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Oral delivery of ivermectin using a fast dissolving oral film: Implications for repurposing ivermectin as a pharmacotherapy for alcohol use disorder

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ABSTRACT

Individuals suffering from an alcohol-use disorder (AUD) constitute a major health concern. Preclinical studies in our laboratory show that acute and chronic intraperitoneal (i.p.) administration of ivermectin (IVM) reduces alcohol intake and preference in mice. To enable clinical investigation to use IVM for the treatment of an AUD, development of an oral formulation that can be used in animals as well as long-term preclinical toxicology studies are required. The present work explores the use of a promising alternative dosage form of IVM, fast-dissolving oral films (Cure Pharmaceutical®), to test the efficacy and safety of oral IVM in conjunction with alcohol exposure. We tested the effect of IVM (0.21 mg) using a fast-dissolving oral film delivery method on reducing 10% v/v alcohol (10E) intake in female C57BL/6 mice using a 24-h access two-bottle choice paradigm for 6 weeks (5 days per week). Differences in ethanol intake, preference for ethanol, water intake, fluid intake, food intake, changes in mouse and organ weights, as well as histological changes to kidney, liver, and brain were analyzed. The IVM group drank significantly less ethanol over the 30-day period compared to the placebo (blank strip) and the no-treatment groups. Organ weights did not differ between the groups. Histological evaluation showed no differences in the brain and kidney between groups. In the liver, there was a slight increase in the incidence of microvesicular fatty and degenerative changes of the animals receiving the thin strips. No overt hepatocellular necrosis or perivascular inflammation was noted. Overall, these data support the use of this novel method of oral drug delivery for longer-term studies and should facilitate FDA required preclinical testing that is necessary to repurpose IVM for treatment of an AUD.

Introduction

There is a large number of individuals suffering from an alcohol-use disorder (AUD) throughout the world (Bouchery, Harwood, Sacks, Simon, & Brewer, 2011; Grant et al., 2004; Harwood, 2000). This disorder is the third leading risk factor for premature death and disabilities and is responsible for 4% of all deaths (World Health Organization, 2011). In the United States, the costs associated with individuals suffering from an AUD account for nearly half of the total cost of untreated addiction, totaling over $185 billion annually (Research Society on Alcoholism, 2011). A significant portion of this cost is due to reduced, lost, and foregone earnings. The remainder of the cost comes from medical and treatment expenses, decline in workforce productivity, accidents, violence, and premature death. Despite the known harm caused by ethanol, current pharmacological treatment strategies are limited and have yielded only modest positive results, as indicated by the continued high prevalence of AUD. Although treatments for AUD have improved somewhat over the past two decades (Miller, Book, & Stewart, 2011), the continued high rate of AUD illustrates the need to develop new, more effective interventions.

Pharmacotherapies for AUD are used less often than psychosocial interventions (Fuller & Hiller-Sturmhöfel, 1999). However, without a pharmacological adjunct to psychosocial therapy, nearly three-quarters of patients resume drinking within 1 year (Johnson, 2008). The limited use of pharmacotherapy for AUD is due, in part, to the relative lack of options to successfully treat these disorders (Edlund, Booth, & Han, 2012). To address this issue, our research group is investigating the utility of ivermectin (IVM) as a novel

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pharmacotherapy for the treatment and/or prevention of AUD (Asatryan et al., 2014; Wyatt et al., 2014; Yardley et al., 2012, 2014).

IVM is a broad-spectrum antiparasitic avermectin (Geary, 2005; Molinari, Soloneski, & Larramendy, 2010; Richard-Lenoble, Chandenier, & Gaxotte, 2003). The current therapeutic potential of IVM is attributed to action on a non-mammalian, glutamate-gated inhibitory chloride channel (Cully et al., 1994; Dent, Davis, & Avery, 1997; Vassilatis et al., 1997). Studies in humans and rodents suggest additional sites of action for IVM not related to these receptors (Sung, Huang, Fan, Lin, & Lin, 2009), including nicotinic acetylcholine receptors (nAChRs) (Krause et al., 1998; Sattelle et al., 2009) and P2X4 purinergic receptors (P2X4Rs) (Asatryan et al., 2010; Wyatt et al., 2014).

Among P2XR family members, IVM is a selective positive modulator of P2X4Rs and has been used to differentiate the role of P2X4Rs from other P2X family members in ATP-mediated processes (Jelinková et al., 2006; Khakh, Proctor, Dunwiddie, Labarca, & Lester, 1999; Silberberg, Li, & Swartz, 2007). Studies from our laboratory using two-electrode voltage clamp methods demonstrate that IVM interferes with and antagonizes the inhibitory effect of ethanol on P2X4Rs (Asatryan et al., 2010). Furthermore, we have shown that IVM decreases ethanol consumption across multiple murine models of ethanol intake (Asatryan et al., 2014; Wyatt et al., 2014; Yardley et al., 2012, 2014) and reduces response for alcohol under an operant self-administration progressive ratio schedule (Kosten, 2011). We found that both acute and multi-day administration of IVM (1.25–10 mg/kg) reliably decreases ethanol intake and preference (Yardley et al., 2012, 2014) without causing overt signs of toxicity and having minimal effect on major domains of neuropsychiatric toxicity (Bortolato et al., 2013). In these aforementioned studies, administration of IVM was achieved using an intraperitoneal (i.p.) injection method.

For translation of a drug from the pre-clinical to clinical arena, efficacy and subsequent safety studies must be performed using the intended clinical route of administration, and it is important that methods used in rodents for such evaluations provide reliable and reproducible results. Current methods of oral delivery, including oral gavage (Brown, Dinger, & Levine, 2000; Germann & Ockert, 1994; Zhang, 2011), peanut butter mixture (Silverman, Oliver, Karras, Gastrell, & Crawley, 2013), gelatin molds (Froehlich, Hausauer, Federoff, Fischer, & Rasmussen, 2013), adding the drug to drinking water or a sucrose solution, and using pre-mixed drug-chocolate pellets (Zhang, 2011), all have significant limitations that can jeopardize the reliability of the data. Taken together, the disadvantages associated with the current methods of drug delivery have prompted interest in developing alternative methods to improve oral delivery in rodents, such as pre-coating the gavage needle with sucrose (Hoggatt, Hoggatt, Honerlaw, & Pelus, 2010).

Fast-dissolving oral films represent another alternative approach for the oral delivery of a drug (for review, see Bala, Pawar, Khanna, & Arora, 2013; Ghodake et al., 2013; Mandeep, Rana, & Nimrata, 2013; Panda, Dey, & Rao, 2012; Saini, Kumar, Sharma, & Visht, 2012). At the present time, the majority of research on the use of fast-dissolving oral films is focused on humans. However, some of the noted benefits are also applicable in preclinical drug delivery studies. For example, fast-dissolving oral films provide a safe passage of oral delivery compared to intragastric oral gavage and offer a less stressful drug delivery experience for the animal.

The present study tests the hypothesis that 0.21 mg IVM, the highest dose tested in previous IVM studies conducted by our laboratory, administered via fast-dissolving oral films, is able to decrease ethanol intake in mice over a 30-day period using a 24-h two-bottle choice paradigm without causing significant pathological changes in the kidney, liver, and brain. Importantly, this study represents the first long-term ethanol/IVM administration study, which is essential if IVM is to be repurposed as a pharmacotherapy for AUD.

Materials and methods

Animals

Studies were performed on drug-naïve C57BL/6 female mice that were approximately 10–12 weeks old at the time of testing, obtained from our internal breeding colony at the University of Southern California (USC). Prior to initiating the study, mice were transferred to the testing room and remained group-housed with 3–5 mice per cage for 1 week. Mice were then single-housed in polycarbonate/polysulfone cages on a 12-h reverse light/dark cycle with lights off at 12:00 noon. The holding room was maintained at approximately 22 °C. All procedures in this study were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and all efforts were made to minimize animal suffering. The USC Institutional Animal Care and Use Committee approved the protocols.

Drugs

Fast-dissolving oral films containing 6 mg of IVM were kindly provided by Cure Pharmaceutical (Oxnard, CA). The film was cut up into smaller sections using a standard single quarter-inch hole-punch to produce pieces containing approximately 0.21 mg IVM each. Ethanol (190 proof USP, Sigma, USA) was diluted in water to achieve a 10% v/v solution (10E). Sucrose (Sigma–Aldrich, St. Louis, MO) was diluted in water to achieve a 4.25% w/v solution.

Alcohol intake study

The 24-h access two-bottle choice paradigm was performed as previously described (Asatryan et al., 2014; Wyatt et al., 2014; Yardley et al., 2012, 2014). Briefly, mice were single-housed and had access to 2 bottles of water for 3 days. Then, one bottle of water was replaced with a bottle of 10E. After 10 days, when drinking stabilized (±10% variability from the mean dose of the previous 3 days), mice were assigned to drug treatment groups so that the average 10E intake on day 10 of exposure to ethanol was similar across groups. There were 3 groups: the IVM group which was administered key lime-flavored oral film with 0.21 mg of IVM ($n = 8$), the placebo group which was administered key lime-flavored oral film with no drug ($n = 8$), and the control group which was handled and presented with the gavage needle dipped in the sucrose solution, but not given any oral film ($n = 6$). We used 0.21 mg because it correlated to the highest dose of IVM previously tested (i.e., 10 mg/kg in a 21-g mouse). Mice were treated for 30 days at 9:30 AM each day, 5 days per week (Monday–Friday) for 6 weeks. Animals continued receiving access to both water and alcohol over the weekend, but animals were not treated and fluid volumes were not measured on these days. Fluid volumes, food weights, and mouse weights were recorded immediately prior to treatment. Bottles were alternated every other day to avoid side preferences. Strips were administered using an animal-feeding needle (Cadence Science, Staunton, VA) dipped in a 4.25% sucrose solution to serve as an adhesive for the film strip. As illustrated in Fig. 1, after being presented with the tip of the gavage needle, mice would self-administer the film.

Analysis of organ tissue samples

The day following the last dose of IVM, 4 animals from the IVM group, 4 from the placebo group, and 3 from the control group were