Drug Treatment Prevents Primary Blast-induced Deficit in Long-term Potentiation in Rat Brain Slice Cultures

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I. INTRODUCTION

Traumatic brain injury (TBI) has been diagnosed in nearly 340,000 US service personnel since 2000, with 82.5% of cases classified as mild [1]. These mild TBIs are most often caused by blast forces [2]. Common symptoms of these injuries include loss of consciousness, loss of spatial navigation, behaviour/mood changes and memory loss [3]. Blast-related TBI poses a significant problem for military personnel. The effect of primary blast loading, caused by shock wave interaction with the skull and brain, remains debated. We have previously reported the damaging effects of primary blast on brain electrophysiological function, specifically in a process known as long-term potentiation (LTP) [4-5]. This process is the functional correlate of memory formation [6]. Currently, there is no clinically approved treatment for TBI. This short communication investigates a potential drug treatment (roflumilast) to prevent primary blast-induced LTP loss in rat brain organotypic hippocampal slice cultures (OHSCs).

II. METHODS

Rat OHSCs (400 µm thick) were excised from the brains of P7-P10 Sprague-Dawley pups, cultured on porous Millipore Millicell cell culture inserts, and fed every two days for 10-14 days. Blast injury was then initiated using a compressed-gas driven shock tube with the cultures placed in a fluid-filled receiver directly below the shock tube exit. Cultures were subjected to either mild blast exposure (336 kPa/0.84 ms/87 kPa·ms) or sham control. Immediately following sham or blast exposure, cultures were placed into full serum media containing either 1µM roflumilast or vehicle (0.07% Dimethyl Sulfoxide).

Electrophysiological recordings were acquired 24 hours following injury. At the time of recording, slices were placed onto 60-channel microelectrode arrays and perfused with artificial cerebral spinal fluid. Basal neuronal firing capacity was evaluated using stimulus-response curves. Stimuli from 0-200 µA (in 10 µA increments) were applied across the Schaffer collateral (SC) pathway to generate S/R curves. Each electrode's response was fit to a sigmoidal curve, defined by specific parameters with physiological meaning: the maximum amplitude of the evoked response (R_{max}), the current necessary to generate a half-maximal response (I_{50}), and the synchronicity of the firing neurons (m). Pre-LTP recordings were captured by application of I_{50} stimuli once a minute for 30 minutes. LTP was induced in CA1 via the SC pathway using 100Hz tetanic constant-current (I_{50}) stimuli. Post-LTP recordings were captured by application of I_{50} stimuli once a minute for 60 minutes post-induction. Potentiation was calculated as the change in average response over the last 10 minutes of post-LTP recordings divided by the average response over the last 10 minutes of pre-LTP recordings.

The effect of drug treatment on cell viability was observed through propidium iodide staining prior to blast or sham exposure and at 24 hours post-exposure.

III. INITIAL FINDINGS

Following blast injury, roflumilast treatment prevented a significant deficit in LTP (69 ± 14%), as compared to vehicle-treated injured cultures (28 ± 11%). Roflumilast treatment did not enhance LTP in sham exposed cultures (78 ± 11%) over vehicle treatment (79 ± 13%). The effects of blast injury and drug treatment were analysed statistically with analysis of variance, where significance was considered $p < 0.05$ (N = 6 cultures). Drug treatment had no significant effect on cell viability (cell death < 5% for all groups) in any anatomical region of interest.

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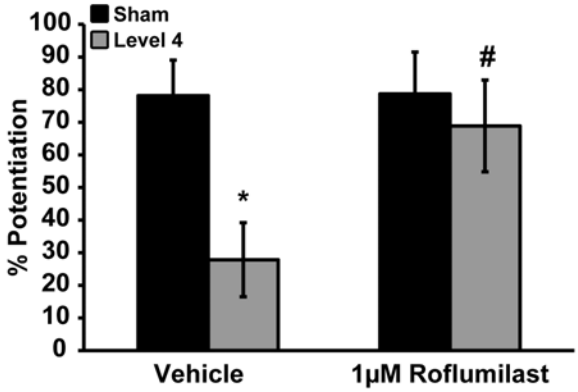

Fig. 1. Roflumilast treatment immediately following injury prevented blast-induced deficits in LTP measured 24 hours post-injury (± SEM). LTP was significantly decreased when cultures were treated with DMSO vehicle immediately following mild blast exposure [Pressure: 336 kPa, Duration: 0.84 ms, Impulse: 87 kPa-ms, n = 6]. Roflumilast treatment immediately post-injury prevented a significant deficit in LTP [n = 6]. A univariate general linear model was used to assess the effect of injury and treatment, with percent potentiation as the unique dependent variable and experimental group as the fixed factor (*p< 0.05 as compared to treatment-matched sham, #p<0.05 as compared to injury-matched vehicle).

IV. DISCUSSION

We report that roflumilast treatment prevented primary blast-induced LTP loss. PDE4 inhibitors have shown promise as a treatment to prevent memory loss after non-blast TBI *in vivo* [7-8]. While the injury biomechanics are different between our blast model and the non-blast TBI models used in previous studies, it is evident that PDE4 inhibitors possess therapeutic potential. Roflumilast is currently clinically approved by the US Food and Drug Administration for treatment of chronic obstructive pulmonary disorder, making it an attractive candidate for clinical trials in TBI. Future work will confirm the cellular mechanisms responsible for this therapeutic effect following primary blast injury.

V. REFERENCES

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