Applications of alcohol clamping in early drug development

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CHAPTER 1

Introduction

Alcohol (or ethanol) is probably the most commonly used drug worldwide (Jang and Harris, 2007), and is mainly consumed for its mild euphoric and disinhibitory effects. For the most part, alcohol is a central nervous system depressant, although it may have biphasic effects (Pohorecky, 1982). Low doses of alcohol often have stimulant properties and increase sociability. In the non-alcoholic, typical effects of low alcohol levels (50 mg/dl) include talkativeness, relaxation and tension reduction. Higher blood levels (above 100 mg/dl) significantly impair mental and cognitive ability including judgement, and there is depression of sensory-motor functioning. As blood ethanol levels exceed 200 mg/dl sensory and cognitive functioning is markedly impaired and at 300 mg/dl most individuals would be stuporous. At higher concentrations (LD_{50} = 400 mg/dl), alcohol is lethal due to severe depression of respiratory function or other complications, such as aspiration of vomit (Pohorecky and Brick, 1988).

The effects of alcohol can partly be explained by its increasing effect on membrane fluidity and its subsequent disturbance of otherwise strictly regulated ion channels and electrolyte balances (Pohorecky and Brick, 1988). Additionally (or consequently), alcohol's central nervous system (CNS) effects are mediated through actions on a variety of neurotransmitters. Dopamine, serotonine, glutamate, noradrenaline and gamma-aminobutyric acid (GABA) are primarily involved. $GABA_A$-receptors are among the most widely distributed neurotransmitter systems in the brain, and alcohol causes an allosteric enhancement of these inhibitory ion channels. This could explain why many of the effects of alcohol are related to CNS-depression. Research shows that alcohol also tends to interfere with opioid receptor binding (Pohorecky and Brick, 1988). There is a complex interplay between these excitatory and inhibitory systems. The numerous transmitters involved in alcohol's action explain its diverse effects and the large number of drug interactions with both prescribed and illicit drugs (McIntosh and Chick, 2004).

These acute CNS-effects have already been extensively investigated. Numerous tests and methods are currently used in such studies to investigate the different effects of alcohol. This diversity reflects the wide range of effects caused by alcohol and its large biomedical and psychosocial impact, but does not explain or justify why so many different tests are used even to study the same effect. The sensitivity of these tests to the effects of alcohol has often not been completely ascertained, and concentration- or dose-effect relationships have only rarely been systematically reported. An overview of the sensitivity and dose responsiveness of different CNS-tests to the effects of alcohol would be useful for future studies focusing on acute alcohol effects or drug-alcohol interaction studies, and could constitute a useful collection of tests to evaluate the acute effects of alcohol on the CNS. Chapter 2 of this thesis contains a systematic review, which attempts to determine the alcohol sensitivity for the large number of CNS tests that is described in the literature.

Another methodological problem in acute alcohol studies is the huge variability in the alcohol exposure profiles that are encountered in such studies. Individuals vary as much as 3- to 4-fold in systemic concentrations and metabolic rates after alcohol administration (Ramchandani *et al.*, 2006). Many factors contribute to this variability, including doses and administration modes, the amount and rate of prior alcohol exposure, drinking history and food intake, differences in gastric emptying, liver volume, blood flow, race, age and gender (Ramchandani *et al.*, 2006). Besides, most of the effects of alcohol are concentration- and time-dependent (Hiltunen *et al.*, 2000; Vogel-Sprott, 1979), and interpretation of the results can be complex if plasma levels change over time, particularly when this is not recorded accurately. This is a problem for many types of alcohol research, including drug interaction studies. A major part of this thesis is devoted to the development and validation of an alcohol administration mode that allows the investigators to attain stable plasma levels of alcohol at a predetermined level in each individual. Few studies of alcohol effects or drug interactions are performed at pseudo-steady state levels, or under other conditions in which these complexities are regulated. In many cases, alcohol exposure is merely controlled by adaptation of the dose to weight, and sometimes to gender or other demographic variables (Wilkinson, 1980). Measurements of the alcohol effects are usually performed at fixed time intervals after intake, and the number of samples is small. Often, breath alcohol levels (BrAC) are measured, but efforts to maintain constant levels are rare. A major reason for this lack of methodological stringency is the complexity and variability of alcohol's pharmacokinetic characteristics.

In an attempt to reduce experimental variance during alcohol studies, O'Connor *et al.* developed a method to keep alcohol plasma levels within close limits using an oral loading dose followed by an intravenous infusion (O'Connor, 1998). The O'Connor method is based upon the theory that for substances with marked saturable elimination in the relevant concentration range (like alcohol), an approximately linear relationship exists between the applied infusion rate and the resulting change in alcohol concentration or BrAC. When alcohol elimination is fully saturated, it is excreted at a constant rate, independent of concentration (Michaelis-Menten kinetics). Therefore, when the input is changed, this will result in a proportional change in alcohol concentration. The change in alcohol level required to achieve the target concentration can then be used to back-extrapolate the infusion rate that corresponds to, and should hence lead to this necessary change. This method was described as the Indiana BrAC clamp.

In this thesis, the original clamping paradigm was modified, with the aim of creating a comparably reliable technique, but one that would be less intensive for the operator and the study subject. In this way, the alcohol administration mode would be more feasible for concomitant CNS research in larger numbers of subjects. The oral loading dose was replaced by an intravenous loading dose to increase experimental control. Also, the time interval between breath samples was increased to facilitate the integration of multiple CNS-measurements into the course of the clamp. Simulations were used to optimize the different phases of the infusion regimen. Additionally, to simplify the execution of the procedure for a larger number of operators after minimal training, the new paradigm was converted into a spreadsheet-based program. These alterations resulted in a new alcohol clamping paradigm, which was tested and simultaneously compared to another procedure for maintaining alcohol at a pre-set plasma level (chapter 3).

Subsequently, a study was performed to investigate whether an intensive battery of CNS measurements could be performed during the course of the new clamping method without interference with or by the alcohol clamping procedure and with the stability of the alcohol levels (chapter 4). This battery consisted of CNS-tests that were highly sensitive to the effects of alcohol and its selection was largely based on our own literature findings (i.e. chapter 2). The CNS-effects of alcohol under pseudo-steady state conditions were quantified in this study, to investigate the profile and time-dependence of CNS-effects during stable (pseudo-steady state) alcohol levels.

The clamping method might also be an appropriate tool to compare groups. Both pharmacodynamic differences and differences in alcohol metabolism can be explored between certain subgroups (e.g. male/female; alcoholics/non-alcoholics; Caucasians/Japanese) without confounding differences in alcohol levels. It is generally assumed that Japanese people are more sensitive to alcohol compared to Caucasians (Shibuya *et al.*, 1989). This is at least partly related to genetic differences in pharmacokinetics, since a high proportion of Japanese have a relative aldehyde dehydrogenase (ALDH) deficiency (Chan, 1986). Differences in body posture and in lifestyle (food and/or use of medication) may further influence the kinetics of alcohol (Duranceaux *et al.*, 2008). Repeated exposure to alcohol may also induce tolerance to its effects (Bennett *et al.*, 1993), and avoidance of alcohol or other differences in habitual use patterns could contribute to an increased sensitivity. Since the alcohol clamp by-passes most of these factors, we tried to directly compare alcohol metabolism and CNS-effects between Caucasians and Japanese subjects in a subsequent study (chapter 5). Besides, efforts were made in this study to expand and to fine-tune the clamping procedure: steady-states were clamped at multiple levels (on different occasions) and initial infusion rates were customized to individual demographic characteristics, to avoid overshoots.

In CNS drug research it is rarely possible to reliably characterize and differentiate drugs on the basis of one single functional test. Profiles of CNS-effects using a test battery usually provide more distinctive information, but this clearly depends on the breadth of scope of the test battery. The information is only reliable if the battery covers the CNS functions that are primarily affected by one drug and much less so or differently by the other. However, CNS-functions are hardly ever determined by a single neuropharmacological mechanism. Hence, functional assessments are bound to show overlap between different drugs classes, and to be affected by non-pharmacological factors like motivation, fatigue and other aspects of

psychological and physical well-being. A generally applicable methodology for repeated measurements of direct drug effects on the entire CNS, without task-related interactions and a priori models, would constitute a major improvement in CNS drug development. Resting-state functional magnetic resonance imaging could satisfy many of these requirement, and was hence hypothesized to be a promising technique for pharmacological research. We investigated whether different psychoactive substances cause drug-specific effects in functional brain connectivity during resting-state (chapter 6). Both alcohol and morphine were selected for this purpose, because of their well-known CNS-effects, which show functional similarities (on attention and mood) as well as distinctions (on respiration, heart rate, motor function and others). Stable drug levels are preferred in this proof-of-concept study to assist in the calibration of the resting state technique. Therefore, the alcohol clamp was used here to produce stable and prolonged alcohol levels with minimal variability, as well as a target-controlled morphine infusion.

Alcohol is a well-know drug in medicine and in society. The effects of alcohol can therefore be used as a frame of reference for compounds that share some functional aspects with alcohol. Some drugs affect performance, and it could be useful to compare these impairments to the effects of alcohol, at a level with a recognized functional impact - for instance based on legal driving limits. Another example is to use a well-known therapeutic effect of alcohol as a benchmark for the effects of a novel drug with a similar putative therapeutic effect. This approach was chosen for the development of two new drugs for essential tremor (ET), which in many patients is improved by alcohol. ET is one of the most common neurological disorders among adults, and is the most common tremor disorder (Louis *et al.*, 1998; Louis, 2001; Louis, 2005). Current pharmacological treatment act symptomatically and have variable effectiveness (Chen and Swope, 2003). Moreover, the occurrence of side effects, like sedation, weight gain and cognitive impairment, limit their use. Estimations indicate that alcohol is effective in approximately 70% of ET patients (Lou and Jankovic, 1991; Koller *et al.*, 1994). This finding is confirmed by controlled studies where alcohol was administered acutely (Growdon *et al.*, 1975; Koller and Biary, 1984). It most likely acts via a reduction of central overactivity, which results in reduced tremor amplitude, whereas the frequency remains unaffected (Koller and Biary, 1984; Koller, 1991). In a search for novel pharmacological treatment options a $GABA_A$ α2,3 subtype-selective partial agonist and a histamine-3 receptor inverse agonist were investigated in two different studies for their efficacy to reduce tremor symptoms (chapter 7 and 8). In both studies, the alcohol clamp was introduced and served as a positive control. In contrast to most controlled trials in which the effect of alcohol on ET is being studied, we attempted to obtain fixed alcohol levels for a prolonged period of time to induce a stable stimulus that would minimize the variability.

An obvious application of the alcohol clamp in drug development, is its use during ethanol interaction studies. The stability and predictability of the clamp reduces the variability that is inherent to most other modes of ethanol administration. This increases the reliability of the statistical analyses of an interaction, and reduces the risks of unexpectedly high pharmacokinetic levels and pharmacodynamic effects in the presence of other drugs with metabolic or central nervous system drug effects. In chapter 9, we employed the alcohol clamp for an interaction study with a novel D_3-antagonist.

This thesis describes the development of a novel alcohol clamp (chapter 3), a new method to obtain stable plasma levels of alcohol and its application in CNS-research. The method might have several advantages that were explored in subsequent studies described in this thesis. The stability of the alcohol clamp was used to examine functional effect profiles and time-dependence of different CNS-effects (chapter 4). The tests to examine these effects were chosen based upon a prior review of the literature, during which the most sensitive CNS-tests were selected (chapter 2). Hereafter, we studied the alcohol clamping method as a tool to compare alcohol disposition capacities between different (ethnic) populations and as a tool to compare their different CNS-responses to *multiple* stable alcohol levels. We also investigated whether the clamping method could be useful as a future benchmarking entity in CNS-research, based on its fMRI effects on the brain at rest (chapter 6) and its efficacy on tremor symptoms (chapter 7 and 8). Finally, we employed the method in an interaction study with a compound that is in development for addictive disorders including alcoholism (chapter 9).

Bennett RH, Cherek DR, Spiga R (1993). Acute and chronic alcohol tolerance in humans - effects of dose and consecutive days of exposure. Alcohol Clin Exp Res 17:740-745

Chan AW (1986). Racial differences in alcohol sensitivity. Alcohol Alcohol 21:93-104.

Chen J J, Swope D M (2003). Essential tremor: diagnosis and treatment. Pharmacotherapy 23: 1105-1122

Duranceaux NC, Schuckit MA, Luczak SE, Eng MY, Carr LG, Wall TL (2008). Ethnic differences in level of response to alcohol between Chinese Americans and Korean Americans. J Stud Alcohol Drugs 69:227-234

Growdon JH, Shahani BT, Young R R (1975). The effect of alcohol on essential tremor. Neurology 25: 259-262

Hiltunen AJ, Saxon L, Skagerberg S, Borg S (2000). Acute tolerance during intravenous infusion of alcohol: comparison of performance during ascending and steady state concentrations – a pilot study. Alcohol 22:69–74

Jang GR, Harris RZ (2007). Drug interactions involving ethanol and alcoholic beverages. Expert Opinion on Drug Metabolism & Toxicology 3:719-731

Koller WC, Biary N (1984). Effect of alcohol on tremors: comparison with propranolol. Neurology 34: 221-222

Koller WC (1991). A new drug for treatment of essential tremor? Time will tell. Mayo Clin Proc 66: 1085-1087

Koller WC, Busenbark K, Miner K (1994). The relationship of essential tremor to other movement disorders: report on 678 patients. Essential Tremor Study Group. Ann Neurol 35: 717-723

Lou JS, Jankovic J (1991). Essential tremor: clinical correlates in 350 patients. Neurology 41: 234-238

Louis ED, Ottman R, Hauser WA (1998). How common is the most common adult movement disorder? estimates of the prevalence of essential tremor throughout the world. Mov Disord 13: 5-10

Louis ED (2001). Essential tremor. New England Journal of Medicine 345: 887-891

Louis ED (2005). Essential tremor. Lancet Neurology 4: 100-110

Mukherjee S, Das SK, Vaidyanathan K, Vasudevan DM (2008). Consequences of alcohol consumption on neurotransmitters -an overview. Curr Neurovasc Res. 5(4):266-72

McIntosh C, Chick J (2004). Alcohol and the nervous system. J Neurol Neurosurg Psychiatry 75(Suppl III):16–21

O'Connor S, Morzorati S, Christian J, Li TK (1998). Clamping breath alcohol concentration reduces experimental variance: application to the study of acute tolerance to alcohol and alcohol elimination rate. Alcohol Clin Exp Res. 22(1):202-10

Pohorecky LA (1982). Influence of alcohol on peripheral neurotransmitter function. Fed Proc. 41(8):2452-5

Pohorecky LA, Brick J (1988). Pharmacology of ethanol. Pharmac. Ther. 36:335-427

Ramchandani VA, O'Connor S, Neumark Y, Zimmermann US, Morzorati SL, de Wit H (2006). The Alcohol Clamp: Applications, Challenges, and New Directions – An RSA 2004 Symposium Summary. Alcoholism: Clinical and Experimental Research 30(1):155-164

Shibuya A, Yasunami M, Yoshida A (1989). Genotypes of alcohol-dehydrogenase and aldehyde dehydrogenase loci in Japanese alcohol flushers and nonflushers. Human Genetics 82:14-16.

Vogel-Sprott MD (1979). Acute recovery and tolerance to low doses of alcohol: differences in cognitive and motor skill performance. Psychopharmacology (Berl) 61:287–91

Wilkinson PK (1980). Pharmacokinetics of ethanol–review. Alcohol Clin Exp Res. 4:6–21