55th Annual Midwest Student Biomedical Research Forum

Saturday, March 2, 2024

ROOM 3042

10:15 a.m. **O-05** ELECTRICAL STIMULATION FOR PRESERVATION OF DENERVATED MUSCLE USING A NOVEL HYDROGEL IONIC CIRCUIT
Presenter: Olawale Alimi, UNMC

10:30 a.m. **O-16** DETRIMENTAL LONG-TERM CONSEQUENCES OF SUBACUTE-PHASE TREATMENT WITH PIOGLITAZONE IN A MOUSE MODEL OF TRAUMATIC BRAIN INJURY
Presenter: L. Daniel Estrella, UNMC

10:45 a.m. **O-24** KETOGENIC DIET IMPROVES CARDIORESPIRATORY FUNCTION AND LONGEVITY IN Kv1.1 KO MICE, A MODEL OF SUDDEN UNEXPECTED DEATH IN EPILEPSY (SUDEP)
Presenter: Shruthi Iyer, Creighton University

11:00 a.m. **O-37** ERK1/2 INHIBITION ALLEVIATES NOISE-INCLUDED HEARING LOSS WHILE TEMPERING DOWN THE IMMUNE RESPONSE
Presenter: Richard Lutze, Creighton University

11:15 a.m. **O-50** THE ROLE OF ASTROCYTE NLRP6-DEPENDENT PYROPTOSIS IN METHAMPHETAMINE-MEDIATED NEUROINFLAMMATION
Presenter: Abiola Oladapo, UNMC

11:30 a.m. **O-53** ALCOHOL USE IMPAIRS NK CELL METABOLISM AND EFFECTOR FUNCTION
Presenter: Ashley Peer, UNMC

11:45 a.m. Break

ELECTRICAL STIMULATION FOR PRESERVATION OF DENERVATED MUSCLE USING A NOVEL HYDROGEL IONIC CIRCUIT

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Background: Skeletal muscle represents approximately 40% of human body weight. It is an important effector of the peripheral nervous system (PNS) and play vital roles in locomotion and respiration. The PNS maintains the muscular structure and function through constant neural signal. When peripheral nerve injury (PNI) occurs, muscles become denervated and there is loss of the neural signals, leading to muscle atrophy. Pending nerve regeneration, electrical stimulation (EStim) is used to preserve muscle physiology mimicking the electrical neural signal from the nerves.

Significance of Problem: An intensity-dependent improved muscle contraction force has been earlier reported with the maximum intensity of 16 mA [1]. This is an indication that muscle functions and structures could be better preserved with a higher intensity EStim. Carbon and stainless-steel electrodes are traditionally used for EStim. However, when used to deliver high intensity EStim, they cause tissue damage due to high temperature and pH change resulting from conversion of electronic current to ionic current at electrode/tissue interface (ETI) [2]. This led to development of hydrogel ionic circuit (HIC) with the ability to absorb and neutralize the temperature and pH changes associated with the electrochemical reaction at ETI [2]. These properties made the HIC suitable for delivering high intensity EStim on tissues without tissue damage.

Hypothesis: The HIC can safely deliver a high intensity EStim to preserve the structure and function of denervated muscle following a long-gap PNI.

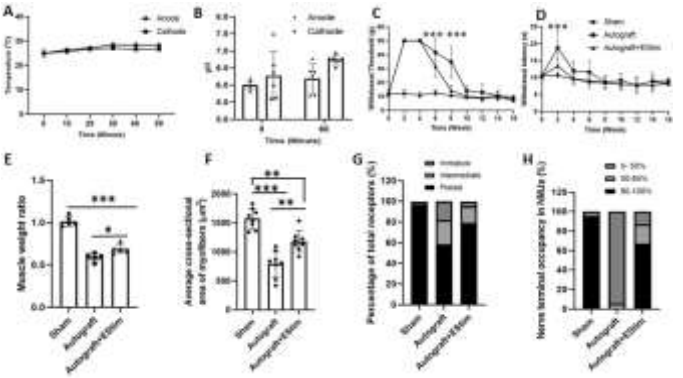

Figure 1. (A) Temperature changes in the electrodes (B) pH changes on the skin where the electrodes were applied (C) von Frey test (D) Hargreaves test (E) Muscle weight ratio (F) Cross-sectional area of myofibers. Percentage of total receptors in the GMs based on maturity (G) and nerve terminal occupancy (H) (n=5-8, *p<0.05, **p<0.01, ***p<0.001)

Experimental Design: The HIC has two key components; a hydrogel and saturated phosphate salt solution. Poly (ethylene glycol) diacrylate (PEGDA) and polyethylene glycol dimethacrylate (PEGDMA 400) are crosslinked with ultraviolet light to prepare the hydrogel. To evaluate the safety of the HIC when used to deliver a high intensity of 32 mA, gastrocnemius muscle (GM) of healthy rats were stimulated over the skin using a frequency of 20 Hz and pulse width of 25 ms for 1 hr. The temperature and pH changes were recorded. After this, a long-gap PNI of 10 mm-long sciatic nerve transection and orthotopically sutured as autograft was used to induce denervation in the right hindlimb. EStim was carried out weekly on the GM beginning from the second week of the surgery for 16 weeks. The sensory functions recovery was evaluated every two weeks using von Frey filament and Hargreaves tests for mechanical and heat responses respectively. After 16 weeks of EStim, muscle contraction test was done after which the rats were euthanized. GMs were isolated to measure their wet weights and were later fixed for hematoxylin and eosin staining (H&E) and immunofluorescence staining for neuromuscular junctions (NMJs). The average cross-sectional area of the myofibers and structures of the neuromuscular junctions were then assessed.

Results: The HIC is safe to deliver to a higher EStim intensity of 32 mA without any tissue damage. There was no significant difference in temperature and pH of the skin over which the EStim was applied before and after the EStim (**Fig 1A, B**). Early sensory functions recovery were observed for mechanical (**Fig 1C**) and thermal (**Fig 1D**) responses as observed in the group of EStim when compared to untreated group. The muscle contraction force was also higher with significance in the treated group compared to untreated (not shown). Following euthanasia and isolation of the GMs, the muscle weight ratio (right/left) showed that the muscles of the EStim group were better preserved following denervation as compared to untreated group (**Fig 1E**). The myofiber cross-sectional area, a hallmark of muscle atrophy, was also preserved significantly in the EStim group when compared to the untreated group (**Fig 1F**). There are also more mature and less immature acetylcholine receptors (AchR) in the EStim group (**Fig 1G**). Furthermore, higher number of AchR in the EStim group have almost complete nerve terminal occupancy when compared to the autograft group (**Fig 1H**).

Conclusions: This study showed that the HIC can safely be used to deliver a higher current of EStim that could reach deeper muscle tissues without tissue damage. The higher EStim current delivered with the HIC can enhance sensory functions recovery while preserving both structure and function of denervated muscle. Therefore, the HIC is a potential substitute for the common electrodes used in muscle stimulation.

Reference: [1] Tamaki, H., et al., *Electrical stimulation of denervated rat skeletal muscle retards trabecular bone loss in early stages of disuse musculoskeletal atrophy.* J Musculoskelet Neuronal Interact, 2014. **14**(2): p. 220-8. [2] Zhao, S., et al., *Programmable Hydrogel Ionic Circuits for Biologically Matched Electronic Interfaces.* Advanced Materials, 2018. **30**(25): p. 1800598.

DETRIMENTAL LONG-TERM CONSEQUENCES OF SUBACUTE-PHASE TREATMENT WITH PIOGLITAZONE IN A MOUSE MODEL OF TRAUMATIC BRAIN INJURY

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Background

Traumatic Brain Injury (TBI) is a disabling neurotraumatic condition and the leading cause of injury-related deaths and disability in the United States. TBI pathology is classified into primary injury, which is the direct physical damage to brain tissue and neurovascular disruption due to drastic acceleration/deacceleration, and secondary injury, which is characterized by the onset of different pathologies downstream from the primary injury. These include chronic neuroinflammation, neurotoxicity, protein buildup, and blood-brain-barrier disruption. Accumulating evidence suggests TBI increases the risk of developing Alzheimer's disease (AD), which is characterized by accumulation of amyloid plaques and Tau tangles, in which Tauopathy correlates with dementia.

Significance of Problem

Current therapeutic approaches primarily focus on ameliorating the effects of secondary injury, like chronic neuroinflammation, such as the use of thiazolidinediones or glitazones, like pioglitazone (an PPAR gamma agonist), to halt the gliosis and chronic neuroinflammatory response that arises from the secondary injury. While previous reports have provided a wide range of data regarding the pathologies that arise after TBI along with the possible therapeutic effects of pioglitazone, these have focused on acute time-points after injury, and the long-term lasting effects that TBI and treatment with pioglitazone have on the brain during the chronic phase after injury remain scarce.

Hypothesis/Purpose

For this reason, the long-term impacts of treatment with pioglitazone during the subacute phase after TBI were interrogated. We hypothesized that halting the critical glial repair response via treatment with pioglitazone in the subacute phase after injury would exacerbate TBI-induced pathology at the chronic phase, and this would be made worse in a Tauopathy mouse model.

Experimental Design

To test this, 2-month-old male and female wild-type (WT) and hTau (express non-mutant human tau in the absence of murine tau) mice were subjected to a unilateral controlled cortical impact or a sham surgery (craniotomy only). Animals were administered 10 mg/kg of pioglitazone or vehicle control 30 minutes post-surgery and every subsequent 24 hours for 5 days (6 days total). Behavioral assessments occurred at 30 DPI (1 month), 152 DPI (5 months), and 274 DPI (9 months) using the tube test and resident intruder paradigm. Neuroinflammatory and pathological parameters were assessed at 247 DPI via histological and immunoblotting techniques.

Results

Pioglitazone treatment during the subacute phase after TBI leads to worsened injury severity (larger injury volume and significant cortical degeneration) and increased territorial and aggressive behavior during the chronic phase after TBI. Histopathological analysis demonstrated marked gliosis and Tau redistribution towards the neuronal soma, a commonly observed pathology in Tau-related neurodegeneration. Further, immunoblotting revealed elevated levels of phosphorylated Tau and known Tau kinases.

Conclusions

While treatment with Glitazones has gained great attention as a possible therapeutic approach to halt TBI-induced neuroinflammation, our data suggests that amelioration of the initial glial repair response during the subacute phase after TBI via pioglitazone treatment can lead to long-term detrimental effects in the brain during the chronic phase after TBI. These include Tau-related pathologies, gliosis, and behavioral alterations. We anticipate this is due to an inhibition of the glial responses that are important for immediate repair following injury and that this subsequently primes glia to be chronically active. This work reveals that timing and long-term consequences of treatment with glitazones must be considered and further studied prior to their use in the clinic for TBI therapy.

Title: KETOGENIC DIET IMPROVES CARDIORESPIRATORY FUNCTION AND LONGEVITY IN Kv1.1 KO MICE, A MODEL OF SUDDEN UNEXPECTED DEATH IN EPILEPSY (SUDEP)

Authors: Shruthi Iyer, Stephanie Matthews, Jodi Hallgren, Lauren Netzel, Samantha Draves, Timothy Simeone, Kristina Simeone.

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Significance: Sudden unexpected death in epilepsy (SUDEP) is one of the leading causes of death in epilepsy affecting 1:1000 epilepsy patients each year. Multicenter clinical studies have reported that severe refractory seizures and cardiorespiratory dysfunction including bradycardia, apneas and severe hypoxia are major risk factors for SUDEP. While several studies are investigating the mitigation of some of these risk factors, there is a critical need to identify treatments that target the overall cardiorespiratory dysfunction in SUDEP. Further, evidence suggests that patients that succumbed to SUDEP, may have experienced seizures and cardiorespiratory dysfunction earlier in their lives; that lead to recovery at the time. However, the mechanisms of this recovery fail right before SUDEP. This suggests a gradual weakening of the cardiorespiratory function. Thus, there may be a critical window for therapeutic intervention, hitherto unexplored.

Background: We have previously reported that the Kv1.1 KO (KO) mice, a model of SUDEP, have increased seizures, bradycardia, apnea and chronic intermittent hypoxia as these mice approach death. This indicates that the KO mice develop a progressive pathophysiology where the seizures and cardiorespiratory dysfunction worsen as they approach SUDEP. We have also reported that treatment with a high fat, low carbohydrate ketogenic diet (KD) reduced severe seizures and increased longevity in the KO mice. Individual studies within and outside of epilepsy have also reported that KD treatment reduced refractory seizures, apneas, hypoxia and improved cardiac function. Here, we tested whether KD ameliorates the progressive cardiorespiratory dysfunction in a preclinical SUDEP model.

Hypothesis: Chronic treatment with ketogenic diet will reduce seizures, improve cardiorespiratory function and thereby increase longevity in the Kv1.1 KO mice.

Experimental Design: Kv1.1 KO mice and wild-type (WT) controls were weaned onto standard diet (SD) or ketogenic diet (KD) cohorts. For both cohorts, cardiorespiratory parameters were measured starting postnatal day (P)21 for every ten days until sudden death. Heart rate and blood oxygen saturation (SaO_2) were determined noninvasively with ECGenie and pulse oximetry respectively. Respiration was determined with noninvasive airway mechanics. Frequency and severity of the seizures were recorded using EEG-video monitoring. Endpoints included survival, heart rate, incidence of bradycardia (heart rate < 600 bpm), intermittent hypoxia (<90% blood O_2 saturation) and apnea. Data were plotted retrospectively from day of SUDEP.

Results: A 100% of the KO mice on standard diet (SDKO) died by P56 ± 2.4 days. KD treatment increased the survival of the KO mice by about 35% to P75 ± 5.9 days ($p < 0.0001$). KD treatment also significantly reduced the seizure burden in the KO mice ($p<0.01$). Compared to the WT controls, SDKO mice had a progressive decrease in heart rate, as these mice approached SUDEP age ($p<0.001$). KD treatment significantly increased the heart rates in the KO mice to WT control level ($p<0.01$). We also found that compared to the WT controls, the SDKO mice experienced more bradycardia. KD treatment significantly reduced the fraction of bradycardia episodes in the KO mice ($p<0.01$). SDKO mice experienced higher incidence of apneas compared to WT controls closer to SUDEP age ($p<0.001$). KD treatment reduced the incidence of apnea in the KO mice. ($p <0.01$) We have previously reported that SDKO mice have increased episodes of intermittent hypoxia as they approach death. Here we found that, KD treatment reduced the fraction of hypoxic episodes experienced by the KO mice from 25% ± 5.5 in the SD group to 6.8% ± 1.9 in the SD group ($p < 0.001$).

Conclusion: These results indicate that ketogenic diet treatment improved cardiorespiratory SUDEP risk factors including seizures, bradyarrhythmia, respiratory dysfunction and thereby increased longevity in this SUDEP animal model. This presents ketogenic diet as a possible chronic dietary treatment to improve longevity in individuals at risk for SUDEP.

ERK1/2 INHIBITION ALLEVIATES NOISE-INDUCED HEARING LOSS WHILE TEMPERING DOWN THE IMMUNE RESPONSE

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Background: Damaging noise exposure is one of the most common causes of hearing loss, yet there are no Food and Drug Administration (FDA)-approved drugs to shield from this common disability. We and others have shown that activation of the mitogen activated protein kinase (MAPK) pathway occurs after noise exposure, and inhibition of this cellular pathway protects from hearing loss. Tizaterkib, formerly AZD-0364, is a novel, highly selective, orally bioavailable ERK1/2 inhibitor that is currently in Phase-1 clinical trials. Infiltration of immune cells following noise exposure could be a possible mechanism that is leading to hearing loss, and some studies suggest that activation of the MAPK pathway could be regulating this immune response. In this study, we show that low doses of the drug that are equivalent to the doses tested now for cancer treatment, protect mice from NIHL and explore the mechanism of protection that the drug is working through.

Significance of Problem: Noise-induced hearing loss afflicts millions of Americans every year, yet there are no FDA drugs to prevent this highly prevalent disability. Additionally, the underlying mechanisms of noise-induced hearing loss are not fully elucidated; therefore, understanding the cellular pathways activated following noise exposure will help develop therapeutics to protect from hearing loss.

Hypothesis: Activation of the MAPK pathway following noise exposure is acting as a cellular stress pathway in these post-mitotic cochlear cells and ERK 1/2 inhibition lowers this stress response and protects mice from hearing loss.

Experimental Design: Different treatment schedules and dosages of tizaterkib were tested to determine the drug's optimal regimen, and minimum effective dose to protect from NIHL. The auditory brainstem response (ABR) was utilized to measure overall hearing function in mice, and distortion product otoacoustic emission (DPOAE) was used to measure outer hair cell function after noise and tizaterkib treatment. Whole mount cochlear sections were stained with anti-myosin VI and anti-Ctbp2 antibodies to measure noise-induced synaptopathy. Cochlear cryosections were stained with anti-CD45 antibody and DAPI, and cochlear western blots were probed with anti-CD45 and anti-CD68 antibodies to determine the effect that tizaterkib has on immune cell infiltration following noise exposure.

Results: Tizaterkib significantly protects 2 different strains of mice from permanent NIHL with a dose of 0.5 mg/kg administered twice a day for 3 days, starting 24 hours after noise exposure of 100-dB SPL or 106-dB SPL for 2 hours. Tizaterkib-treated mice have significantly lower ABR (average 20-25 dB in three frequencies) and DPOAE threshold shifts compared to noise alone mice. Additionally, mice treated with tizaterkib have more ctbp2 puncta per IHC and larger ABR wave 1 amplitudes compared to noise alone mice. Furthermore, tizaterkib treatment significantly lowers the number of CD45 and CD68 positive cells in the cochlea on days 4 and 6 following noise exposure. Tizaterkib treatment was also confirmed to protect mice from NIHL through inhibition of the MAPK pathway by utilizing the KSR1 KO genetic mouse model.

Conclusions: 0.5 mg/kg given twice a day is the mouse equivalent to the doses that humans are currently receiving in Phase-1 clinical trials. Tizaterkib has a therapeutic window greater than 50 in mice to protect from NIHL. Tizaterkib offers significant protection from NIHL while lowering the number of immune cell infiltrates into the cochlea which could be a possible protective mechanism of MAPK inhibition.

ABSTRACT TITLE: THE ROLE OF ASTROCYTE NLRP6-DEPENDENT PYROPTOSIS IN METHAMPHETAMINE-MEDIATED NEUROINFLAMMATION.

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Background: Methamphetamine (Meth) is a highly addictive and widely abused psychostimulant that causes severe global and economic challenges. Meth abuse impair normal CNS hemostasis with accompanying neuroinflammation and neuropathology by activating glial cells and neurons in the brain. There is mounting evidence suggesting that inflammasome proteins play a crucial role as mediators of cellular reactivation, impacting various essential biological processes.

Significance of Problem: However, it is unclear how Meth interacts with inflammatory processes, contributing to neuroinflammation, and exacerbating brain damage.

Hypothesis, Problem, or Question: We study the involvement of the astrocyte-specific inflammasome, NOD-like receptor family, pyrin domain-containing protein 6 (NLRP6), in Meth-induced astrocytic reactivation and ensuring neuroinflammation.

Experimental Design: We determine the dose and time-dependent expression of astrocyte reactivation and NLRP6-dependent pyroptosis in mouse primary astrocytes using western blot. Also, we assessed the expression of NLRP6 protein and its signaling mediators in mouse primary astrocytes by western blot, ASC Oligomerization assay, qPCR, ELISA and propidium fluorometry assay. We utilized genetic silencing approach to determine the role of NLRP6-inflammasome in Meth-induced cellular reactivation and pyroptotic cell death. Additionally, we analyzed the expression levels of brain-enriched miR-152-3p and its regulatory role in NLRP6 expression in Meth-exposed astrocytes using miScript PCR assay, qPCR, and western blot. In vivo experiments were conducted by administering Meth to mice and analyzing the frontal cortices, striatum, and hippocampus to validate NLRP6 inflammasome signaling.

Results/Data: Meth exposure resulted in dose-dependent upregulation of GFAP and NLRP6-dependent pyroptosis in astrocytes. Time-dependent upregulation of NLRP6 protein and its signaling mediators was also observed. Gene silencing experiments confirmed the involvement of NLRP6-inflammasome signaling in Meth-induced cellular activation and pyroptotic cell death. Meth-exposed astrocytes exhibited decreased miR-152-3p expression and increased NLRP6 levels, thereby suggesting epigenetic regulation of NLRP6 in both astrocyte activation and pyroptosis. In vivo analysis of Meth-exposed mice corroborated astrocyte reactivation and NLRP6-dependent cascade in the frontal cortex, striatum and hippocampus, thereby implicating Meth-impaired effects.

Conclusion: The study underscores the importance of astrocyte-specific NLRP6 inflammasome in Meth-induced neuroinflammation. Targeting this pathway could mitigate the detrimental effects of Meth on CNS pathology.

ALCOHOL USE IMPAIRS NK CELL METABOLISM AND EFFECTOR FUNCTION

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Departments of [1]Internal Medicine-Pulmonary Division, [2]Pharmacy Practice and Science, and [3]Environmental, Agricultural and Occupational Health, University of Nebraska Medical Center, Omaha, NE, USA; [4]Department of Veterans Affairs Nebraska, Western Iowa Health Care System, Omaha, NE, USA. [5]Nebraska Food for Health Center, University of Nebraska-Lincoln, Lincoln, NE, USA.

Introduction: Alcohol misuse accounts for approximately 3.3 million deaths per year globally (~5.3% of all deaths). Alcohol misuse is a well-established independent risk factor for bacterial pneumonia. Our lab was the first to determine that gut dysbiosis due to alcohol misuse increases bacterial pneumonia risk. Our previous studies have demonstrated attenuated natural killer (NK) cell recruitment to the lungs during bacterial infection in binge-on-chronic alcohol-fed mice. Additionally, supplementation with indole, a metabolite produced by gut bacteria, restored NK cell recruitment to the lung. Furthermore, we discovered that the NK cell aryl hydrocarbon receptor (AhR) and transforming growth factor-β1 (TGF-β1) signaling pathways are significantly modified under the effect of alcohol. However, the mechanism in which NK cell function is altered via these pathways is still currently unknown. Our current hypothesis is that alcohol modulates NK cell metabolism, resulting in decreased effector function (antimicrobial peptide production and trafficking), as well as decreased bacterial clearance and increasing mortality.

Methods: Utilizing both *in vitro* and *in vivo* models we sought to evaluate the effects of alcohol on NK cell effector function. Briefly, 8 to 12-week-old female C57BL/6 mice were placed onto a 5% vol/vol ethanol containing liquid diet or control-liquid diet. Control mice were pair-fed to account for any variation in caloric consumption by ethanol-fed mice. Ethanol-fed mice will be administered 4 g/kg (24.03% vol/vol) ethanol by gavage (binge) following 5 and 10 days of chronic-ethanol consumption. Mouse splenic NK cells were then isolated via a negative-selection based magnetic bead kit and cultured at 37°C in 5% CO_2 for 6 days in RPMI 1640 media containing 5% FBS, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, 100 IU/ml penicillin, 100ml/ml streptomycin, 0.05 mM 2-mercaptoethanol, and 1000 IU/ml of mouse IL-2. NK cell bactericidal capacity agianst *K. pneumoniae*, as well as NK cell migratory capacity was then assessed. Finally, NK cell metabolism was assessed using the XFe96 extracellular flux analyzer.

Results: We found that NK cell bactericidal activity was dependent on the production of α-defensin and was decreased in NK cells isolated from alcohol-fed mice. We also discovered that NK cells treated with alcohol exhibited a significant decrease in oxygen consumption rate (OCR) compared to untreated NK cells, in addition to reduced basal respiration, maximal respiration, spare respiratory capacity, non-mitochondrial oxygen consumption, ATP-coupled respiration, as well as increased proton leak. Likewise, we observed decreases in both percent coupling efficiency and percent spare respiratory capacity and observed a distinct decrease in extracellular acidification rate (ECAR) in alcohol-treated NK cells. Most striking, we found that supplementation with indole in alcohol treated NK cells partially recovered both OCR and ECAR.

Conclusion: Together these data suggest that NK cell effector function and metabolism are significantly impaired by alcohol. These data also suggests that alcohol misuse increases bacterial pneumonia risk, in part, via metabolic disarrangement of NK cells. However, the mechanism by which NK cell metabolism is impaired by alcohol, alcohol-related gut dysbiosis, and TGF-β1/AhR signaling requires additional investigation.