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Treatment of traumatic brain injury with 17α-ethinylestradiol-3-sulfate in a rat model

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OBJECTIVE 17α-ethynylestradiol-3-sulfate (EE-3-SO_4) is a highly water-soluble synthetic estrogen that has an extended half-life (~ 10 hours) over that of naturally occurring estrogen (~ 10 minutes). In this study, EE-3-SO_4 was evaluated in a lateral fluid percussion–induced traumatic brain injury (TBI) model in rats.

METHODS A total of 9 groups of Sprague-Dawley rats underwent craniectomy. Twenty-four hours later, lateral fluid percussion was applied to 6 groups of animals to induce TBI; the remaining 3 groups served as sham control groups. EE-3-SO_4 (1 mg/kg body weight in 0.4 ml/kg body weight) or saline (vehicle control) was injected intravenously 1 hour after TBI; saline was injected in all sham animals. One day after EE-3-SO_4/saline injection, intracranial pressure (ICP), cerebral perfusion pressure (CPP), and partial brain oxygen pressure ($PbtO_2$) were measured in Groups 1–3 (2 TBI groups and 1 sham group), and brain edema, diffusion axonal injury, and cerebral glycolysis were assessed in Groups 4–6 using MRI T2 mapping, diffusion tensor imaging (DTI), and FDG-PET imaging, respectively. Four days after dosing, the open-field anxiety of animals was assessed in Groups 7–9 by measuring the duration that each animal spent in the center area of an open chamber during 4 minutes of monitoring.

RESULTS EE-3-SO_4 significantly lowered ICP while raising CPP and $PbtO_2$, compared with vehicle treatment in TBI-induced animals ($p < 0.05$). The mean size of cerebral edema of TBI animals treated with EE-3-SO_4 was 25 ± 3 mm^3 (mean ± SE), which was significantly smaller than that of vehicle-treated animals (67 ± 6 mm^3, $p < 0.001$). Also, EE-3-SO_4 treatment significantly increased the fractional anisotropy of the white matter in the ipsilateral side ($p = 0.003$) and cerebral glycolysis ($p = 0.014$). The mean duration that EE-3-SO_4-treated animals spent in the center area was 12 ± 2 seconds, which was significantly longer than that of vehicle-treated animals (4 ± 1 seconds; $p = 0.008$) but not different from that of sham animals (11 ± 3 seconds; $p > 0.05$).

CONCLUSIONS These data support the clinical use of EE-3-SO_4 for early TBI treatment.

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KEY WORDS TBI; traumatic brain injury; intracranial pressure; T2 mapping; diffusion tensor imaging; positron emission tomography; behavioral test

Traumatic brain injury (TBI) is a worldwide leading cause of mortality and long-term disability.[13] TBI often causes cerebral edema, which leads to an increase in intracranial pressure (ICP) and a decrease in cerebral perfusion pressure (CPP).[16,46,49] These changes result in cerebral ischemia (or hypoxia first and then eventually ischemia) and neuronal degeneration,[10] which is associated with low partial brain oxygen pressure ($PbtO_2$).[33] In our previous study, we described the efficacy of 17β-estradiol (E2) in its sulfate-conjugated form (17β-estradiol sulfate, E2-SO_4) to treat experimental TBI.[25] E2-SO_4 is a naturally occurring derivative of E2, created by the addition of a sulfate moiety via a steroid sulfotransferase enzyme. The physiological sulfate conjugation process allows excretion of this soluble form of E2 via the kidneys, which regulates the hormone levels. In our case, the solubility of E2-SO_4 enables intravenous delivery of supraphysiological quantities of this hormone, which would not be possible with the highly hydrophobic native E2.

E2 is highly neuroprotective.[2,9,25,29] It is synthesized in the brain with both autocrine and paracrine activities[2] and freely passes the blood-brain barrier; thus, endocrine E2

ABBREVIATIONS CPP = cerebral perfusion pressure; DAI = diffuse axonal injury; DTI = diffusion tensor imaging; EE= 17α-ethinyl estradiol; EE-3-SO_4 = 17α-ethynylestradiol-3-sulfate; E2 = 17β-estradiol; E2-SO_4 = 17β-estradiol sulfate; ICP = intracranial pressure; $PbtO_2$ = partial brain oxygen pressure; SUV = standardized uptake value; TBI = traumatic brain injury.

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is likewise at play in the CNS. While estrogen's role as a female sex hormone is well known, it is also present and synthesized normally in males, albeit at a lower level than in females. In fact, the enzyme aromatase, which is constitutive in many tissues including vertebrate brain, converts testosterone into E2.[7] This process can occur in a rapid, nongenomic fashion in the brain, which supports the notion that estrogen plays a major role in brain physiology and homeostasis.[40] E2 is nominally found only in neurons, but after penetrating brain injury, there is rapid de novo upregulation of aromatase in hippocampal astrocytes.[12] However, E2 has a short plasma half-life (about 10 minutes), which may limit its therapeutic efficacy.

17α-ethinyl estradiol (EE) is a synthetic derivative of E2 and has a longer plasma half-life (about 10 hours) than E2.[35] EE has been used for decades and has an extensive track record for efficacy and safety when used for hormone replacement therapy and birth control.[1,18] However, since EE is also hydrophobic, we had 17α-ethynylestradiol-3-sulfate (EE-3-SO_4) custom synthesized. EE-3-SO_4 treatment in the absence of fluid resuscitation significantly increased survival of minipigs and rats that underwent severe hemorrhage (60% blood loss).[17,31] These data led us to adopt EE-3-SO_4 as the drug of choice for TBI treatment studies, made more relevant by a dearth of effective pharmaceuticals available to treat TBI.[6]

Rather than EE-3-SO_4 having a lower rate of degradation or excretion, it is possible that it can be "recycled." It has been observed that when orally administered ethinyl estradiol is conjugated and excreted, the portion that is passed in urine is completely cleared, while some of the portion entering the gut can be taken up and returned to the periphery, where the sulfate conjugate exceeds the quantity of unconjugated ethinyl estradiol.[3,14] There is insufficient information on the fate of intravenously administered ethinyl estradiol (conjugated or unconjugated) to draw conclusions for the clearance and resorption, but a gut reuptake of EE-3-SO_4 seems at least plausible.

TBI can also induce behavioral changes.[39] Of interest, it has been reported that behavioral changes in female rats were significantly milder than those in male rats after TBI.[36] This might indicate that female gonadal hormones, most likely estradiol, may alleviate sexually dimorphic stress. In fact, the improved behavioral activity of TBI models in animals was observed after estrogen treatment.[5,50]

In this study, we used physiological and imaging techniques to measure the salutary effect of EE-3-SO_4 in a TBI rat model. The physiological measurements used were ICP, CPP, and $PbtO_2$. The imaging methods used were diffusion tensor imaging (DTI) to assess diffuse axonal injury (DAI),[4] T2 mapping to determine the size of the edematous region,[24] and FDG-PET to measure brain glycolysis.[11] In addition, an open-field test was performed to assess the effect of EE-3-SO_4 on spontaneous exploratory behavior as a secondary injury of TBI.

Methods

Animal Study Design

Nine groups of male Sprague-Dawley rats were used (mean weight 318 ± 3 g; 10 ± 2 weeks old [± SE]); Groups 1–3 (4–5 rats per group) were used for measuring ICP, CPP, and $PbtO_2$, Groups 4–6 (5–6 per group) were used for in vivo imaging study, and Groups 7–9 (9–11 per group) were used for open-field anxiety measurement. The groups are summarized in Table 1. Craniectomy was performed in all animals as described in our previous study.[25] Groups 1, 4, and 7 were used as sham groups; in the remaining groups, TBI was induced using a lateral fluid percussion method 24 hours after completion of the craniectomy, as previously described.[9] The lateral fluid percussion force was monitored and recorded from an inline transducer for quality control purposes. One hour after TBI (or 25 hours after craniectomy), each animal in Groups 3, 6, and 9 was intravenously injected with EE-3-SO_4 (1 mg/kg body weight in 0.9% NaCl; 0.4 ml/kg body weight), while the other animals were intravenously injected with saline (0.9% NaCl; 0.4 ml/kg body weight). At 22 hours after dosing (or 47 hours after craniectomy), ICP, CPP, and $PbtO_2$ levels were monitored in Groups 1–3 for 2 hours at 15-minute intervals. T2-weighted imaging, DTI, and FDG-PET/CT imaging were performed in Groups 4–6 during the same time. MRI was performed using a 9.4-T MR scanner dedicated to small animals (Bruker BioSpin Corp.), and PET/CT imaging was performed using a microPET/CT system, Triumph (GE). Animals were anesthetized using 1%–2% isoflurane during craniectomy, physiological parameter monitoring, dosing, and imaging. At 96 hours after dosing (or 121 hours after craniectomy), the open-field anxiety of animals in Groups 7–9 was assessed by measuring the duration that each animal spent in the center region of an open chamber. To reduce pain, all animals including sham animals received carprofen (intraperitoneal 5 mg/kg) and a prophylactic antibiotic (Baytril, intraperitoneal 1 mg/kg) after TBI twice per day until all experiments ended. All animal experiments were approved by the Institutional Animal Care and Use Committee at the University of Alabama at Birmingham.

Measurement of Physiological Parameters

Physiological parameters (ICP, CPP, and $PbtO_2$) were measured using similar methods introduced in our previous study.[25] Briefly, ICP and $PbtO_2$ were measured on the brain exposed by craniectomy using fiberoptic-based pressure transducer probes (FISO LS-10 signal conditioner and 0.9-F probe, Harvard Apparatus) and a Licox oxygen catheter microprobe (Integra Neuroscience), respectively. CPP was obtained by subtracting ICP from the mean arterial pressure, with mean arterial pressure measured using a blood pressure analyzer (Digi-Med BPA blood pressure analyzer, MicroMed Inc.) from the cannulated left femoral artery.

T2 Mapping

T2 mapping was performed to detect cerebral edema. Conventional T2-weighted images can be also used to identify the edema region, but due to nonuniform sensitivity of a surface coil over the field of view, automatic segmentation is difficult. The T2 map was obtained using the multiple TE approach.[48] A multi-slice multi-echo sequence was employed for 10 1-mm-thick slices and 10 different TE

TABLE 1. Time schedule of TBI induction, dosing, ICP/$PbtO_2$/CPP measurement, imaging or open-field measurement after craniectomy in each group

Group No.	Time After Completing Craniectomy (hrs)				
	0	24	25	47–49	121–122
1 (n = 4)	Craniectomy		Vehicle	ICP/$PbtO_2$/CPP	
2 (n = 5)	Craniectomy	TBI	Vehicle	ICP/$PbtO_2$/CPP	
3 (n = 5)	Craniectomy	TBI	EE-3-SO_4	ICP/$PbtO_2$/CPP	
4 (n = 5)	Craniectomy		Vehicle	T2/DTI/PET/CT	
5 (n = 6)	Craniectomy	TBI	Vehicle	T2/DTI/PET/CT	
6 (n = 6)	Craniectomy	TBI	EE-3-SO_4	T2/DTI/PET/CT	
7 (n = 9)	Craniectomy		Vehicle		Open-field measurement
8 (n = 11)	Craniectomy	TBI	Vehicle		Open-field measurement
9 (n = 9)	Craniectomy	TBI	EE-3-SO_4		Open-field measurement

values (14, 28, 42, 56, 70, 84, 98, 112, 126, and 140 msec) were used. The other detail parameters were as follows: TR 5000 msec, FOV 30 × 30 mm, and matrix size 256 × 256. The region in the ipsilateral side having T2 values larger than the mean T2 value plus 2 standard deviations of the contralateral side was determined as the edematous region, and the edema volume was calculated by the sum of all edematous regions in 10 slices. T2 values were calculated from the equation SI = Kexp(–TE/T2)t, where SI is the MR signal, K is constant, and t is time, using our own custom computer software made with MATLAB (version 7.11.0, MathWorks, Inc.). On the T2-weighted image (TE 14 msec) at the center of craniectomy, the area of the ipsilateral brain was compared with that of the contralateral side to determine whether brain morphology was changed after TBI.

Diffusion Tensor Imaging

DTI was conducted using a modified Stejskal and Tanner spin-echo diffusion-weighted sequence[47] with the following imaging parameters: TR 3001 msec, TE 32 msec, FOV 30 × 30 mm, and matrix size 128 × 128. Six 1-mm-thick slices were obtained across the injured brain region. First, imaging with a b value of 0 sec/mm^2 was conducted, and then diffusion gradients along 6 different directions were applied to quantitate fractional anisotropy (FA) as shown in our previous study.[25] In FA maps, the region having higher FA values than the surrounding tissue was determined as the region of white matter. In each slice, the mean FA value of white matter in the ipsilateral side was divided by that in contralateral side, and the relative FA value was calculated by averaging all of these values from 6 slices. FA values were quantitated using laboratory-made computer software with MATLAB (version 7.11.0, MathWorks, Inc.).

FDG-PET/CT Imaging

The animals were not fasted prior to imaging. Animals were intravenously injected with FDG (54 ± 2 MBq in 300 μl of phosphate-buffered saline), and PET/CT imaging was applied 43 ± 1 minutes after dosing. CT imaging was conducted to identify the bone region that underwent craniectomy, as described previously.[44] CT imaging was completed in 1.07 minutes, and 10 minutes of PET imaging followed. The maximum likelihood expectation maximization algorithm (10 iterations) was employed for PET image reconstruction. The field of view of each PET image slice was 46 × 46 mm, and 31 1.175-mm-thick slices were obtained. The matrix size of each PET image slice was 184 × 184. CT images were coregistered with PET images using vendor software. The animals' body temperatures were regulated to 37°C during the entire imaging process. The standardized uptake value (SUV) was calculated as (C × W)/D, where C is the tissue radioactivity per unit volume (MBq/ml), W is animal body weight (g), and D is the injected dose (MBq). In the 5 slices showing the opening for the skull, the mean SUV of the pixels in the upper half of the brain region was divided by that in the central region (about 10 mm^2), and the relative SUV was determined by averaging all of these values from 5 slices. SUV was quantitated using lab-made computer software with LabVIEW (version 2010, National Instruments Co.).

Open-Field Anxiety Measurement

Open-field anxiety of animals was measured at the Behavioral Assessment Core of the University of Alabama at Birmingham. Each animal was placed at the center of a square chamber that had transparent plastic walls (width × length × height: 70 × 70 × 35 cm) and no top cover. Animal behavior was recorded using a WV-CP-484 CCD Color Surveillance camera (Panasonic) for 4 minutes. The animal track in the video was retrieved using EthoVision XT (Noldus Information Technology Inc.), a video tracking software package. The inner square (30 × 30 cm) at the center of the chamber was defined as the center region, and the duration that each animal spent in the center region was calculated. To determine whether motor and/or sensory cortex was damaged, the total distance that each animal traveled was also measured.

Statistical Analysis

SAS statistical software (version 9.4, SAS Institute Inc.) was used to conduct statistical analysis. Physiological parameters (ICP, CPP, and $PbtO_2$) or open-field anxiety measurements among 3 groups (sham, vehicle-treated, and EE-3-SO_4-treated groups) were compared using 1-way

ANOVA.[34] Imaging quantitated values (edema size, relative FA value, and relative SUV value) among 3 groups were compared using 1-way ANOVA as well. After Bonferroni correction for multiple comparison,[34] $p < 0.05$ was considered statistically significant. When the p value became larger than 1 after Bonferroni correction, it was truncated to 1. In this article, the mean value is represented as the mean ± standard error.

Results

EE-3-SO_4 Significantly Decreased ICP and Increased $PbtO_2$ and CPP

Figure 1 presents the mean ICP, $PbtO_2$, and CPP in the sham, vehicle-treated, and EE-3-SO_4-treated groups, which were monitored for 2 hours (23–25 hours after dosing). The ICP of the EE-3-SO_4-treated group was about 40% lower than that of the vehicle-treated group, and the difference was statistically significant for the entire 2 hours ($p < 0.05$). Meanwhile, $PbtO_2$ and CPP values in the EE-3-SO_4-treated group were about 60% and 10% higher than those in the vehicle-treated group, respectively; this difference was also statistically significant ($p < 0.05$).

EE-3-SO_4 Significantly Decreased Cerebral Edema

Figure 2A shows T2-weighted images (TE 14 msec) of the brain and T2 maps of representative sham, vehicle-treated, and EE-3-SO_4-treated animals after TBI in the same color scale. The ipsilateral brain regions of vehicle- and EE-3-SO_4-treated animals were 4.0% ± 2.4% and 2.5% ± 1.5% larger than the contralateral brain regions, respectively, but no statistically significant difference was found between the groups ($p = 0.648$). Figure 2B presents the size of cerebral edema of each group. Cerebral edema was not observed in sham animals, but the mean edema size in the vehicle-treated group was 67 ± 6 mm^3. The edema size of EE-3-SO_4-treated animals was 25 ± 3 mm^3, significantly smaller than that of vehicle-treated animals ($p < 0.001$).

EE-3-SO_4 Significantly Alleviated DAI

Figure 3A presents cerebral FA maps of representative sham, vehicle-treated, and EE-3-SO_4-treated animals after TBI. In sham animals, the FA values of the ipsilateral white matter were not different from those of the contralateral side. However, the relative FA value of TBI-induced animals treated with vehicle was only 82.4% ± 2.2%, significantly lower than that of sham animals ($p < 0.001$) (Fig. 3B). When the animals were treated with EE-3-SO_4, the relative FA value was increased to 90.8% ± 1.4%, which was significantly higher than that of the vehicle-treated group ($p = 0.003$), but lower than that of sham animals ($p = 0.001$).

EE-3-SO_4 Significantly Increased Cerebral Glycolysis

Figure 4A presents FDG-PET/CT images of representative sham, vehicle-treated, and EE-3-SO_4-treated animals after TBI, normalized to the intensity of central brain region. Figure 4B shows the relative SUV in the cerebral region of the 3 groups. The relative SUV of vehicle-treated

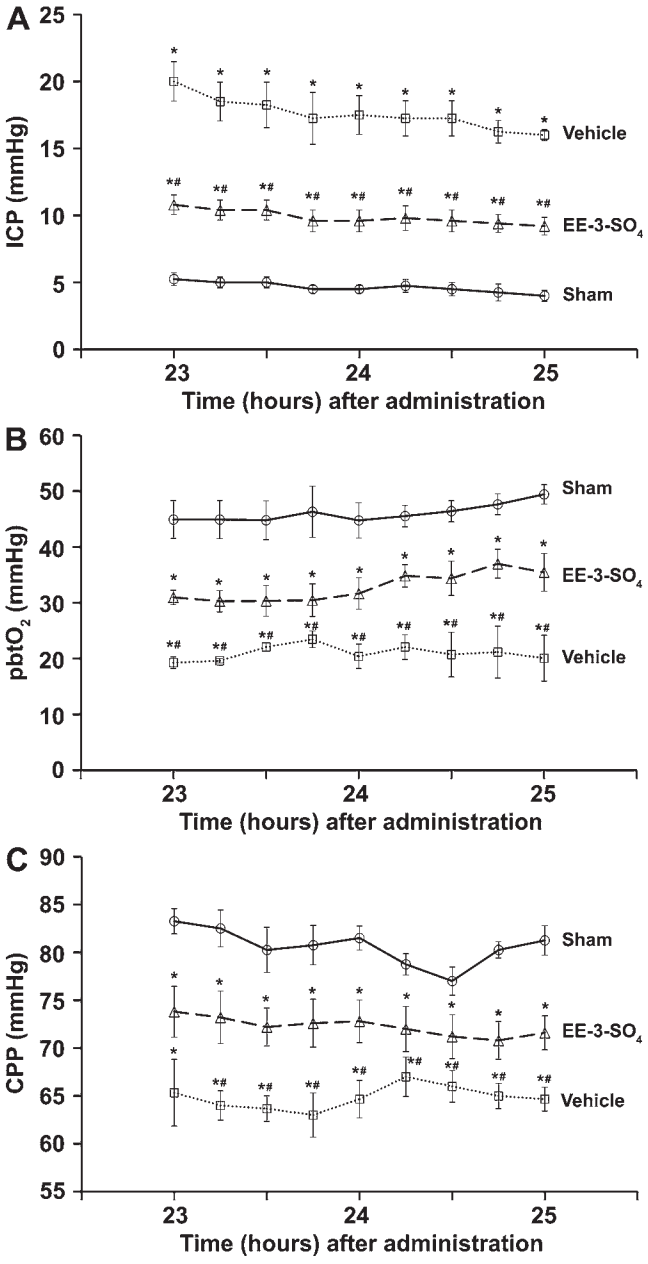

FIG. 1. Effect of EE-3-SO_4 treatment on physiological parameters following TBI. ICP (**A**), $PbtO_2$ (**B**), and CPP (**C**) of sham, vehicle-treated, and EE-3-SO_4-treated groups at 9 different time points from 23 hours to 25 hours after administration. *Asterisks* and *hash marks* represent statistically significant differences from sham and vehicle-treated groups (4–5 per group), respectively.

group after TBI was 84.9% ± 1.1%, which was significantly lower than that of the sham group (100.2% ± 0.8%, $p < 0.001$) and the EE-3-SO_4-treated group (88.9 ± 0.7%, $p = 0.014$). The relative SUV of EE-3-SO_4-treated group, however, was significantly lower than that of the sham group ($p < 0.001$).

EE-3-SO_4 Significantly Relieved Open-Field Anxiety Induced by TBI

Figure 5A shows the representative images of the ani-

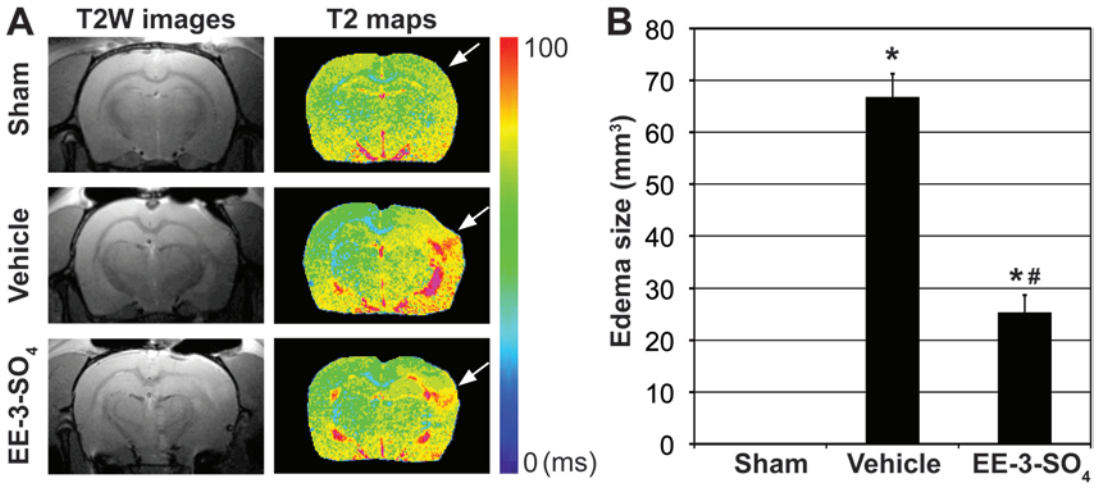

FIG. 2. Cerebral edema assessed by T2 mapping. **A:** Representative T2-weighted (T2W) images (TE 14 msec) and T2 maps of sham, vehicle-treated, and EE-3-SO_4-treated animals 1 day after administration. The side that underwent craniectomy is indicated by the *arrows*. Edema is represented by higher T2 values (in *red*). **B:** Mean edema size in sham, vehicle-treated, and EE-3-SO_4-treated groups (5–6 rats per group) at 1 day after administration. *Asterisks* and the *hash mark* represent statistically significant differences from sham and vehicle treated groups, respectively. *Whiskers* represent SE. Figure is available in color online only.

mal track. The mean distances traveled by sham, vehicle-treated, and EE-3-SO_4-treated animals were 1772 ± 109, 1712 ± 117, and 1721 ± 97 cm, respectively, and no statistically significant difference was found between groups ($p > 0.05$). Figure 5B shows the duration that animals in each group spent in the center region. Sham animals spent 11 ± 3 seconds in the center region, whereas the vehicle-treated animals spent only 3 ± 1 seconds in the region ($p = 0.018$). Of note, EE-3-SO_4-treated animals spent a mean of 12 ± 2 seconds in the center region, which was significantly longer than that of vehicle-treated animals ($p = 0.008$), and not different from that of sham animals ($p = 1$).

Discussion

The early salutary effects of EE-3-SO_4 against TBI were confirmed using a rat model in this study. EE-3-SO_4 significantly increased $PbtO_2$, CPP, and glycolic metabolism in the brain, and decreased ICP and cerebral edema as early as 1 day after treatment compared with vehicle. These results are similar to those of estrogen sulfate (E2-SO_4) in our previous study.[25] Estrogen decreases vascular permeability in the injured area, increasing the transport of water molecules out of the edematous region back into circulation.[30,38,45] This leads to a decrease in edema size and ICP. Estrogen also increases blood pressure during

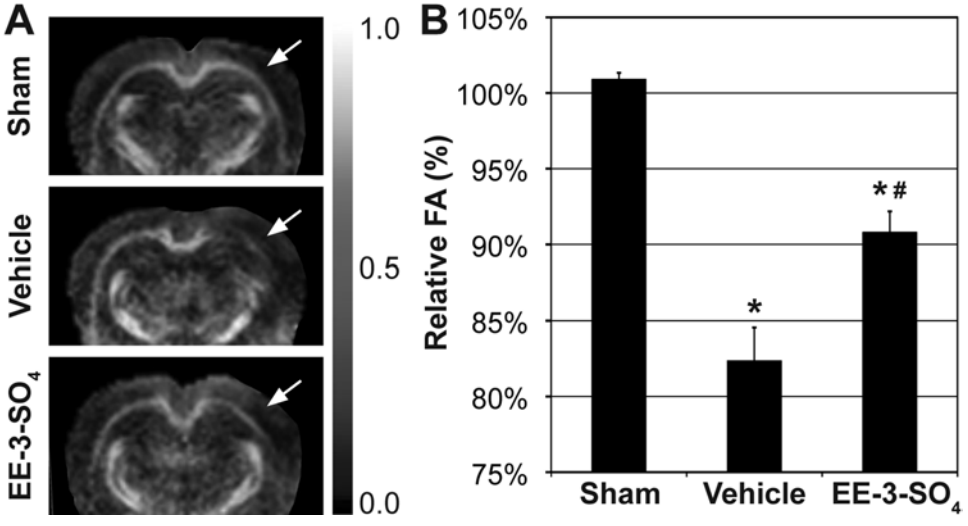

FIG. 3. DAI assessed by FA mapping. **A:** Representative FA maps of sham, vehicle-treated, and EE-3-SO_4-treated animals 1 day after administration. The white matter adjacent to the craniotomy site is indicated by the *arrows*. **B:** The mean relative FA (ratio of FA value in the white matter of the ipsilateral side to that of the contralateral side) of sham, vehicle-treated, and EE-3-SO_4-treated groups (5–6 rats per group) 1 day after administration. *Asterisks* and the *hash mark* represent statistically significant differences from sham and vehicle-treated groups, respectively. *Whiskers* represent SE.

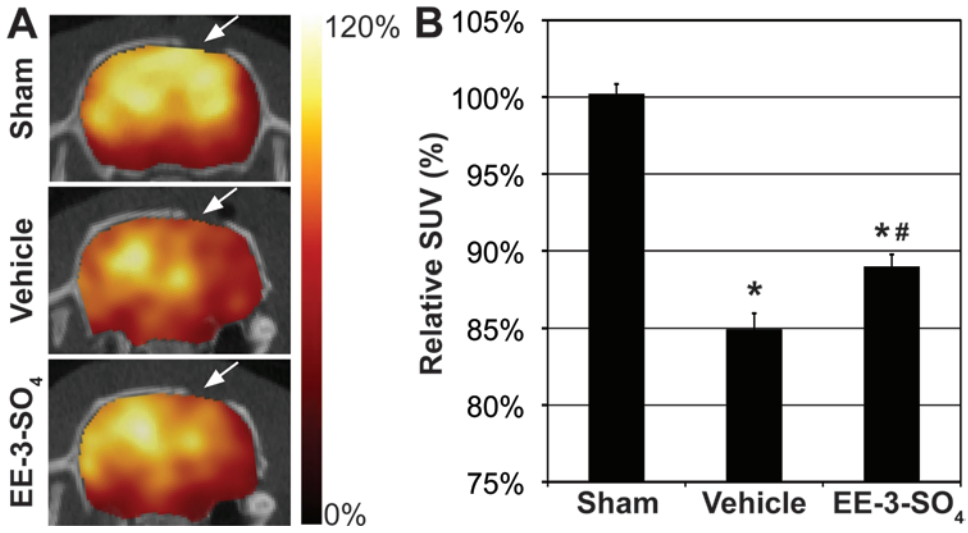

FIG. 4. Cerebral glycolysis assessed by FDG-PET/CT imaging. **A:** Representative ^{18}F-FDG PET/CT images of sham, vehicle-treated, and EE-3-SO_4-treated animals 1 day after administration. The intensity of PET images is normalized to that of the central brain region. The area that underwent craniectomy is indicated by the *arrows*. **B:** The mean relative SUV (ratio of SUV averaged in the upper half of the brain to that in the central brain region) of sham, vehicle-treated, and EE-3-SO_4-treated groups (5–6 rats per group) 1 day after administration. *Asterisks* and the *hash mark* represent statistically significant differences from sham and vehicle-treated groups, respectively. *Whiskers* represent SE. Figure is available in color online only.

permissive hypotension,[31] which may be associated with the increase of CPP and $PbtO_2$ seen in this study. All of these factors lead to increased brain cell metabolism.

We also examined the therapeutic potential of EE-3-SO_4 on behavior related to anxiety. We conducted a video tracking test of spontaneous exploratory behavior in a fenced open field, which provides a measurement of fear-related emotionality and the evolution of overall locomotor activity. The total distances that the animals traveled were not statistically different between groups, which represents that the damage to the motor and/or sensory cortex by TBI was minimal. The duration that the animals spent in the center region is associated with the ability to cope with anxiety. The EE-3-SO_4-treated animals made significantly more visits to the center region than vehicle-treated animals, which indicates that EE-3-SO_4 treatment decreased TBI-induced anxiety. These data are consistent with the results of a previous study, demonstrating the salutary effect of estrogen to relieve TBI-induced anxiety.[36]

EE-3-SO_4 significantly alleviated DAI 1 day after administration; E2-SO_4 was not able to do this.[25] This might be related to the long plasma half-life of 17α-ethinyl estradiol (EE). EE can change its metabolism via altering enzymatic activities such as glucuronidation and sulfation in the liver,[15,37,41] and it also can increase its bioavailability via interaction with specific binding proteins in the plasma.[15] In addition, the binding affinity of EE to the estrogen receptors is about 2-fold higher than that of E2,

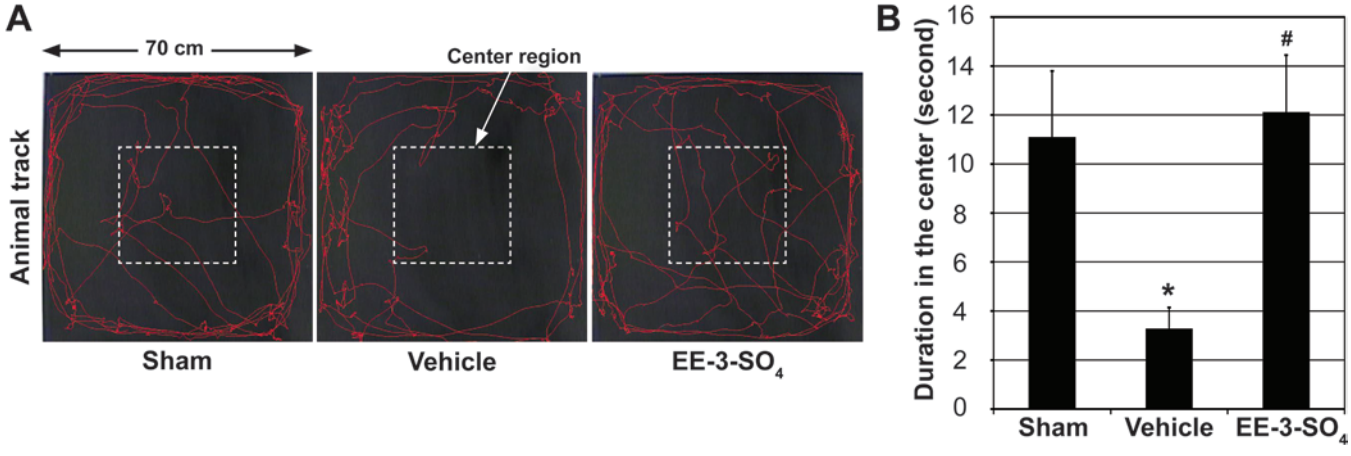

FIG. 5. Open-field anxiety measurement. **A:** Representative images of the animal track in sham, vehicle-treated, and EE-3-SO_4-treated groups 4 days after administration. The track is indicated by a *red solid line* in each subfigure. Animal movement was monitored for 4 minutes in an open box (width × length × height: 70 × 70 × 35 cm). The *inner dotted square* (30 × 30 cm) is defined as the center region. **B:** The mean duration that animals spent in the center region in each group (9–11 rats per group). *The asterisk* and *hash mark* represent statistically significant differences from sham and vehicle treated groups, respectively. *Whiskers* represent SE. Figure is available in color online only.

which induces stronger intercellular signals.[21,26,27] Furthermore, sulfated ethinyl estrogens are highly soluble in the water, which is essential for effective drug distribution in tissues.[17,27,31]

The rationale of DAI recovery by EE-3-SO_4, however, is still not clear. We presume that EE-3-SO_4 alleviates traumatic axonal injury via increasing glucose uptake to stabilize ion channels. After TBI, the intracellular pH balance and ion concentrations should be restored immediately to prevent further membrane breakdown in the neurons and axons,[19] and glucose uptake is important for regulating energy-dependent ion transport proteins (especially Na/K ATPase-dependent transport proteins).[52] To meet the increased needs of glucose, cerebral circulation should be enhanced.[42] EE-3-SO_4 can increase the cerebral circulation via its vasodilatory effects.[17,31,38] Neutralizing free radicals in the damaged area using antioxidants during the early stage of injury can result in significant reduction of inflammation, edema, and axonal breakdown.[51] Estrogens have strong antiinflammatory and antioxidant effects.[30,45] Since EE presents higher binding affinity to estrogen receptors than natural estrogens,[21,26,27] EE-3-SO_4, a sulfated EE, may present higher potency against DAI, as demonstrated in this study.

In addition, a long half-life of EE-3-SO_4 may effectively mitigate the secondary brain injury from TBI, reducing the likelihood of permanent brain damage and disability. TBI has primary and secondary injury phases. At the time of injury, the immediate parenchymal damages occur in the neuronal, glial, and vascular tissues, and disruptions of those cell membranes cause the disturbance of ionic homeostasis,[10,46,49] which is the unavoidable primary injury. The secondary injury occurs minutes to days after the accident; cytokines and chemokines are released from immune cells after the traumatic injury, thereby promoting neuroinflammation, which triggers a cascade of physiological deterioration in tissues.[8] In addition, axons within a white matter tract undergo dynamic deformations as neurons are seen to endure in both the primary and secondary injury phases, and the axonal and neuronal damages aggravate inflammation in the surrounding tissue.[28,43] Antiinflammatory effects of estrogens are well established,[45] and therefore EE-3-SO_4 may be able to successfully prevent the secondary injury as it presents increased efficacy and gives longer exposure. EE-3-SO_4 may also be able to prevent cerebral vasospasm and edema that occur during the secondary injury phase.

It should be noted that serious injuries on the battlefield and in civilian settings are often polytraumatic, and thus TBI may present simultaneously with hemorrhage. This offers the intriguing prospect that pharmacological estrogen treatment could serve a dual purpose. Since hemorrhage is obvious, our proposed treatment could be given immediately for the treatment of hemorrhage and in lieu of a TBI diagnosis, where TBI may or may not be obvious. In so doing, both TBI and hemorrhage would benefit from exposure to estrogen as early as possible, preferably within the "golden hour." In addition, previous work supports the use of estrogen as prophylaxis or treatment for sepsis,[22,23] which is another likely component of polytrauma, especially in cases of penetrating wounds. Also, it should be noted that plans are under way to conduct clinical trials with EE-3-SO_4 for treating severe hemorrhage, beginning with the DARPA (Defense Advanced Research Agency) Surviving Blood Loss initiative, which specified that large and small animals be treated with a drug without immediate fluid resuscitation. In those studies,[18,34] we were able to demonstrate a 6-hour survival with no interventions other than EE-3-SO_4 administration in rats and pigs, delivered in a very small, nonresuscitative volume (0.4 ml/kg body weight).

One additional prospect for administration of exogenous estrogen is a secondary or "knock on" effect. An interesting example in this context is the stimulation of the pituitary to secrete prolactin by exogenous estrogen administration, which has been demonstrated in healthy women,[3] as well as with cultured pituitary cells.[20] It has been found that prolactin is also neuroprotective, where it minimizes the damage from excitotoxicity in the hippocampus. This has been modeled in rats with kainic acid, which induces seizures. The investigators observed that prolactin greatly reduces the number and progression of seizures.[32]

Although our study has shown salutary effects of EE-3-SO_4 treatment after TBI on various parameters including edema reduction, it should be noted that the earliest measurements were carried out 23 hours posttreatment. Thus, it remains unknown if the salutary effects are observed even earlier than 23 hours after treatment. Likewise, it remains unknown if the salutary effects persist longer than the time frame in this study. It would appear, however, that the salutary effects of EE-3-SO_4 do persist after 24 hours since these parameters do normalize after a week or so in this model of mild to moderate TBI, which is supported by the results of the open-field anxiety test performed 4 days after TBI in this study. Thus, it appears that the use of EE-3-SO_4 after TBI accelerates the recovery of all the measured parameters. It should also be noted that only a single dose of EE-3-SO_4 (i.e., 1 mg/kg body weight) was used in this study. Thus, it remains unknown if a higher dose of EE-3-SO_4 would be even more efficacious in faster recovery of the altered parameters to normal.

Conclusions

EE-3-SO_4 as a drug will be stable, highly soluble, and have minimal space requirements, which will facilitate easy transport of the agent for military medics and civilian first responders. An intraosseous autoinjector will allow for battlefield self- or "buddy" administration as well. Our hope is that this drug will prove to be lifesaving and may prevent long-term disability from TBI. However, as our study was based on a single TBI rodent model and histological analyses were not implemented, more extensive preclinical studies and clinical trials will need to follow to confirm the utility of EE-3-SO_4 in battlefield or with civilian trauma settings.

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Disclosures

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

Author Contributions

Conception and design: Chaudry, Kim, Yu, van Groen, Hubbard. Acquisition of data: Kim, Yu, Cam-Etoz. Analysis and interpretation of data: all authors. Drafting the article: Kim, Yu, Cam-Etoz, Hubbard. Critically revising the article: all authors. Reviewed submitted version of manuscript: all authors. Approved the final version of the manuscript on behalf of all authors: Chaudry. Statistical analysis: Kim. Administrative/technical/material support: Chaudry, Kim, Yu, van Groen, Hubbard. Study supervision: Chaudry, Kim, van Groen, Hubbard.

Supplemental Information

Previous Presentations

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