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TOPIC HIGHLIGHT

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In vitro and *in vivo* models of acute alcohol exposure

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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.

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]. 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[9]. Humans use and abuse alcohol acutely or chronically, when alcohol consumption is frequent and dependence has developed[10]. 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.

ACUTE ALCOHOL ABUSE (AAA): HOW BIG THE PROBLEM REALLY IS?

In the USA, a "drink" is defined as an equivalent of 14 g alcohol, which equals roughly 1 shot [1.25 oz of 40% (80-proof) liquor], 1 (12 oz) beer (4.2 mL/L, Ethanol), or 1 (4 oz) glass of wine (12 mL/L, Ethanol)[14]. In other countries, the alcohol content of a serving is measured in "units". One unit (about 25 mL of a 40% 80-proof liquor) contains 7.9 g of pure ethanol[8,15]. However, in many countries the "standard drink" is used to quantify alcohol intake. More importantly, the standard drink varies significantly from country to country, from 10 mL (7.9 g) of alcohol in the UK to as high as 25 mL (19.75 g) in Japan[16]. Current use includes at least one drink in the past 30 d; binge drinking is defined as five or more drinks on the same occasion within 2 h at least once in the past 30 d; and heavy use is defined as five or more drinks on the same occasion on at least 5 different days in the past 30 d[11-13]. The 0.08% blood alcohol level (BAL) is the legal limit for most states in the US and it is achieved with consumption of five or more drinks for an adult male and four or more drinks for an adult female[11-13].

Traditionally medical research focuses on the mechanisms of chronic alcohol abuse; this is due to the significant financial burden that society encountered primarily from chronic alcohol abusers[1-13]. However, more recently acute alcohol abuse has emerged as a

Table 1 The characteristics of *in vitro* and *in vivo* models of AAA

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

social problem[17]. The National Survey on Drug Use and Health (NSDUH) estimated that in the USA about 4.4 million persons had used alcohol for the first time in 2004, which lead to about 12 000 "new recruits" per day; this was significantly greater than in 2002 (3.9 million) and 2003 (4.1 million). Most (86.9%) of the 4.4 million recent alcohol initiates were younger than 21 years of age at the time of encounter. More than one fifth (22.%) of people age 12 or older participated in binge drinking at least once in the 30 d prior to the survey in 2004[12]. Acute alcohol intake in the form of binge drinking in 2004 was highest for the 18- to 25-year-old age group compared with other age groups, with the peak rate occurring at age 21[1,5-7,11-13]. The statistics also show that illness and death among young adults primarily result from lifestyle choices and behaviors, including excessive alcohol use[18,19].

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[20,21]. AAA leads to impaired CNS activity due to alcohol's effect on synthesis[22], release[23] and signaling[23,24] of neurotransmitters, including serotonin[25,26], glutamate[27], GABA[28], endocannabinoids[29,30] and their receptors. AAA causes damage and functional impairment of the gastrointestinal (GI) tract, including luminal GI[31-38], liver[39-55], and pancreas[56-62]; it also affects the protein, carbohydrate, and fat metabolism[58,63-66]. AAA leads to insufficient immune system responses to infections; such deficiency was observed both in organ-specific[67-69] and systemic infections[70-72]. Acute alcohol intoxication impairs the ability of the host to counteract hemorrhagic shock[73], augments corticosteroid release[74] and delays wound healing[75-78], thus contributing to higher morbidity and mortality[79] and prolonged recovery from trauma[80]. 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[81-84].

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[85], 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[86-97]. 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[97].

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[98-106]. *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[11,12,17,18]. Thus any model using consumption of biologically active amounts of alcohol within 2 h is considered an acceptable model of AAA[81,107-121]. (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[7,11,12,98-106]. For the *in vivo* studies an 0.08% BAL or above this level yields

signs of intoxication and it is employed in the majority of biomedical studies[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[122]. Among most frequently used questionnaires are those that incorporate the AUDIT and CAGE tests[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[40,100]. At lower concentrations (< 100 mmol/L), alcohol changes the redox status of the cells and alters intercellular junctions[33,126], increases the membrane fluidity of cells[127-129] and affects the composition of lipid rafts[106,130,131], all of which may contribute to alcohol-mediated increase in transcellular and paracellular permeability[132,133] and thus affect cell function[106,130-134]. Alcohol also affects the expression of adhesion molecules[135], 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[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℃ with gas mixture and an alcohol atmosphere[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[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[76,134].

The *in vitro* AAA model offers the possibility of primary *in vitro* exposure of alcohol-naive cells to alcohol alone or its combinations with diverse pharmacological or naturally-derived substances[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[110,113,115] 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[134,139-142] or co-culture of several cell types[143]; 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[100], organ slices[144] or organ explants[145]; 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 due to the availability of systemic alcohol metabolism. Currently there are human and non-human models of AAA, and the later include use of invertebrates[146-147] and vertebrates[21,25,37,44,46,47,53,65,72,86,93,94,98,104,110,111]. The invertebrate models (Drosophila melanogaster[146,147], Caenorhabditis elegans[105]) and those using lower vertebrates (Zebra fish Danio rerio)[98] 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[87,92,107,110-113,115]. 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[92,107,110-113], which based on recent NIAAA and NSDUH classification qualifies as binge drinking[11-13,17]. 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[5,7,11-13,17-19].

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[107,110]. 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[107]. The human model of AAA is currently used for research in physiology[86,92,111,122], hematology[107,128] and immunology[110,113,115].

CONSUMPTION OF DISTILLED ETHANOL MODEL

In this model the study individuals drink distilled alcohol (usually 80-proof vodka) in amounts of about 0.5-0.6 g/kg body weight, which is an equivalent of about 2 mL vodka/kg body weight in a standardized total volume of liquid (300-450 mL of water or orange juice)[92,107,110-113].

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*[107] 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 calorie 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[107].

NON-HUMAN AAA MODELS

Among non-human vertebrates commonly involved in alcohol research are primates[90,91,148], pigs[104,120], dogs[114,121], mice[70,72,74,86,89,96,109,118,119,141], rats[88,94,108,149,150] and rabbits[132]. 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[88,89,96,109]. Most non-human AAA models currently in use[93,95] examine relative oral self-administration from a bottle containing alcohol versus one[86,94,108] or multiple bottles[119] containing water (preference drinking) or administration of alcohol against the will, either by physiological (by mouth using gavage)[54,71,72] or by non-physiological (parenteral)[67,68] 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[97]. 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[54,71,72,132,149].

Table 2 The effect of acute alcohol abuse on GI system

GI segment	Effect of acute alcohol exposure
Oral cavity	Unknown
Esophagus	Low concentrations of alcohol (up to 5%) cause alterations in ion transports and affect the barrier function
	Concentrations of alcohol of 10% and above cause injury of mucosa
	Co-carcinogenic potency
	Motor dysfunction: decrease in lower esophageal sphincter pressure and amplitude
Stomach	Motor dysfunction: Inhibition of gastric emptying
	Mucosal damage, impaired barrier function, increased epithelial permeability
	Pro-inflammatory reaction: decreased gastric blood flow, vascular damage, polymorphonuclear neutrophils (PMN) dependent- and independent-mucosal damage
	Aggravation of *H pylori* infection
Intestine	Disruption of barrier function
	Epithelial apoptosis
	Enhanced bioavailability of some alcohol-soluble drugs and impaired absorption of key nutrients
	Increased paracellular intestinal permeability to toxins
Liver	Hepatocytes:
	Amplification of Fas-mediated hepatocyte death
	Generation of oxidative stress
	Hepatic mitochondrial dysfunction
	Increased free iron levels
	Imbalanced fatty acid metabolism
	Inhibition of IFN-α-induced antiviral response towards hepatotropic viruses including hepatitis C virus favors hepatitis C virus repli con expression
	Induced histone H3 acetylation leading to increased gene expression in the liver
	Limited hepatic protein synthesis
	Arrest of liver regeneration early after partial hepatectomy and suppression of hepatic stimulator substance (HSS) activity by induc tion of liver cell cycle arrest
	Kupffer cells:
	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
	Desensitized HIV-1 gp120-induced CC-chemokine production
	Downregulates HIV-1 glycoprotein 120-induced KC and RANTES production
	Regulates production of reactive oxygen species
	Modulate the tolerance to LPS
	Stellate cells:
	Imbalanced redox potential owed to increased generation of reactive oxygen species upon GSH depletion
Pancreas	Stimulates islet blood flow, amplifies insulin secretion, induces hypoglycemia
	Lower baseline amylase output of acinar pancreatic cells, with the difference being significantly exacerbated by cerulein stimulation
	Interference with release of oxidized proteins in acinar cells
	Predisposes the pancreas to postprandial cholinergic stimulation that triggers cellular events leading to pancreatic inflammation
	Impaired apical exocytosis and redirected exocytosis to less efficient basolateral plasma membrane sites
	Augments elevated-[Ca^{2+}]-induced trypsin activation in pancreatic acinar zymogen granules, leading to premature activation of trypsin and tissue damage.

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 routs, 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 known to affect the erythrocytes when present in high concentrations[128]. Thus alcohol administration by intravenous route is currently limited to creating an acute alcohol exposure during treatment of alcohol withdrawal symptoms[151], 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[53,67-72,74,89,90,96,109,116,119,121,132,135,141,152] or alcohol-containing beverages, such as wine[152,153] and beer[46]; 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[23-30,116], gastrointestinal [38-44,46-48,64-66,154], vascular[73,153], muscle[97] and immune[68-72,74] systems.

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[155,156] 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[45] 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[45,159]. 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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