**Supplemental Digital Content to:**

**Pifithrin-μ Neuroprotection after Cardiac Arrest in a Rodent Model**

**1. Detailed Description of Surgery, Induction of Cardiac Arrest and Resuscitation**

The surgery and resuscitation procedures have been described previously (1, 2), here we provide a short description and minor changes to the aforementioned protocol. Anesthesia was induced with sevoflurane (Sevorane, Abbott Switzerland) and fentanyl 20 μg/kg ip (Fentanyl, Janssen-Cilag, Switzerland), followed by orotracheal intubation with a 2.0 mm diameter angiocath (Venflon, BD, Heidelberg, Germany) under direct vision. After confirmation of proper tube placement with capnography anesthesia was maintained with sevoflurane. Mechanical ventilation (KTR-5, Hugo-Sachs Elektronik, March, Germany) was started with 50 breaths per minute, an inspiratory pressure of 12 cm H2O and a PEEP of 3 cm H2O. FiO2 was set at 0.3. The ventilation was adapted after a first blood gas analysis to keep CO2 in the normal range. A thermometer was placed in the esophagus and the animal’s core temperature was kept at 36 - 36.5°C with a n operation table perfused with 31°C heated water and an infrared bulb. ECG electrodes were placed on standard positions subcutaneously. The right femoral artery was surgically exposed and a PE 50 catheter for blood pressure monitoring and blood sampling was inserted. Another PE 50 catheter for drug administration was inserted into the right jugular vein. The whole procedure took in median 23 minutes in length [interquartil range IQR 20 – 28.25 minutes]. Recordings of blood pressure, ECG and temperature were performed with a standard anesthesia monitor (Datex S/5 Anaesthesia Monitor, GE Healthcare, Finland) and the Wincollect data logger (Wincollect, Datex Ohmeda, GE Healthcare, Finland) at a rate of 100 Hz.

For cardiac arrest, the rats were paralyzed with 2 mg/kg vecuronium (Norcuron, MSD, Switzerland), then 1 ml of a mixture of potassium chloride and esmolol (0.5 ml of 2 mmol/ml KCl + 7.5 ml of 10 mg/ml esmolol, Esmolol-Orpha, OrPha Swiss, Switzerland), resulting in 0.125 mmol potassium and 9.375 mg esmolol per animal (corresponding to 0.363 mmol/kg body weight potassium and 27.3 mg/kg body weight esmolol) was injected via the jugular vein catheter. The cardiac arrest was indicated by ECG and a drop of the mean arterial blood pressure below 15 mmHg. After 7’30’’, ventilation was resumed with 100% oxygen. At 8 minutes, manual metronome guided chest compressions with 2 fingers at a rate of 220/min was started. Diluted adrenaline 15 μg/kg (Adrenalin Bichsel, Switzerland) and 0.2 ml of a diluted calcium solution containing 0.1125 mmol Ca/ml (Calcium-Sandoz, Sandoz, Switzerland) were injected, separated by 0.1 ml of normal saline. If ROSC was not achieved within 60 seconds, adrenaline was repeated with 5 μg/kg every 30 seconds until ROSC. Calcium was repeated after 2 minutes of cardiopulmonary resuscitation. ROSC was defined as regular cardiac activity > 200 min-1 with a mean arterial pressure of ≥ 60 mmHg, sustained for at least 20 seconds (one monitor length). If ROSC was not achieved within 4 minutes, no further resuscitation attempts were made.

After successful resuscitation, ventilation was increased, the rats received 50 mg/kg ampicillin (Clamoxyl, GlaxoSmithKline, Switzerland) for perioperative prophylaxis once, and 20µg/kg fentanyl intramuscularly for analgesia. At this time point, the animals received pifithrin in vehicle or vehicle only as described above intraperitoneally.

Blood for arterial blood gas analysis (aBGA) was drawn at ROSC +5 min and ROSC + 15 min and ventilation (pressure and rate) was adapted consequently. After a final aBGA at ROSC + 60 minutes, the catheters were withdrawn and wounds closed. Sevoflurane was added at a minimal dose to avoid self-extubation (0 to max 0.8%), and the animals received 20µg/kg fentanyl every hour for 3 hours. 3.5 hours post ROSC 20 µg/kg buprenorphine (Temgesic, Reckitt Benckiser, Switzerland) was administered sc, and 4 hours post-ROSC rats were weaned from mechanical ventilation. Temperature was managed to maintain a temperature of 37.5°C ± 0.5°C until extubation.

After extubation, the animals were placed in a single cage beneath a heating lamp until the next day, then they returned to their standard cage; to avoid injuries from rank hierarchy fights, the rats were kept single.

In series 2., cardiac arrest time was prolonged to 10 minutes, and to spare blood, arterial blood gas analysis were done before cardiac arrest, 5 minutes post ROSC, and 20 minutes post ROSC. Postresuscitation care was the same as in series 1., including temperature management to maintain a temperature of 37.5°C ± 0.5°C.

**2. Details of the Neurobehavioral Tests**

Neuro Deficit Score (3) : NDS evaluates general behavior and respiration (maximum, 40 points), cranial nerves function (maximum, 20 points), sensitivity to tactile stimuli (maximum, 10 points), motor function (maximum, 10 points), and coordination (maximum, 20 points).

Open Field Task (4): We used the Open Field Task to determine general activity levels and exploration habits. The animals were assessed by placing them in the middle of a square (0.75 x 0.75 m) wooden box, with a mounted video recorder for later analysis with a video tracking system (Ethovision® XT-11, Noldus Information Technology, Wageningen, Netherlands). The animals were placed in the middle of the arena and allowed to freely move for 4 minutes while being recorded. The test was performed before cardiac arrest, and on day 4 and day 5. We analyzed the footage and mobility/immobility ratio.

In series 1., two additional neurobehavioural tests were performed:
Sensorimotor integration was tested by the Tape-Removal Test (5), where the time to successfully remove a standard (10x12 mm) adhesive tape from the front paws was measured. The animals were habituated to the test daily from 5 days before the experiment, and the test was repeated daily from baseline to day 5; and then by day 10.

For testing the spatial memory, a standard water maze test was performed after wound healing (day 10 to day 15) as described in (6). The tank had a diameter of 1.8 m (surface 2,54 m2) and was filled with water and dark food coloring. The swim patterns of the rats were registered with a video tracking system (Ethovision® XT-11, Noldus Information Technology, Wageningen, Netherlands) mounted at the ceiling above the tank. The water was blackened and its surface was virtually divided into four inner quadrants. An adjustable platform measuring 16 x 13 cm was placed in the center of the first quadrant, 0.5 cm below the water surface. Four entry zones, situated each between two quadrants, were marked outside the pool. Three posters of 0,6 x 0,3 m with different black-and-white patterns (horizontal and diagonal stripes, circles) were placed on three different walls to serve as visual cues. All animals performed 5 training trials per day for 5 days with the invisible platform in quadrant 1, and one probe trial in which the platform was removed. The purpose of the probe trial was to observe the animal’s performance without chance-encounters with the platform. The rats were put into the water with their head directed towards the wall of the tank. If an animal found the platform within 90 seconds, it was allowed to stay on it for 15 seconds before it was put back to the cage. If the rat did not find the platform within 90 seconds, it was guided there by hand and was allowed to stay on it for 15 seconds. Between trials, animals rested 45 minutes. Entry zones were randomized with the function RANDBETWEEN of MS Excel 2010 for each trial. Tracks were recorded by Ethovision® with 5 samples per second. Parameters evaluated for each probe trial were the time to first virtual platform crossing, time spent in the quadrant which have contained the platform during the training sessions and the mean distance swum until the animal reached the center of the platform.

Reference

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**3. Results: Supplemental Tables**

**Table suppl 1a. Blood gas analysis series 1. (8 min cardiac arrest).**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| variable | time | pifithrin | control | post-hoc | ANOVA |
| *Glucose[mmol/l]* | *Baseline(Before)* | *11.3 [10.2 – 12.2]* | *10.5 [9.9 – 11.4]* | *p: 0.110* | *Shapiro-Wilk failed:ANOVA na* |
| *ROSC + 5 min* | *16.1 [14.4 – 17.6]* | *16.7 [15.2 – 18.6]* | *p: 0.164* |
| *ROSC + 15 min* | *14.5 [12.9 – 15.9]* | *13.9 [12.9 – 16.8]* | *p: 0.773* |
| *ROSC + 60 min* | *7.4 [6.5 – 8.6]* | *7.3 [6.7 – 8.3]* | *p: 0.906* |
| *Friedman* | *p: <0.001* | *p: <0.001* |  |  |
|  |
| *Lactate[mmol/l]* | *Baseline(Before)* | *1.2 [1.0 – 1.5]* | *1.2 [1.1 – 1.5]* | *p: 0.966* | *Shapiro-Wilk failed:ANOVA na* |
| *ROSC + 5 min* | *7.7 [7.0 – 8.4]* | *7.5 [6.8 – 8.0]* | *p: 0.364* |
| *ROSC + 15 min* | *4.0 [3.7 – 4.8]* | *4.3 [3.8 – 4.9]* | *p: 0.410* |
| *ROSC + 60 min* | *0.7 [0.6 – 1.1]* | *0.7 [0.6 – 1.0]* | *p: 0.908* |
| *Friedman* | *p: <0.001* | *p: <0.001* |  |  |
|  |
| *pH* | *Baseline(Before)* | *7.48 [7.46 – 7.50]* | *7.49 [7.46 – 7.54* | *p: 0.339* | *Shapiro-Wilk failed:ANOVA na* |
| *ROSC + 5 min* | *7.12 [7.08 – 7.16]* | *7.12 [7.04 – 7.20]* | *p: 0.938* |
| *ROSC + 15 min* | *7.28 [7.21 – 7.32]* | *7.24 [7.23 – 7.32]* | *p: 0.755* |
| *ROSC + 60 min* | *7.34 [7.23 – 7.42]* | *7.36 [7.32 – 7.39]* | *p: 0.683* |
| *Friedman* | *p: <0.001* | *p: <0.001* |  |  |
|  |
| *pO2[mmHg]* | *Baseline(Before)* | *115 [97 – 128]* | *113 [95 – 127]* | *p: 0.623* | *Shapiro-Wilk failed:ANOVA na* |
| *ROSC + 5 min* | *292 [256 – 330]* | *283 [235 – 327 ]* | *p: 0.384* |
| *ROSC + 15 min* | *175 [132 – 193]* | *173 [144 – 212]* | *p: 0.328* |
| *ROSC + 60 min* | *196 [162 – 217]* | *216 [163 – 222]* | *p: 0.322* |
| *Friedman* | *p: <0.001* | *p: <0.001* |  |  |
|  |
| *pCO2 [mmHg]* | *Baseline(Before)* | *32 [28 – 35]* | *30 [27 – 33]* | *p: 0.131* | *Shapiro-Wilk failed:ANOVA na* |
| *ROSC + 5 min* | *37 [31 – 41]* | *34 [28 – 44]* | *p: 0.848* |
| *ROSC + 15 min* | *30 [28 – 34]* | *32 [27 – 37]* | *p: 0.736* |
| *ROSC + 60 min* | *35 [31 – 48]* | *36 [32 – 43]* | *p: 0.622* |
| *Friedman* | *p: <0.001* | *p: <0.001* |  |  |
|  |
| *temp[°C]* | *Baseline(Before)* | *36.2 [36.0 – 36.6]* | *36.5 [36.1 – 36.7]* | *p: 0.123* | *Shapiro-Wilk failed:ANOVA na* |
| *ROSC + 5 min* | *35.0 [34.5 – 35.3]* | *35.0 [34.7 –35.4]* | *p: 0.532* |
| *ROSC + 15 min* | *36.0 [35.3 – 36.4]* | *36.1 [35.8 – 36.6]* | *p: 0.216* |
| *ROSC + 60 min* | *37.0 [36.9 – 37.5]* | *37.3 [37.0 – 37.5]* | *p: 0.152* |
| *Friedman* | *p: <0.001* | *p: <0.001* |  |  |

The parameters from the arterial blood gas analysis in the 8 minutes trial have a non-parametric distribution, therefore we compared the baseline values to recognize a difference, and the respective time row. We did not find any differences in the baseline, and both groups have significant changes within time, pointing towards the same direction. So we assume important changes within time, but not between the groups.

**Table suppl 1b. Blood gas analysis series 2. (10 min cardiac arrest).**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| variable | time | pifithrin | control | post-hoc | ANOVA |
| Glucose[mmol/l] | Baseline(Before) | 11.1 ± 2.2 | 11.0 ± 1.5 |  | p group: 0.388p time: < 0.001p group x time:0.431 |
| ROSC + 5 min | 17.1 ± 2.5 | 16.4 ± 2.1 |
| ROSC + 20 min | 15.1 ± 2.1 | 15.0 ± 2.6 |
| post-hoc | BL to 5 p: <0.001BL to 20 p: <0.001 | BL to 5 p: <0.001BL to 20 p: <0.001 |  |
|  |
| Lactate[mmol/l] | Baseline(Before) | 1.2 ± 0.4 | 1.2 ± 0.4 |  | p group: 0.324p time: < 0.001p group x time:0.510 |
| ROSC + 5 min | 7.3 ± 0.7 | 7.6 ± 0.8 |
| ROSC + 20 min | 2.9 ± 0.4 | 2.9 ± 0.6 |
| post-hoc | BL to 5 p: <0.001BL to 20 p: <0.001 | BL to 5 p: <0.001BL to 20 p: <0.001 |  |
|  |
| pH | Baseline(Before) | 7.45 ± 0.06 | 7.47 ± 0.06 |  | p group: 0.940p time: < 0.001p group x time:0.527 |
| ROSC + 5 min | 7.10 ± 0.12 | 7.07 ± 0.07 |
| ROSC + 20 min | 7.31 ± 0.10 | 7.29 ± 0.08 |
| post-hoc | BL to 5 p: <0.001BL to 20 p: <0.001 | BL to 5 p: <0.001BL to 20 p: <0.001 |  |
|  |
| *pO2[mmHg]* | *Baseline(Before)* | *146 [111 – 165]* | *135 [104 – 163]* | *p: 0.600* | *Shapiro-Wilk failed:ANOVA na* |
| ROSC + 5 min | *288 [232 – 346]* | *309 [272 – 356 ]* | *p: 0.303* |
| ROSC + 20 min | *123 [110 – 139]* | *123 [112 – 165]* | *p: 0.871* |
| *Friedman* | *p: <0.001* | *p: <0.001* |  |  |
|  |
| pCO2 [mmHg] | Baseline(Before) | 37 ± 7.1 | 34 ± 5.8 |  | p group: 0.444p time: < 0.001p group x time:0.948 |
| ROSC + 5 min | 50 ± 15.7 | 50 ± 11.2 |
| ROSC + 20 min | 39 ± 11.5 | 38 ± 10 |
| post-hoc | BL to 5 p: <0.001BL to 20 p: 0.792 | BL to 5 p: <0.001BL to 20 p: 0.649 |  |
|  |
| temp[°C] | Baseline(Before) | 35.9 ± 0.6 | 35.7 ± 0.5 |  | p group: 0.970p time: < 0.001p group x time:0.462 |
| ROSC + 5 min | 35.0 ± 0.6 | 35.3 ± 0.5 |
| ROSC + 20 min | 36.6 ± 0.8 | 36.7 ± 0.8 |
| post-hoc | BL to 5 p: <0.001BL to 20 p: 0.003 | BL to 5 p: 0.070BL to 20 p: 0.001 |  |

The parameters from the arterial blood gas analysis in the 10 minutes trial do not show differences between the groups, but important changes throughout time, indicating extensive stress and injury during cardiac arrest.

**Table suppl. 2a Mean Arterial Pressure and Heart Rate series 1. (8 min cardiac arrest).**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| variable | time | pifithrin | control | post-hoc | ANOVA |
| *MAP[mmHg]* | *Baseline(Before)* | *93 [80 – 105]* | *96 [86 – 104]* | *p: 0.664* | *Shapiro-Wilk failed:ANOVA na* |
| *ROSC - 5 min* | *115 [98 – 131]* | *108 [99 – 123]* | *p: 0.114* |
| *5 - 20 min* | *67 [54 – 79]* | *64 [54 – 73]* | *p: 0.544* |
| *20 – end art cath (≈60 min)* | *111 [97 – 118]* | *108 [93 – 119]* | *p: 0.918* |  |
| *Friedman* | *p: <0.001* | *p: <0.001* |  |  |
|  |
| *HR[bpm]* | *Baseline(Before)* | *390 [372 – 435]* | *390 [372 – 420]* | *p: 0.863* | *Shapiro-Wilk failed:ANOVA na*  |
| *ROSC - 5 min* | *288 [264 – 315]* | *282 [261 – 290]* | *p: 0.149* |
| *5 - 20 min* | *309 [276 – 336]* | *294 [264 – 336]* | *p: 0.388* |
| *20 – end art cath (≈60 min)* | *480 [323 – 495]* | *468 [408 – 492]* | *p: 0.646* |
| *Friedman* | *p: <0.001* | *p: <0.001* |  |  |

No differences between groups are found in blood pressure or heart rate. Note the reduced heart rate in the first minutes after successful resuscitation are probably caused by the esmolol.

**Table suppl. 2b Hemodynamics and perioperative temperature series 2. (10 min cardiac arrest).**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| variable | time | pifithrin | control | ANOVA |
| MAP[mmHg] | Baseline(Before) | 87 ± 11 | 86 ± 15 | p group: 0.871p time: < 0.001p group x time:0.930 |
| ROSC - 5 min | 74 ± 13 | 75 ± 10 |
| 5 - 20 min | 98 ± 14 | 99 ± 11 |
| 20 – end art cath (≈30 min) | 71 ± 16 | 69 ± 9 |  |
| post-hoc | BL vs 5 p: 0.002BL vs 20 p: 0.061BL vs end p: 0.008 | BL vs 5 p: 0.006BL vs 20 p: 0.028BL vs end p: 0.006 |  |
|  |
| HR[bpm] | Baseline(Before) | 322 ± 36 | 322 ± 37 | p group: 0.881p time: < 0.001p group x time:0.923 |
| ROSC - 5 min | 259 ± 20 | 266 ± 33 |
| 5 - 20 min | 302 ± 19 | 300 ± 40 |
| 20 – end art cath (≈30 min) | 328 ± 44 | 327 ± 49 |
| post-hoc | BL vs 5 p: <0.001BL vs 20 p: 0.387BL vs end p: 1 | BL vs 5 p: <0.001BL vs 20 p: 0.711BL vs end p: 1 |  |
|  |
| cont.temp[°C] | Baseline(Before) | 35.8 ± 0.81 | 36.0 ± 0.89 | p group: 0737p time: < 0.001p group x time:0.644 |
| ROSC - 5 min | 33.9 ± 0.61 | 34.0 ± 0.50 |
| 5 - 20 min | 35.4 ± 0.59 | 35.4 ± 0.66 |
| 20 – end art cath (≈30 min) | 36.4 ± 0.66 | 36.3 ± 0.53 |
| 20 – end temp monitoring (≈40 min) | 36.4 ± 0.56 | 36.3 ± 0.46 |
| post-hoc | BL vs 5 p: <0.001BL vs 20 p: 0.359BL vs end art cath p: 0.006BL vs end tempp: 0.013 | BL vs 5 p: <0.001BL vs 20 p: 0.021BL vs end art cath p: 1BL vs end tempp: 1 |  |

No differences between groups are found in blood pressure or heart rate. Note the reduced heart rate in the first minutes after successful resuscitation are probably caused by the esmolol.

**Table suppl 3 Administered doses of adrenaline and calcium during resuscitation**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| variable | drug | pifithrin | control | Mann–Whitney U test |
| *adrenaline[µg/kg body weight]* | *Series 1.**[8 min cardiac arrest]* | *24.26 [20.33 – 26.23]* | *21.26 [20.60 – 25.91]* | *p: 0.636* |
| *Series 2.**[10 min cardiac arrest]* | *15.67 [15.21 – 20.28]* | *15.85 [15.38 – 20.05]* | *p: 0.242* |
| *Mann–Whitney U test* | *p: <0.001* | *p: <0.001* |  |
|  |
| *calcium[mmol/kg body weight]* | *Series 1.**[8 min cardiac arrest]* | *0.055 [0.055 – 0.059]* | *0.056 [0.056 – 0.058]* | *p: 0.432* |
| *Series 2.**[10 min cardiac arrest]* | *0.057 [0.054 – 0.057]* | *0.058 [0.055 -0.058]* | *p: 0.849* |
| *Mann–Whitney U test* | *p: 0.516* | *p: 0.921* |  |

The drug dosages during resuscitation do not differ between the groups. This was expected since resuscitation was performed before randomization and pifithrin/placebo administration. The difference of adrenaline dosage between Series 1 and Series 2 is due to the shorter resuscitation time (see text).

**Table suppl. 4 Histological examinations series 1., day 1**

|  |  |  |
| --- | --- | --- |
| variable | pifithrin-µ | control |
| Fluoro-Jade in CA1 [numbers of stained cells/mm] | 0 | 0 |
|  |
| Cresyl Violet stain: pyknotic cells in CA1 [%] | 0[0– 0.01] | 0[0 – 0] |

No Fluoro-Jade stained cells could be detected on day one after cardiac arrest, the same is true for CV staining, indication delayed cell death.

**Table suppl 5 Measurement of the sectional area surface of the CA1 segment, series 1**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| variable | time | pifithrin | control | ANOVA |
| CA1 cell layer atrophy (normalized: surface/length) [mm2/mm] | Day 1 | 0.069 ± 0.011 | 0.073 ± 0.009 | p group: 0.425p time: <0.001p group x time:<0.001 |
| Day 5 | 0.064 ± 0.012 | 0.0572 ± 0.008 |
| post-hoc | p: 0.011 | p: <0.001 |  |

The area or breadth of the CA1 segment is an indirect marker of neuronal damage, cell death results in a thinner cell layer. Both groups have significant reduction of cell layer breadth over time, but the significant interaction term indicates a difference in the reduction over time between groups. The hippocampus is a 3-dimensional structure, so delineating the borders in the microscope is difficult to achieve. We decided not to perform this measure in the second series.

**Table suppl 6 Histological assessment of the non-ischemic sham animals of the series 1. on day 1 (n=3)**

|  |  |
| --- | --- |
| variable | Day 1 |
| Fluoro-Jade in CA1 [numbers of stained cells/mm] | 0 |
| pyknotic cells in CA1 [%] | 0  |
| CA1 cell layer atrophy (normalized: surface/length) [mm2/mm] | 0.081 ± 0.001 |

The 3 animals represent normal values

**Table 7 a Results of neurobehavioral tests series 1.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| variable | time | pifithrin | control | post-hoc | ANOVA |
| *Weight [g]* | Baseline(Before) | *303 [295-313]* | *301 [294-312]* | *p: 0.626* | *Shapiro-Wilk failed:ANOVA na* |
| Day 1 | *298 [288-304]* | *296 [287-302]* | *p: 0.453* |
| Day 2 | *284 [275-298]* | *285 [275-293]* | *p: 0.835* |
| Day 3 | *288 [277-300]* | *286 [280-294]* | *p: 0.691* |
| Day 4 | *289 [281-303]* | *287 [279-297]* | *p: 0.382* |
| Day 5 | *294 [285-307]* | *289 [284-298]* | *p: 0.524* |
| Day 10 | *324 [310-342]* | *319 [301-324]* | *p: 0.294* |  |
| *Friedman* | *p: <0.001* | *p: <0.001* |  |  |
|  |
| *Neuro Deficit Score[Range0 – 100]0 =no deficit100 = brain death* | *Baseline(Before)* | *0 [0-0]* | *0 [0-0]* | *p: 1* | *Shapiro-Wilk failed:ANOVA na* |
| *Day 1* | *0 [0 – 5]* | *0 [0 – 10]* | *p: 0.172* |
| *Day 2* | *0 [0-0]* | *0 [0-4]* | *p: 0.019* |
| *Day 3* | *0 [0-0]* | *0 [0-0]* | *p: 0.069* |
| *Day 4* | *0 [0-0]* | *0 [0-0]* | *p: 0.205* |
| *Day 5* | *0 [0-0]* | *0 [0-0]* | *p: 0.205* |
| *Friedman* | *p: <0.001* | *p: <0.001* |  |  |
|  |
| *Tape Removal Test[sec]* | *Baseline(Before)* | *9 [7-12]* | *8 [7-12]* | *p: 0.546* | *Shapiro-Wilk failed:ANOVA na* |
| *Day 1* | *30 [17 – 62]* | *43 [15 – 114]* | *p: 0.531* |
| *Day 2* | *18 [8-33]* | *16 [9-41]* | *p: 0.847* |
| *Day 3* | *15 [8-32]* | *16 [9-30]* | *p: 0.759* |
| *Day 4* | *13[8-22]* | *11 [7-32]* | *p: 0.927* |
| *Day 5* | *12 [6-22]* | *10 [5-25]* | *p: 1* |
| *Friedman* | *p: <0.001* | *p: <0.001* |  |  |
|  |
| OFT distance[cm] | Baseline(Before) | 1393 ± 368 | 1387 ± 359 |  | p group: 0.149p time: 0.250p group x time:0.182 |
| Day 4 | 1290 ± 431 | 1194 ± 459 |
| Day 5 | 1302 ± 391 | 1044 ± 507 |
|  |
| *OFT mob/immob ratio* | *Baseline(Before)* | *6.7 [4.3-11.4]* | *8.6 [5.2-14.4]* | p: 0.374 | *Shapiro-Wilk failed:ANOVA na* |
| *Day 4* | *3.7 [2.6-6.3]* | *3.5 [2.3-6.7]* | p: 0.606 |
| *Day 5* | *3.6 [2.1-5.0]* | *3.0 [1.3-7.5]* | p: 0.830 |
| *Friedman* | *p: 0.055* | *p: <0.001* |  |  |
|  |
| Water Maze time to first crossing[sec] | Day 1 | 55 ± 12.1 | 47 ± 20.4 |  | p group: 0.419p time: <0.001p group x time:0.643 |
| Day 2 | 39 ± 19.0 | 41 ± 24.0 |
| Day 3 | 37 ± 22.0 | 28 ± 19.8 |
| Day 4 | 30 ± 23.9 | 23 ± 16.8 |
| Day 5 | 26 ± 18.9 | 30 ± 24.3 |
|  |
| Water Maze mean proximity to virtual platform[cm] | Day 1 | 87 ± 5.3 | 79 ± 11.6 | p: 0.042 | p group: 0.436p time: <0.001p group x time:0.016 |
| Day 2 | 70 ± 6.2 | 72 ± 9.2 | p: 0.569 |
| Day 3 | 64 ± 11.5 | 58 ± 12.3 | p: 0.264 |
| Day 4 | 62 ± 9.6 | 56 ± 10.1 | p: 0.158 |
| Day 5 | 57 ± 12.4 | 65 ± 12.3 | p: 0.147 |
|  |
| Water Maze time in platform quadrant[sec] | Day 1 | 6.9 ± 3.15 | 12.1 ± 3.72 |  | p group: 0.210p time: <0.001p group x time:0.070 |
| Day 2 | 14.0 ± 4.57 | 14.5 ± 4.70 |
| Day 3 | 15.8 ± 5.47 | 18.8 ± 8.52 |
| Day 4 | 15.6 ± 6.24 | 20.6 ± 7.12 |
| Day 5 | 18.1 ± 9.88 | 14.4 ± 7.07 |
| post-hoc | BL vs 4 p: 0.003BL vs 5 p: <0.001 | BL vs 4 p: 0.006BL vs 5 p: 1 |  |

**Table 7 b Results of neurobehavioral tests series 2.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| variable | time | pifithrin | control | post-hoc | ANOVA |
| Weight [g] | Baseline(Before) | 434 ± 19 | 429 ± 18 |  | p group: 0.355p time: < 0.001p group x time:0.820 |
| Day 1 | 421 ± 18 | 410 ± 22 |
| Day 2 | 406 ± 21 | 398 ± 21 |
| Day 3 | 403 ± 27 | 397 ± 20 |
| Day 4 | 405± 29 | 397 ± 23 |
| Day 5 | 407 ± 33 | 404 ± 24 |
| post-hoc | p: <0.001 | p: <0.001 |  |
|  |
| *Neuro Deficit Score[Range0 – 100]0 =no deficit100 = brain death* | *Baseline(Before)* | *0 [0-0]* | *0 [0-0]* | *p: 1* | *Shapiro-Wilk failed:ANOVA na* |
| *Day 1* | *10 [10 – 15]* | *10 [5 – 15]* | *p: 0.339* |
| *Day 2* | *0 [0-5]* | *0 [0-5]* | *p: 0.835* |
| *Day 3* | *0 [0-1.25]* | *0 [0-0]* | *p: 0.223* |
| *Day 4* | *0 [0-0]* | *0 [0-0]* | *p: 0.126* |
| *Day 5* | *0 [0-0]* | *0 [0-0]* | *p: 0.126* |
| *Friedman* | *p: <0.001* | *p: <0.001* |  |  |
|  |
| OFT distance[cm] | Day 4 | 2387 ± 852 | 2050 ± 693 |  | p group: 0.347p time: <0.001p group x time:0.440 |
| Day 5 | 1855 ± 736 | 1708 ± 590 |
| post-hoc | p: 0.002 | p: 0.037 |  |
|  |
| OFT mob/immob ratio | Day 4 | 2.5 ± 1.15 | 1.9 ± 0.81 |  | p group: 0.304p time: <0.001p group x time:0.064 |
| Day 5 | 1.3 ± 0.60 | 1.2 ± 0.55 |
| post-hoc | p: <0.001 | p: 0.002 |  |
|  |