DR. RANIA ABUTARBOUSH (Orcid ID : 0000-0002-6077-6199)

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Brain Oxygenation with a Non-vasoactive Perfluorocarbon Emulsion in a Rat Model of Traumatic Brain Injury

Running title: Brain oxygenation and microcirculation after TBI

RANIA ABUTARBOUSH,^{*,†} SAAD H. MULLAH,^{*,†} BISWAJIT K. SAHA,[†] ASHRAFUL HAQUE,[†] PETER B. WALKER,[†] CHIOMA ALIGBE,[‡] GEORGINA PAPPAS,[‡] LAM THUY VI TRAN HO,[†] FRANCOISE G. ARNAUD,^{†,‡} CHARLES R. AUKE,[†] RICHARD M. MCCARRON,^{†,‡} ANKE H. SCULTETUS,^{†,‡} AND PAULA MOON-MASSAT[†]

*Authors made equal contributions to the paper.

[†]Naval Medical Research Center, NeuroTrauma Department, Silver Spring, MD, USA

[‡]Uniformed Services University of the Health Sciences, Department of Surgery, Bethesda, MD, USA

Address for correspondence: Rania Abutarboush, Naval Medical Research Center, NeuroTrauma Department 503 Robert Grant Avenue, Silver Spring, MD, USA. *E-mail:* rania.abutarboush.ctr@mail.mil.

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ABSTRACT

Objective: The aim of this study was to assess, in two experiments, the safety and efficacy of the PFC emulsion Oxycyte as an oxygen therapeutic for traumatic brain injury (TBI) to test the hypothesis that early administration of this oxygen-carrying fluid post-TBI would improve brain tissue oxygenation ($P_{bt}O_2$).

Methods: The first experiment assessed effects of Oxycyte on cerebral vasoactivity in healthy, uninjured rats using intravital microscopy. The second experiment investigated the effect of Oxycyte on cerebral $P_{bt}O_2$ using the phosphorescence quenching method (PQM) in TBI model. Animals in the Oxycyte group received a single injection of Oxycyte (6 ml/kg) shortly after TBI, while NON animals received no treatment.

Results: Oxycyte did not cause vasoconstriction in small- (<50 μm) or medium- (50-100 μm) sized pial arterioles nor did it cause a significant change in blood pressure.

Treatment with Oxycyte while breathing 100% O_2 did not improve $P_{bt}O_2$. However, in rats ventilated with ~40% O_2 , $P_{bt}O_2$ improved to near pre-TBI values within 105 minutes after Oxycyte injection.

Conclusions: Although Oxycyte did not cause cerebral vasoconstriction, its use at the dose tested while breathing 100% O_2 did not improve $P_{bt}O_2$ following TBI. However, Oxycyte treatment while breathing a lower enriched oxygen concentration may improve $P_{bt}O_2$ after TBI.

KEY WORDS: controlled cortical impact, intravital microscopy, oxygen therapeutic, phosphorescence quenching method, pial microcirculation.

Abbreviations used:

Perfluorocarbon, PFC; traumatic brain injury, TBI; controlled cortical impact, CCI; brain tissue oxygenation, $P_{bt}O_2$; phosphorescence quenching method, PQM;

INTRODUCTION

TBI is a major public health problem worldwide. In the United States, the incidence of TBI-related hospital visits and deaths is 824 per 100,000 population [10] with an annual cost of \$48.3 billion [7]. Reduction of brain tissue oxygenation and hypoxic processes associated with TBI are believed to contribute to brain trauma presumably due to reduction in cerebral blood flow (CBF) [28].

Signal transmission in the brain is a high energy cost process [46] and an estimated 20% of the body's systemic oxygen is consumed by the brain owing to its high metabolic rate [11]. Reduction in cerebral tissue oxygen partial pressure following TBI could trigger a cascade of immediate and delayed pathological events that lead to neuronal and other cellular damage and death. Specifically, disruption of oxidative phosphorylation leads to a decline in ATP levels inhibiting ATP-dependent neuronal activity [23]. In some cases, when reduction in CBF and cerebral tissue oxygen deprivation after TBI reach ischemic levels [7], the sequelae include reduced reuptake and increased release of excitatory neurotransmitters, which eventually lead to apoptotic cell death [8]. In addition, reoxygenation following restoration of blood flow (reperfusion) leads to oxidative stress damage caused by the release of reactive oxygen species which damage cellular components [44].

Since oxygen deprivation secondary to TBI is one mechanism of trauma and neuronal damage [6,28], improving oxygen delivery and brain tissue oxygenation to affected tissue immediately post-TBI may prevent further damage and reduce morbidity and mortality. Several therapeutic agents are under development as adjuvants that enhance the oxygen-carrying capability of blood following hypoxic or ischemic events. These oxygen therapeutics fall into two classes: perfluorocarbon emulsions (PFC) and hemoglobin-based oxygen carriers. PFCs are fluorinated hydrocarbons that are entirely synthetic and can theoretically be produced in abundant quantities [40]. Emulsifying factors are used to make the hydrophobic hydrocarbon suitable for intravenous (IV) injection. Of first-generation PFCs, only Fluosol-DA (20% w/v PFC; Alpha Therapeutics, CA) reached the marketplace with a limited indication (coronary angioplasty) [19] and was eventually

withdrawn due to storage and production complications and undesirable side-effects, including pulmonary hypertension [19,54]. The next generation formulations had a higher PFC content and greater oxygen-carrying capacity than Fluosol-DA. Oxygent (60% w/v PFC; Alliance Pharmaceutical Corp., CA) reached Phase III clinical trials but failed to receive FDA approval [47]. Currently, there are no PFCs approved for use as oxygen therapeutics in the United States.

Oxycyte (Tenax Therapeutics, NC) is an injectable PFC that has been studied in preclinical models for the treatment of stroke [14,58] and decompression sickness [25,52]. Unlike earlier generation perfluorocarbons tested as oxygen therapeutics, Oxycyte (molecular formula $C_{10}F_{20}$) does not contain any bromine or chlorine. Fluid percussion TBI models showed that injured animals treated with Oxycyte had less neuronal damage and improved cognitive outcome [61]. Even though Phase II clinical trials of Oxycyte for treatment of TBI have been reported, the undertaking was recently abandoned due to low patient enrollment.

Regulatory approval of earlier oxygen therapeutics was hindered by concerns over side effects purportedly caused by vasoconstriction [33]. This screening study was carried out to answer two specific questions; with the expectation that a more comprehensive study should be undertaken if results were promising. The first question, a safety question, was whether Oxycyte caused cerebral vasoconstriction in a normal, healthy rat model. If it did, then the second study would not be undertaken and our investigations into the product would cease. The second question, an efficacy question, was whether administration of Oxycyte increased brain interstitial tissue oxygenation after TBI. In the first study, changes in diameter of cerebral pial arterioles were measured directly using intravital microscopy, while changes in systemic blood pressures were used as an indirect indicator of possible systemic vasoconstriction. The second study evaluated brain tissue oxygenation following TBI in a controlled cortical impact (CCI) model.

MATERIALS AND METHODS

The study protocol was reviewed and approved by the Walter Reed Army Institute of Research/Naval Medical Research Center Institutional Animal Care and Use Committee in compliance with all applicable federal regulations governing the protection of animals in research.

Test solutions

The PFC Oxeye is perfluorinated tert-butylcyclohexane (60% w/v) emulsified in egg yolk lecithin surfactant. A vehicle solution (lecithin surfactant) to serve as a control was not available from the manufacturer. Because the PFC emulsion has negligible colloidal pressure (0.3 mmHg), 0.9% normal saline (NS; Abbott Laboratories, Chicago, IL) was used as a volume control solution.

Pial microvessel experiments: healthy animals

Healthy male Sprague-Dawley rats (n = 22; 300-450 g; Charles River Laboratories, Wilmington, MA) were anesthetized with an intraperitoneal injection of a mixture of ketamine and acepromazine (72 and 4 mg/kg, respectively) and 0.01 mg/kg of subcutaneous buprenorphine for analgesia. Core body temperature was maintained at 37°C with a warming pad (Harvard Apparatus, Cambridge, MA). The right and left femoral veins were cannulated with PE-50 catheters for intravenous (IV) infusion of the PFC and control solutions and the femoral artery was cannulated for blood sampling and arterial blood pressure and heart rate (HR) monitoring (Datascope Corporation, Montvale, New Jersey). Because pilot studies showed that rats breathing room air (21% oxygen) became hypoxemic, all animals were intubated and mechanically ventilated with a small animal ventilator (RSP1002, Kent Scientific Corp., Lichtfield, CT) using 40% oxygen and 60% nitrogen with settings adjusted to maintain normal blood gases (PaCO₂ of 35- 45 mm Hg and PaO₂ ≥ 80 mm Hg). Pial microcirculation was accessed as described in Levasseur *et al.* [22]. Briefly, a rectangular craniotomy (~3 x 4 mm) was prepared in the right parietal bone and the dura was cut and reflected to the side to allow visualization and imaging of pial surface microvasculature. The surface of the brain was

superfused with artificial cerebrospinal fluid (Na 150 mM, K 3.0 mM, Ca 1.4 mM, Mg 0.8 mM, P 1.0 mM, Cl 155 mM; Harvard Apparatus, MA) to maintain electrolyte balance and was covered with a glass cover slip to prevent desiccation. A stereomicroscope (SZ16, Olympus, Japan) equipped with a DP-73 digital camera was used for direct visualization and imaging of pial microvessels at a total 80X magnification and a resolution of 0.8063 pixel/micrometer for both X and Y dimensions. Pial arterioles were measured at the same locus throughout the experiment using the computer program CellSens (Olympus, 2010). At the end of the experiment, a 5% aqueous BaCl₂ solution was applied topically as a positive control to confirm vessel responsiveness and ability to constrict [41].

The experimental design for the healthy animal experiments is summarized in Table 1. Rats were randomized to the experimental (Oxycyte, n = 9) or control (NS, n = 13) groups. Baseline measurements (T0) were collected and then animals received four 30-minute IV infusions of Oxycyte or NS, with 10 minutes between each infusion. Oxycyte was administered at 1.5 ml/kg for infusions 1-4, for a cumulative dose of 6 ml/kg over 2.5 hours, the duration of the experiment. The Oxycyte animals were administered additional NS via a separate IV catheter to bring the total fluid volume per infusion to 4 ml/kg/h, equivalent to the volume and rate of the NS control animals. Pial arteriolar vessel diameters, hemodynamic parameters (systolic, diastolic and mean arterial pressures), and vital signs (HR and temperature) were recorded at the beginning and end of each infusion. Arterial blood samples were obtained at baseline and at the end of each infusion. Arterial blood samples were analyzed on an automated blood gas system (ABL 700, Radiometer, Copenhagen, Denmark) for pH, partial pressures of oxygen (PaO₂) and carbon dioxide (PaCO₂), lactate, and bicarbonate (HCO₃⁻).

P_{bt}O₂ measurements: TBI model

Similar to the healthy rat experiments, male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) were initially anesthetized with intraperitoneal injection of ketamine and acepromazine (72 and 4 mg/kg, respectively). Body temperature was

maintained at 37 °C using a homoeothermic blanket (Kent Scientific, CT). The femoral artery was cannulated for invasive blood pressure and HR monitoring and for blood sampling and the femoral vein was cannulated for Oxycyte or NS infusion. To maintain normocapnia and normoxia (PaCO_2 of 35- 45 mm Hg and $\text{PaO}_2 \geq 80$ mm Hg, respectively), animals were mechanically ventilated (MouseVent, Kent Scientific, CT) via an endotracheal tube with a mixture of 40% oxygen and 60% nitrogen until onset of TBI. At T15 (15 min after TBI, immediately before fluid infusion), inspired oxygen was increased to 100% oxygen in the Oxycyte-treated group (Oxycyte 100) and the NS group (NON 100) until the end of the study based on the manufacturer's recommendations that it would optimize the amount of oxygen carried by the PFC. Due to inadvertent entrainment of room air in the pre-ventilator tubing that did not affect ventilator function, a group of Oxycyte-treated TBI rats ($n=5$), received 40% oxygen (Oxycyte 40). However, due to the resulting $\text{P}_{\text{bt}}\text{O}_2$ in these rats (described below), they were analyzed post-hoc as their own group and the data were compared to a NON group ventilated with 40% oxygen (NON 40, $n=8$). A rectangular craniotomy (~6 X 3 mm) was prepared in the right parietal bone for brain $\text{P}_{\text{bt}}\text{O}_2$ and a ~6 mm diameter round craniotomy was produced in the left parietal bone for TBI induction. Due to the size of the phosphorescence quenching fiber optic wand used for recording $\text{P}_{\text{bt}}\text{O}_2$ and the size of the CCI device impactor tip relative to the dimensions of the rat head, it was not possible to measure $\text{P}_{\text{bt}}\text{O}_2$ at or near the site of injury. Therefore, $\text{P}_{\text{bt}}\text{O}_2$ was measured contralateral to the injury site. As previously shown [29,32], the non-penetrating CCI TBI model used in this study resulted in a global cerebral injury as evidenced by histological injury at the site of impact as well as a significant reduction in $\text{P}_{\text{bt}}\text{O}_2$ contralateral to the impact site. Therefore, the model was deemed acceptable for determining if Oxycyte had a beneficial effect on cerebral brain oxygenation following TBI.

Oxygen-dependent quenching of phosphorescence was used to enable *in vivo* real-time measurement of $\text{P}_{\text{bt}}\text{O}_2$ [56,57]. The technical basis for measuring tissue oxygen pressure using phosphorescence quenching method (PQM) has been described elsewhere [13,15,55,56]. An oxygen sensitive phosphor (Oxyphor, G4; Oxygen Enterprises, Ltd., Philadelphia, PA) with absorption and phosphorescence in the red spectrum was used

[15]. Measurements were made using a frequency domain phosphorescence lifetime instrument (PMOD 5000; Oxygen Enterprises) and the data were analyzed using software developed by Vinogradov and colleagues [55]. The technique has been successfully used in our laboratory to evaluate $P_{bt}O_2$ after TBI [29,31,32]. Briefly, the validity of PQM was established in pilot animals that reliably showed $P_{bt}O_2$ values 1) increased with increasing fraction of inspired oxygen (FiO_2), 2) decreased with increase in TBI severity, and 3) decreased to near zero after euthanasia. In addition, sham animals were used to assess the stability of PQM measurements of $P_{bt}O_2$ over time.

The experimental design of the TBI study is summarized in Table 2. As previously described [32], G4 was injected under the dura (in the subarachnoid space) contralateral to the $P_{bt}O_2$ monitoring side to avoid possible injury by the injection and $P_{bt}O_2$ was continuously monitored at 15 seconds intervals. Each $P_{bt}O_2$ value was the average of eight measurements over a 2 min period. The $P_{bt}O_2$ reading obtained 30 minutes after G4 injection was defined as baseline. Immediately following baseline readings, animals were assigned to sham (no TBI), TBI with Oxycyte, or TBI with no treatment (NON) groups. The time of TBI onset was defined as T0. At T0, TBI was induced using a stereotaxic controlled cortical injury device (Leica Impact One®, Leica Biosystems, IL). The left parietal lobe was impacted over the intact dura with a 5 mm impactor tip at 4 m/s velocity, 1 mm impact depth, and 100 ms dwell time. A microprobe thermometer temperature probe (BAT-12, Physitemp®, Kent Scientific, CT) was placed near the brain injury site to monitor brain surface temperature. At T15, animals in the Oxycyte groups received 6 ml/kg IV Oxycyte at a rate of 1 ml/min followed by maintenance saline (4 ml/kg/h) until the end of the study (T155). Animals in the NON groups received only maintenance saline (4 ml/kg/h) post-TBI for the duration of the study. Temperature, blood pressures, HR and $P_{bt}O_2$ data were recorded at baseline (before TBI), immediately post-TBI (T0), before Oxycyte or NS administration (T15), and every 15 minutes for the remainder of the study. Blood samples were collected before TBI, before Oxycyte or NS injection (T15), and every 30 minutes thereafter until the end of the study. Arterial blood samples were obtained at baseline and at the end of each infusion. Blood samples were

analyzed with an automated blood gas system (ABL 700) for pH, PaO₂, PaCO₂, lactate, and HCO₃⁻.

At the end of the experiment, the brain was rapidly collected and fixed in 10% formalin. A matrix-guided trimming protocol for the rat brain was used to select the injury site and the hippocampus, the latter being a brain region considered to be the most vulnerable to ischemic injury. Brain tissue was embedded in paraffin and 5 µm coronal sections were cut with a microtome, mounted on glass slide, and stained with hematoxylin and eosin (H&E) using standard methodology. A board certified veterinary pathologist, blinded to treatment, evaluated the slides using an Olympus BX-41 (Olympus, Japan) light microscope. Relevant microscopic findings included meningeal congestion, superficial cortical damage, and superficial tissue loss at the site of impact. The severity of findings was overall similar across the groups. Pathological findings were rated using the following 5-point system: 0 = normal, 1 = minimal, 2 = mild, 3 = moderate, 4 = marked and 5 = severe damage. No animals in this study were assessed a grade of 4 (marked) or 5 (severe). Due to nearly identical scoring between the Oxycyte and NON groups, histopathological data were not analyzed any further.

Statistical analysis

The data was assessed graphically for normality prior to the inferential statistical analysis. Data analyses were performed using IBM SPSS Statistics 21.0 (IBM Corporation, 2012). Body weights for all animals were analyzed using an independent sample t-test. For the pial microvessel (healthy animals) experiments and as previously described [2,29], mixed general linear models were used for analyzing heart rate, blood pressure, vessel diameter, temperature, blood gases, methemoglobin and lactate data. Rat body weights were analyzed using a one-way analysis of variance (ANOVA). Pial arteriolar vessel diameters were grouped into three categories based on size at baseline measurement [3,37,38]: (1) “small” vessels having a diameter < 50 µm, (2) “medium” vessels with diameters of 50 to 100 µm, and (3) “large” vessels with diameters > 100 µm. The anatomical differences between small and larger microvessels and, specifically, the

increase in the amount of smooth muscle with vessel size justify the choice of the size categories [42]. For vessel diameters, percent change from baseline was compared among the three groups. Percent change was determined using pre- and post-infusion data for each infusion separately. Least square means were used for post hoc t-tests. For all parameters, a P-value ≤ 0.05 was considered statistically significant. Large-sized vessels were not analyzed due to small sample size (n = 3 for NS and n = 5 for Oxycyte rats). Presented data are mean $\pm$ SEM.

For the TBI studies, sham animals were used to validate the model and only within group statistical analysis were performed to determine the stability of the PQM $P_{bt}O_2$ readings. To determine whether the differences between the Oxycyte and NON groups differed as a function of infusion and observation period, an omnibus 2 (Group) x 15 (Infusion Period) mixed-model ANOVA was used to compare percent change in $P_{bt}O_2$ from the pre-TBI baseline, HR, blood pressures, temperature, blood gases, pH, Hb and lactate data between the Oxycyte and NON group. Following this analysis, a one-way repeated measures ANOVA was performed on the two groups across the infusion and observation period to determine whether measurements of $P_{bt}O_2$ changed over time. Finally, post hoc comparisons using LSD were performed to find the effect of drug treatment over time. The Oxycyte 40% O_2 (Oxycyte 40) data were compared to the Oxycyte 100% O_2 (Oxycyte 100), the NON 100% O_2 (NON 100) and NON 40% O_2 (NON 40) data. To further understand the three-way interaction between group, oxygen level, and time, a separate 2-way repeated measures ANOVA was performed for both 40% and 100% O_2 groups. Finally, for all parameters, a P-value ≤ 0.05 was considered statistically significant. Presented data are mean $\pm$ SEM.

RESULTS

Healthy Rats Experiments

Animals in the NS (n = 13, 404 ± 52 g) and Oxycyte (N = 9; 410 ± 55 g) groups survived the protocol without any observable adverse events. Body temperatures were not different between the two groups (36.7 ± 0.4 °C for the Oxycyte animals and 36.6 ± 0.5 °C for the NS animals) over the duration of the study.

Blood Pressures and Heart Rate. Mean arterial blood pressure (MAP) data are presented in Figure 1. Overall, there were no cumulative changes in MAP (comparing T0 to T150) within the NS and Oxycyte groups. There were also no differences in MAP between the two groups after any of the infusions. MAP was selected as the representative parameter as results with systolic and diastolic blood pressure (data not shown) followed the same trends as those observed in MAP. HR significantly increased in both the NS and Oxycyte groups only after the third infusion but was not different between the two groups at any point during the course of the experiment. This increase in HR was within physiologically normal limits in both groups (Table 3).

Cerebrovascular diameters. Overall, small and medium-sized pial arterioles in the Oxycyte group did not exhibit vasoconstriction as compared to the NS group. Vessels in the two size categories (small, Figure 2a, and medium, Figure 2b) showed significant vasoconstriction after topical application of BaCl₂ at the end of the experiments, indicating vascular reactivity was present throughout the study. Small-sized (< 50 µm) pial arterioles exhibited significant vasoconstriction in the NS group during the second infusion but not during any of the other infusions or cumulatively (i.e., comparing T0 to T150). In the Oxycyte group, small-sized arterioles dilated after the third infusion and constricted after the fourth infusion, with no overall cumulative change in diameter (i.e., comparing T0 to T150). For medium-sized vessels (50-100 µm), there was a significant cumulative decrease in vessel diameters (9 ± 3% vasoconstriction comparing T0 to T150) in the NS group but not the Oxycyte group. There was significant vasodilation in Oxycyte-treated rats during the third infusion but no significant changes in vessel diameter after any of the other infusions. In saline-treated rats, there was significant vasoconstriction during both the first and second infusions and no further changes during the third and fourth infusions. Representative images of pial arterioles before and after

infusion of Oxycyte are shown in Figure 2c as is the reaction of arterioles to topical administration of BaCl₂, the positive control for vasoconstriction.

In vitro analyses. Arterial blood data (hemoglobin, pH, lactate, PaO₂ and PaCO₂) were within normal physiological values (Table 4). Methemoglobin values were too low to be detected in the two groups. Compared to the NS control, pH was higher after the fourth infusion in the Oxycyte group but was still within physiologically normal values and was not indicative of disruption of acid-base homeostasis. PaO₂ values were higher in the Oxycyte-treated animals although the difference between the two groups only became significant at T150.

TBI Experiments

All animals (Sham n = 5, Oxycyte n = 8, and NON n = 8) survived the experiment. Mean body temperature over the course of the study was not significantly different between the two treatment groups (37.1± 0.02 ° C and 37.0± 0.02 °C for the Oxycyte and NON rats, respectively).

Blood pressures and heart rate. MAP data for the TBI Oxycyte and NON groups are illustrated in Figure 3. MAP was not different between the groups at baseline, immediately before TBI (T0), or at T15 before beginning of the infusion. The difference in overall MAP between the two groups was not significant; however, the change in MAP over time was statistically significant (P< 0.05). In both the Oxycyte and NON groups, there was a transient increase in MAP immediately following TBI followed by a decrease 10 minutes later (at T25). A post-hoc one-way repeated measures ANOVA showed a significant change in MAP over time in the Oxycyte group. After completion of Oxycyte injection, a moderate (8 mm Hg) decrease in MAP was noted at T25 (P< 0.05). This decrease was followed by a similarly sized (~10-12 mm Hg) increase in MAP that was sustained between T45 and T95 (P< 0.05). From T95 until the end of the experiment, blood pressure tended to decrease slightly while still remaining higher than the T15 value. In the NON group, there were no changes in MAP compared to T15. Overall,

HRs during the experiment were 419 ± 17 and 400 ± 73 beats/min in Oxycyte 100 and NON 100 groups, respectively, and did not change from the pre-infusion value (T15) within either group nor were they different between the two groups.

Brain oxygenation. $P_{bt}O_2$ of sham rats was 25 ± 5 mm Hg and did not change over the course of the observation period. For the experimental groups, $P_{bt}O_2$ before TBI was not different between the Oxycyte 100 (30 ± 3 mm Hg) and NON 100 (28 ± 2 mm Hg) groups. Percent change in $P_{bt}O_2$ from pre-TBI values for the Oxycyte and NON groups are presented in Figure 4a. At 15 min after TBI (T15), $P_{bt}O_2$ decreased $54.4 \pm 4\%$ and $51.8 \pm 7\%$ in the Oxycyte and NON groups, respectively. $P_{bt}O_2$ continued to decrease in both groups, decreasing to $\sim 60\%$ and $\sim 55\%$ of the pre-TBI value in the Oxycyte and NON groups, respectively, by the end of the study. As seen in Figure 4a, there was relatively little difference in $P_{bt}O_2$ measurements as a function of time ($P = 0.08$) or across groups ($P > 0.05$). Further, the interaction between group and time in $P_{bt}O_2$ measurements was not significant. Collectively, these analyses indicate that there was no improvement in $P_{bt}O_2$ with administration of 6 ml/kg IV Oxycyte as a single dose injected at a rate of 1 ml/min in conjunction of 100% O₂.

In the Oxycyte 40 group, PaO_2 was expectedly lower than that of rats breathing 100% oxygen (279.0 ± 18.3 mm Hg). The mean PaO_2 at T15 in this group (181 ± 9.5 mm Hg) was similar to the PaO_2 of NON rats ventilated with 40% oxygen (154 ± 4.4 mm Hg, $n=8$ rats; ANOVA followed by Tukey's multiple comparisons test). All hemodynamic and in vitro parameters in the Oxycyte 40 group were similar to the 100% O₂ group and, therefore, comprehensive statistical analyses were performed only on $P_{bt}O_2$ values. The 3-way analysis of group, oxygen level and time showed that the interaction between group and oxygen level was significant (Figure 4b, $P < 0.001$). A 2-way repeated measures ANOVA showed a significant increase in $P_{bt}O_2$ over time in the Oxycyte 40 compared to the NON 40 group and a lack of an increase in $P_{bt}O_2$ with 100% oxygen. Finally, a one-way repeated measures ANOVA showed that the only experimental group that exhibited a change in $P_{bt}O_2$ over time ($P < 0.001$) was the Oxycyte 40 group.

As shown in Figure 4b, $P_{bt}O_2$ AT T35 in the Oxycyte 40 group was already significantly higher than the pre-treatment TBI value (T15). $P_{bt}O_2$ continued to increase progressively until the end of the experiment and approached baseline, pre-injury values (P 's < 0.05 for all time points from T35 until the end of the experiment). By T125, $P_{bt}O_2$ was 25.8 ± 4.3 mm Hg in the Oxycyte 40 group, which is not different from the pre-TBI value of 26.7 ± 3.6 mm Hg. The data suggest that treatment with a combination of Oxycyte and oxygen levels of 40% O_2 may be a successful strategy for increasing cerebral tissue oxygenation.

In vitro analyses. Statistical analysis of biosamples was performed only on the two primary study cohorts (i.e., NON 100 and Oxycyte 100). Arterial blood data were within normal physiological values (Table 5) in spite of sporadic statistically significant differences. Similar to the healthy rat study, methemoglobin values were undetectable. In both groups, pH and lactate values changed over the course of the study; however, both parameters were within physiologically normal ranges and did not reflect disruption of acid-base homeostasis. PaO_2 values were different between the two groups over time ($P < 0.0001$), group ($P = 0.003$), and time and group interaction ($P < 0.001$). The change in PaO_2 over time was due to increasing the inspired oxygen from 40% pre-injury to 100% post-injury. Higher PaO_2 values in the Oxycyte-treated rats were previously reported by other investigators [51,52,60] and could possibly be a treatment effect.

Brain histopathology. Histopathological assessment of tissue sections revealed meningeal congestion, superficial cortical hemorrhage and superficial tissue loss at the site of impact. There were no differences in the severity of damage between the groups. Hemorrhage was minimal or mild in all animals indicating consistency in TBI severity. There was no evidence of neuronal necrosis in any of the animals due to the short duration of the experiment.

DISCUSSION

The present study investigated the effect of treatment with the PFC Oxycyte on cerebral arteriolar diameters and blood pressure in healthy rats and on brain tissue oxygenation

and cellular damage following TBI. Specifically, the data showed that IV administration of the PFC Oxycyte to normovolemic healthy rats is not associated with cerebral vasoconstriction in small- and medium-sized pial arterioles nor is it associated with overall changes in systemic blood pressures. Treatment with Oxycyte along with breathing 100% O₂ after moderate CCI TBI did not improve brain tissue oxygenation compared to injured untreated rats although, unexpectedly, it appears there may be a benefit to Oxycyte treatment following TBI when breathing 40% O₂.

Some of the early PFCs, specifically Fluosol-DA, were associated with cerebral vasoconstriction [30] which was postulated to be due to either increased oxygen delivery or lower viscosity. Our model sought to directly assess any cerebral vasoconstrictive effects in healthy vessels of animals that received supplemental oxygen (40%), only to prevent hypoxemia, but did not receive 100% oxygen because that alone might have caused vasoconstriction of the cerebral vessels of these uninjured animals [16,35,39]. If the physical properties of Oxycyte (such as its viscosity or particle size which could change the rheological properties of blood [4]) were to cause vasoconstriction, our experiments would reveal this effect.

The overall decrease in pial arteriolar diameter in the NS group was previously reported by our lab [3] and is believed to be associated with the healthy rat cranial window model and may be caused by the surgery, anesthesia, or changes in intracranial pressure (ICP). This pial arteriolar vasoconstriction was abated by the administration of Oxycyte, which suggests that Oxycyte may even have a vasodilatory effect (albeit statistically insignificant) on pial arterioles although this was not specifically tested in our study. In addition to its lack of cerebral vasoconstrictive properties, our data show that Oxycyte may not cause systemic vasoconstriction as there was no overall change in MAP with the administration of Oxycyte to healthy rats. Cabrales and Briceno [9] have shown a similar lack of Oxycyte-induced vasoconstriction in a systemic (hamster dorsal skin) vascular bed using an exchange hemodilution anemia model. Taken together, these findings suggest that Oxycyte lacks the vasoconstrictive effects that raised concerns over the safety of some of the earlier generation oxygen therapeutics.

While our results in healthy rats address the effects of Oxycyte on pial vessels in isolation from a clinical insult, it is possible that pial vessels may behave differently in a TBI model. Ideally, cerebral vasoconstriction with Oxycyte treatment would have also been assessed in the TBI model. However, it was technically not possible to perform intravital microscopy to obtain vessel measurements (which requires cutting the dura) in a CCI-TBI model because of the small size of the skull of the rat. In pilot animals (data not shown), the impact of TBI, induced on the contralateral side to the cranial window over intact dura, caused hemorrhage and swelling of the brain surface (damaging the pial vessels) at the cranial window site after cutting the dura.

The transient increase in blood pressure observed immediately following brain impact (T0-T15) in TBI animals is similar to previous reports [21]. A brief period of hypotension lasting 10 minutes was observed after Oxycyte administration. The mechanism of this observation is unclear but we suspect it could be, at least in part, due to a compensatory mechanism following the rapid injection of the product (that caused an immediate transient rise in MAP which is not shown in the figure). The subsequent rise in MAP in the Oxycyte-treated TBI animals was not seen in the injured NON animals although there was a tendency (not statistically significant) for blood pressure to increase in the healthy Oxycyte-treated rats after each Oxycyte infusion. Other investigators reported increases in MAP after PFC treatments that occur in a dose-dependent fashion [12,61]. A mechanism for this blood pressure change cannot be determined by our study as direct hemodynamic variables (i.e., cardiac output and central venous pressures) were not assessed. It could be that the PFC particles were retained within the vasculature leading to a small volume expansion that resulted in an increase in blood pressure. The rise in blood pressure could also be caused by changes in blood viscosity due to the presence of the PFC. Future studies designed to further investigate this phenomenon are needed.

Treatment with oxygen-carrying PFCs has been shown to reduce neuronal ischemic injury in various models of experimental cerebral ischemia. Specifically, animal studies showed that IV administration of PFCs reduced infarct volume in a stroke model (middle

cerebral artery occlusion) [36,50] and provided neuroprotection during brain stem ischemia [17]. Significant enhancement of brain oxygenation and mitochondrial function [12], and improvement in cognitive function [1,61] were reported after PFC treatment in fluid percussion TBI models in rats. In particular, treatment with Oxycyte, in conjunction with breathing 100% O₂, was shown to have a therapeutic effect on cognitive function as assessed by the Morris Water Maze after moderate lateral fluid percussion injury in rats as well as reduced injury-induced cell death in the hippocampus [61]. Our data did not show a difference in brain tissue oxygenation as measured by the PQM between injured animals treated with 6 ml/kg Oxycyte breathing 100% O₂ and untreated NON animals breathing 100% O₂. Neither treatment with Oxycyte with 100% O₂ nor NS with 100% O₂ resulted in improvement in P_{bt}O₂ over the 2.5 hour observation period, but treatment with Oxycyte in conjunction with a ~40% O₂ led to a significant improvement in P_{bt}O₂. In addition, there was no difference in hippocampal histology between the two groups, but this negative finding may have been due to the short survival time (2.5 hours) following TBI [61].

The prior work by Daugherty *et al.* [12] is different from the present study in several ways; despite both studies examining the effects of PFC treatment on brain oxygenation. First, Daugherty *et al.* used modified intraparenchymal microelectrode sensors (Licor, Integra) to measure P_{bt}O₂ in a fluid percussion TBI model, while the present study used the PQM for measuring P_{bt}O₂ in a CCI TBI model. The PQM has been successfully used in our laboratory to detect changes in P_{bt}O₂ in uninjured (healthy) and TBI animals treated with HBOCs [29] and PFC [32] and have demonstrated that the method is sensitive enough to detect changes in P_{bt}O₂ following TBI. Therefore, the difference in the methodology for measuring oxygen tension in the Daugherty study and our study does not account for the majority of variance in the data. Second, Daugherty *et al.* [12] assessed brain oxygenation following treatment with a different PFC (Oxygent) ipsilateral to the injury site, while in this study the side contralateral to TBI was studied following treatment with Oxycyte. Although both Oxycyte and Oxygent are 60% weight/volume PFC, they are chemically different and Oxycyte does not contain bromine; therefore, some of the difference in P_{bt}O₂ data could be due to differences in

the properties of the drugs tested. Perhaps more significantly, the injured side of the brain which suffers a greater hypoxic insult due to direct physical injury most likely responds differently to PFC treatment while breathing 100% O₂ compared to the contralateral side that only experiences the secondary effects of trauma, which include reduced blood flow and hypo-perfusion. It is well documented that CBF auto-regulation is impaired or absent after TBI [49,53]. Thus, cerebral auto-regulation may be greatly diminished (if not absent) on the ipsilateral side of the impact site, whereas auto-regulation is probably still present, to a certain extent, on the contralateral side. One can speculate that, in our TBI study, cerebral auto-regulation led to vasoconstriction due to over-oxygenation resulting from the circulating oxygen-carrying PFC and exposure to 100% O₂ resulting in no detectable improvement in P_{bt}O₂. On the other hand, in the Oxycyte-treated rats that were breathing 40% O₂, any oxygen-induced vasoconstriction that might have occurred may not have reduced CBF to the same extent which allowed for higher P_{bt}O₂. This speculative reasoning could also explain why, in the absence of auto-regulation, treatment with a PFC while breathing 100% O₂ would increase P_{bt}O₂ in the ipsilateral side of injury as demonstrated by Daugherty *et al* [12].

Rosenthal and colleagues [43] showed that while increasing FiO₂ leads to an increase in cerebral tissue oxygenation, it also causes a significant reduction in CBF. Other studies have also demonstrated a decrease in CBF with elevated arterial oxygen (PaO₂) [20] and that the increase in P_{bt}O₂ in the ischemic brain during hyperoxia is blunted compared to the non-ischemic brain [18]. Thus, while using a higher FiO₂ increases the amount of oxygen dissolved in the PFC thereby increasing the oxygen gradient for diffusion into hypoxic tissue, it may simultaneously decrease CBF and thereby limit oxygen delivery. As such, using a lower FiO₂ in conjunction with Oxycyte may be more conducive to enhancing tissue oxygenation. In support of this hypothesis, P_{bt}O₂ of the TBI Oxycyte 40 was significantly higher than both NON- and Oxycyte-treated rats ventilated using 100% O₂. There is also evidence that using PFCs with a FiO₂ of 1.0 increases free radical formation [12]. Additionally, hyperoxia may exacerbate damage to ischemic brain tissue resulting from reperfusion/reoxygenation- induced mitochondrial oxidative stress [34].

These observations may explain the benefit of using FiO_2 lower than 1.0 in conjunction with Oxycyte to restore brain tissue oxygenation.

In our TBI study, only the contralateral side to the injury was used for the PQM measurement of $\text{P}_{\text{bt}}\text{O}_2$ and not the ipsilateral side. The PQM is an optical method in which a light sensitive phosphor is injected in the subarachnoid space to obtain $\text{P}_{\text{bt}}\text{O}_2$ by measuring the phosphorescence quenching decay lifetimes. The method is less invasive than electrode-based methods (such as Licox®, Integra, or OxyLite™ and OxyFlo™, Oxford Optronix), because it does not involve insertion of electrodes into the brain parenchyma, which could injure small blood vessels. Since the PQM technique is based on excitation and detection of phosphorescence, the presence of blood interferes with the readings. Therefore, due to hemorrhage at the injury site, and the small size of the rat skull, only contralateral $\text{P}_{\text{bt}}\text{O}_2$ could be measured reliably using the PQM in our rat model. It is important to study $\text{P}_{\text{bt}}\text{O}_2$ after treatment with Oxycyte in the penumbra of the injury site and future work should investigate the effect of Oxycyte on that region. It is also of importance, however, to understand the effects of TBI and treatment with oxygen therapeutics on brain regions removed from the injury site. As shown in this study and others [29,31,32], $\text{P}_{\text{bt}}\text{O}_2$ changes significantly post-TBI and the changes are detectable immediately and persist for at least two hours.

Since our model was a normovolemic “top-load” TBI model (with no blood loss), the total amount of oxygen carried by circulating red blood cells (RBC) should be similar in the Oxycyte and the NS treatment groups, with any differences being due to a change in oxygen delivery (where oxygen delivery is the product of oxygen content times blood flow). We measured interstitial tissue oxygenation (i.e., $\text{P}_{\text{bt}}\text{O}_2$), the end result of cerebral oxygen delivery. Thus, the increase in brain oxygen could be due to changes in either or both parameters (oxygen content and/or blood flow) and additional work, beyond the capabilities provided in this screening rat model, is warranted. Measurement of CBF was technically not feasible in our model due to the small size of the head and the presence of the PQM fiberoptics. We postulate, however, that it is unlikely that the observed increase in $\text{P}_{\text{bt}}\text{O}_2$ was due entirely to changes in only CBF. The literature shows that Oxycyte is

an oxygen carrier [48] and, thus, the PFC itself should play a role in any increase in oxygenation. Therefore, it is reasonable to conclude that the increase in $P_{bt}O_2$ in the Oxycyte 40 group is at least in part due to the presence of the oxygen-carrying PFC. This assumption would need to be confirmed with direct measurement of regional CBF to eliminate the possibility of changes in CBF due to the presence of Oxycyte as a cause of the increase in $P_{bt}O_2$. Regardless of the mechanism by which improvement in $P_{bt}O_2$ occurs, our data are clinically relevant. The data suggest that treatment with Oxycyte in conjunction with milder hyperoxia than 100% O_2 may be beneficial to $P_{bt}O_2$ following TBI.

Treatment with Oxycyte has been shown to improve outcome following pathophysiological conditions other than TBI where damage to the vasculature and/or alterations in microvascular function occur and result in hypoxic injury. Specifically, in a model of spinal cord contusion, Oxycyte was shown to increase tissue oxygenation [45], to have neuroprotective properties, and to reduce apoptotic cell death [59]. In addition, Oxycyte may also be a promising treatment for decompression sickness, which is believed to be associated with the formation of gas bubbles during depressurization that lead to arterial embolization with various symptoms including, neurological manifestations and spinal cord injury [5,26]. Treatment with Oxycyte in swine models of decompression sickness improved survival and reduced tissue injury [24,27]. Specifically, IV injection of Oxycyte was found to significantly reduce spinal cord lesion size after severe experimental decompression sickness presumably due to improvement in oxygen transport under low intravascular flow conditions [24].

In this study, normal saline was used as a volume-matched crystalloid control with similar oncotic pressure to Oxycyte. Although Oxycyte is emulsified in egg-yolk lecithin, a lecithin control was not used in this study as the purpose of this study was to assess the safety and efficacy of Oxycyte as a therapeutic fluid for TBI and not the mechanistic contribution of the different components of Oxycyte. Even though lecithin may have its own effects on the microcirculation, from a clinical and regulatory perspective, Oxycyte (all components included), as a therapeutic fluid, would either be safe (not vasoactive)

and efficacious (improve brain tissue oxygenation) or it would be unsafe and inefficacious. Therefore, the study was designed to compare Oxycyte at the manufacturer recommended therapeutic dose to a clinically-relevant control.

The findings of this study should be interpreted with acknowledgment of some limitations. The serendipitous finding of this study, that Oxycyte-treated TBI rats breathing 40% O₂ showed improved P_{bt}O₂ compared to untreated animals or those treated with Oxycyte but breathing 100% oxygen, must be evaluated in a separate experiment where FiO₂ is varied systematically. Furthermore, follow-up studies using larger animal models are necessary to monitor additional critical parameters, including ICP, CBF, cerebral perfusion pressure, systemic and pulmonary blood pressures and vascular resistances, and cardiac output as well as evaluation of P_{bt}O₂ and brain histology at later time points post-TBI (24 hours and later). This information would be important for a better understanding of the effects of using Oxycyte as an oxygen therapeutic.

Conclusion

Our findings show that Oxycyte IV infusion does not cause constriction of cerebral pial arterioles when administered to healthy uninjured rats. The United States FDA has shown that “oxygen therapeutic” products will be placed on clinical hold until they show lack vasoconstrictive effects. The FDA indicated that this safety milestone should be reached for all oxygen therapeutics being developed to treat hemorrhage, TBI or stroke compared to the standard of care. Thus, our findings show that Oxycyte passed this initial safety milestone in our rat model and was, therefore, evaluated in a TBI model for efficacy. In TBI animals, administration of Oxycyte while breathing 100% O₂ (FiO₂ = 1) does not improve P_{bt}O₂ brain tissue oxygenation as compared to untreated injured animals.

However, there is some evidence that treatment with Oxycyte in conjunction with a lower FiO₂ might lead to improvement in P_{bt}O₂. This finding suggests that a lower FiO₂ may be the optimal combination for correcting cerebral oxygen deficiency after TBI, although a more systematic evaluation using different levels of FiO₂ is needed. It is important to perform more comprehensive follow up experiments using a larger number of animals.

PERSPECTIVE

Future research focusing on using Oxycyte while breathing a lower FiO_2 and investigations of the effects of oxygen toxicity and free radicals as they relate to the optimal use of PFCs are needed. Furthermore, investigations into the effects of IV administration of oxygen carrying PFCs with varying levels of FiO_2 on CBF are important for fully understanding resulting changes in $\text{P}_{\text{bt}}\text{O}_2$. The use of an oxygen-carrying fluid to correct reduction in brain tissue oxygenation after TBI may be a useful therapeutic strategy.

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Author Disclosure Statements

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Table 1. Experimental design for the pial microvessel experiments (healthy rats). NS: 0.9% normal saline.

Time (min)	Treatment	Group	
		Oxycyte	NS
0-30	Infusion 1	3 ml/kg/h PFC+ 1 ml/kg/h NS	4 ml/kg/h NS
40-70	Infusion 2	3 ml/kg/h PFC+ 1 ml/kg/h NS	4 ml/kg/h NS
80-110	Infusion 3	3 ml/kg/h PFC+ 1 ml/kg/h NS	4 ml/kg/h NS
120-150	Infusion 4	3 ml/kg/h PFC+ 1 ml/kg/h NS	4 ml/kg/h NS
155	Vasoconstriction positive control	Topical BaCl ₂	Topical BaCl ₂

Table 2. Experimental design for the TBI and brain tissue oxygenation experiments. NS: 0.9% normal saline.

Time (min)	Treatment	Group	
		Oxycyte	NON
0-15	TBI followed by 15 min delay		
15-18	drug Infusion	6 ml/kg PFC at 1 ml/min	4 ml/kg/h NS
18-155	Observation and maintenance fluids	4 ml/kg/h NS	4 ml/kg/h NS

Table 3. Blood pressure and heart rate parameters (mean $\pm$ SEM) for Oxycyte and saline (NS) groups. # Post-infusion value (T30, T70, T110 or T150) is significantly different ($P \leq 0.05$) from its corresponding within-group pre-infusion value (T0, T40, T80, and

T120). * Post-infusion value (T30, T70, T110 or T150) is significantly different ($P \leq 0.05$) from its corresponding within-group value at T0. There were no statistically significant differences between the two treatment groups at any time point.

Time	Treatment	MAP (mm Hg)	HR (beats/min)
T0 (Pre-treatment)	Oxycyte	99.1 $\pm$ 5.8	303 $\pm$ 80
	NS	93.5 $\pm$ 13.0	305 $\pm$ 61
T30 (After Infusion 1)	Oxycyte	96.9 $\pm$ 6.9	318 $\pm$ 95
	NS	92.5 $\pm$ 7.4	308 $\pm$ 42
T40 (Before Infusion 2)	Oxycyte	93.2 $\pm$ 8.9	301 $\pm$ 71
	NS	95.2 $\pm$ 11.5	338 $\pm$ 36
T70 (After Infusion 2)	Oxycyte	97.2 $\pm$ 8.2	321 $\pm$ 66
	NS	92.0 $\pm$ 12.6	318 $\pm$ 44
T80 (Before Infusion 3)	Oxycyte	92.8 $\pm$ 9.3	308 $\pm$ 69
	NS	92.6 $\pm$ 17.5	336 $\pm$ 65
T110 (After Infusion 3)	Oxycyte	99.4 $\pm$ 12.4	341 $\pm$ 62 [#]
	NS	94.3 $\pm$ 17.8	331 $\pm$ 45*
T120 (Before Infusion 4)	Oxycyte	94.0 $\pm$ 15.0	331 $\pm$ 75
	NS	91.5 $\pm$ 16.0	351 $\pm$ 48
T150 (After Infusion 4)	Oxycyte	94.2 $\pm$ 10.0	339 $\pm$ 57
	NS	93.5 $\pm$ 14.5	325 $\pm$ 57

Table 4. Arterial blood chemistry and gas parameters (mean $\pm$ SEM). [§] Indicates that the values were significantly ($P \leq 0.05$) different between the Oxycyte and NS group. * Indicates within group differences between post-infusion values (T30, T70, T110, and T150) and T0.

Time	Group	Hb	pH	PaCO ₂	PaO ₂	Lactate
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		(g/dL)		(mm Hg)	(mm Hg)	(mmol/L)
T0 (Pre-treatment)	Oxycyte	12.4 ± 0.5	7.383 ± 0.02	44 ± 2	122 ± 20	0.5 ± 0.1
	NS	12.5 ± 0.9	7.349 ± 0.08	47 ± 12	110 ± 41	0.6 ± 0.09
T30 (After Infusion 1)	Oxycyte	12.3 ± 0.5	7.394 ± 0.02	41 ± 2	143 ± 20	0.8 ± 0.1*
	NS	12.4 ± 0.9	7.352 ± 0.07	46 ± 10	116 ± 34	0.7 ± 0.3
T70 (After Infusion 2)	Oxycyte	12.1 ± 0.5	7.383 ± 0.02	39 ± 2*	152 ± 20	0.6 ± 0.1
	NS	12.3 ± 1.3	7.354 ± 0.06	42 ± 9	118 ± 29	0.7 ± 0.3
T110 (After Infusion 3)	Oxycyte	12.2 ± 0.5	7.372 ± 0.02	39 ± 2*	160 ± 20*	0.7 ± 0.1*
	NS	12.1 ± 1.1	7.340 ± 0.06	43 ± 9	111 ± 31	0.7 ± 0.2
T150 (After Infusion 4)	Oxycyte	12.5 ± 0.5	7.37 ± 0.02 [§]	41 ± 2	165 ± 20* [§]	1 ± 0.1*
	NS	12.6 ± 1.1	7.323 ± 0.05 [§]	47 ± 8	106 ± 29 [§]	0.7 ± 0.2

Table 5. Arterial blood chemistry and gas parameters (mean ± SEM) in TBI Oxycyte and NON animals mechanically ventilated using 100% oxygen. * Indicates values were significantly different from T0 within the group.

Time	Group	Hb (g/dL)	pH	PaCO ₂ (mm Hg)	PaO ₂ (mm Hg)	Lactate (mmol/L)
T0 (Pre-injury)	Oxycyte	11.5 ± 0.6	7.362 ± 0.01	39 ± 1	150 ± 7	0.6 ± 0.1
	NON	12.9 ± 0.7	7.390 ± 0.01	40 ± 1	130 ± 9	0.7 ± 0.1
T15	Oxycyte	11.8 ± 0.5	7.362 ± 0.02	41 ± 2	153 ± 7	0.7 ± 0.2
	NON	12.2 ± 0.7	7.373 ± 0.01	38 ± 2	133 ± 11	0.7 ± 0.1
T45	Oxycyte	11.4 ± 0.6	7.343 ± 0.01	40 ± 1	432 ± 48*	0.7 ± 0.1
	NON	13.0 ± 0.7	7.348 ± 0.01*	41 ± 1	346 ± 45*	0.8 ± 0.08
T75	Oxycyte	11.7 ± 0.8	7.326 ± 0.01*	40 ± 3	444 ± 28*	0.8 ± 0.1*
	NON	13.4 ± 0.8	7.327 ± 0.02*	41 ± 2	342 ± 37*	0.9 ± 0.1
T105	Oxycyte	11.6 ± 0.9	7.312 ± 0.01*	42 ± 2	438 ± 42*	0.8 ± 0.1*
	NON	13.2 ± 0.5	7.328 ± 0.01*	39 ± 3	343 ± 38*	0.9 ± 0.1

T135	Oxycyte	12.1 ± 0.8	7.318 ± 0.01	40 ± 2	482 ± 27*	0.8 ± 0.2*
	NON	14.1 ± 0.7	7.322 ± 0.02*	41 ± 1.5	299 ± 44*	0.9 ± 0.2
T155	Oxycyte	12.4 ± 0.4	7.319 ± 0.02*	42 ± 3	463 ± 17*	0.9 ± 0.1*
	NON	13.4 ± 0.6	7.318 ± 0.02*	38 ± 2	372 ± 25*	0.8 ± 0.1

Figure Legends

Figure 1. Mean blood pressure (MAP; mean ± SEM). Animals were treated with Oxycyte or NS as four-30 min IV infusions represented as grey vertical bars. There were no significant differences in MAP between the two groups.

Figure 2. Pial arteriolar diameters. Diameter of (a) small-sized (<50 µm) and (b) medium-sized (50- 100 µm) pial arterioles (mean ± SEM) from rats treated with Oxycyte or NS. Test solutions were administered as four-30 min IV infusions represented as grey vertical bars. Final data point for each treatment is the vasoconstrictive effect of topical BaCl₂. # and * Indicate a significant (P ≤ 0.05) change in vessel diameters comparing pre- vs. post-infusion values in the Oxycyte and NS groups, respectively. ## and ** indicate a significant cumulative change (T0 vs. T150) in Oxycyte and NS groups, respectively. There were no differences in percent change of either small or medium-sized vessel between the two treatment groups for any of the infusions. (c) Representative images of pial microvessels before infusion of Oxycyte (left), after Oxycyte infusion (middle), and at the end of the experiment after application of BaCl₂ as a positive control for constriction. Arrows point to pial arterioles. Scale bars = 100 µm.

Figure 3. Mean blood pressure (MAP; mean ± SEM). TBI was induced immediately after T0 (arrow) and Oxycyte animals were treated with 6 ml/kg Oxycyte at T15 (Oxycyte 100), while NON animals (NON 100) did not receive any treatment. Both groups were mechanically ventilated using 100% oxygen. MAP was higher (asterisk: P < 0.05) in Oxycyte-treated animals from T45-T155.

Figure 4. Percent change in $P_{bt}O_2 \pm$ SEM from pre-injury. **(a)** $P_{bt}O_2$ decreased significantly following TBI (arrow) in both the Oxycyte and NON groups. Both groups were ventilated using 100% oxygen. There was no difference in $P_{bt}O_2$ between Oxycyte-treated (Oxycyte 100) and untreated (NON 100) for the duration of the experiment. **(b)** $P_{bt}O_2$ in a group of Oxycyte-treated animals ventilated with 40% oxygen increased significantly (*) at T35 compared to T15 and continued to increase until the rest of the observation period. $P_{bt}O_2$ was significantly higher in the Oxycyte and 40% oxygen (Oxycyte 40) than $P_{bt}O_2$ in the Oxycyte and 100% oxygen (Oxycyte 100), NON and oxygen 100% (NON 100), and NON and 40% oxygen (NON 40) groups.

Table 1. Experimental design for the pial microvessel experiments (healthy rats). NS: 0.9% normal saline.

Time (min)	Treatment	Group	
		Oxycyte	NS
0-30	Infusion 1	3 ml/kg/h PFC+ 1 ml/kg/h NS	4 ml/kg/h NS
40-70	Infusion 2	3 ml/kg/h PFC+ 1 ml/kg/h NS	4 ml/kg/h NS
80-110	Infusion 3	3 ml/kg/h PFC+ 1 ml/kg/h NS	4 ml/kg/h NS
120-150	Infusion 4	3 ml/kg/h PFC+ 1 ml/kg/h NS	4 ml/kg/h NS
155	Vasoconstriction positive control	Topical BaCl ₂	Topical BaCl ₂

Table 2. Experimental design for the TBI and brain tissue oxygenation experiments. NS: 0.9% normal saline.

Time (min)	Treatment	Group	
		Oxycyte	NON
0-15	TBI followed by 15 min delay		
15-18	drug Infusion	6 ml/kg PFC at 1 ml/min	4 ml/kg/h NS
18-155	Observation and maintenance fluids	4 ml/kg/h NS	4 ml/kg/h NS

Table 3. Blood pressure and heart rate parameters (mean $\pm$ SEM) for Oxycyte and saline (NS) groups. # Post-infusion value (T30, T70, T110 or T150) is significantly different ($P \leq 0.05$) from its corresponding within-group pre-infusion value (T0, T40, T80, and T120). * Post-infusion value (T30, T70, T110 or T150) is significantly different ($P \leq 0.05$) from its corresponding within-group value at T0. There were no statistically significant differences between the two treatment groups at any time point.

Time	Treatment	MAP (mm Hg)	HR (beats/min)
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T70 (After Infusion 2)	Oxycyte	97.2 $\pm$ 8.2	321 $\pm$ 66
	NS	92.0 $\pm$ 12.6	318 $\pm$ 44
T80 (Before Infusion 3)	Oxycyte	92.8 $\pm$ 9.3	308 $\pm$ 69
	NS	92.6 $\pm$ 17.5	336 $\pm$ 65
T110 (After Infusion 3)	Oxycyte	99.4 $\pm$ 12.4	341 $\pm$ 62 [#]
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T120 (Before Infusion 4)	Oxycyte	94.0 $\pm$ 15.0	331 $\pm$ 75
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	NS	12.3 $\pm$ 1.3	7.354 $\pm$ 0.06	42 $\pm$ 9	118 $\pm$ 29	0.7 $\pm$ 0.3
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	NS	12.1 $\pm$ 1.1	7.340 $\pm$ 0.06	43 $\pm$ 9	111 $\pm$ 31	0.7 $\pm$ 0.2
T150 (After Infusion 4)	Oxycyte	12.5 $\pm$ 0.5	7.37 $\pm$ 0.02 [§]	41 $\pm$ 2	165 $\pm$ 20* [§]	1 $\pm$ 0.1*

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	NON	12.2 $\pm$ 0.7	7.373 $\pm$ 0.01	38 $\pm$ 2	133 $\pm$ 11	0.7 $\pm$ 0.1
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	NON	13.0 $\pm$ 0.7	7.348 $\pm$ 0.01*	41 $\pm$ 1	346 $\pm$ 45*	0.8 $\pm$ 0.08
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	NON	13.4 $\pm$ 0.8	7.327 $\pm$ 0.02*	41 $\pm$ 2	342 $\pm$ 37*	0.9 $\pm$ 0.1
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Author/s:

Abutarboush, R;Mullah, SH;Saha, BK;Haque, A;Walker, PB;Aligbe, C;Pappas, G;Tran Ho, LTV;Arnaud, FG;Auker, CR;McCarron, RM;Scultetus, AH;Moon-Massat, P

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