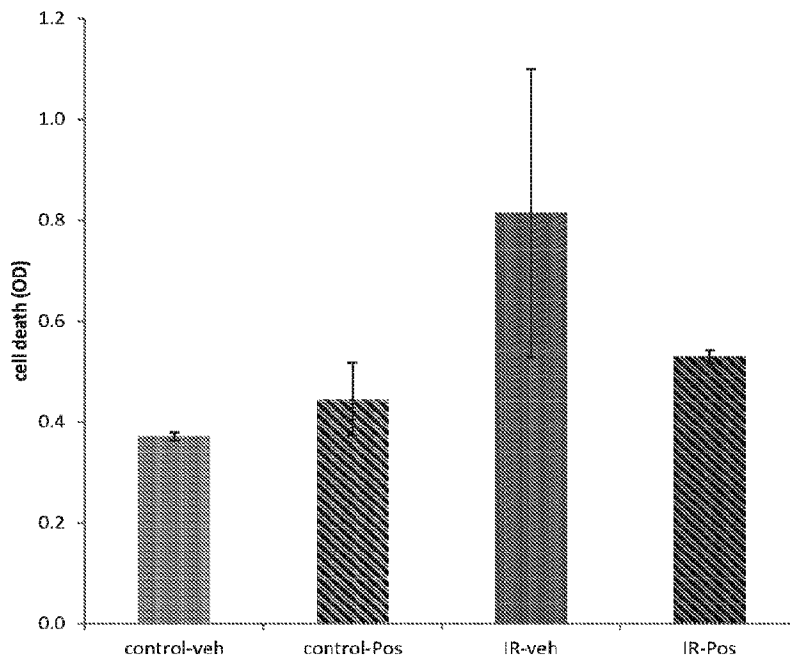




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(72) Inventeur/Inventor:
MACCECCHINI, MARIA, US
(73) Propriétaire/Owner:
ANNOVIS BIO, INC., US
(74) Agent: BORDEN LADNER GERVAIS LLP

(54) Titre : METHODES DE TRAITEMENT OU DE PREVENTION DE LESIONS AIGUES DU CERVEAU OU DES NERFS
(54) Title: METHODS OF TREATMENT OR PREVENTION OF ACUTE BRAIN OR NERVE INJURIES



(57) Abrégé/Abstract:

One of the aspects of the invention is directed toward a method of treating or preventing acute brain or nerve injury in a subject, comprising administering an effective amount of posiphen, or a pharmaceutically acceptable salt of posiphen, to the subject in need thereof. Similarly, another aspect of the invention is directed toward a use of posiphen, or a pharmaceutically acceptable salt of posiphen, for the treatment or prevention of acute brain or nerve injury. Preferably, the posiphen in the method or use of the invention is posiphen tartrate.

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- (71) Applicant: **QR PHARMA** [US/US]; 1055 Westlakes Drive, #300, Berwyn, PA 19312 (US).
- (72) Inventor: **MACCECCHINI, Maria**; 1223 Fox Glove, West Chester, PA 19380-5854 (US).
- (74) Agent: **WONG, King, Lit**; Seyfarth Shaw LLP, 975 F Street, N.W., Washington, DC 20004 (US).
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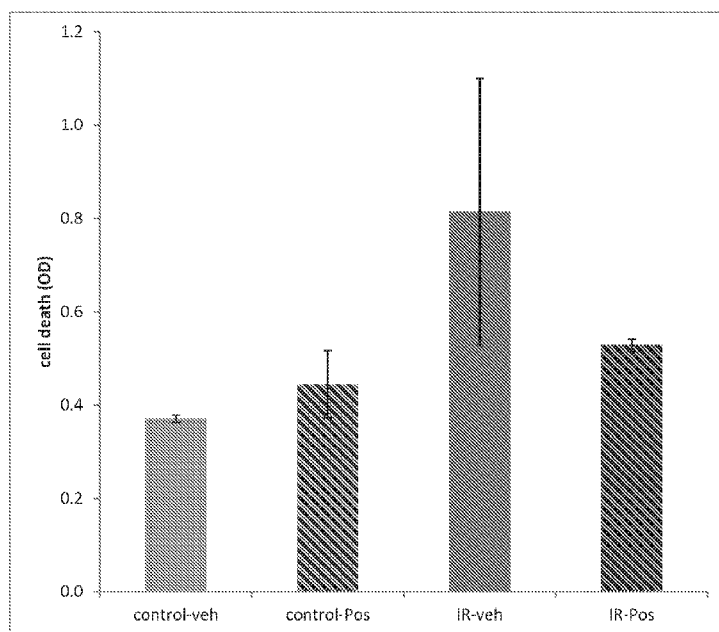


Figure 1

(57) Abstract: One of the aspects of the invention is directed toward a method of treating or preventing acute brain or nerve injury in a subject, comprising administering an effective amount of posiphen, or a pharmaceutically acceptable salt of posiphen, to the subject in need thereof. Similarly, another aspect of the invention is directed toward a use of posiphen, or a pharmaceutically acceptable salt of posiphen, for the treatment or prevention of acute brain or nerve injury. Preferably, the posiphen in the method or use of the invention is posiphen tartrate.

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Methods of Treatment or Prevention of Acute Brain or Nerve Injuries

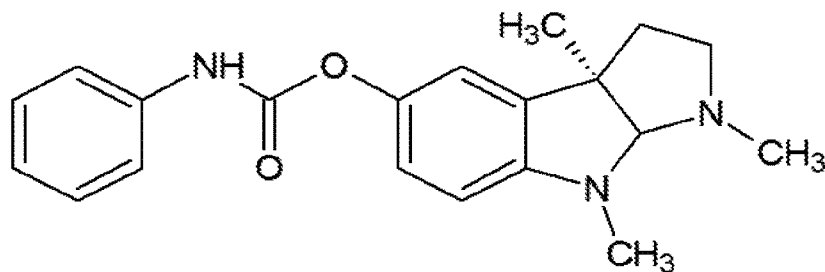
The present patent application concerns methods of treating or preventing acute brain or nerve injuries.

5 **Background**

10 The functioning of the brain and nerves is sensitive to abnormal biochemical and/or structural changes. Abnormally low level of oxygen in the brain could adversely affect the brain function, and could even lead to structural injuries in the brain. As a result, the brain is very sensitive to any changes in the blood supply. For instance, an interruption, or reduction, of the blood supply to an area of the brain could lead to an infarct in the area and/or neuronal loss. The brain and nerves are also structurally fragile and are susceptible to structural damage upon violent physical impact. These injuries or damage to the brain and/or nerves are pathologically different from the chronic neurodegeneration associated with diseases such as Parkinson's disease and Alzheimer's disease. In terms of the temporal pattern, these injuries or damage to the
15 brain and/or nerves are more acute than Parkinson's disease and Alzheimer's disease.

There are no drugs currently approved for the prevention or treatment of acute injuries to the brain and/or nerves. With sports and outdoor activities becoming more and more popular, and automobile accidents becoming more widespread, the incidence of acute brain or nerve injuries is higher than before. As acute brain or nerve injuries become more common, there is an
20 increased need for a drug to prevent and/or treat acute injuries to the brain and/or nerves.

The present invention shows that posiphen is effective in the prevention or treatment of acute brain or nerve injuries. Posiphen has been known to be effective in preventing or treating neurotoxicity or neurodegenerative processes (US 2012/0225922A1). Posiphen has a chemical name of (3aR)-1,3a,8-trimethyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-b]indol-5-yl
25 phenylcarbamate. The chemical structure of posiphen is represented with Formula I.



Formula I

5 Summary of the Invention

The present invention is directed toward methods of treating or preventing an acute brain or nerve injury in a subject comprising administering an effective amount of posiphen, or a pharmaceutically acceptable salt of posiphen, to a subject in need thereof.

10 Similarly, the invention is directed to the use of posiphen, or a pharmaceutically acceptable salt of posiphen, for the treatment or prevention of an acute brain or nerve injury. Alternatively, the invention is directed to the use of posiphen, or a pharmaceutically acceptable salt of posiphen, in the manufacture of a medicine for the treatment or prevention of an acute brain or nerve injury.

15 Brief Description of the Drawings

Figure 1 shows the extent of dead nerve cells in the retina of an eye subjected to transient ischemia reperfusion (IR) and the retina of the opposite eye not subjected to IR (serving as the control) in two groups of rats: one group was treated with posiphen (the "IR-Pos" and "control-Pos") and the other group was treated with a vehicle (the "IR-veh" and "control-veh") in
 20 Example 1.

Figure 2 shows the intensity of tyrosine hydroxylase (TH) immunoreactivity in the whole striatum of sham operated rats and rats subjected to fluid percussion injury (FPI) and treated with saline in Example 2, wherein the ipsilateral side was the side of the brain subjected to the fluid percussion injury in the rats treated with saline (the vehicle-treated group).

25 Figure 3 shows the intensity of tyrosine hydroxylase (TH) immunoreactivity in the whole striatum of rats subjected to fluid percussion injury (FPI) treated with saline, posiphen at 2.5

mg/kg, posiphen at 5 mg/kg or posiphen at 10 mg/kg in Example 2, wherein the ipsilateral side was the side of the brain subjected to the fluid percussion injury.

Figure 4 shows the percentage of IBA-1⁺ microglial cells in the substantia nigra of rats subjected to fluid percussion injury (FPI) and treated with saline (the vehicle), or posiphen at 10 mg/kg in Example 3, demonstrating that posiphen decreased brain inflammation after mild traumatic brain injury, wherein IBA-1⁺ represents positive for ionized calcium adaptor binding protein. Data on the horizontal axis are diameters of IBA positive cell bodies in substantia nigra ipsilateral to the fluid percussion injury (FPI), an index of inflammation.

Figure 5 shows the latency for rats to reach a platform in a Morris water maze in Example 4. The rats were subjected to mild traumatic brain injury and were divided into sham-operated group, vehicle-treated group, and posiphen-treated groups at a dose of 2.5 mg/kg of body weight, 5 mg/kg of body weight or 10 mg/kg of body weight.

Detailed Description of the Invention

As used herein, the term “posiphen” refers to (3aR)-1,3a,8-trimethyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-b]indol-5-yl phenylcarbamate, with the chemical structure shown in Formula I, at a chemical purity of at least 90%, preferably at least 95%, at least 98%, at least 99%, at least 99.5%, at least 99.9% or 100%.

The term “chemical purity” as applied to (3aR)-1,3a,8-trimethyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-b]indol-5-yl phenylcarbamate or a pharmaceutically acceptable salt of posiphen means the percent by weight of (3aR)-1,3a,8-trimethyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-b]indol-5-yl phenylcarbamate or the pharmaceutically acceptable salt of posiphen in terms of (3aR)-1,3a,8-trimethyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-b]indol-5-yl phenylcarbamate or the pharmaceutically acceptable salt of posiphen and other chemical impurities, e.g., its (-)-enantiomer, that may be present.

Examples of the pharmaceutically acceptable salt of posiphen include acid addition salts prepared from a suitable acid. The suitable acid can be hydrobromic acid, hydrochloric acid, hydroiodic acid, sulfuric acid, carbonic acid, nitric acid, phosphoric acid, tetrafluoroboric acid, perchloric acid, 1-hydroxy-2-naphthoic acid, 2,2-dichloroacetic acid, methanesulfonic acid, trifluoromethanesulfonic acid, 2-hydroxyethanesulfonic acid, benzenesulfonic acid, p-toluenesulfonic acid, cyclohexylaminosulfonic acid, 2-oxoglutaric acid, 4-acetamidobenzoic acid, acetic acid, phenylacetic acid, propionic acid, formic acid, succinic acid, glycolic acid, gluconic acid, malic acid, lactic acid, tartaric acid, citric acid, glucuronic acid, ascorbic acid, maleic acid, fumaric acid, pyruvic acid, aspartic acid, glutamic acid, benzoic acid, 4-hydroxybenzoic acid, anthranilic acid, 4-hydroxybenzoic acid, mandelic acid, pantoic acid, pantothenic acid, sulfanilic acid, stearic acid, alginic acid, β -hydroxybutyric acid, salicylic acid,

galactaric acid and galacturonic acid. Preferably, the pharmaceutically acceptable salt is posiphen tartrate, i.e., the acid addition salt of tartaric acid.

Examples of the acute brain or nerve injury treated or prevented with the methods or uses of the invention include traumatic brain injury, stroke, acute brain injury induced by brain ischemia, acute brain injury induced by insufficient oxygen supply to the brain, acute brain injury induced by anoxia or hypoxia, micro infarcts, acute brain injury induced by concussion, post-operative cognitive decline resulting from anesthesia or surgery-induced inflammation, acute brain injury induced by drowning, acute brain injury associated with whip lash, acute brain injury associated with bicycle crashes, acute brain injury associated with automobile accidents, shaken baby syndrome, acute brain injury induced by falling, e.g., falling out of windows, acute brain injury associated with physical impact of the head, and acute angle-closure glaucoma. The acute brain or nerve injury treated or prevented with the methods or uses of the invention excludes neurodegeneration associated with dementia of Alzheimer's disease, Parkinson's disease, Huntington's disease, Prion's disease, Amyloid Lateral Sclerosis, Tauopathy, Frontotemporal dementia, and chronic encephalopathy.

In the methods or uses of the invention, posiphen can be administered parenterally or enterally. Examples of the route of administration of posiphen are intravenous, intraocular, intramuscular, subcutaneous, topical, oral, sublingual and buccal. Preferably, posiphen is administered intravenously. For the treatment of acute angle-closure glaucoma, posiphen is preferably administered intraocularly or intravenously, with the intraocular route more preferred.

Posiphen, or a pharmaceutically acceptable salt of posiphen, can be administered once or repetitively. In the methods or uses of the invention for treating the acute brain or nerve injury, posiphen or a pharmaceutically acceptable salt of posiphen can be administered after an episode of the acute brain injury or acute angle-closure glaucoma has occurred. For example, in some of the embodiments of the methods or uses of the invention for treating the acute brain or nerve injury, posiphen or a pharmaceutically acceptable salt of posiphen can be administered once, twice, three times or four times within 24 hours after an episode of the acute brain injury or acute angle-closure glaucoma has occurred, starting as early as one minute, or within 0.5, 1, 1.5, 2, 3, 6, 12, 18, 20 or 22 hours after the episode of the acute brain or nerve injury such as acute angle-closure glaucoma has occurred. Posiphen, or a pharmaceutically acceptable salt of posiphen, can also be administered once, twice, three times or four times daily for up to one month after the episode of the acute brain injury or acute glaucoma has occurred. For instance, posiphen or a pharmaceutically acceptable salt of posiphen can be administered once, twice, three times or four times daily several days in a row, like 2, 3, 4, 5, 6 or 7 days, or daily for 2 or 3 weeks, or daily up to one month, after the episode of the acute brain injury or acute glaucoma has occurred. In the methods or uses of the invention to prevent the acute brain or nerve injury such as post-operative cognitive decline or a traumatic brain injury, posiphen or the pharmaceutically acceptable salt of posiphen is administered before an episode of the acute brain or nerve injury is expected to occur such as within 1 hour, 2 hours, 3 hours, 6 hours, 12 hours, 1 day, 2 days or 3 days before when

the episode of the acute brain or nerve injury is expected to occur, for preventing the acute brain or nerve injury. For example, in some of the embodiments of the methods or uses of the invention for preventing the acute brain or nerve injury, posiphen or a pharmaceutically acceptable salt of posiphen can be administered once, twice, three times or four times within the first day, second day or third day before an episode of the acute brain injury or acute glaucoma occurs. In some of the embodiments of the methods or uses of the invention for preventing the acute brain or nerve injury, posiphen or a pharmaceutically acceptable salt of posiphen can be administered once, twice, three times or four times within the first day, and administered once, twice, three times or four times within the second day before an episode of the acute brain injury or acute glaucoma occurs. In some of the embodiments of the methods or uses of the invention for preventing the acute brain or nerve injury, posiphen or a pharmaceutically acceptable salt of posiphen can be administered once, twice, three times or four times within the first day, administered once, twice, three times or four times within the second day, and administered once, twice, three times or four times within the third day before an episode of the acute brain injury or acute glaucoma occurs.

Depending on the routes of administration, posiphen can be administered in different dose ranges. For example, in some of the embodiments of the methods or uses of the invention, posiphen is administered intravenously at 0.01 to 2 mg/kg body weight, preferably at 0.05 to 1 mg/kg, 0.05 to 0.5 mg/kg or 0.1 to 0.5 mg/kg body weight. In some of the embodiments of the methods or uses of the invention, posiphen is administered orally at 0.1 to 10 mg/kg body weight, preferably at 0.2 to 5 mg/kg body weight or 0.5 to 1 mg/kg body weight. In some of the embodiments of the methods or uses of the invention, posiphen is administered intraocularly at 0.001 to 0.2 mg/kg body weight, preferably at 0.002 to 0.1 mg/kg body weight, or 0.005 to 0.05 mg/kg body weight. When a pharmaceutically acceptable salt of posiphen is administered in a method or use of the invention, the appropriate dose of the pharmaceutically acceptable salt of posiphen administered can be calculated based on the dose of posiphen disclosed herein using the ratio of the molecular weight of posiphen and the molecular weight of the pharmaceutically acceptable salt of posiphen so that the amount of the pharmaceutically acceptable salt of posiphen administered would deliver a dose equivalent to the dose of posiphen disclosed herein.

In the methods or uses of the invention, the subject treated is a mammal, preferably a human. For example, the subject treated in the methods or uses of the invention is a mammal, or human, suffering from the acute nerve or brain injury.

The present invention includes the following embodiments.

Embodiment 1: A method of treating or preventing acute brain or nerve injury in a subject, comprising administering an effective amount of posiphen, or a pharmaceutically acceptable salt of posiphen, to the subject in need thereof.

Embodiment 2: The method according to Embodiment 1, wherein the posiphen, or a pharmaceutically acceptable salt of posiphen, administered to the subject is the pharmaceutically acceptable salt of posiphen.

Embodiment 3: The method according to Embodiment 2, wherein the pharmaceutically acceptable salt of posiphen is posiphen tartrate.

Embodiment 4: The method according to Embodiment 1, wherein posiphen or the pharmaceutically acceptable salt of posiphen administered to the subject has a chemical purity of at least 98%.

Embodiment 5: The method according to Embodiment 4, wherein posiphen or the pharmaceutically acceptable salt of posiphen administered to the subject has a chemical purity of at least 99%.

Embodiment 6: The method according to Embodiment 1, wherein the acute brain or nerve injury is traumatic brain injury, stroke, acute brain injury induced by brain ischemia, acute brain injury induced by insufficient oxygen supply to the brain, acute brain injury induced by anoxia or hypoxia, micro infarcts, acute brain injury induced by concussion, post-operative cognitive decline resulting from anesthesia or surgery-induced inflammation, acute brain injury induced by drowning, acute brain injury associated with whip lash, acute brain injury associated with bicycle crashes, acute brain injury associated with automobile accidents, shaken baby syndrome, acute brain injury induced by falling, acute brain injury associated with physical impact of the head, or acute angle-closure glaucoma.

Embodiment 7: The method according to Embodiment 1, wherein the acute brain or nerve injury treated is an acute brain injury.

Embodiment 8: The method according to Embodiment 7, wherein the acute brain injury treated is traumatic brain injury, stroke, acute brain injury induced by brain ischemia, acute brain injury induced by insufficient oxygen supply to the brain, acute brain injury induced by anoxia or hypoxia, micro infarcts, acute brain injury induced by concussion, post-operative cognitive decline resulting from anesthesia or surgery-induced inflammation, acute brain injury induced by drowning, acute brain injury associated with whip lash, acute brain injury associated with bicycle

crashes, acute brain injury associated with automobile accidents, shaken baby syndrome, acute brain injury induced by falling, or acute brain injury associated with physical impact of the head.

Embodiment 9: The method according to Embodiment 1, wherein the acute brain or nerve injury treated is an acute nerve injury.

- 5 Embodiment 10: The method according to Embodiment 9, wherein the acute nerve injury treated is acute angle-closure glaucoma.

Embodiment 11: The method according to Embodiment 1, wherein posiphen or the pharmaceutically acceptable salt of posiphen is administered intravenously.

- 10 Embodiment 12: The method according to Embodiment 10, wherein posiphen or the pharmaceutically acceptable salt of posiphen is administered intraocularly.

Embodiment 13: The method according to Embodiment 1, wherein posiphen is administered orally at a dose of 0.1 to 10 mg/kg body weight, or the pharmaceutically acceptable salt of posiphen is administered orally at a dose that is equivalent to a posiphen dose of 0.1 to 10 mg/kg body weight.

- 15 Embodiment 14: The method according to Embodiment 11, wherein posiphen is administered intravenously at a dose of 0.01 to 2 mg/kg body weight, or the pharmaceutically acceptable salt of posiphen is administered intravenously at a dose that is equivalent to a posiphen dose of 0.01 to 2 mg/kg body weight.

- 20 Embodiment 15: The method according to Embodiment 12, wherein posiphen is administered intraocularly at a dose of 0.001 to 0.2 mg/kg body weight, or the pharmaceutically acceptable salt of posiphen is administered intraocularly at a dose that is equivalent to a posiphen dose of 0.001 to 0.2 mg/kg body weight.

- 25 Embodiment 16: The method according to Embodiment 1, wherein posiphen or the pharmaceutically acceptable salt of posiphen is administered after an episode of the acute brain or nerve injury has occurred for treating the acute brain or nerve injury.

Embodiment 17: The method according to Embodiment 16, wherein posiphen or the pharmaceutically acceptable salt of posiphen is administered once, twice, three times or four

times within 24 hours after the episode of the acute brain injury or acute angle-closure glaucoma has occurred, starting as early as one minute, or within 0.5 , 1, 1.5, 2, 3, 6, 12, 18, 20 or 22 hours after the episode of the acute brain or nerve injury has occurred, or administered once, twice, three times or four times daily for up to one month after the episode of the acute brain injury or acute glaucoma has occurred.

Embodiment 18: The method according to Embodiment 1, wherein posiphen or the pharmaceutically acceptable salt of posiphen is administered before an episode of the acute brain or nerve injury is expected to occur such as within 1 hour, 2 hours, 3 hours, 6 hours, 12 hours, 1 day, 2 days or 3 days before the episode of the acute brain or nerve injury, for preventing the acute brain or nerve injury.

Embodiment 19. The method of Embodiment 18, wherein posiphen or a pharmaceutically acceptable salt of posiphen is administered once, twice, three times or four times within the first day, second day or third day before the episode of the acute brain or nerve injury occurs

EXAMPLES

Example 1

Prevention of Neuronal Cell Death in Retinal Ischemia-Reperfusion

Posiphen was demonstrated to be effective in protecting the retina from neuronal cell death after transient ischemia-reperfusion (IR) in rats. IR is a well-established experimental model of glaucoma as well as neuronal cell death in other retinal diseases. IR was induced on one eye of each rat while the opposite eye served as an internal control. Transient IR was induced by raising the intraocular pressure by a controlled micro-injection of saline into the anterior chamber of the eye. This approach is well established to induce large amounts of apoptotic cell death in the retina, beginning within hours after the reperfusion and continuing for several days, leading to a loss of about 50% of the retina. There would also be a dramatic and persistent increase in the permeability of the vasculature of the inner retina during this period, which may lead to edema.

Adult male Sprague-Dawley rats (approx. 450 g, n=8) were housed under normal 12hr light/dark conditions with free access to food and water. Rats were given posiphen tartrate (30 mg/kg body weight, delivered in 15 mg/ml water) or an equivalent volume of water (vehicle) by oral gavage once per day. Gavage was performed daily over three days and then on the fourth day IR was induced in anesthetized rats (ketamine : xylazine, 100 : 10 mg/kg, i.p.). IR was induced by slow injection of saline into the anterior chamber of the eye using a syringe pump (Harvard Instruments) which raised the intraocular pressure above that required for retinal perfusion, which rendered the retina ischemic (no blood flow, indicated by loss of the pink color of the fundus). The eye pressure was held constant for 50 min, followed by the removal of the cannulation needle which caused rapid reperfusion of the retina. The rats were sacrificed a further week later (14 days post IR). Retinas were rapidly dissected and snap frozen for storage. The retinas were homogenized and fractionated to obtain a cytoplasmic sample which was assessed for nucleosome content in duplicate, using the Cell Death ELISA (Roche). Data were averaged and expressed as optical density (OD).

Results: Retinal Cell Death Assay

The number of dead nerve cells in the retina of the eye subjected to transient IR and in the retina of the opposite eye (the control retina) in the rats treated with posiphen or the vehicle are shown in Figure 1, wherein IR-veh was the eye subjected to transient IR in the rats administered with the vehicle; control-veh was the opposite eye in the rats administered with the vehicle; IR-Pos was the eye subjected to transient IR in the rats treated with posiphen; and control-Pos was the opposite eye in the rats treated with posiphen.

In the rats treated with the vehicle, approximately twice as many dead nerve cells were observed in the retina of the eye subjected to transient IR (IR-veh) than in the retina of the opposite eye not subjected to transient IR (control-veh). In contrast, in the rats treated with posiphen, the number of dead nerve cells in the retina of the eye subjected to transient IR (IR-Pos) was only slightly more than the number of dead nerve cells in the retina of the opposite eye not subjected to transient IR (control-Pos). Posiphen rescued 72% of the retinal neurons in the

treated rats (IR-Pos). N=6. Thus, posiphen was effective in treating the acute nerve cell injury induced by the transient IR.

Example 2

5 Traumatic Brain Injury (TBI)

Rats were subjected to fluid percussion injury (FPI) or sham operation to one side of the brain using the same procedures described in Griesbach et al (Brain Research, 1288:105-115, 2009; PMID:PMC2735616) and Hutson et al (J. Neurotrauma, June 6, 2011; PMID:21644813). The fluid percussion resulted in mild traumatic brain injury (TBI) in that side of the brain. About
 10 5 to 30 minutes after FPI or sham operation, the rats were administered with saline or posiphen tartrate at a dose of 2.5, 5 or 10 mg/kg body weight intraperitoneally and also daily for the next 28 to 32 days. At 90 minutes after the last saline or posiphen treatment, all the rats were sacrificed and sections of the brain of each of the rats were stained for tyrosine hydroxylase (TH), wherein TH stains only live cells. The amounts of TH immunoreactivity in the whole
 15 striatum of the brain slices were measured. Stereological analysis of dopaminergic (DA) neurons in the substantia nigra was conducted to determine whether posiphen would block the TBI-induced loss of DA neurons ipsilateral and contralateral to the FPI injury, using procedures described in Johnson et al (Brain Pathol. June 29, 2011, doi: 10.1111/j.1750-3639.2011.00513.x) and Hoshino et al (Neurol. Med. Chir. (Tokyo), 2003, Apr; 43(4):165-74). The results are shown
 20 in Figures 2 and 3. The groups of rats included the rats with LOC above/at the median value. In Figure 2, the sham operated group was compared with the vehicle group with two-way ANOVA, Bonferroni comparisons, wherein * $p < 0.05$. In Figure 3, the vehicle group was compared with the posiphen-treatment groups with one-way ANOVA, Bonferroni comparisons, wherein # $p < 0.05$.

Compared with the rats in the sham operated group, in the rats subjected to FPI in the
 25 vehicle group, a 25% death of the nerve cells in the contralateral area of the striatum and a 42% death in the ipsilateral area were detected, wherein the ipsilateral side was the side of the brain subjected to FPI. In the posiphen group, the rats treated with 3 doses of posiphen showed a statistically significant increase over the sham operated animals in the number of surviving cells in the ipsilateral area of the brain at all 3 doses and an increase in the number of nerve cells of

the striatum in the contralateral part of the brain at the highest dose of 10 mg/kg. Posiphen protected the striatum in fluid percussion injury at all 3 doses tested.

The data are shown in Figures 2 and 3 as the mean $\pm$ S.E.M. (standard error of the mean). A Student's t-test between the sham-operated group and vehicle-treated group revealed a significant decrease in TH+ fluorescence intensity in the striatum of the vehicle-treated group (5 $t=2.470$, $*p=0.0295$). Based on the data in the ipsilateral striatum (the ipsilateral side was the side of the brain subjected to FPI), posiphen treated animals showed full protection of the TH+ cells in all parts of the striatum (whole, rostral, medial and caudal). A one-way ANOVA with Bonferroni post-hoc comparisons revealed a significantly higher TH+ fluorescence intensity in all the posiphen-treated groups when compared with the vehicle-treated group (10 $F=5.499$, $^{*}p<0.05$). Posiphen attenuated the FPI-induced decrease in the TH+ terminals in the striatum.

Although no significant effect of the TBI on the TH+ fluorescent intensity was seen in any subregion of the substantia nigra, a very strong trend toward a decreased mean number of TH+ neurons in the ventral subregion was detected, when the data were analyzed with Student's (15 t test, one tailed ($p=0.059$)).

Example 3

Because FPI can induce microglial activation according to Hutson et al (J. Neurotrauma, June 6, 2011; PMID:21644813), whether posiphen would reverse this pathological response using quantitative measures was tested in the vehicle or posiphen tartrate treated (10 mg/kg) rats (20 from Example 2 (part of the brain from each of the euthanized rat was stained for tyrosine hydroxylase in Example 2 and another part of the brain was stained for microglia in Example 3). Microglial activation was assessed by quantitative measure of the diameter of IBA-1-positive cells, wherein IBA-1 stands for ionized calcium adaptor binding protein. Microglia with cell (25 body diameters less than 5 μm had a resting morphology characterized by multiple ramified processes. Hyper-ramified microglia/partially activated microglia had a mean cell body diameter of 5-6 μm . Fully activated amoeboid microglia had a mean cell body diameter of 7-14 μm , with an activated morphology characterized by amoeboid cell bodies with few, short processes. Analysis was done on a Leica DM-LB microscope with a Ludl XYZ motorized stage and z-axis (30 microcator (MT12, Heidenheim, Traunreut, Germany) using StereoInvestigator software

(MicroBrightField, Colchester, VT). A contour was drawn to delineate the substantia nigra under the 5x objective lens to ensure anatomical accuracy. Following delineation, the diameters of microglial cell bodies were measured in the first counting frame (100 μ m) and then in every fifth counting frame at 40x magnification. This sampling frequency was chosen in order to count approximately 30-50 IBA-1+ cells in each side of the substantia nigra. The first counting frame was always positioned in the upper left corner of the contour and systematically moved from left to right and from top to bottom until the entire delineated contour region was sampled. The number of IBA-1+ microglia cells with cell diameters ranging from 1 μ m to 14 μ m was then normalized to the total number counted in each section and expressed as a percentage of total microglia. The results are shown in Figure 4 (wherein the percent of IBA-1+ cells was plotted on the vertical axis, and the microglial cell diameter in μ m was plotted on the horizontal axis). In Figure 4, the blank portion of each data bar represents the mean % of IBA-1+ cells with the solid or striped portion at the top of the bar representing the 95% confidence interval. In each pair of data bars in Figure 4, the bar on the left having a solid portion at the top represents data from the vehicle-treated group, while the bar on the right having a striped portion at the top represents data from the 10 mg/kg posiphen-treated group, wherein the data of the posiphen-treated group (the bar on the right) were compared with the data of the vehicle-treated group (the bar on the left) with Bootstrapping method. The asterisks (*) at the top of some of the bars of the 10 mg/kg posiphen-treated group indicate statistical significance at $p < 0.05$ when compared with the data of the corresponding bar of the vehicle-treated group as analyzed with the Bootstrapping method. Posiphen increased the number of resting microglia and reduced the number of activated microglia.

The data show that FPI increased microglia activation in the substantia nigra ipsilateral to the injury, an effect attenuated by posiphen at a dose of 10 mg/kg in a statistically significant manner. The diameters of IBA positive cell bodies in substantia nigra ipsilateral to the injury were an index of inflammation. The data show that posiphen decreased brain inflammation after traumatic brain injury.

Example 4

The objective of this example was to establish the efficacy of posiphen as a treatment of traumatic brain injuries (TBIs). In a mild model of TBI, posiphen was shown to improve water maze performance in rats subjected to TBI as described below.

5 *Posiphen Improved Performance of Working Memory in Morris Water Maze*

The vehicle or posiphen tartrate treated rats from Example 2 were also subjected to working memory test in Morris water maze. Each of the rats was individually tested in the Morris water maze, which had 4 release points for putting the rat into the water and 4 platforms for the rat to swim to. For a total of 3 consecutive days (on one and two days before the last vehicle or posiphen treatment; and also on the day of the last vehicle or posiphen treatment before euthanasia), the rats were tested on their ability to find the 4 platform positions, using paired trials (4 paired trials per day). In each pair of the trials, the first trial was the learning trial, for the rat to learn the position of a platform, and the second trial was the memory trial for testing the memory of the rat to swim to the platform that the rat learned in the learning trial. In the learning trial, each of the rats was allowed a maximum swim time of 90 seconds to swim to the platform, and guided to the platform if needed after 90 seconds. The memory trial was started 5 seconds after the completion of the learning trial and the time for the rat to reach the platform by swimming in the maze in the memory trial was recorded. The 4 platform positions were switched after every pair of trials. The time interval between the learning trials of two consecutive pairs of trials in a day was 5 minutes. The data were averaged over the 3 days. The mean duration of time (and +/- standard error of the mean) for 5 groups of the rats to reach the platform in the memory trials in the Morris water maze are shown in Figure 5. Based on a one-way ANOVA with Bonferroni post-hoc comparisons between the vehicle-treated group and 10 mg/kg posiphen-treated group (depicted with the bar marked with the # symbol), a statistically significant difference was found ($t=2.209$ and $p=0.0335$). The other two posiphen doses, 2.5 mg/kg and 5 mg/kg, revealed a non-significant trend toward a reduction in latency to find the platform ($F=2.192$; $p=0.0961$). Thus, posiphen at a dose of 10 mg/kg rescued FPI injury-induced impairment as tested in the working memory Morris water maze.

The experiment showed a significant decrease in performance in the vehicle-treated group when compared to the sham group indicating that the FPI induced a performance deficit in

the vehicle-treated rats as tested with the working memory Morris water maze. The experiment also showed an amelioration of this deficit in the 10 mg/kg posiphen group when compared to the vehicle-treated group.

CLAIMS:

1. A use, for treating or preventing traumatic brain injury in a subject and for reducing nerve cell death of said subject, prior to said subject experiencing a traumatic brain injury, of posiphen, or a pharmaceutically acceptable thereof, wherein the posiphen or the pharmaceutically acceptable salt thereof is for use within about 3 days before the traumatic brain injury, and thereafter for at least 7 days, wherein the posiphen or the pharmaceutically acceptable salt thereof is for use once, twice, three times or four times within the first day, second day or third day before the traumatic brain injury occurs.
2. The use according to claim 1, wherein the posiphen or the pharmaceutically acceptable salt thereof has a chemical purity of at least 98%.
3. The use according to claim 1, wherein the posiphen or the pharmaceutically acceptable salt thereof has a chemical purity of at least 99%.
4. The use according to any one of claims 1 to 3, wherein the posiphen or the pharmaceutically acceptable salt of posiphen is for intravenous use.
5. The use according to claim 2, wherein the posiphen or the pharmaceutically acceptable salt thereof is for intraocular use.
6. The use according to claim 1, wherein the posiphen is for oral use at a dose of 0.1 to 10 mg/kg body weight, or the pharmaceutically acceptable salt thereof is for oral use at a dose that is equivalent to a posiphen dose of 0.1 to 10 mg/kg body weight.
7. The use according to claim 4, wherein the posiphen is for intravenous use at a dose of 0.01 to 2 mg/kg body weight, or the pharmaceutically acceptable salt thereof is for intravenous use at a dose that is equivalent to a posiphen dose of 0.01 to 2 mg/kg body weight.
8. The use according to claim 5, wherein the posiphen is for intraocular use at a dose of 0.001 to 0.2 mg/kg body weight, or the pharmaceutically acceptable salt thereof is for intraocular use at a dose that is equivalent to a posiphen dose of 0.001 to 0.2 mg/kg body weight.

9. The use according to claim 1, wherein the posiphen or the pharmaceutically acceptable salt thereof is for use once, twice, three times or four times within 24 hours after the traumatic brain injury or has occurred, starting as early as one minute, or within 0.5, 1, 1.5, 2, 3, 6, 12, 18, 20 or 22 hours after the traumatic brain injury.
10. The use according to any one of claims 1 to 9, comprising use of the pharmaceutically acceptable salt of posiphen.
11. The use according to claim 10, wherein the pharmaceutically acceptable salt of posiphen is posiphen tartrate.
12. A use of posiphen, or a pharmaceutically acceptable salt thereof, for treatment or prevention of acute brain or nerve injury.
13. The use according to claim 12, wherein the posiphen, or a pharmaceutically acceptable salt thereof, is the pharmaceutically acceptable salt of posiphen.
14. The use according to claim 13, wherein the pharmaceutically acceptable salt thereof is posiphen tartrate.
15. The use according to claim 12, wherein the posiphen or the pharmaceutically acceptable salt thereof has a chemical purity of at least 98%.
16. The use according to claim 12, wherein the posiphen or the pharmaceutically acceptable salt thereof has a chemical purity of at least 99%.
17. The use according to claim 12, wherein the posiphen or the pharmaceutically acceptable salt thereof is for intravenous use.
18. The use according to claim 12, wherein the posiphen or the pharmaceutically acceptable salt thereof is for intraocular use.
19. The use according to claim 12, wherein the posiphen is for oral use at a dose of 0.1 to 10 mg/kg body weight, or the pharmaceutically acceptable salt thereof is for oral use at a dose that is equivalent to a posiphen dose of 0.1 to 10 mg/kg body weight.

20. The use according to claim 12, wherein the posiphen is for intravenous use at a dose of 0.01 to 2 mg/kg body weight, or the pharmaceutically acceptable salt thereof is for intravenous use at a dose that is equivalent to a posiphen dose of 0.01 to 2 mg/kg body weight.
21. The use according to claim 18, wherein the posiphen is for intraocular use at a dose of 0.001 to 0.2 mg/kg body weight, or the pharmaceutically acceptable salt thereof is for intraocular use at a dose that is equivalent to a posiphen dose of 0.001 to 0.2 mg/kg body weight.
22. The use according to claim 12, wherein the posiphen or the pharmaceutically acceptable salt thereof is for use once, twice, three times or four times within 24 hours after the acute brain or nerve injury has occurred, starting as early as one minute, or within 0.5, 1, 1.5, 2, 3, 6, 12, 18, 20 or 22 hours after the acute brain or nerve injury has occurred; or for use once, twice, three times or four times daily for up to one month after the acute brain or nerve injury has occurred.
23. The use according to claim 12, wherein the posiphen or the pharmaceutically acceptable salt thereof is for use before the acute brain or nerve injury is expected to occur.
24. The use according to claim 23, wherein the posiphen or a pharmaceutically acceptable salt thereof is for use once, twice, three times or four times within the first day, second day or third day before the acute brain or nerve injury occurs.
25. The use according to claim 23, wherein the posiphen or the pharmaceutically acceptable salt thereof is for use within 1 hour, 2 hours, 3 hours, 6 hours, 12 hours, 1 day, 2 days or 3 days before the acute brain or nerve injury is expected to occur.
26. A use, for preparation of a medicament for treating or preventing traumatic brain injury in a subject and for reducing nerve cell death of said subject, prior to said subject experiencing a traumatic brain injury, of posiphen, or a pharmaceutically acceptable thereof, wherein the posiphen or the pharmaceutically acceptable salt thereof is for use within about 3 days before the traumatic brain injury, and thereafter for at least 7 days, wherein the posiphen or the pharmaceutically acceptable salt thereof is for use once, twice, three times or four times within the first day, second day or third day before the traumatic brain injury occurs.
27. The use according to claim 26, wherein the posiphen or the pharmaceutically acceptable salt thereof has a chemical purity of at least 98%.

28. The use according to claim 26, wherein the posiphen or the pharmaceutically acceptable salt of thereof has a chemical purity of at least 99%.
29. The use according to any one of claims 26 to 28, wherein the medicament is for intravenous use.
30. The use according to claim 27, wherein the medicament is for intraocular use.
31. The use according to claim 26, wherein the medicament is for oral use at a dose that provides 0.1 to 10 mg/kg body weight of the posiphen, or a dose of the pharmaceutically acceptable salt thereof that is equivalent to a posiphen dose of 0.1 to 10 mg/kg body weight.
32. The use according to claim 26, wherein the medicament is for intravenous use at a dose that provides 0.01 to 2 mg/kg body weight of the posiphen, or a dose of the pharmaceutically acceptable salt thereof that is equivalent to a posiphen dose of 0.01 to 2 mg/kg body weight.
33. The use according to claim 26, wherein the medicament is for intraocular use at a dose that provides 0.001 to 0.2 mg/kg body weight of the posiphen, or a dose of the pharmaceutically acceptable salt thereof that is equivalent to a posiphen dose of 0.001 to 0.2 mg/kg body weight.
34. The use according to claim 26, wherein the medicament is for use once, twice, three times or four times within 24 hours after the traumatic brain injury or has occurred, starting as early as one minute, or within 0.5, 1, 1.5, 2, 3, 6, 12, 18, 20 or 22 hours after the traumatic brain injury.
35. The use according to claim 26, comprising use of the pharmaceutically acceptable salt of posiphen.
36. The use according to claim 35, wherein the pharmaceutically acceptable salt of posiphen is posiphen tartrate.
37. A use of posiphen, or a pharmaceutically acceptable salt thereof, for preparation of a medicament for treatment or prevention of acute brain or nerve injury.
38. The use according to claim 37, wherein the posiphen, or a pharmaceutically acceptable salt thereof, is the pharmaceutically acceptable salt of posiphen.

39. The use according to claim 38, wherein the pharmaceutically acceptable salt thereof is posiphen tartrate.
40. The use according to claim 37, wherein the posiphen or the pharmaceutically acceptable salt thereof has a chemical purity of at least 98%.
41. The use according to claim 37, wherein the posiphen or the pharmaceutically acceptable salt thereof has a chemical purity of at least 99%.
42. The use according to claim 37, wherein the medicament is for intravenous use.
43. The use according to claim 37, wherein the medicament is for intraocular use.
44. The use according to claim 37, wherein the medicament is for oral use at a dose that provides 0.1 to 10 mg/kg body weight of the posiphen, or a dose of the pharmaceutically acceptable salt thereof that is equivalent to a posiphen dose of 0.1 to 10 mg/kg body weight.
45. The use according to claim 37, wherein medicament is for intravenous use at a dose that provides 0.01 to 2 mg/kg body weight of the posiphen, or a dose of the pharmaceutically acceptable salt thereof that is equivalent to a posiphen dose of 0.01 to 2 mg/kg body weight.
46. The use according to claim 43, wherein the medicament is for intraocular use at a dose that provides 0.001 to 0.2 mg/kg body weight of the posiphen, or a dose of the pharmaceutically acceptable salt thereof that is equivalent to a posiphen dose of 0.001 to 0.2 mg/kg body weight.
47. The use according to claim 37, wherein medicament is for use once, twice, three times or four times within 24 hours after the acute brain or nerve injury has occurred, starting as early as one minute, or within 0.5, 1, 1.5, 2, 3, 6, 12, 18, 20 or 22 hours after the acute brain or nerve injury has occurred; or for use once, twice, three times or four times daily for up to one month after the acute brain or nerve injury has occurred.
48. The use according to claim 37, wherein the medicament is for use before the acute brain or nerve injury is expected to occur.

49. The use according to claim 48, wherein the medicament is for use once, twice, three times or four times within the first day, second day or third day before the acute brain or nerve injury occurs.

50. The use according to claim 48, wherein the medicament is for use within 1 hour, 2 hours, 3 hours, 6 hours, 12 hours, 1 day, 2 days or 3 days before the acute brain or nerve injury is expected to occur.

51. The use according to claim 1, wherein the posiphen or the pharmaceutically acceptable salt of posiphen is for oral use.

52. The use according to claim 12, wherein the posiphen or the pharmaceutically acceptable salt of posiphen is for oral use.

53. The use according to claim 26, wherein the posiphen or the pharmaceutically acceptable salt of posiphen is for oral use.

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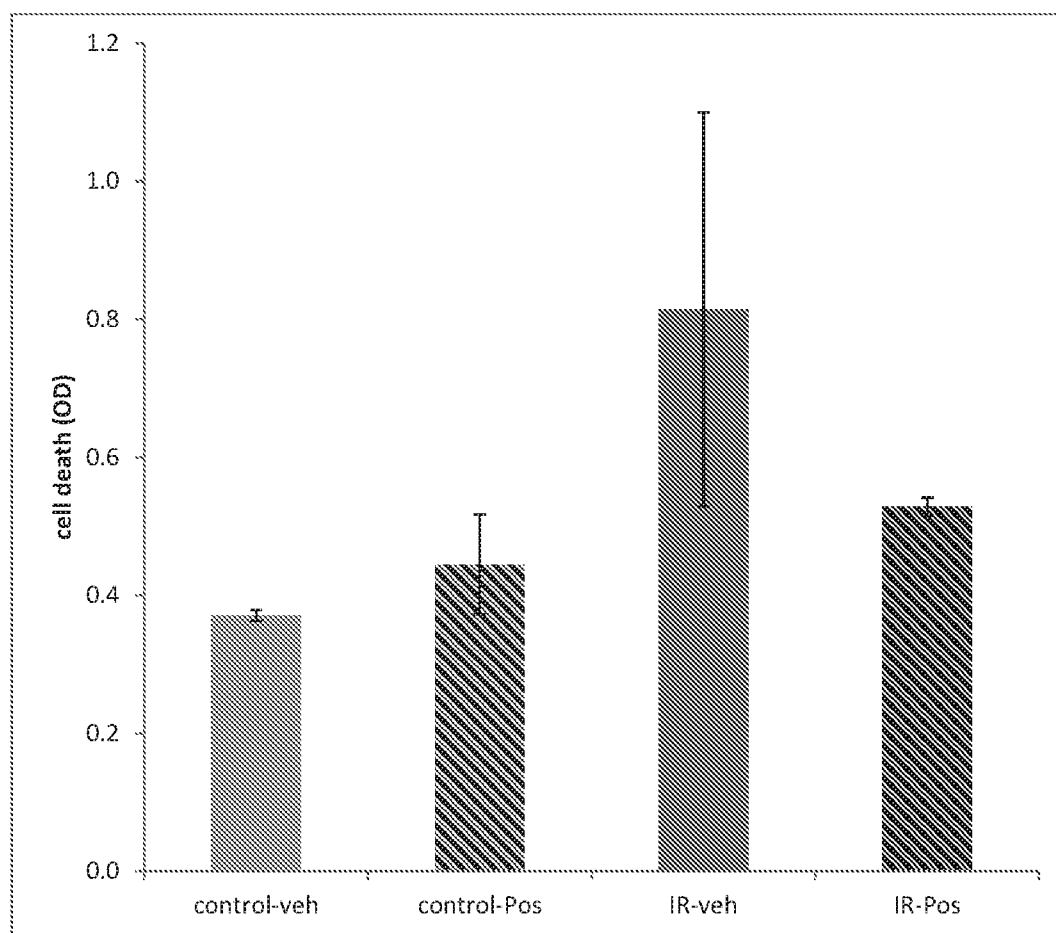


Figure 1

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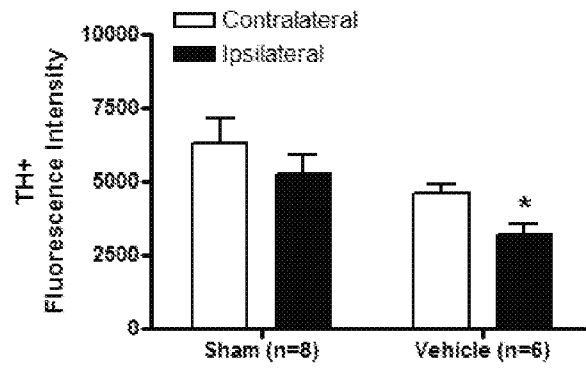


Figure 2

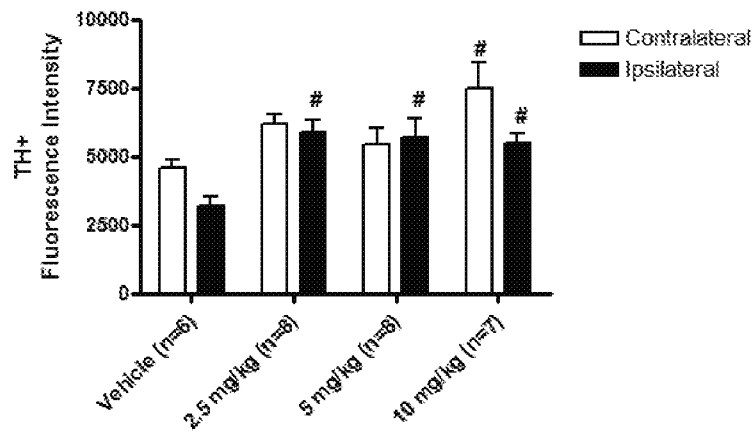
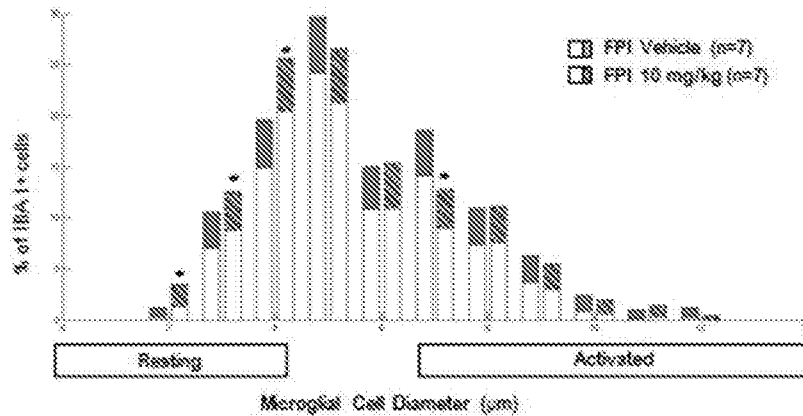


Figure 3

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Posiphen decreases brain inflammation after traumatic brain injury



Rats were subjected to mild traumatic brain injury with lateral fluid percussion and treated with Posiphen for 31 days. Data are diameters of IBA1 positive cell bodies in substantia nigra ipsilateral to injury, an index of inflammation.

Figure 4

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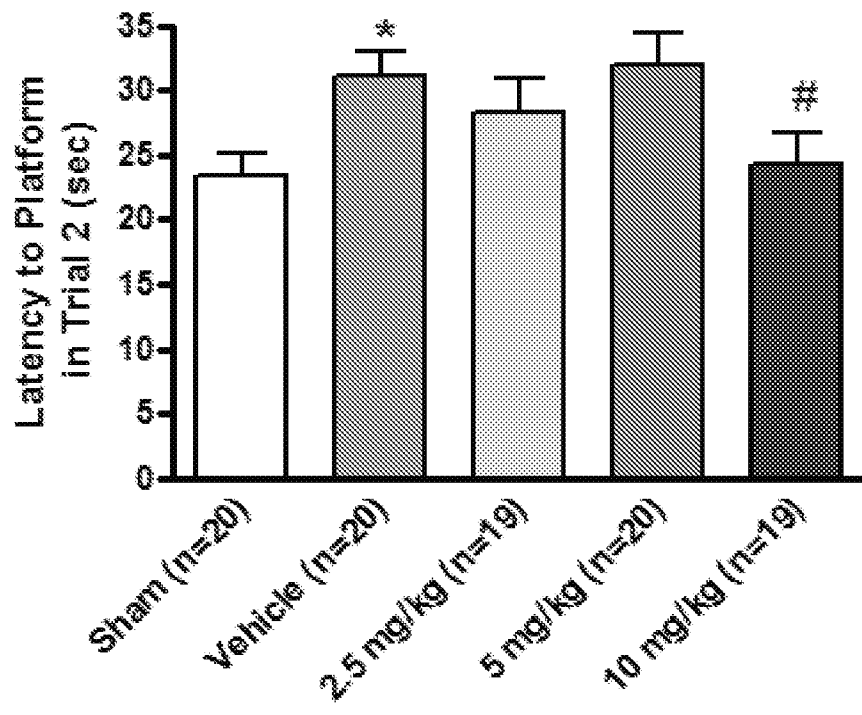


Figure 5

