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The effect of resveratrol on neurodegeneration and blood brain barrier stability surrounding intracortical microelectrodes

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ABSTRACT

The current study seeks to elucidate a biological mechanism which may mediate neuroinflammation, and decreases in both blood–brain barrier stability and neuron viability at the intracortical microelectrode-tissue interface. Here, we have focused on the role of pro-inflammatory reactive oxygen species. Specifically, adult rats implanted within intracortical microelectrodes were systemically administered the anti-oxidant, resveratrol, both the day before and the day of surgery. Animals were sacrificed at two or four weeks post-implantation for histological analysis of the neuroinflammatory and neurodegenerative responses to the microelectrode. At two weeks post-implantation, we found animals treated with resveratrol demonstrated suppression of reactive oxygen species accumulation and blood–brain barrier instability, accompanied with increased density of neurons at the intracortical microelectrode-tissue interface. Four weeks post-implantation, animals treated with resveratrol exhibited indistinguishable levels of markers for reactive oxygen species and neuronal nuclei density in comparison to untreated control animals. However, of the neurons that remained, resveratrol treated animals were seen to display reductions in the density of degenerative neurons compared to control animals at both two and four weeks post-implantation. Initial mechanistic evaluation suggested the roles of both anti-oxidative enzymes and toll-like receptor 4 expression in facilitating microglia activation and the propagation of neurodegenerative inflammatory pathways. Collectively, our data suggests that short-term attenuation of reactive oxygen species accumulation and blood–brain barrier instability can result in prolonged improvements in neuronal viability around implanted intracortical microelectrodes, while also identifying potential therapeutic targets to reduce chronic intracortical microelectrode-mediated neurodegeneration.

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1. Introduction

Intracortical microelectrodes allow the activity of many individual neurons from specific structures within the brain to be analyzed over time [1]. Chronic microelectrode recordings in paralyzed humans provide a way for neural signals to be used to directly control various useful assistive devices, or to directly restore motor or sensory function with high sensitivity [2]. Additionally, chronic recordings in animals can advance our fundamental understanding of brain function in various normal and disease states. Unfortunately, the signal quality and the length of time that useful signals can be recorded are inconsistent [3–6]. Thus, intracortical microelectrodes have yet to be widely clinically implemented.

Saxena et al. recently provided the first direct evidence suggesting a strong impact of the chronic blood–brain barrier breach on intracortical microelectrode function [5]. Rennaker et al. also demonstrated that anti-inflammatory treatment both reduced the neuroinflammatory response to intracortical microelectrodes, and increased the longevity and quality of functional neural recordings [4]. However, neither directly addressed the fluctuating instability of chronic intracortical recordings.

The signal to noise ratio and the mean number of driven channels obtained from chronic intracortical microelectrodes can rapidly decrease over the first two weeks post-implantation [5,7,8]. Additionally, the quality of the neural recordings temporarily

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improve around four weeks post-implantation [3,5]. However, Bellamkonda recently reported a second significant decline in the quality of neural recordings between thirteen and sixteen weeks post-implantation [5]. Interestingly, the time course of the fluctuations in the stability of neural recordings is also consistent with our recent observations that the long-term neuroinflammatory response to traditional intracortical microelectrodes is multi-phasic [9]. Specifically, we found that neuronal populations around the electrode interface were greatly reduced at both two and sixteen weeks post-implantation despite neuronal recovery between four and eight weeks. Therefore, the above studies collectively provided important information regarding how the biphasic neuroinflammatory response to microelectrodes directly correlates with the instability of neural recordings.

Several groups have investigated possible strategies for reducing the inflammatory response to microelectrodes, with varying degrees of success [4,10–15]. Some of the most successful approaches to mitigate the neuroinflammatory response have indicated a dominant role of reactive microglia cells and infiltrating macrophages, as well as the stability of the local blood–brain barrier [5,16,17]. The release of pro-inflammatory cytokines, chemokines and reactive oxygen species by activated inflammatory cells can directly result in the breakdown of the blood–brain barrier and decreased neuron health [18]. However, only a causal role of monocyte chemotactic protein-1 (MCP-1) and tumor necrosis factor-alpha (TNF-α) have been suggested to facilitate blood–brain barrier instability and neuronal loss at the microelectrode-tissue interface [13,19]. Further, an area that has been particularly under explored in the context of microelectrode-mediated neurodegeneration is the role of reactive oxygen species.

Inflammatory-generated reactive oxygen species serve as signaling molecules to facilitate pro-inflammatory events. Reactive oxygen species have been implicated to facilitate blood–brain barrier stability, both directly [20], and indirectly through the initiation of matrix metalloproteinases release [21]. Further, reactive oxygen species accumulation leads to oxidative stress on local cells. Hallmark neurotoxic events of oxidative stress include: reactive oxygen species and nitric oxide species formation, damage of DNA, proteins, and lipids, and mitochondrial dysfunction [22]. Thus, reactive oxygen species can self-perpetuate the chronic neuroinflammatory response by inducing secondary blood–brain barrier breach and cellular damage.

Sarker et al. have demonstrated that induced blood–brain barrier permeability can be fully prevented by sequestering reactive oxygen species with administration of anti-oxidative enzymes [23,24]. Additionally, McAuthur et al. demonstrated that apoptotic neurons can be rescued if the pro-inflammatory stimulus is removed [25]. Therefore, it is likely that reduction of the accumulation of reactive oxygen species at the intracortical microelectrode-tissue interface could result in a direct improvement in blood–brain barrier stability and neuronal health.

Consequently, the current study is designed to investigate the feasibility of targeting reactive oxygen species accumulation, as a means to preserve blood–brain barrier stability and neuronal health. The current study focused on role of reactive oxygen species during only the initial instability in the biphasic neuroinflammatory response to microelectrodes. We hypothesized that reactive oxygen species formation is a key mediator in blood brain barrier stability and neuronal health at the intracortical microelectrodes–tissue interface. Specifically, using a rat intracortical microelectrode implantation model, we have investigated the use of resveratrol, an anti-oxidant derived from grapes [26], in mitigating oxidative stress and neuroinflammatory events after device implantation. Evaluation of inflammatory events around the microelectrode included neuroinflammatory cells profiles, neuronal nuclei density and expression of key enzymes involved in reactive oxygen species breakdown, at both two and four weeks after implantation. Finally, we also investigated the effect of mitigating reactive oxygen species accumulation on a stab wound model to explore the potential consequences on wound healing.

2. Materials and methods

2.1. Animals and surgical implantation

Sixty-five male Sprague Dawley rats (225–300 g) (Charles River) were used in this study and allowed to survive for either two or four weeks. A minimum of four animals was used for each implanted condition, for each staining paradigm, at each time point. In addition to surgical implantation, a minimum of two non-implanted age-matched and condition-matched controls were assessed for all time points. Surgical procedures closely followed our established protocols [9,27]. All procedures and animal care practices were performed in accordance with the Louis Stokes Department of Veterans Affairs and Case Western Reserve University Institutional Animal Care and Use Committees.

Briefly, initial anesthesia for animals was obtained using an intraperitoneally (IP) injected mixture of ketamine (80 mg/kg) and xylazine (10 mg/kg). Isoflurane (0.5–2%) was used to maintain anesthesia for the duration of the procedure. Depth of anesthesia was monitored using both vitals (heart rate and blood-oxygen content) and toe-pinch reflex. Prior to surgical preparation, the animal received cefazolin (16 mg/kg) and meloxicam (1 mg/kg) subcutaneously (SQ) to prevent infection and to manage pain. Once anesthetized, the surgical area was thoroughly shaved and then the animal was mounted onto a stereotaxic frame. Local anesthesia was provided using an SQ injection of Marcaine (0.25%) at the incision site. Prior to incision, a sterile surgical field was obtained by scrubbing the shaved area with alternating passes of betadine solution and 70% isopropanol. Animal temperature was maintained using a circulating water heating pad, placed below the stereotaxic frame. Ophthalmic ointment was used throughout the procedure to prevent retinal drying.

For surgical implantation, first, the skull was exposed with a one-inch incision down midline and surrounding tissue retracted. To expose the brain tissue, a 3-mm hole was created by hand in the skull using a biopsy punch (PSS Select) approximately 3 mm lateral to midline and 4 mm caudal to bregma. The dura was gently reflected using a 45° dura pick (Fine Science Tools) to minimize vascular damage. Then, a sterile 2 mm × 123 μm × 15 μm non-functional single shank 'Michigan-style' electrode containing a 1 mm × 1 mm tab (APT Center) was inserted carefully by hand until the base of the tab reached the cortical tissue, avoiding visible vasculature. In the case of stab wounds, the electrode was inserted for ~10 s and then removed from the tissue space. All animals received both a chronic implant and a control stab wound in contralateral hemispheres. Prior to addition of dental acrylic, silicone gel (Kwik-Sil, World Precision Instruments) was applied over both craniotomies to prevent cortical tissue dehydration. Chronically implanted electrodes were then securely anchored to the skull using either ultra-violet (UV) curing dental acrylic (Fusio/Flow-it ALC, Pentron Dental) or self-curing dental acrylic (Stoelting Co.). In the case of self-curing acrylic, acrylic was tethered to 3 screws that were placed directly into the bone, without extending into the cortical space, 3 mm rostral to bregma or 3 mm rostral to lambda. The surgical site was closed using 5-0 monofilament polypropylene suture. Triple antibiotic ointment was applied following suturing to prevent drying and localized infection around the incision. To minimize variability, the same surgeon performed all implantation surgeries.

2.2. Resveratrol preparation and administration

To investigate effects of resveratrol in our electrode implantation model, three conditions were investigated in chronically implanted and stab wounded animals: (1) no dose (control), (2) dosed with resveratrol and (3) dosed with diluent. In addition, to account for any alterations that resveratrol could have on normal cortical homeostasis, non-implanted sham controls (control 3) were also administered resveratrol. Following established protocols [28–30], animals received either resveratrol or diluent both 16–24 h before surgical implantation as well as directly following the craniotomy. In the case of non-implanted sham controls, two doses of resveratrol were given within 16–24 h of each other.

Here, resveratrol (Astatech, Inc.) was administered IP at a dose of 30 mg/kg. Injections were prepared by first dissolving resveratrol at 50 mg/mL in 50% ethanol (diluted from 95% in water) (Fisher) at 50 °C. Then, the appropriate amount of resveratrol stock solution per animal was collected in a syringe and directly diluted to a final concentration of 2% ethanol with sterile saline solution. Injections were prepared immediately before use and slightly heated prior to injection to increase solubility. In the case of diluent controls, injections were prepared identically, without resveratrol.

2.3. Tissue extraction and processing

To gain a detailed understanding of the role oxidative stress contributes to acute neuroinflammatory and neurodegenerative events at the cortical tissue–device