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In vitro and in vivo models of acute alcohol exposure

Angela Dolganiuc, Gyongyi Szabo

Alcohol is the third leading cause of preventable death in the United States and the third leading cause of healthy years lost to death and disability in developed nations[8]. Humans use and abuse alcohol acutely or chronically, when alcohol consumption is frequent and dependence has developed[16]. Although significant progress was made in the area of alcohol research during the last decades, the pathogenesis of alcohol use and abuse is not fully understood. Further, most research was focused on alcoholism, which is an advanced stage of alcohol abuse, involving chronic alcohol consumption, alcohol dependence and severe health and social consequences[1-13]. Thus, research models are emergent in order to detail what drives human desire to consume alcohol, how the body responds to alcohol, and most important, what are the beneficial and harmful effects of acute alcohol consumption on the human body.

ABSTRACT

Alcohol abuse is a global problem due to the financial burden on society and the healthcare system. While the harmful health effects of chronic alcohol abuse are well established, more recent data suggest that acute alcohol consumption also affects human wellbeing. Thus, there is a need for research models in order to fully understand the effect of acute alcohol abuse on different body systems and organs. The present manuscript summarizes the interdisciplinary advantages and disadvantages of currently available human and non-human models of acute alcohol abuse, and identifies their suitability for biomedical research.

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Key words: Models; Acute alcohol abuse; Human; Non-human; Progress

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INTRODUCTION

Alcohol abuse is widely spread around the globe[1-9].
Advantages and their receptors. After 4-5 drink equivalents the length of acute alcohol treatment is variable studies an 0.08% BAL or above this level yields Availability of physiological routes of alcohol administration Area of research. studies the 10-100 mmol/L, and their relationship to blood, augments corticosteroid release, (2). For the and their consumption of Research of acute alcohol consumption/abuse is entirely health is not fully understood. trauma morbidity and mortality delays wound healing shock and systemic infections such deficiency was observed both in organ-specific and systemic infections and prolonged recovery from trauma. The pathogenesis of AAA effects on human health is not fully understood.

AAA: BIOMEDICAL IMPACT

The known biological effects of AAA include those of the central nervous system (CNS) and non-CNS origin. Alcohol use is characterized by symptoms of CNS intoxication, impaired brain activity, poor motor coordination, and behavioral changes. AAA leads to impaired CNS activity due to alcohol's effect on synthesis, release and signaling of neurotransmitters, including serotonin, glutamate, GABA, endocannabinoids and their receptors. AAA causes damage and functional impairment of the gastrointestinal (GI) tract, including luminal GI, liver, and pancreas; it also affects the protein, carbohydrate, and fat metabolism. AAA leads to insufficient immune system responses to infections; such deficiency was observed both in organ-specific and systemic infections and prolonged recovery from trauma. The pathogenesis of AAA effects on human health is not fully understood.

MODELS OF AAA

Research of acute alcohol consumption/abuse is entirely based on models, due to their advantage of controlled settings. Currently there are in vitro and in vivo models of AAA; their characteristics are defined in Table 1. In contrast to chronic alcohol abuse, the research of AAA has not benefited from population studies due to recall bias.

One important feature of AAA models is the definition of biologically meaningful levels of alcohol, either in vitro or in vivo, and their relationship to blood alcohol levels (BAL) in humans. This is an important requirement of the research models of AAA, because BAL can be detected as soon as minimal amounts of alcohol are ingested, however measurable affects of alcohol on physiology and/or behavior is established at 0.08% or above this level, with individual variations depending on the species, metabolic particularities, age, gender and genetic background. It is also important to identify that AAA models differ by their route of alcohol delivery to achieve alcohol intoxication, some of them being physiological, such as oral administration, while others being non-physiological, when ethanol is administered by parenteral routes. Nevertheless, current research shows that the BAL levels, rather than the route of alcohol administration play a major role in the establishment of the biological effects of alcohol.

Thus, optimal AAA models should fulfill several criteria: (1) Define the length of alcohol exposure. In vitro the length of acute alcohol treatment is variable in diverse published experimental settings and range from seconds to hours; it is currently accepted that treatment with alcohol for up to 24 h is considered as an acute setting. In vivo the consumption of alcohol in one setting implies that the entire dose of alcohol is consumed at once, while a ‘binge’ is defined by NIAAA as an excessive pattern of alcohol drinking that produces BAL greater than 0.08% within a 2-h period and may, or may not, be associated with dependence. Thus any model using consumption of biologically active amounts of alcohol within 2 h is considered an acceptable model of AAA. (2) Establish an exposure to an accurate concentration of ethanol. For in vitro studies the 10-100 mmol/L ethanol range is considered physiological, with 25 mmol/L ethanol being close to 0.08% BAL achieved in vivo after 4-5 drink equivalents. For the in vivo studies an 0.08% BAL or above this level yields

<table>
<thead>
<tr>
<th>AAA model</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Area of research</th>
</tr>
</thead>
<tbody>
<tr>
<td>In vitro</td>
<td>Low cost</td>
<td>Limited alcohol metabolism</td>
<td>Behavioral and biomedical</td>
</tr>
<tr>
<td></td>
<td>Technically easy to perform</td>
<td>Limited complexity at cellular and tissue levels</td>
<td>Behavioral and biomedical</td>
</tr>
<tr>
<td></td>
<td>Large number of experimental groups</td>
<td>Limited areas of research, not suitable for behavioral and social studies.</td>
<td>Behavioral and biomedical</td>
</tr>
<tr>
<td></td>
<td>Pure cell populations</td>
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<td></td>
<td>Single cell type or multi-cell type co-culture</td>
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<tr>
<td></td>
<td>Strictly controlled settings yielding reproducible results</td>
<td>Ethical concerns</td>
<td>All areas of research including biomedical, behavioral and social.</td>
</tr>
<tr>
<td></td>
<td>Availability of physiological routes of alcohol administration</td>
<td>High cost</td>
<td></td>
</tr>
<tr>
<td>In vivo</td>
<td>Complex interactions of all bodily organs and systems, including complex metabolism</td>
<td>Limited information about the effect on one separate cell population.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Controlled settings, caloric and composition controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Indications to individual and population variability</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1 The characteristics of in vitro and in vivo models of AAA
signs of intoxication and it is employed in the majority of biomedical studies\textsuperscript{[107-121]}. (3) Recruit individuals who are currently not and never have been alcohol abusers for in vivo studies and employ alcohol-naïve primary cells or cell lines for in vitro studies. Alcohol use habits of the study participants are usually determined by questionnaires\textsuperscript{[122]}. Among most frequently used questionnaires are those that incorporate the AUDIT and CAGE tests\textsuperscript{[123-125]}; the study parameters are usually permissive for males who had alcohol use of fewer than nine drinks/week, females < 6 drinks/week.

**IN VITRO AAA MODEL**

The in vitro alcohol treatment model is based on supplementation of culture media with pure alcohol, usually 200-proof ethanol. Currently supplementation of cell culture with a wide variety of alcohol concentrations, ranging from 1 to 500 mmol/L, is reported in the bio-medical literature. One of the major concerns with the in vitro alcohol treatment using concentrations above 100 mmol/L is the direct cytotoxic effect of alcohol on cells\textsuperscript{[49,100]}. At lower concentrations (< 100 mmol/L), alcohol changes the redox status of the cells and alters intercellular junctions\textsuperscript{[83,126]}, increases the membrane fluidity of cells\textsuperscript{[127-129]} and affects the composition of lipid rafts\textsuperscript{[106,130-134]}, all of which may contribute to alcohol-mediated increase in transcellular and paracellular permeability\textsuperscript{[83,123,130]} and thus affect cell function\textsuperscript{[106,130-134]}. Alcohol also affects the expression of adhesion molecules\textsuperscript{[139]}, which may be a concern when using adherent cell types due to possible cell detachment. Additional concerns arise from the possibility of modified ex vivo function of some primary cells, including hepatocytes, stellate cells and their precursors, due to limited ex vivo environment compared to in vivo conditions\textsuperscript{[136-138]}.

From a technical point, the acute alcohol exposure of cells in vitro may be hampered by alcohol evaporation. To avoid the fluctuation of alcohol concentration due to evaporation, investigators used settings where ethanol was added into the culture media and the cell culture plates were maintained for the entire duration of stimulation in a microclimate chamber at 37°C with gas mixture and an alcohol atmosphere\textsuperscript{[139]}. For example, if the desired alcohol concentration in the cell culture is 25 mmol/L, a Petri dish with 2 × the alcohol amount (50 mmol/L) was placed on the bottom of the chamber to ensure the saturation of the gas in the chamber); such conditions maintain the initial alcohol concentration ± 15% over a 24 h period\textsuperscript{[139]}. However, depending on the scientific question of the study, the declining alcohol levels in vitro may be desired to mimic the alcohol elimination in vivo; in these situations the in vitro experiments are disadvantaged by the absence/limitation of alcohol metabolism\textsuperscript{[6,134]}.

The in vitro AAA model offers the possibility of primary in vitro exposure of alcohol-naïve cells to alcohol alone or its combinations with diverse pharmacological or naturally-derived substances\textsuperscript{[24,28,31,35,36,42,55,68,71,72,96,103]}, but also the investigation of the effects of in vivo exposure to alcohol followed by ex vivo exposure to other stimulants\textsuperscript{[109,113,115]} or vice versa. One other main characteristic of the in vitro AAA model is its simplicity, often considered as an advantage or disadvantage depending on the research goal. Most of the in vitro research involves culture of a single cell type\textsuperscript{[134-139,142]} or co-culture of several cell types\textsuperscript{[44]}, while such an approach brings forward the differential effect of alcohol on pure cell populations, and/or their intercellular interaction; it lacks the systemic alcohol metabolism and intercellular interactions. More recently significant efforts were invested in establishment of more complex in vitro systems, such as culture of cells in three dimensional systems\textsuperscript{[108]}, organ slices\textsuperscript{[114]} or organ explants\textsuperscript{[142]}; while such systems are informative in the setting of chronic alcohol exposure to date there is no report of their use as an AAA model.

**IN VIVO AAA MODELS**

The in vivo models of AAA are more informative compared to the in vitro model due to complex physiological impact of alcohol on all bodily organs and systems, but also to the availability of systemic alcohol metabolism. Currently there are human and non-human models of AAA, and the later include use of invertebrates\textsuperscript{[146-147]} and vertebrates\textsuperscript{[21,23,37,44,46,47,53,65,72,80,93,94,98,104,110,111]}. The invertebrate models (Drosophila melanogaster\textsuperscript{[146,147]}, Caenorhabditis elegans\textsuperscript{[109]}) and those using lower vertebrates (Zebra fish Danio rerio\textsuperscript{[89]}) are invaluable for research of the effect of alcohol on behavior, development and maintenance of memory, and on basic signaling mechanisms. These models offer the advantage of a well-defined genetic background, high-turnover rate of experiments due to short life cycle and relatively low-cost; in light of these advantages they constitute an excellent resource for research of signaling pathways and are highly desirable for their drug-screening capacity. On the downside, significant differences in the structure and function of organs and systems compared to humans limit the informative value of invertebrate and lower vertebrate models of AAA.

The vertebrate models are preferred to those using invertebrates due to closer resemblance of their bodily structure, function, and metabolism to that of humans. However, because of intrinsic differences between humans and other vertebrates, no single non-human model is perfect since none of the models can represent all features of the complex human trait, such as motivation for social occasional or binge alcohol consumption, development of alcohol dependence and establishment of the impact on health. Further, the controlled setting of research models may not be completely satisfactory for psychology and social research, since they may not fully reproduce the social component, the motivation and the spontaneity of alcohol abuse. However, research models are invaluable for the understanding of the effects of alcohol and its
mechanisms of action on hardwired bodily systems, including the brain and all other organs and systems.

HUMAN MODELS OF AAA

Human alcohol intake in the experimental setting is the best available model of AAA, because it offers the advantage of the physiological route of alcohol consumption, the possibility to investigate human pathobiology and the availability of relatively large amounts of physiological bodily fluids for research. The disadvantages of human models of AAA include ethical concerns related to potential harmful health effects due to excessive or repeated intoxication, and the theoretical possibility of development of dependence or tolerance even after a one-time drinking session. Published models of human AAA are based on consumption of alcoholic beverages containing either distilled ethanol or wine; these models are physiological, as they involve alcohol drinking, and achieve a biologically meaningful BAL. The majority of the reported in vivo models of human AAA strictly control for the amount of alcohol based on the constant volume of alcohol per kg body weight, includes placebo-treated age and gender matched controls. However, most of these studies design the consumption of the alcohol beverage during a 2 h period of time, which based on recent NIAAA and NSDUH classification qualifies as binge drinking. Thus the major disadvantage of the human models of AAA is that they (1) do not clearly distinguish between one-time and the binge alcohol consumption pattern, and, (2) for ethical reasons, do not allow longer binge sessions which are often observed in real-life and account for the majority of the heavy alcohol intake in young adults.

To fulfill the requirement for an AAA model, the human studies usually include nonalcoholic individuals, who did not drink any alcohol at least 24 h prior to the study. Depending on the study design, some AAA human models require that the study participants did not take any medication, while others accept individuals taking moderate doses of anti-hypertensive medication and oral contraceptives. The study participants are usually required to abstain from food for at least 6 h before alcohol consumption and are allowed free access to water and a light meal before or shortly after the study. The human model of AAA is currently used for research in physiology, hematology, and immunology.

CONSUMPTION OF NON-DISTILLED ETHANOL MODEL

In this model the study individuals drink wine to an equivalent of a pre-determined amount of ethanol/kg BW (for example, Fehr et al. reported use of 4.36 mL of red wine/kg of body weight as an equivalent of 0.5 g ethanol/kg BW to lead to a peak BAL of about 15 mmol/L in the first 2 h), while the control individuals are exposed to the same volume of fluid by mouth (usually water) per individual in a randomized way. The major disadvantage of this model is the use of controlled volumes of liquids that are not matched by caloric intake or by composition, which is technically challenging to achieve due to restricted availability of equivalent alcohol-free compounds. To bypass the bias concern some studies employ a cross-over approach, where each subject serves as its own control and repeats the study at least 2 wk after the first experiment with either alcohol or placebo consumption according to the cross-over design.

NON-HUMAN AAA MODELS

Among non-human vertebrates commonly involved in alcohol research are primates, pigs, dogs, mice, rats, and rabbits. The rodent AAA models (mice and rats) are used most frequently due to their relatively well-defined genetic background and the availability of diverse genetic traits, including those coding for high or low alcohol consumption. Most non-human AAA models currently in use examine relative oral self-administration from a bottle containing alcohol versus or multiple bottles containing water (preference drinking) or administration of alcohol against the will, either by physiological (by mouth using gavage) or by non-physiological (parenteral) routes. Voluntary consumption of alcohol may be an optimal animal model of AAA, due to physiological route and pattern of alcohol consumption. However, in the self-administration models it is not clear when or if the animals drink to pharmacologically significant levels because the drinking is episodic and often occurs over a 24-h period. Nevertheless, these models are invaluable for research of neurobiology of acute intoxication with alcohol and for establishment of mechanisms of addiction. The AAA models using administration of alcohol against-the-will bypass all the above-mentioned inconveniences of AAA models using voluntary consumption. Alcohol administered either by physiological (by mouth using gavage) or by non-physiological (parenteral) routes yields comparable physiological effects on the central nervous system and on organs/systems that are not affected directly by the route of alcohol administration, such as muscle and brain. However, administration of alcohol per os is more physiological compared to administration via parenteral routes, yields meaningful levels of BAL, and shows signs of acute alcohol intoxication.

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Among disadvantages of administration of alcohol per os are technical challenges, time consumption and high cost of the procedures. In contrast, alcohol administration by parenteral routes is relatively easy to perform technically and offers controlled settings (time, amount); on the downside, they may be less suitable for research of the effects of alcohol on organs/systems that are affected directly by the routes of alcohol administration. In this context, administration of alcohol by intraperitoneal route may be less suitable for research using peritoneal macrophages, or even liver and intestines, compared to other administration routes, such as intravenous or enteral. Further, some parenteral methods of alcohol administration are preferred over others, owing to differences in the level of technical difficulty of the procedure and the effect of alcohol on different cell types. For example, alcohol administration by intravenous route is currently limited to creating an acute alcohol exposure during treatment of alcohol withdrawal symptoms, while administration by intraperitoneal injections is widely preferred in research settings.

Similar to human AAA models, the non-human in vivo models employ either distilled alcohol or alcohol-containing beverages, such as wine and beer, the control groups are usually treated with alcohol-free caloric and composition equivalents. The vertebrate AAA models are widely used in research of biomedical effects of AAA, including brain, gastrointestinal, vascular, muscle and immune systems.

Table 2  The effect of acute alcohol abuse on GI system

<table>
<thead>
<tr>
<th>GI segment</th>
<th>Effect of acute alcohol exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral cavity</td>
<td>Unknown</td>
</tr>
<tr>
<td>Esophagus</td>
<td>Low concentrations of alcohol (up to 5%) cause alterations in ion transports and affect the barrier function</td>
</tr>
<tr>
<td></td>
<td>Concentrations of alcohol of 10% and above cause injury of mucosa</td>
</tr>
<tr>
<td></td>
<td>Co-carcinogenic potency</td>
</tr>
<tr>
<td></td>
<td>Motor dysfunction: decrease in lower esophageal sphincter pressure and amplitude</td>
</tr>
<tr>
<td>Stomach</td>
<td>Motor dysfunction: Inhibition of gastric emptying</td>
</tr>
<tr>
<td></td>
<td>Mucosal damage, impaired barrier function, increased epithelial permeability</td>
</tr>
<tr>
<td></td>
<td>Pro-inflammatory reaction: decreased gastric blood flow, vascular damage, polymorphonuclear neutrophils (PMN) dependent- and independent-mucosal damage</td>
</tr>
<tr>
<td>Intestine</td>
<td>Disruption of barrier function</td>
</tr>
<tr>
<td></td>
<td>Epithelial apoptosis</td>
</tr>
<tr>
<td></td>
<td>Enhanced bioavailability of some alcohol-soluble drugs and impaired absorption of key nutrients</td>
</tr>
<tr>
<td></td>
<td>Increased paracellular intestinal permeability to toxins</td>
</tr>
<tr>
<td>Liver</td>
<td>Hepatocytes: Amplification of Fas-mediated hepatocyte death</td>
</tr>
<tr>
<td></td>
<td>Generation of oxidative stress</td>
</tr>
<tr>
<td></td>
<td>Hepatic mitochondrial dysfunction</td>
</tr>
<tr>
<td></td>
<td>Increased free iron levels</td>
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<tr>
<td></td>
<td>Imbalanced fatty acid metabolism</td>
</tr>
<tr>
<td></td>
<td>Inhibition of IFN-α-induced antiviral response towards hepatotropic viruses including hepatitis C virus favors hepatitis C virus replication</td>
</tr>
<tr>
<td></td>
<td>Induced histone H3 acetylation leading to increased gene expression in the liver</td>
</tr>
<tr>
<td></td>
<td>Limited hepatic protein synthesis</td>
</tr>
<tr>
<td></td>
<td>Arrest of liver regeneration early after partial hepatectomy and suppression of hepatic stimulator substance (HSS) activity by induction of liver cell cycle arrest</td>
</tr>
<tr>
<td>Kupffer cells</td>
<td>Suppressed LPS-mediated priming for enhanced CC-chemokine release in vitro; up-regulated expression of CC-chemokine mRNA; primed the KC for enhanced RANTES release</td>
</tr>
<tr>
<td></td>
<td>Desensitized HIV-1 gp120-induced CC-chemokine production</td>
</tr>
<tr>
<td></td>
<td>Downregulates HIV-1 glycoprotein 120-induced KC and RANTES production</td>
</tr>
<tr>
<td></td>
<td>Regulates production of reactive oxygen species</td>
</tr>
<tr>
<td></td>
<td>Modulate the tolerance to LPS</td>
</tr>
<tr>
<td>Stellate cells</td>
<td>Imbalanced redox potential owed to increased generation of reactive oxygen species upon GSH depletion</td>
</tr>
<tr>
<td>Pancreas</td>
<td>Stimulates islet blood flow, amplifies insulin secretion, induces hypoglycemia</td>
</tr>
<tr>
<td></td>
<td>Lower baseline amylase output of acinar pancreatic cells, with the difference being significantly exacerbated by cerulein stimulation</td>
</tr>
<tr>
<td></td>
<td>Interference with release of oxidized proteins in acinar cells</td>
</tr>
<tr>
<td></td>
<td>Predisposes the pancreas to postprandial cholinergic stimulation that triggers cellular events leading to pancreatic inflammation</td>
</tr>
<tr>
<td></td>
<td>Impaired apical exocytosis and redirected exocytosis to less efficient basolateral plasma membrane sites</td>
</tr>
<tr>
<td></td>
<td>Augments elevated-[Ca²⁺]-induced trypsin activation in pancreatic acinar zymogen granules, leading to premature activation of trypsin and tissue damage</td>
</tr>
</tbody>
</table>

THE PARTICULARITIES OF AAA MODELS FOR RESEARCH IN GASTROENTEROLOGY

In contrast to the abundance of the literature about the
effects of chronic alcohol abuse on the gastrointestinal system, research of the effects of acute alcohol abuse on the gastrointestinal (GI) tract is limited to certain cell types, as outlined in Table 2.

Currently the state of scientific knowledge suggests a tight interplay between organs and systems. The GI system is dependent on blood circulation and systemic availability of metabolites, is closely governed by both the central and the autonomous nervous system\(^{[153,154]}\) and contains a hallmark of resident and recruited immune cells\(^{[157,158]}\). Thus, it is conceivable that the direct effects of alcohol on either of these systems will indirectly affect the function of the gastrointestinal system; this area is currently largely unexplored.

From a technical point, the GI research may take advantage of both in vitro and in vivo AAA models; however some in vivo models, such as those using parenteral administration of alcohol by the intraperitoneal route, may be less suitable due to the non-physiological direct contact between high concentrations of alcohol and GI tissues.

Alcohol use/abuse is associated with acute life-threatening conditions, including acute alcoholic hepatitis\(^{[49]}\) or acute pancreatitis\(^{[159]}\). The majority of these patients report acute alcohol abuse, which is often overlapping with withdrawal from or even discontinued chronic alcohol abuse, or it follows an episode of binge drinking\(^{[49,159]}\). As such, it is difficult to associate these diseases with the single-occasion AAA, yet they do not fit into the classic chronic alcohol abuse picture. This category of alcohol abuse, defined as “acute-on-chronic”, is in need of modeling for GI research.

In prospective, we currently lack in-depth knowledge in regards to the effects of acute alcohol abuse on different segments of the luminal GI tract, on liver functions, and on pancreas, including its endocrine and exocrine functions. Further, we do not know if acute alcohol consumption affects the GI stem cells and/or is involved in development of GI-derived tumors.

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