Title

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RECENT ADVANCES IN THE DISCOVERY AND PRECLINICAL TESTING OF NOVEL COMPOUNDS FOR THE PREVENTION AND/ OR TREATMENT OF ALCOHOL USE DISORDERS

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Abstract

Alcohol abuse and dependence have a staggering socio-economic impact, yet current therapeutic strategies are largely inadequate to treat these disorders. Thus, the development of new strategies that can effectively prevent alcohol use disorders is of paramount importance. Currently approved medications attempt to deter alcohol intake by blocking EtOH metabolism or by targeting the neurochemical systems downstream of the cascades leading to craving and dependence. Unfortunately, these medications have provided only limited success as indicated by the continued high rates of alcohol abuse and alcoholism. The lack of currently available effective treatment strategies is highlighted by the urgent call by the NIAAA to find new and paradigm-changing therapeutics to either prevent or treat alcohol-related problems. The present mini-review highlights recent findings from four laboratories with a focus on compounds that have the potential to be novel therapeutic agents that can be developed for the prevention and/or treatment of alcohol use disorders.

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INTRODUCTION

Alcohol (ethanol) use disorders (AUDs) have a major national impact in the United States affecting nearly 18 million people, causing over 100,000 deaths, and costing $235 billion annually (Bouchery et al., 2011; Grant et al., 2004; Harwood, 2000). Worldwide, alcohol abuse and misuse is the third leading risk factor for premature death and disabilities (World Health Organization). The few drugs currently approved for AUD management attempt to deter alcohol intake by blocking its metabolism or by targeting the neurochemical systems in the downstream cascades leading to craving and dependence (Colombo et al., 2007; Gewiss et al., 1991; Johnson et al., 2007; Steensland et al., 2007). Presently there are three FDA-approved oral medications and one FDA-approved injectable medication to treat alcohol dependence. These include: disulfiram (1949), naltrexone (1994 oral; 2006 injectable), and acamprosate (2004) (Johnson et al., 2007). Disulfiram (Antabuse) blocks the enzyme acetaldehyde dehydrogenase, preventing formation of acetic acid from acetaldehyde (Heilig and Egli, 2006). By blocking this step in the normal metabolism of alcohol, there is an increase in the concentration of acetaldehyde, which causes violent nausea and in some instances can be life-threating if the patient is unable to resist consuming alcohol. Naltrexone (oral-Revia; inejctable-Vivitrol and Naltrel) blocks the mu opioid receptors thought to be responsible for the rewarding effects of alcohol, thereby decreasing alcohol craving (Bouza et al., 2004). However, high cost, nausea, as well as compliance issues due to the fact that the drug inhibits the rewarding properties to natural rewards have limited the usefulness of naltrexone. Furthermore, the drug was shown to be effective only on a subpopulation of recovering alcoholics. Lastly, acamprosate (Campral) is thought to act by decreasing glutamate activity, ultimately lessening the negative effects associated with alcohol withdrawal. Although there have been no studies in the U.S. showing efficacy, FDA approval was based on results from European clinical trials (Johnson et al., 2007). However, large and frequent dosing requirements make adherence to this therapy difficult for patients.

Unfortunately, because of these limitations, even with currently available combination pharmacological-psychological strategies, rates of uncontrolled heavy drinking remain high. In large urban areas such as Los Angeles, Oakland and Portland the high incidences of alcohol abuse and misuse are linked to significant increases in crime, family disruption, underemployment, and Medicare and Medicaid costs. The lack of effective therapies is highlighted by the urgent call by the National Institute on Alcohol Abuse and Alcoholism (NIAAA) to bring in new therapeutics to either prevent or treat alcohol-related problems. As such, development of effective treatments for AUDs represents an important public health goal (Bouchery et al., 2011; Heilig and Egli, 2006; Johnson, 2010; Johnson et al., 2007; Steensland et al., 2007).

This mini-review highlights recent findings from several laboratories with a focus on compounds that have the potential to be developed as novel therapeutic agents for the prevention and/or treatment of AUDs. A central theme of this mini-review is that many of the compounds presented have activity on ligand-gated ion channels (LGIC) and result in changes in the actions of alcohol with potential therapeutic treatment for AUDs. Drs. Olsen and Finn discuss agents directly modulating GABA$_A$ receptors, perhaps interfering, and certainly modifying, alcohol actions. Drs. Barak and Ron's work on glial cell line-derived neurotrophic factor (GDNF) shows that this growth factor suppresses alcohol seeking,

drinking and relapse, by normalizing alcohol-induced alterations in dopamine transmission. Finally, Dr. Davies discusses investigations focusing on ivermectin potentiation of P2×4 receptors and suggest that potentiating agent(s) acting on this target could be beneficial in reducing alcohol use disorders. Together, these studies are consistent with the idea that alcohol acts on LGIC proteins with structural homology that might include ethanol (EtOH) modulatory sites [e.g., (Howard et al., 2011)].

NOVEL COMPOUNDS FROM HERBAL MEDICINES FOR ALCOHOL DISORDERS AS POTENTIAL THERAPEUTIC ALCOHOL DRUGS

Drs. Olsen and Liang have screened Chinese herbal medicines with reported alcohol uses for the ability to interfere with chronic EtOH-induced GABA$_A$ receptor (GABA$_A$R) plasticity in animal models of alcoholism. They found activity in plant compounds and identified a purified flavonoid from *Hovenia* that prevents alcohol tolerance and withdrawal in rats (Shen et al., 2012). The compound, dihydromyricetin (DHM), a) enhances GABA$_A$Rs in CNS neurons, and b) antagonizes i) acute alcohol intoxication; ii) EtOH-induced potentiation of GABA$_A$Rs; iii) EtOH-induced GABA$_A$R plasticity, as well as iv) withdrawal signs including alcohol tolerance and increased anxiety and seizure susceptibility in an animal model of alcohol withdrawal, assessed one day after a single pre-exposure to an intoxicating high dose of EtOH. The behavioral effects are accompanied by plastic alterations in GABA$_A$R function involving restructured GABA$_A$R subunit composition with resulting physiological and pharmacological changes consistent with signs of alcohol dependence (Liang et al., 2007). These plastic changes are transient but mimic persistent plastic changes described by these workers in a rat model of human alcoholism, the rat chronic intermittent EtOH (CIE) model (Cagetti et al., 2003; Liang et al., 2006, Olsen & Spigelman, 2012). DHM is also capable of reducing voluntary consumption of EtOH in rats using the limited access two bottle choice paradigm: co-administration of DHM with EtOH in the bottle prevents the accelerated drinking, whereas administration of DHM to rats already trained to drink heavily using the two bottle choice brings their drinking down to control levels (Shen et al., 2012). The authors suggest that these properties of DHM are likely to prove therapeutic for AUDs.

Flavonoids are abundant ingredients in plants and herbal medicines. Analogues of DHM have been described to show antioxidant and neuroprotective activity, to stimulate alcohol metabolism *in vivo* and to reduce voluntary alcohol consumption in animals and humans (Arolfo et al., 2009; Lukas et al., 2005). Some of these agents have activity at GABA$_A$R's (Karim et al., 2011). We tested DHM's ability to reduce alcohol action by promoting metabolism *in vivo* and found that it did reduce alcohol uptake rate into rat blood and brain, especially at low doses of EtOH and early times, but that it was unable to account for the action of DHM to block EtOH action *in vivo* (Shen et al., 2012). DHM had weaker antioxidant and free radical scavenger activity than many other flavones; conversely, none of the several other flavonoids we examined (myricetin, quercetin) including some active on GABA$_A$R's (daidzin, puerarin) were as active as DHM to block EtOH's actions [(Shen et al., 2012), Olsen and Liang, unpublished].

This new potential "magic bullet" DHM appears to work via the GABA system in brain, in particular at GABA$_A$R's. The actions of DHM *in vivo* and *in vitro* are blocked by flumazenil, an antagonist of the benzodiazepine (BZ) site on GABA$_A$R's. Further, DHM enhances GABA$_A$R function, like BZs and EtOH, and it competitively inhibits radioligand binding of [^{3}H]flunitrazepam to the BZ sites in brain membrane homogenates (Shen et al., 2012). How DHM is able to block EtOH action *in vivo* and *in vitro* on GABA$_A$R, is not yet understood. It does not appear to behave exactly like classical BZs such as diazepam, nor does it resemble very much the 'alcohol antagonist' BZ ligand Ro15-4513 (Paul, 2006;

Wallner et al., 2006). In particular, DHM is not an excitatory drug and lacks partial inverse agonist efficacy on GABA$_A$Rs, a problem with Ro15-4513. The DHM story is consistent with the idea that the inhibitory neurotransmitter GABA is involved in the acute and chronic actions of EtOH on the brain, and suggests strongly that the GABA system is a fruitful area of investigation for drugs manipulating AUDs.

REGULATION OF ETHANOL INTAKE AND ETHANOL SEEKING BY NEUROSTEROIDS AND ACTIVATION OF EXTRASYNAPTIC GABA$_A$ RECEPTORS

EtOH has many pharmacodynamic properties, but its ability to potentiate the action of GABA at GABA$_A$Rs appears to be integral for its reinforcing and discriminative stimulus effects [e.g., (Chester and Cunningham, 2002)]. Given that some steroid derivatives exhibit similar pharmacological properties to EtOH and exert rapid nongenomic actions as positive allosteric modulators of GABA$_A$Rs, studies in the laboratory manipulated GABAergic steroid levels and determined the impact on EtOH's rewarding properties. Initial manipulations focused on the progesterone metabolite allopregnanolone (ALLO), because it is the most potent positive endogenous modulator of GABA$_A$Rs identified [reviewed in (Belelli and Lambert, 2005)]. We found that ALLO produced a biphasic effect on EtOH intake and promoted reinstatement of EtOH seeking in male C57BL/6J mice (Finn et al., 2008; Ford et al., 2007; Ford et al., 2005), consistent with work in male rats (Janak and Gill, 2003; Nie and Janak, 2003). In contrast, female C57BL/6J mice were resistant to the effects of exogenous ALLO challenge on EtOH intake (Ford et al., 2008). It is possible that sex differences in endogenous neurosteroid tone may interact with the EtOH-induced plasticity of GABA$_A$Rs and contribute to the discrepancies between sexes in the effects of neurosteroid manipulations on EtOH drinking-related behaviors [reviewed in (Finn et al., 2010)].

Dr. Finn and Ms. Ramaker conducted studies in male C57BL/6J mice to further examine the manner in which neurosteroids altered the consumption and pattern of EtOH drinking. Studies utilized ganaxolone (GAN), a synthetic neurosteroid analog of ALLO that is in Phase II clinical trials for the treatment of migraine, epilepsy, and post-traumatic stress disorder. Because GAN is more metabolically stable than ALLO (Hogenkamp et al., 1997), we predicted that GAN would lead to similar but more prolonged changes in EtOH consumption as that seen with ALLO. Further, given that neurosteroids such as ALLO have high affinity for extrasynaptic GABA$_A$Rs, similar studies were performed using gaboxadol (THIP), a GABA$_A$R agonist with selectivity for extrasynaptic receptors (Meera et al., 2011) that is in Phase III clinical trials for the treatment of insomnia and major depressive disorder. We reasoned that similar results with THIP and GAN would provide insight regarding the regulation of EtOH drinking and self-administration via the activation of extrasynaptic GABA$_A$Rs.

Initial studies utilized lickometers to determine whether the drugs were leading to a shift in EtOH drinking patterns across 24 hrs (Ramaker et al., 2011), and subsequent studies examined effects of GAN and THIP on operant self-administration of EtOH (10% v/v; 10E) in 30 min sessions. Lickometer studies indicated that both GAN and THIP produced biphasic effects on continuous access EtOH intake, with differences in time course and persistence of the effects (Ramaker et al., 2011). For instance, analysis of 24 hour EtOH intake revealed that both the 5 and 10 mg/kg doses of GAN significantly decreased 10E intake, but both doses significantly increased EtOH intake during hour 1 of 10E access and suppressed EtOH intake during hour 3 of 10E access. This biphasic effect was similar to what was observed with ALLO (Ford et al., 2007; Ford et al., 2005), but longer lasting.

GAN (5 and 10 mg/kg) also produced a dose-dependent increase in operant EtOH self-administration during a 30 min session. This result is consistent with the recent report of a biphasic effect of GAN on operant EtOH self-administration (increase with 1 mg/kg, decrease and behavioral suppression with 30 mg/kg) in male rats (Besheer et al., 2010). With regard to THIP, the biphasic effect on 10E intake was evident in the analysis of 24 hr EtOH intake: significant increase in 10E licks following the 2 and 4 mg/kg doses, and significant decrease in 10E licks following the 8 and 16 mg/kg doses. Analysis of hourly 10E intake revealed a significant reduction in EtOH intake following the 8 and 16 mg/kg THIP doses during hours 1 – 5 of 10E access. In contrast, the 4 mg/kg THIP dose significantly decreased operant EtOH self-administration, and the 8 mg/kg dose produced behavioral suppression so that the majority of animals were not able to complete the lever press requirement to gain access to the 10E solution. In general, the suppression of EtOH intake with the higher THIP doses is consistent with recent work (Moore et al., 2007). Importantly, based on evidence that THIP doses   10 mg/kg produced CNS concentrations that selectively activated extrasynaptic GABA$_A$Rs (Cremers and Ebert, 2007), it is likely that the changes in EOH intake and self-administration following the 2 – 8 mg/kg THIP doses in the present study were via an activation of extrasynaptic GABA$_A$Rs [discussed in (Ramaker et al., 2011).

Collectively, the similarities in the biphasic effects of GAN and THIP on EtOH intake to previous results with ALLO are consistent with the hypothesis that neurosteroid levels and activation of extrasynaptic GABA$_A$Rs are important determinants of EtOH intake and reinforcement. Evidence stemming from this work will likely generate a new target site for therapeutics aimed at AUDs.

GDNF; A NOVEL TREATMENT STRATEGY FOR ALCOHOL ABUSE DISORDERS

Glial cell line-derived neurotrophic factor (GDNF) is an essential growth factor for the survival, regeneration and maintenance of midbrain dopamine (DA) neurons (Airaksinen and Saarma, 2002; Lin et al., 1993). GDNF was shown to regulate dopamine transmission in the nigrostriatal dopamine pathway in the adult brain (Airaksinen and Saarma, 2002). Moreover, GDNF was also suggested to plays an important role in the regulation of the mesolimbic DAergic system (Airavaara et al., 2004), originating in the ventral tegmental area (VTA) of the midbrain, and projecting to the nucleus accumbens (NAc), which has been implicated in reward processing (Diana et al., 2003).

Activation of the GDNF pathway in the VTA has recently been highlighted as a promising approach to treat addiction to drugs of abuse, including EtOH (Carnicella and Ron, 2009; Ghitza et al., 2010). Specifically for the latter, Dr. Ron's group have shown that activation of the GDNF pathway in the VTA of rats very rapidly reduces EtOH operant self-administration and abolishes EtOH seeking during relapse in rats (Carnicella et al., 2008). Specifically, infusion of recombinant GDNF into the VTA suppressed lever presses for either 10% or 20% EtOH solutions, in rats without or with a history of excessive EtOH consumption, respectively (Carnicella et al., 2008), suggesting that the growth factor suppresses both moderate and excessive EtOH intake. Moreover, intra-VTA GDNF infusion also abolished reacquisition of rats' operant EtOH self-administration after the response was extinguished, implying that the growth factor decreases EtOH seeking during relapse (Carnicella et al., 2008). In addition, GDNF rapidly suppressed home cage EtOH intake in rats trained to consume excessive level of EtOH in the intermittent access to 20% EtOH 2-bottle choice procedure (Carnicella et al., 2009c), further suggesting that GDNF is a potent suppressor of excessive EtOH consumption and relapse. Importantly, GDNF had no effects on the self-administration of sucrose (Carnicella et al., 2008), suggesting that the growth

factor does not affect the general motivation to seek and consume natural rewards. Finally, to determine whether endogenous GDNF plays a protective role against the development of EtOH abuse disorders, Ron and colleagues compared the sensitivity of GDNF heterozygote knockout (GDNF HET) mice and wildtype (WT) littermates to EtOH. The group showed that GDNF HET mice exhibit higher levels of conditioned place preference (CPP) to EtOH and increased EtOH intake following a period of abstinence, compared to WT mice (Carnicella et al., 2009b). Taken together, these findings strongly suggest that GDNF negatively regulates EtOH-drinking behaviors.

Dr. Ron and colleagues recently showed that GDNF activation in the VTA rapidly increases the spontaneous activity of DAergic neurons in this brain region, causing an increase in the extracellular DA levels in the NAc, and that these effects are mediated by the activation of the mitogen-activated protein kinase (MAPK) pathway (Wang et al., 2010). Since the rewarding effects of virtually all drugs of abuse are associated with elevation of DA levels in the NAc (Gonzales et al., 2004), Drs. Barak and Ron (2011b) tested whether GDNF in the VTA possesses intrinsic reinforcing effects on its own, and as a consequence GDNF potentially substituting for EtOH. Using the CPP paradigm, the authors showed that GDNF on its own does not induce place preference, suggesting that the growth factor is not rewarding (Barak et al., 2011b). However, GDNF blocked the acquisition as well as the expression of EtOH-induced CPP, suggesting that GDNF suppresses the rewarding effects of EtOH. Barak et al. (2011b) further confirmed this conclusion, by showing that GDNF infusion into the VTA can shift the dose-response curve for EtOH self-administration downwards, suggesting that the growth factor acts to suppress the motivation to seek and consume EtOH, rather than substituting for the rewarding effects of the latter.

Next, Drs. Barak and Ron elucidated the mechanism by which GDNF in the VTA suppresses EtOH seeking and intake. Withdrawal from chronic exposure to high levels of EtOH leads to a substantial reduction in the activity of DAergic VTA neurons projecting to the NAc (Diana et al., 1993; Shen et al., 2007), resulting in a reduction in DA levels in the NAc, which has been associated with EtOH craving during relapse (Diana et al., 1993; Rossetti et al., 1992; Weiss et al., 1996). Therefore, Drs. Barak and Ron hypothesized that by increasing DA levels in the NAc, GDNF may reverse EtOH withdrawal-associated DA deficiency in this brain region, and consequently suppress EtOH seeking during withdrawal.

Using *in vivo* microdialysis, Drs. Barak and Ron (Barak et al., 2011b) showed that following long-term excessive EtOH consumption in the intermittent access to 20% EtOH in 2-bottle choice procedure (7 weeks; average consumption 5.47 ± 0.37 g/kg/24-hrs), 24-hrs withdrawal from EtOH causes a substantial reduction in NAc DA overflow. This DA deficiency was reversed by a single infusion of GDNF into the VTA (Barak et al., 2011b). Interestingly, although DA deficiency was not detected when rats were tested immediately after a 24-hr EtOH drinking session, DA levels in these rats declined within 2 hrs to levels similar to those of their counterparts measured after 24-hr withdrawal (Barak et al., 2011b). Taken together, these results suggest that GDNF can reverse allostatic changes in the mesolimbic DA system that are associated with withdrawal from prolonged excessive EtOH consumption.

The findings above provide a possible to mechanism to the rapid suppressive effects of GDNF on EtOH intake, as the changes in the DA system induced by the growth factor occur within minutes following GDNF activation. However, GDNF was also shown to induce suppression of EtOH intake that lasts 24 hrs and even longer (Carnicella et al., 2009c; Carnicella and Ron, 2009). Therefore, Drs. Barak and Ron elucidated the mechanisms by which GDNF acts to induce the long-lasting suppression of EtOH consumption. Specifically, they showed that infusion of GDNF into the VTA increases the levels of

GDNF mRNA and protein for at least 48 hrs (Barak et al., 2011a). They further showed the long term upregulation in the levels of *GDNF* was eliminated by the inhibition of protein synthesis, as well as by the downregulation of the *GDNF* transcript (Barak et al., 2011a). These findings suggest that the long-lasting positive autoregulation of GDNF depends upon *de novo* transcription and translation of the growth factor. Importantly, Barak et al. (2011a) further showed that the GDNF-mediated positive autoregulatory loop accounts for the long-lasting suppressive actions of GDNF on excessive EtOH consumption. Specifically, the long-lasting inhibitory effects on excessive EtOH consumption, induced by a single GDNF infusion into the VTA, were prevented by the inhibition of protein synthesis (Barak et al., 2011a). Moreover, these long-lasting effects on EtOH intake were also prevented when the upregulation of GDNF expression was countered by a focal downregulation in GDNF mRNA levels in the VTA (Barak et al., 2011a). Together, these findings suggest that GDNF can amplify and prolong its own signal the VTA, resulting in long-lasting suppression of EtOH intake.

The results of Dr. Ron's group highlight GDNF as a promising candidate strategy to treat EtOH use and abuse disorder. However, GDNF itself does not cross the blood brain barrier (BBB), making in unsuitable as an orally available treatment. Therefore, Ron et al. have been interested in identifying small organic compounds that cross the BBB and that increase the expression of GDNF in the brain. One of the compounds that adhere to these criteria is Cabergoline (Dostinex, Cabaser) a dopamine D2 receptor-like agonist, which is an FDA-approved drug for the treatment of hyperprolactinemia (Colao et al., 2006).

Dr. Ron and colleagues found that systemic administration of cabergoline in rodents increases the expression of *GDNF* in the VTA, which results in the activation of the GDNF pathway within this brain region (Carnicella et al., 2009a). Furthermore, cabergoline treatment suppressed EtOH intake and EtOH seeking, and reduced relapse, and its action to reduce EtOH consumption was localized to the VTA (Carnicella et al., 2009a). Finally, the increase in GDNF expression and the decrease in EtOH consumption by cabergoline were observed in wild-type mice but were abolished in GDNF heterozygous knockout littermates (Carnicella et al., 2009a) mice, confirming that the suppressive effects on EtOH-drinking and -seeking behaviors were mediated by the growth factor. Together, these results strongly suggest that drugs that activate the GDNF pathway may be promising novel treatments for EtOH use and abuse disorders.

REPURPOSING IVERMECTIN FOR USE AS A THERAPEUTIC AGENT FOR ALCOHOL-RELATED DISORDERS

In the search to identify therapeutic targets for AUDs, the Davies group and others have found that the P2×4 receptor (P2×4R), a member of the P2X family of ATP-gated channels abundantly expressed in the CNS, plays a role in alcohol-induced behaviors (Asatryan et al., 2011; Kimpel et al., 2007; Tabakoff et al., 2009). *In vitro,* P2×4Rs are inhibited by EtOH concentrations as low as 5 mM (Asatryan et al., 2010; Davies et al., 2005; Davies et al., 2002; Popova et al., 2010; Xiong et al., 2005). P2×4Rs are expressed in key brain regions implicated in the reinforcing properties of alcohol and other drugs, thus, supporting a role for P2×4Rs in alcohol addiction possibly via modulation of P2×4Rs in the mesolimbic DA system. Additional evidence supporting the hypothesis comes from recent studies reporting that the *p2rx4* gene may be linked to alcohol intake and/or preference (Kimpel et al., 2007; Tabakoff et al., 2009). Overall, these findings suggest that alcohol intake may be modulated by EtOH acting on P2×4Rs and that pharmacological activation of P2×4Rs may reduce alcohol consumption and preference.

While no selective agonists of P2×4Rs have been developed to date, a large body of evidence has shown that ivermectin (IVM), a broad-spectrum antiparasitic used worldwide in humans and animals (Geary, 2005; Molinari et al., 2010), acts as a potent and selective positive allosteric modulator of P2×4Rs. IVM's selectivity affinity (between P2XR subtypes) provides the basis for it to be routinely employed to determine the contribution of P2×4Rs in ATP-mediated processes (Khakh et al., 1999).

We recently reported that IVM antagonized the inhibitory effects of EtOH on P2×4Rs (Asatryan et al., 2010). Taken in context with evidence suggesting that P2×4Rs play a role in alcohol-induced behaviors (Asatryan et al., 2011; Kimpel et al., 2007; Tabakoff et al., 2009), we proposed that IVM may be able to block or antagonize some effects of EtOH *in vivo*. Support for this hypothesis comes from our groups' discovery that IVM significantly reduced alcohol intake in male and female C57BL/6J mice (Yardley et al., under revision). We found that IVM (1.25–10 mg/kg, intraperitoneal) significantly reduced 24-h alcohol consumption and intermittent limited access (4-h) binge drinking, and operant alcohol self-administration (1-h). Moreover, IVM (1.25 g/kg/day × 7 days) was effective at decreasing 24-h EtOH intake in a short-term chronic model. Pharmacokinetic studies demonstrated that the effects of IVM on EtOH intake correlated with the presence of drug in brain. Consistent with these results, acute administration of IVM reduced maintenance of alcohol self-administration in rats, but results from this work were inconclusive (Kosten, 2011). Collectively, these findings indicated that IVM reduces alcohol intake across several different models of self-administration and suggest that IVM may be useful in the treatment of AUDs.

To assess the therapeutic potential of IVM on the comorbid psychiatric disorders associated with AUDs, we evaluated its effects on a set of murine behavioral paradigms aimed at capturing complementary aspects of perceptual, emotional and cognitive functions. The results of these experiments indicated that the doses of IVM that significantly reduced alcohol consumption (2.5–10 mg/kg, i.p.) also elicited a complex array of behavioral changes (manuscript in preparation). In particular, IVM reduced anxiety-like behaviors in the elevated plus-maze and marble-burying paradigms. Furthermore, IVM reduced sensorimotor gating, as assessed by the prepulse inhibition (PPI) of the acoustic startle reflex, but did not affect mnemonic encoding in the novel object recognition paradigm. IVM did not have any intrinsic effects on conditioned place preference. This complex set of outcomes reflected a multifaceted profile of IVM in behavioral regulation, plausibly via the combined activation of P2×4Rs and $GABA_A$Rs. In particular, the recent finding that P2×4Rs might counter the effects of $GABA_A$Rs suggested that the concomitant stimulation of both targets could reduce the impact of behavioral effects typically associated with GABAergic agonists or positive modulators - such as sedation and preference - while preserving anxiolytic-like properties. Although the biological significance of the reduction in sensorimotor gating needs to be carefully evaluated in further studies, the present results, together with clinical data on the high tolerability and safety of IVM, point to this agent as a potentially attractive platform for the development of novel drugs for AUDs and anxiety-spectrum disorders, with little to no risk of addiction liability.

The potential translation of the present findings and repurposing of IVM for use in humans as a therapeutic for AUDs is aided by the twenty plus year history of IVM's use to treat parasitic diseases in millions of humans [reviewed in (Omura, 2008). Moreover, despite earlier reports to the contrary, IVM has been shown to pass the BBB and is known to cause CNS effects in humans (Guzzo et al., 2002). Taken together, the overall safety of IVM, coupled with the data presented above point to IVM as an attractive agent for the treatment of AUDs, with good tolerability and limited side effects. Notably, the use of IVM (i.e., repurposing) for the prevention and/or treatment of AUD could be implemented in a shorter

time frame and at lower cost than what would be required for the development of new chemical entity. However, before clinical investigations can be initiated, key preclinical pharmacokinetic and pharmacodynamic studies are necessary to extend the investigation to demonstrate appropriate dosing and efficacy in longer term chronic studies and to conduct initial toxicity studies under these paradigms.

SUMMARY AND CONCLUSION

As mention above, the available pharmacotherapies for alcohol use and abuse disorders is very limited, and compliance is a serious issue due to adverse side effects such as nausea, sedation, and anhedonia (Johnson et al., 2007). Here, we discussed four CNS targets that, according to findings in animal models, provide a new hope for achieving improved beneficial effects, i.e., suppression of alcohol consumption and relapse, while producing less side effects. Importantly, a common feature of all four therapeutic directions is the availability of compounds shown to work through these targets, which are already in advanced clinical trials or in clinical use. Thus, $GABA_AR$ can be targeted *via* the herbal medicine-derived compound DHM (Shen et al., 2012) or *via* neurosteroid analogs of ALLO (Hogenkamp et al., 1997), currently in Phase II clinical trials for the treatment of migraine, epilepsy, and post-traumatic stress disorder; activation of the GDNF system which can be induced by the FDA-approved drug cabergoline (Carnicella et al., 2009a); and P2×4R, which is activated by IVM, another FDA-approved drug (Geary, 2005; Molinari et al., 2010). Therefore, by advancing these therapeutic strategies, the use of existing drugs or the development of new efficient drugs for alcohol abuse disorders may be closer than it seems.

Acknowledgments

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