[147]. To date, the studies on the effects of *Kudzu* root extract on alcohol consumption have shown contradictory results. A study on voluntary drinking and withdrawal showed that *Kudzu* root extract suppressed voluntary alcohol intake and alcohol withdrawal symptoms in alcohol-preferring (P) and non-preferring rats[150–152]. However, human clinical studies showed that compared with placebo, *Kudzu* root extract did not significantly reduce alcohol cravings and consumption[153]. A recent pilot study has shown that a single isoflavone, puerarin, found in the *Kudzu* root can alter alcohol drinking in humans, suggesting that alcohol consumption patterns are influenced by puerarin administration and that this botanical medication may be a useful adjunct in the treatment of excessive alcohol intake[154]. Clearly, systematic studies are needed for understanding *Kudzu*'s effects on alcohol dependence.

### Hovenia

*Hovenia* is listed among the premier anti-alcohol intoxication herbal medicines in the Compendium of Materia Medica. *Hovenia* has been frequently used for protecting liver injuries[155–159]. Its therapeutic effects on alcohol intoxication and alcohol dependence have been observed[160, 161]. In the past three decades, effort has been made to evaluate the efficacy

of *Hovenia* on alcoholism and to understand the mechanisms of actions of its active constituents. There is evidence that *Hovenia* markedly decreases alcohol concentration in the blood and promotes the clearance of alcohol. *Hovenia* extract enhances ALDH activity more than ADH activity[161–163], suggesting that *Hovenia* may relieve alcohol intoxication effectively by decreasing acetaldehyde concentration quickly in the liver and blood[164]. These studies showed that *Hovenia* eliminated excessive free radicals induced by drinking alcohol[161] and block lipoperoxidation[165], alleviating alcoholic liver injury and consequently avoiding alcohol-induced dysfunction. *Hovenia* also showed neuroprotective activity against glutamate-induced neurotoxicity in the mouse hippocampus[166].

Studies in purifying *Hovenia* show that it contains a number of useful flavonoids, including myricetin, dihydromyricetin, hovenitin, laricitrin, and quercetin. Their unique characteristics have attracted the attention of scientists. In-depth studies are underway that focus on the effects of these flavonoids as anti-cancer agents[167–170], in protecting against liver injury[171, 172], as anti-diabetes agents[173–175], in protecting against neurodegeneration[176, 177], and as anti-alcohol intoxication blockers.

### Dihydromyricetin

The plant *Hovenia* has been used in traditional herbal medicine as a treatment for alcohol hangovers for hundreds of years. In a recent study conducted with animals, researchers found that dihydromyricetin (DHM), a flavonoid compound isolated from *Hovenia* and teas, blocked acute alcohol intoxication and alcohol tolerance and prevented signs of withdrawal when co-administered with ethanol. DHM also greatly reduced voluntary alcohol drinking in rats. At the cellular level, the researchers found that DHM inhibited the effect of alcohol on GABA$_A$Rs in the brain. DHM anti-alcohol effects were blocked by the BZ antagonist flumazenil, and DHM competitively inhibited BZ-site [$^3$H]flunitrazepam binding, suggesting that DHM interaction with EtOH involves the BZ sites on GABA$_A$Rs. Importantly, unlike BZs, DHM blocks acute EtOH intoxication within the effective dosage range, but does not cause sedation, sleep, or tolerance. These findings provide a foundation for further preclinical and clinical evaluation of DHM as a pharmacotherapy for alcohol dependence[175, 178, 179].

Great progress in the study of traditional herbal medicines has been made, especially the potential therapeutics for anti-alcohol abuse. This progress not only includes new experimental findings but also demonstrates a method to utilize the treasure of traditional Eastern medications.

Alcohol use disorders continue to be a health concern worldwide. The need for continuing research into treatments for AUD is urgent to provide patients with more effective options having limited side effects. The goal of drug development for AUD can be targeted to reduce alcohol intoxication, reduce AWS, and reduce cravings. The potential options for producible medications include traditional medicines and alternative medications for the management of alcohol dependence.

## Abbreviations

AUD, alcohol use disorders; AWS, alcohol withdrawal syndrome; BAC, blood alcohol concentration; EtOH, alcohol, ethanol; CNS, central nervous system; WHO, World Health Organization; FDA, the US Food and Drug Administration; CDC, the Centers for Disease Control and Prevention; DA, dopamine; VTA, ventral tegmental area; NAcc, nucleus accumbens; HPA, hypothalamic-pituitary-adrenal; PTZ, pentylenetetrazol.

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