Neuroscience Letters 532 (2013) 1–6

Contents lists available at SciVerse ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Plenary article

Effects of early rolipram treatment on histopathological outcome after controlled cortical impact injury in mice

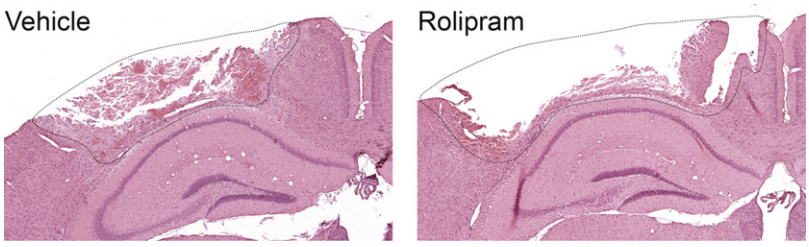
Coleen M. Atkins*, Maria L. Cepero, Yuan Kang, Daniel J. Liebl, W. Dalton Dietrich

The Miami Project to Cure Paralysis, Department of Neurological Surgery, University of Miami Miller School of Medicine, Miami, FL 33136-1060, United States

HIGHLIGHTS

- We investigated the effects of rolipram on controlled cortical impact (CCI) injury.
- Rolipram treatment increased cortical contusion volume.
- Rolipram did not affect neuronal loss in the hippocampus resulting from CCI.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Cyclic AMP
Contusion
Controlled cortical impact
Mice
Phosphodiesterase
Rolipram

ABSTRACT

Traumatic brain injury (TBI) pathology includes contusions, cavitation, cell death, all of which can be exacerbated by inflammation. We hypothesized that an anti-inflammatory drug, rolipram, may reduce pathology after TBI, since in several CNS injury models rolipram reduces inflammation and improves cell survival and functional recovery. Adult male C57BL/6 mice received a craniotomy over the right parietotemporal cortex. Vertically directed controlled cortical impact (CCI) injury was delivered. Naïve controls were used for comparison. At 30 min post-surgery, animals were treated with vehicle or rolipram (1 mg/kg), and then once per day for 3 days. On day 3, the brains were systematically sectioned and stained to visualize the resulting pathology using hematoxylin and eosin (H&E) staining and NeuN immunocytochemistry. Total parietotemporal cortical contusion and cavity volume were significantly increased in rolipram-treated as compared to vehicle-treated CCI animals. Contusion areas at specific bregma levels indicated a significant effect of drug across bregma levels. Neuronal cell loss in the dentate hilus and area CA3 of the hippocampus were similar between vehicle and rolipram-treated animals. Although rolipram is well known to reduce pathology and inflammation in several other CNS injury models, the pathology resulting from CCI was worsened with rolipram at this particular dose and administration schedule. These studies suggest that consideration of the unique characteristics of TBI pathology is important in the extrapolation of promising therapeutic interventions from other CNS injury models.

© 2012 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Traumatic brain injury (TBI) is a serious health problem affecting approximately 1.7 million Americans annually [8,43]. Experimental and clinical research has delineated the etiology of many pathomechanisms of TBI and several potential therapeutics have been identified [23,25,31]. However, limited clinical studies have reported beneficial effects of various pharmacological treatments [22]. Thus, there is a need to evaluate novel therapeutic interventions that target a range of cellular and molecular events and

Abbreviations: BBB, blood–brain barrier; CCI, controlled cortical impact; cAMP, cyclic AMP; H&E, hematoxylin and eosin; i.p., intraperitoneal; PDE, phosphodiesterase; Sur1, sulfonylurea receptor 1; TBI, traumatic brain injury.

* Corresponding author at: Department of Neurological Surgery, The Miami Project to Cure Paralysis, University of Miami Miller School of Medicine, 1095 NW 14th Terrace, 3-17, Miami, FL 33136-1060, United States. Tel.: +1 305 243 4698; fax: +1 305 243 3914.
E-mail address: catkins@med.miami.edu (C.M. Atkins).

0304-3940/$ – see front matter © 2012 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.neulet.2012.10.019

promote protection and repair in the complex, heterogeneous TBI patient population.

One of the major injury mechanisms investigated in animal models of brain injury is activation of inflammatory pathways [26]. After trauma, blood–brain barrier (BBB) dysfunction is acutely observed as well as activation of inflammatory cells including microglia, invading neutrophils, and macrophages/monocytes [21]. Activation and recruitment of inflammatory cells into the injured brain generates inflammatory cytokines, free radicals and other damaging molecules [16,42]. Clinical studies have corroborated the increases in pro-inflammatory cytokines and chemokines using biomarkers [11,39]. Thus, a major area of continued therapeutic development is the identification and testing of novel drugs that target excessive inflammatory events acutely after injury [25].

Previous brain and spinal cord injury studies have investigated drug candidates that modulate cyclic AMP (cAMP) signaling by targeting type 4 phosphodiesterase (PDE) isoforms found in inflammatory cells and neurons [1,14,33]. Inhibition of PDE4 with rolipram decreases inflammation after CNS injury [4,12,30]. Recently, our laboratories have reported that rolipram reduced levels of the proinflammatory cytokines TNFα and IL-1β when given immediately prior to fluid-percussion brain injury [3]. In that study, pretreatment with rolipram significantly reduced histopathological damage by decreasing contusion volume, hippocampal cell death and axonal injury. To move this potential therapeutic to a more translational model of TBI, the effects of post-injury treatment with rolipram were next evaluated. Rolipram treatment initiated 30 min after fluid-percussion brain injury again reduced TNFα levels, but did not significantly reduce contusion volume [2]. Histopathological outcome was actually worsened and associated with an increase in cortical hemorrhage and BBB permeability. This outcome could not be explained by any rolipram-induced systemic effects including acute hypotension. These results suggest that caution is required when considering rolipram as a post-injury therapeutic and indicates a critical need to further clarify the neuroprotective and anti-inflammatory potential of PDE4 inhibitors.

TBI is a highly heterogeneous clinical problem with a wide range of pathologies and behavioral consequences [22]. To investigate the different types of TBI, models of diffuse and focal injury are used with several animal species [23,38]. In previous TBI studies with rolipram, we utilized the parasagittal fluid-percussion brain injury model which produces a cortical contusion associated with selective neuronal vulnerability and diffuse axonal pathology [3]. This model is treatable with several pharmacological agents, environmental enrichment, and therapeutic hypothermia [24,28,41]. Another model of brain injury is controlled cortical impact (CCI) injury [6]. This model produces well-demarcated cortical infarcts that mimic the contusions commonly observed in severe TBI patients. Because pathophysiological injury cascades are known to be dependent on impact severity and location, it is important to determine whether post-injury rolipram treatment would alter histopathological outcome in a second TBI model with distinct histopathological characteristics. The overall purpose of this study was therefore to evaluate whether rolipram, at a dose previously found to improve outcome after CNS injury, would reduce histopathological damage when given 30 min after CCI injury in mice [4,15,19].

2. Materials and methods

All surgical procedures were in compliance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the University of Miami Animal Care and Use Committee. Adult C57BL/6 male mice (2–3 months, Charles River Laboratories, Wilmington, MA, USA) were anesthetized with ketamine and xylazine (intraperitoneal, i.p.). Body temperature was monitored with a rectal probe and maintained at 37 °C. A 5 mm craniotomy was made over the right parietotemporal cortex (−2.5 mm posterior, 2.0 mm lateral from bregma). Injury was induced using the ECCI-6.3 device (Custom Design & Fabrication, Richmond, VA, USA) with a 3 mm impounder at 6 m/s velocity, 0.5 mm depth, 150 ms impact duration [6,40].

Rolipram (Sigma–Aldrich, St. Louis, MO, USA) was administered at 1 mg/kg (i.p.) 30 min post-injury [2]. Rolipram or vehicle (6 ml/kg, 5% DMSO in saline) were given once per day for 3 days ($n = 6$ mice/treatment).

At 3 days after TBI, animals were anesthetized and perfused transcardially with 4% paraformaldehyde and brains were paraffin embedded and sectioned at 10 μm thick. Serial sections (150 μm apart) were stained with hematoxylin and eosin (H&E). Contusion and cavity volumes were assessed by an investigator blinded to the treatments by tracing H&E stained sections using Neurolucida 10.50 (MicroBrightField, Williston, VT, USA) and an Olympus BX51TRF microscope (Olympus America, Center Valley, PA, USA) with a 4× objective. Cavity boundaries were determined by tracing the contralateral cortex, and projecting the inverted contour over the ipsilateral cortex. Contusion boundaries were demarcated by pyknotic neurons, reactive astrocytes, hemorrhage, and edema. Contusion areas were calculated for four coronal levels at or around the epicenter (−1.1, −1.6, −2.7, −3.3 mm posterior from bregma). Images were taken at 20×.

To determine hippocampal neuronal survival, adjacent sections (150 μm apart) were immunostained with NeuN (1:500, Millipore, Temecula, CA, USA). Immunostaining was developed with anti-mouse IgG (1:1000, Vector Laboratories, Burlingame, CA, USA), ABC elite (Vector Laboratories) and NiDAB (2.5%, nickel ammonium sulfate acetate–imidasole buffer, 0.05% DAB, 0.001% H_2O_2).

NeuN-positive cells were quantified using stereology and an Axiovert 200 M microscope (Carl Zeiss MicroImaging, Inc., Thornwood, NY, USA). The dentate hilus and area CA3 of the hippocampus were contoured at 5× magnification between bregma levels −1.6 to −2.7 mm (dentate hilus) or between bregma levels −1.9 to −2.1 mm (CA3) [3]. Using StereoInvestigator (10.50, MicroBrightField), a 40 μm × 65 μm grid was placed over the hilus and a 55 μm × 110 μm grid was placed over area CA3. Neurons were counted within a 35 μm × 35 μm frame at 100× (NA 1.30). Q values for the dentate hilus were 69–192 (mean 122 ± 6.6), the CE^2/CV^2 ratio for vehicle-treated animals was 0.20 (ipsilateral) and 0.33 (contralateral), and the CE^2/CV^2 ratio for rolipram-treated animals was 0.82 (ipsilateral) and 0.14 (contralateral). Q values for CA3 were 80–343 (mean 173 ± 10.4), the CE^2/CV^2 ratio for vehicle-treated animals was 0.17 (ipsilateral) and 0.05 (contralateral), and the CE^2/CV^2 ratio for rolipram-treated animals was 0.30 (ipsilateral) and 1.24 (contralateral). Images were taken at 20×.

Data are mean ± SEM. Contusion volumes were analyzed using Student's t-test. Contusion areas were analyzed with a repeated measures two-way ANOVA (factors: group × bregma level), followed by Bonferroni t-tests. Dentate hilus and CA3 cell counts were analyzed using a two-way ANOVA with hemisphere (ipsilateral vs contralateral) and drug (vehicle vs rolipram) as the factors and post hoc Bonferroni t-tests.

3. Results

At 3 days after CCI injury, a well demarcated contusion and cavity were observed in both treated and non-treated TBI animals. Rolipram increased contusion volume after CCI injury as compared to vehicle-treated controls (Fig. 1). In addition, contusion areas at specific bregma levels (−1.1, −1.6, −2.7, −3.3 mm) were analyzed to evaluate the treatment effects at multiple brain regions. A main

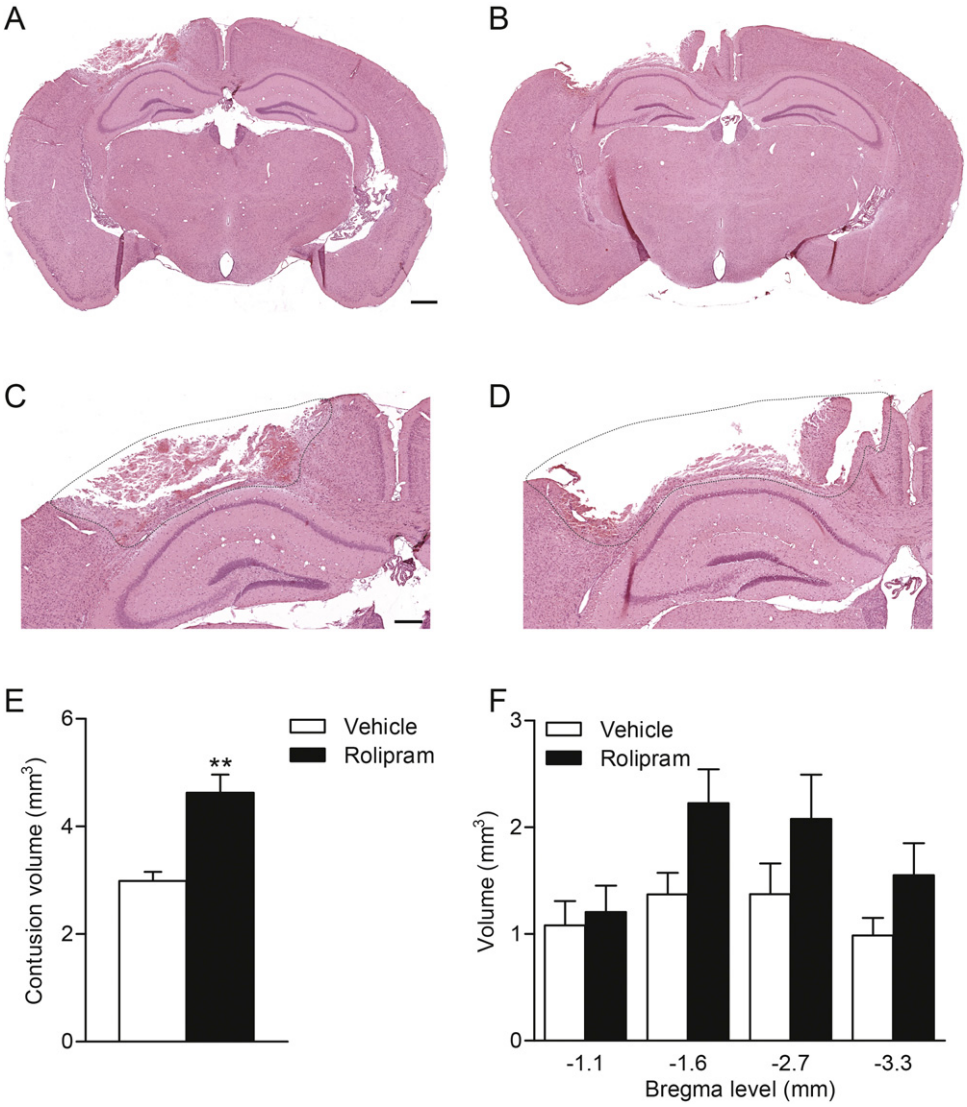


Fig. 1. Cortical contusions after CCI injury in mice. Representative images of H&E stained sections at −2.4 mm posterior from bregma for vehicle-treated (A) and rolipram-treated (B) mice. Scale bar 500 μm. Magnification of the contused cortex for vehicle (C) and rolipram (D) treatment. The contoured contusion and cavity areas are denoted by the dashed lines. Scale bar 250 μm. Total contusion volume quantification (E) and contusion areas across bregma levels (F). Mean ± SEM, $n = 6$/group, $^{**}p < 0.01$ for CCI + vehicle vs CCI + rolipram.

effect of drug ($F_{(1,10)} = 7.42$, $P = 0.0214$) was observed. No main effect of bregma level and no significant interaction of bregma level and drug were detected.

A consistent pathology associated with CCI injury is the selective vulnerability of dentate hilar neurons. Quantitative assessment of dentate hilar cells demonstrated a significant decrease in the number of NeuN-positive cells in the ipsilateral vs contralateral hemispheres (Fig. 2). There was a main effect of hemisphere ($F_{(1,20)} = 22.48$, $P = 0.0001$), but no main effect of drug and no significant interaction of drug and hemisphere. These results indicate that rolipram did not affect overall dentate hilus cell loss.

Area CA3 of the hippocampal region is another area of vulnerability after CCI injury. Small pockets of neuronal loss were observed with CCI injury on the ipsilateral side, and this was unaffected by rolipram treatment (Fig. 3). A main effect of hemisphere was observed ($F_{(1,20)} = 17.85$, $P = 0.0004$), and no significant main effect of drug or interaction of drug and hemisphere was detected. These results indicate that rolipram treatment after CCI injury has no significant effect on neuronal loss in area CA3 and dentate hilus in the hippocampus, but did exacerbate contusional size in the cortex.

4. Discussion

In this study, we report that the PDE4 inhibitor rolipram in a mouse model of CCI injury does not improve histopathological outcome when given posttrauma. This negative study is consistent with our previous report showing that posttraumatic rolipram treatment after moderate fluid-percussion brain injury also fails to protect against neuronal damage [2]. Together, these studies emphasize the continued need to examine the potential role of phosphodiesterase inhibition in preclinical models of TBI.

Previous studies in cerebral ischemia and spinal cord injury reported beneficial effects of rolipram even in the post-injury setting [4,19,27,30]. However, with any pharmacological study, there could be side effects that abrogate the beneficial effects. In the case of rolipram, its effects on multiple PDE4 isoforms may have participated in the negative results of the present and previous TBI studies. For example, rolipram can potentially affect hemodynamic injury responses that complicate the usefulness of this drug [5,36,45]. In previous studies utilizing rats, rolipram did not significantly affect mean arterial blood pressure, blood

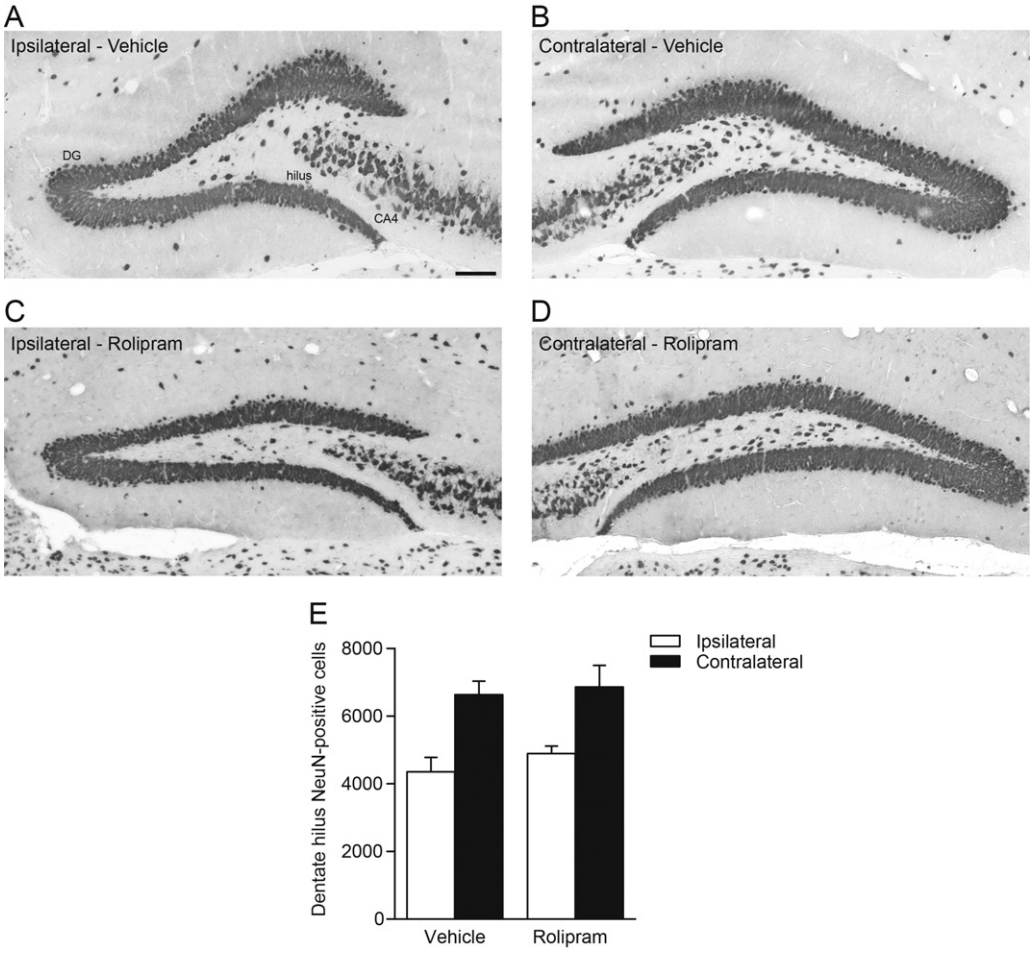


Fig. 2. Rolipram did not affect dentate hilar cell loss. Representative images of the dentate hilus immunostained with NeuN at bregma level −2.1 mm for vehicle-treated (A and B), and rolipram-treated (C and D) mice. Scale bar 100 μm. Estimated numbers of dentate hilus NeuN-positive cells between bregma levels −1.6 to −2.7 mm (E). Mean ± SEM, $n = 6$/group.

pO_2 levels or cerebral blood flow [2,3]. However, a previous study using mice found that rolipram decreased systemic arterial blood pressure [36]. Thus, it is possible rolipram may have exacerbated the cortical contusion by decreasing local cerebral blood flow to the vulnerable cortex [34]. Alternatively, rolipram may have contributed to progressive expansion of the hemorrhagic contusion. Upregulation of sulfonylurea receptor 1 (Sur1) in microvessels has been linked to hemorrhage expansion after TBI and experiments exploring the connection of PDE4 signaling and Sur1 may shed light on the molecular mechanisms of the increase in cortical contusion expansion observed with rolipram [29,37]. More basic research is needed to understand which PDE4 isoforms target vascular integrity and how rolipram could produce adverse effects in neurotrauma that lead to vascular vulnerability [45].

Rolipram affects multiple PDE4 isoforms and therefore may target multiple cell types and biological processes [10]. The PDE4 family includes 20 isozymes encoded by four genes (PDE4A, B, C and D) [13]. Loss of specific PDE4 subtypes enhances memory and promotes neurogenesis [20]. The identification of novel PDE4 inhibitors that selectively inhibit specific isoforms may be more suitable for pharmacological protection after TBI and other CNS injuries [10]. For example, PDE4 inhibition is currently being investigated as a therapeutic for depression and age-related cognitive decline [32,44]. Published data has emphasized that the PDE4A and B isoforms may be the most relevant for targeting these quality of life issues [10]. Because higher cortical functions including cognition and depression are important consequences of TBI, it will be important in future studies to test more selective PDE4 inhibitors to determine their efficacy in TBI [7,9].

In this study, we utilized a standard model of TBI with differential characteristics compared to the fluid-percussion brain injury. CCI produces a well demarcated, focal contusion that extends from the pial surface down to deep cortical layers [6]. This is associated with severe perfusion deficits at the cortical contusion [18]. We also used mice in this study in contrast to our published work with rat fluid-percussion brain injury [2,3]. The testing of various therapeutics in different injury models as well as species are important considerations for translation to the clinic [17]. The fact that post-traumatic treatment with rolipram in two models using different animal species produced negative effects emphasizes the need for additional work in this area.

In the present study, we used a dose and therapeutic route that we have found in our previous TBI study and has been shown in other CNS injury paradigms to improve histological outcome [4,12,15,19]. A recent study in spinal cord injury assessed a wide range of rolipram doses for efficacy in improving pathology [35]. In that study, rolipram doses ranging from 0.1 to 5 mg/kg improved neuronal survival with 1 mg/kg being the most efficacious, which is the dose used in this study. In addition, it is possible that rolipram may be more effective in other therapeutic routes. In our previous TBI study and in other studies of cerebral ischemia, intraperitoneal administration of rolipram significantly improved histopathological outcome and behavioral recovery [3,4,12,15]. However, a recent spinal cord injury study reported that although rolipram improved

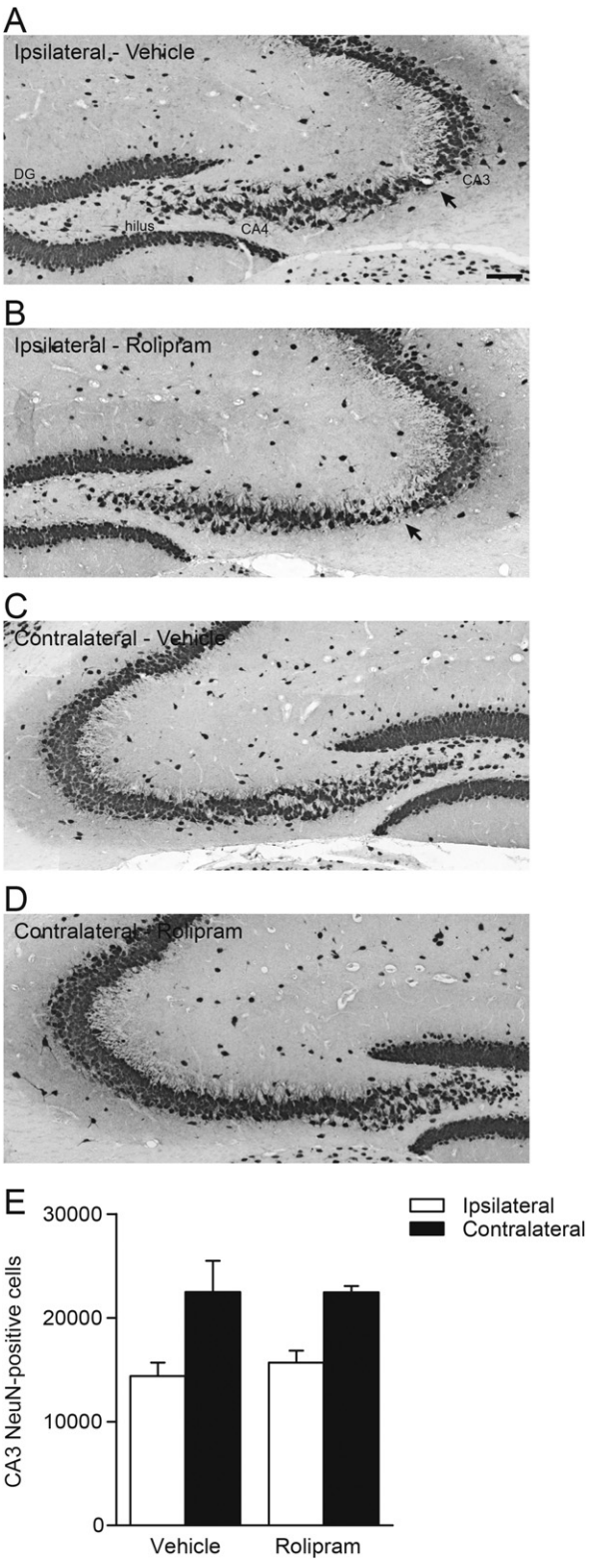


Fig. 3. Neuronal loss in area CA3 after CCI injury is not affected by rolipram treatment. Images taken at bregma level −1.9 mm for the ipsilateral side of vehicle-treated (A) and rolipram-treated animals (B) indicate small areas of neuronal loss (*arrows*). No significant cell loss was observed on the contralateral side with either vehicle (C) or rolipram treatment (D). Scale bar 100 μm. Quantification of CA3 cell loss between bregma levels −1.9 to −2.1 mm indicated a significant loss of neurons in both vehicle- and rolipram-treated animals on the ipsilateral side (E). Mean ± SEM, $n = 6$/group.

neuronal survival when given intravenously, orally, or subcutaneously, the most effective route was intravenous [35]. Additional studies are needed to test extensive dose responses and other administrative routes to maximize the chances of rolipram or other PDE4 inhibitors having a beneficial effect on their targeted pathomechanisms especially when given in the post-injury setting.

5. Conclusions

In summary, rolipram treatment failed to improve outcome after CCI injury in mice. The fact that the treatment actually increased some degree of histopathological damage in this model is important to consider as PDE4-related therapies move forward for CNS injury. The investigation of more selective PDE4 isoform-specific inhibitors is an important area for further studies. It is anticipated that as more information regarding the cellular and biochemical response to TBI is clarified, new selective blockers will be developed related to cAMP-related cell signaling cascades critical to cell survival and neuronal function.

Acknowledgements

This work was supported by NIH/NINDS NS069721 (C.M.A.), NS056072 (W.D.D.), and NS030291 (W.D.D., D.J.L.), and The Miami Project to Cure Paralysis. We thank David Sequiera for technical support.

References

[1] M. Ariga, B. Neitzert, S. Nakae, G. Mottin, C. Bertrand, M.P. Pruniaux, S.L. Jin, M. Conti, Nonredundant function of phosphodiesterases 4D and 4B in neutrophil recruitment to the site of inflammation, Journal of Immunology 173 (2004) 7531–7538.
[2] C.M. Atkins, Y. Kang, C. Furones, J.S. Truettner, O.F. Alonso, W.D. Dietrich, Postinjury treatment with rolipram increases hemorrhage after traumatic brain injury, Journal of Neuroscience Research 90 (2012) 1861–1871.
[3] C.M. Atkins, A.A. Oliva Jr., O.F. Alonso, D.D. Pearse, H.M. Bramlett, W.D. Dietrich, Modulation of the cAMP signaling pathway after traumatic brain injury, Experimental Neurology 208 (2007) 145–158.
[4] F. Block, A. Tondar, W. Schmidt, M. Schwarz, Delayed treatment with rolipram protects against neuronal damage following global ischemia in rats, Neuroreport 8 (1997) 3829–3832.
[5] Y.C. Chang, C.C. Huang, P.L. Hung, H.M. Huang, Rolipram, a phosphodiesterase type IV inhibitor, exacerbates periventricular white matter lesions in rat pups, Pediatric Research 64 (2008) 234–239.
[6] C.E. Dixon, G.L. Clifton, J.W. Lighthall, A.A. Yaghmai, R.L. Hayes, A controlled cortical impact model of traumatic brain injury in the rat, Journal of Neuroscience Methods 39 (1991) 253–262.
[7] J.R. Fann, T. Hart, K.G. Schomer, Treatment for depression after traumatic brain injury: a systematic review, Journal of Neurotrauma 26 (2009) 2383–2402.
[8] M. Faul, L. Xu, M.M. Wald, V.G. Coronado, Traumatic Brain Injury in the United States: Emergency Department Visits, Hospitalizations and Deaths, Centers for Disease Control and Prevention, National Center for Injury Prevention and Control Atlanta, GA, 2010.
[9] L.K. Gorman, B.L. Shook, D.P. Becker, Traumatic brain injury produces impairments in long-term and recent memory, Brain Research 614 (1993) 29–36.
[10] M.E. Gurney, A.B. Burgin, O.T. Magnusson, L.J. Stewart, Small molecule allosteric modulators of phosphodiesterase 4, Handbook of Experimental Pharmacology (2011) 167–192.
[11] A. Helmy, C.A. Antoniades, M.R. Guilfoyle, K.L. Carpenter, P.J. Hutchinson, Principal component analysis of the cytokine and chemokine response to human traumatic brain injury, PLoS One 7 (2012) e39677.
[12] T. Imanishi, A. Sawa, Y. Ichimaru, M. Miyashiro, S. Kato, T. Yamamoto, S. Ueki, Ameliorating effects of rolipram on experimentally induced impairments of learning and memory in rodents, European Journal of Pharmacology 321 (1997) 273–278.
[13] S.L. Jin, S.L. Ding, S.C. Lin, Phosphodiesterase 4 and its inhibitors in inflammatory diseases, Chang Gung Medical Journal 35 (2012) 197–210.
[14] S.L. Jin, L. Lan, M. Zoudilova, M. Conti, Specific role of phosphodiesterase 4B in lipopolysaccharide-induced signaling in mouse macrophages, Journal of Immunology 175 (2005) 1523–1531.
[15] H. Kato, T. Araki, Y. Itoyama, K. Kogure, Rolipram, a cyclic AMP-selective phosphodiesterase inhibitor, reduces neuronal damage following cerebral ischemia in the gerbil, European Journal of Pharmacology 272 (1995) 107–110.
[16] K. Kinoshita, K. Chatzipanteli, E. Vitarbo, J.S. Truettner, O.F. Alonso, W.D. Dietrich, Interleukin-1b messenger ribonucleic acid and protein levels after

fluid-percussion brain injury in rats: importance of injury severity and brain temperature, Neurosurgery 51 (2002) 195–203.
[17] P.M. Kochanek, H. Bramlett, W.D. Dietrich, C.E. Dixon, R.L. Hayes, J. Povlishock, F.C. Tortella, K.K. Wang, A novel multicenter preclinical drug screening and biomarker consortium for experimental traumatic brain injury: operation brain trauma therapy, Journal of Trauma 71 (2011) S15–S24.
[18] P.M. Kochanek, K.S. Hendrich, C.E. Dixon, J.K. Schiding, D.S. Williams, C. Ho, Cerebral blood flow at one year after controlled cortical impact in rats: assessment by magnetic resonance imaging, Journal of Neurotrauma 19 (2002) 1029–1037.
[19] L.X. Li, Y.F. Cheng, H.B. Lin, C. Wang, J.P. Xu, H.T. Zhang, Prevention of cerebral ischemia-induced memory deficits by inhibition of phosphodiesterase-4 in rats, Metabolic Brain Disease 26 (2011) 37–47.
[20] Y.F. Li, Y.F. Cheng, Y. Huang, M. Conti, S.P. Wilson, J.M. O'Donnell, H.T. Zhang, Phosphodiesterase-4D knock-out and RNA interference-mediated knockdown enhance memory and increase hippocampal neurogenesis via increased cAMP signaling, Journal of Neuroscience 31 (2011) 172–183.
[21] G. Lotocki, J.P. de Rivero Vaccari, E.R. Perez, J. Sanchez-Molano, O. Furones-Alonso, H.M. Bramlett, W.D. Dietrich, Alterations in blood–brain barrier permeability to large and small molecules and leukocyte accumulation after traumatic brain injury: effects of post-traumatic hypothermia, Journal of Neurotrauma 26 (2009) 1123–1134.
[22] A.I. Maas, D.K. Menon, Traumatic brain injury: rethinking ideas and approaches, The Lancet Neurology 11 (2012) 12–13.
[23] N. Marklund, L. Hillered, Animal modelling of traumatic brain injury in preclinical drug development: where do we go from here? British Journal of Pharmacology 164 (2011) 1207–1229.
[24] Y. Matsushita, H.M. Bramlett, O. Alonso, W.D. Dietrich, Posttraumatic hypothermia is neuroprotective in a model of traumatic brain injury complicated by a secondary hypoxic insult, Critical Care Medicine 29 (2001) 2060–2066.
[25] K.W. McConeghy, J. Hatton, L. Hughes, A.M. Cook, A review of neuroprotection pharmacology and therapies in patients with acute traumatic brain injury, CNS Drugs 26 (2012) 613–636.
[26] M.C. Morganti-Kossmann, M. Rancan, P.F. Stahel, T. Kossmann, Inflammatory response in acute traumatic brain injury: a double-edged sword, Current Opinion in Critical Care 8 (2002) 101–105.
[27] E. Nikulina, J.L. Tidwell, H.N. Dai, B.S. Bregman, M.T. Filbin, The phosphodiesterase inhibitor rolipram delivered after a spinal cord lesion promotes axonal regeneration and functional recovery, Proceedings of the National Academy of Sciences of the United States of America 101 (2004) 8786–8790.
[28] M.J. Passineau, E.J. Green, W.D. Dietrich, Therapeutic effects of environmental enrichment on cognitive function and tissue integrity following severe traumatic brain injury in rats, Experimental Neurology 168 (2001) 373–384.
[29] A.D. Patel, V. Gerzanich, Z. Geng, J.M. Simard, Glibenclamide reduces hippocampal injury and preserves rapid spatial learning in a model of traumatic brain injury, Journal of Neuropathology and Experimental Neurology 69 (2010) 1177–1190.
[30] D.D. Pearse, F.C. Pereira, A.E. Marcillo, M.L. Bates, Y.A. Berrocal, M.T. Filbin, M.B. Bunge, cAMP and Schwann cells promote axonal growth and functional recovery after spinal cord injury, Nature Medicine 10 (2004) 610–616.
[31] R. Raghupathi, T.K. McIntosh, Pharmacotherapy for traumatic brain injury: a review, Proceedings of the Western Pharmacology Society 41 (1998) 241–246.
[32] O.A. Reneerkens, K. Rutten, H.W. Steinbusch, A. Blokland, J. Prickaerts, Selective phosphodiesterase inhibitors: a promising target for cognition enhancement, Psychopharmacology (Berl.) 202 (2009) 419–443.
[33] E. Reyes-Irisarri, A.J. Sanchez, J.A. Garcia-Merino, G. Mengod, Selective induction of cAMP phosphodiesterase PDE4B2 expression in experimental autoimmune encephalomyelitis, Journal of Neuropathology and Experimental Neurology 66 (2007) 923–931.
[34] K. Rutten, E.L. Van Donkelaar, L. Ferrington, A. Blokland, E. Bollen, H.W. Steinbusch, P.A. Kelly, J.H. Prickaerts, Phosphodiesterase inhibitors enhance object memory independent of cerebral blood flow and glucose utilization in rats, Neuropsychopharmacology 34 (2009) 1914–1925.
[35] S.M. Schaal, M.S. Garg, M. Ghosh, L. Lovera, M. Lopez, M. Patel, J. Louro, S. Patel, L. Tuesta, W.M. Chan, D.D. Pearse, The therapeutic profile of rolipram, PDE target and mechanism of action as a neuroprotectant following spinal cord injury, PLoS One 7 (2012) e43634.
[36] M.K. Shah, P.J. Kadowitz, Cyclic adenosine monophosphate-dependent vascular responses to purinergic agonists adenosine triphosphate and uridine triphosphate in the anesthetized mouse, Journal of Cardiovascular Pharmacology 39 (2002) 142–149.
[37] J.M. Simard, M. Kilbourne, O. Tsymbalyuk, C. Tosun, J. Caridi, S. Ivanova, K. Keledjian, G. Bochicchio, V. Gerzanich, Key role of sulfonylurea receptor 1 in progressive secondary hemorrhage after brain contusion, Journal of Neurotrauma 26 (2009) 2257–2267.
[38] K.D. Statler, L.W. Jenkins, C.E. Dixon, R.S. Clark, D.W. Marion, P.M. Kochanek, The simple model versus the super model: translating experimental traumatic brain injury research to the bedside, Journal of Neurotrauma 18 (2001) 1195–1206.
[39] D.M. Stein, A.L. Lindel, K.R. Murdock, J.A. Kufera, J. Menaker, T.M. Scalea, Use of serum biomarkers to predict secondary insults following severe traumatic brain injury, Shock 37 (2012) 563–568.
[40] M.H. Theus, J. Ricard, J.R. Bethea, D.J. Liebl, EphB3 limits the expansion of neural progenitor cells in the subventricular zone by regulating p53 during homeostasis and following traumatic brain injury, Stem Cells 28 (2010) 1231–1242.
[41] A. Utagawa, H.M. Bramlett, L. Daniels, G. Lotocki, G.A. Dekaban, L.C. Weaver, W.D. Dietrich, Transient blockage of the CD11d/CD18 integrin reduces contusion volume and macrophage infiltration after traumatic brain injury in rats, Brain Research 1207 (2008) 155–163.
[42] E.A. Vitarbo, K. Chatzipanteli, K. Kinoshita, J.S. Truettner, O.F. Alonso, W.D. Dietrich, Tumor necrosis factor α expression and protein levels after fluid percussion injury in rats: the effect of injury severity and brain temperature, Neurosurgery 55 (2004) 416–424.
[43] E. Zaloshnja, T. Miller, J.A. Langlois, A.W. Selassie, Prevalence of long-term disability from traumatic brain injury in the civilian population of the United States, 2005, Journal of Head Trauma Rehabilitation 23 (2008) 394–400.
[44] H.T. Zhang, Cyclic AMP-specific phosphodiesterase-4 as a target for the development of antidepressant drugs, Current Pharmaceutical Design 15 (2009) 1688–1698.
[45] J. Zhang, R.D. Snyder, E.H. Herman, A. Knapton, R. Honchel, T. Miller, P. Espandiari, F.M. Goodsaid, I.Y. Rosenblum, J.P. Hanig, F.D. Sistare, J.L. Weaver, Histopathology of vascular injury in Sprague-Dawley rats treated with phosphodiesterase IV inhibitor SCH 351591 or SCH 534385, Toxicologic Pathology 36 (2008) 827–839.