THE NEUROIMMUNOPHARMACOLOGY OF ALCOHOL

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Table of Contents

Abstract

Background and purpose

Alcohol exposure induces glial toll-like receptor 4 (TLR4) signalling, while morphine administration leads to both TLR2 and TLR4 signalling in the central nervous system. However, the acute behavioural consequences of such immune activation remain unknown. This thesis aimed to examine: (a) the role of microglia, TLR2, TLR4, MyD88, and IL-1 receptor signalling in sedation and motor impairment following acute alcohol administration in mice; (b) the relationship between these observed behavioural effects and the changes in central and peripheral alcohol pharmacokinetic profiles; (c) the effect of alcohol on MAPK (ERK, JNK, and p38) and NFκB (IκBα) pathways and the alteration of such effects by attenuating microglial, TLR4, MyD88, and IL-1 receptor signalling *ex vivo* and *in vitro*; (d) the role of TLR2, TLR4, MyD88, IL-1 receptor, and μ opioid receptor (MOR) in the interaction between alcohol and morphine as assessed by sedation in mice; and (e) the association between the *TLR4* Asp299Gly SNP and opioid or alcohol dependence in humans.

Experimental approach

In mouse studies and mouse cellular studies, pharmacological blockade of microglial signalling, TLR4, IL-1 receptor, or both MOR and TLR4 by minocycline, (+)-naloxone (the MOR-inactive isomer), IL-1 receptor antagonist, or (-)-naloxone (the MOR-active isomer), respectively, was utilised. Mice deficient in TLR2, TLR4, both TLR2 and TLR4, or MyD88 were used. The sedative effect of alcohol and the interaction between alcohol and morphine were assessed by the sleep time (loss of righting reflex) test, and alcohol dose-induced motor impairment was

determined by the rotarod test. The activation of MAPK cascade was determined by ERK, JNK, and p38 phosphorylation using a cytometric bead array assay, and the alteration in NFκB cascades was characterised via cellular IκBα protein levels utilising western blotting experiments.

In the human pharmacogenetic study, *TLR4* Asp299Gly SNP genotypes were determined by a polymerase chain reaction (PCR)-restriction fragment length polymerase (RFLP) assay in 99 opioid dependent subjects, 100 alcohol dependent subjects, and 56 non-dependent healthy controls.

Key results

Pharmacological or genetic inhibition of microglial activation, TLR2, TLR4, both TLR2 and TLR4, MyD88, or IL-1 receptor signalling attenuated alcohol dose-induced sedation and motor impairment in mice. The modification of IκBα protein levels by alcohol exposure *in vitro* was time-dependent, and the increase in such protein levels was attenuated by inhibiting proinflammatory microglial activation, TLR4, MyD88, or IL-1 receptor signalling. In contrast, blocking the activities of TLR2, both TLR2 and TLR4, and MyD88, but not TLR4 or IL-1 receptor, inhibited the enhancement of alcohol's sedative effect by morphine. The human genetic data showed a lack of association between alcohol or opioid dependence and *TLR4* Asp299Gly polymorphisms.

Conclusions and implications

Collectively, these data suggest that in mice, alcohol activates microglial and TLR2- and TLR4-MyD88-NFκB-IL-1 receptor signalling rapidly, and this activation subsequently contributes to sedation and motor impairment induced by alcohol administration. However, TLR2-MyD88, but

not TLR4 and IL-1 receptor, cascade is involved in the interaction between alcohol and morphine. Such behavioural preclinical data provide novel insights into the immune mechanisms of the effects of alcohol and opioids.

Declaration

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Statement of Authorship

Attenuation of microglial and IL-1 signaling protects mice from acute alcohol-induced sedation and/or motor impairment

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Inhibiting the TLR4-MyD88 signalling cascade by genetic or pharmacologic strategies reduces acute alcohol dose-induced sedation and motor impairment in mice

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TLR2 and MyD88 mediate the sedative effect of alcohol and interaction between alcohol and morphine in mice

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Abbreviations

ADH	alcohol dehydrogenase
ALDH	aldehyde dehydrogenase
ATP	adenosine triphosphate
B2M	β-2-microglobulin
BBB	blood-brain barrier
cAMP	cyclic adenosine monophosphate
CCL	chemokine (C-C) motif ligand
CCR	chemokine (C-C) motif ligand receptor
CD	cluster of differentiation
CI	confidence interval
CNS	central nervous system
COPD	chronic obstructive pulmonary disease
COX	cyclooxygenase
CTSF	cathepsin F
CTSS	cathepsin S
CXCL	chemokine (C-X-C) motif ligand
CYP	cytochrome P450
DA	dopamine
ERK	extracellular regulated kinase
GABA	gamma-aminobutyric acid
GFAP	glial fibrillary acidic protein
HBV	hepatitis B virus
IFN	interferon
IL	interleukin
IL-1RI	interleukin-1β receptor type I
IL-1ra	IL-1 receptor antagonist
iNOS	inducible nitric oxide synthase

IRAK4	interleukin-1 receptor-associated kinase 4
IRF3	IFN regulatory factor 3
IκBα	NFκB inhibitor α
JNK	c-Jun N-terminal kinase
LBP	LPS binding protein
LORR	loss of righting reflex
LPS	lipopolysaccharide
MAL	myelin and lymphocyte protein
MAPK	mitogen-activated protein kinase
MD-2	myeloid differentiation factor 2
MHC	major histocompatibility complex
MOR	μ opioid receptor
mRNA	messenger ribonucleic acid
MyD88	myeloid differentiation primary response gene 88
NAc	nucleus accumbens
NAD	nicotinamide adenine dinucleotide
NFκB	nuclear factor kappa-light-chain-enhancer of activated B cells
NMDA	N-methyl-D-aspartate
NO	nitric oxide
OR	odds ratio
PCR	polymerase chain reaction
PI3K	phosphoinositide 3 kinase
PKA	protein kinase A
PKC	protein kinase C
SN	substantia nigra
SNP	single nucleotide polymorphism
TIR	toll/IL-1 receptor
TLR	toll-like receptor
TNF-α	tumor necrosis factor-α

TRAM	TRIF-related adaptor molecule
TRIF	toll/IL-1R domain containing adaptor inducing interferon-β
VTA	ventral tegmental area

Human gene symbols

Gene symbol	Gene name	Protein
ABCB1	ATP-binding cassette, sub-family B, member 1	P-glycoprotein
ADH1B	alcohol dehydrogenase 1B (class I), beta polypeptide	ADH1B
ADH4	alcohol dehydrogenase 4 (class II), pi polypeptide	ADH4
ALDH1A1	aldehyde dehydrogenase 1 family, member A1	ALDH1A1
ALDH2	aldehyde dehydrogenase 2 family (mitochondrial)	ALDH2
CYP2B6	cytochrome P450, family 2, subfamily B, polypeptide 6	CYP2B6
CYP2E1	cytochrome P450, family 2, subfamily E, polypeptide 1	CYP2E1
CYP3A4	cytochrome P450, family 3, subfamily A, polypeptide 4	CYP3A4
GABRA6	gamma-aminobutyric acid (GABA) A receptor, alpha 6	$GABA_A\alpha6$
IL10	interleukin 10	IL-10
IL1B	interleukin 1, beta	IL-1β
IL1RN	interleukin 1 receptor antagonist	IL-1ra
LY96	lymphocyte antigen 96	MD-2
NFKB1	nuclear factor of kappa light polypeptide gene enhancer in B-cells 1	NFκB
OPRM1	opioid receptor, mu 1	MOR
TLR2	toll-like receptor 2	TLR2
TLR4	toll-like receptor 4	TLR4