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Minocycline attenuates brain tissue levels of TNF-α produced by neurons after prolonged hypothermic cardiac arrest in rats

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Abstract

Neuro-cognitive disabilities are a well-recognized complication of hypothermic circulatory arrest. We and others have reported that prolonged cardiac arrest (CA) produces neuronal death and microglial proliferation and activation that are only partially mitigated by hypothermia. Microglia, and possibly other cells, are suggested to elaborate tumor necrosis factor alpha (TNF-α) which can trigger neuronal death cascades and exacerbate edema after CNS insults. Minocycline is neuroprotective in some brain ischemia models in part by blunting the microglial response. We tested the hypothesis that minocycline would attenuate neuroinflammation as reflected by brain tissue levels of TNF-α after hypothermic CA in rats. Rats were subjected to rapid exsanguination, followed by a 6 min normothermic CA. Hypothermia (30 °C) was then induced by an aortic saline flush. After a total of 20 min CA, resuscitation was achieved via cardiopulmonary bypass (CPB). After 5 min reperfusion, minocycline (90 mg/kg; n=6) or vehicle (PBS; n=6) were given. Hypothermia (34 °C) was maintained for 6 h. Rats were sacrificed at 6 or 24 h. TNF-α was quantified (ELISA) in four brain regions (cerebellum, CEREB; cortex, CTX; hippocampus, HIP; striatum, STRI). Naïve rats (n=6) and rats subjected to the same anesthesia and CPB but no CA served as controls (n=6). Immunocytochemistry was used to localize TNF-α. Naïve rats and CPB

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controls had no detectable TNF-α in any brain region. CA markedly increased brain TNF-α. Regional differences were seen, with the highest TNF-α levels in striatum in CA groups (10-fold higher, P<0.05 vs. all other brain regions). TNF-α was undetectable at 24 h. Minocycline attenuated TNF-α levels in CTX, HIP and STRI (P<0.05). TNF-α showed unique co-localization with neurons. In conclusion, we report region-dependent early increases in brain TNF-α levels after prolonged hypothermic CA, with maximal increases in striatum. Surprisingly, TNF-α co-localized in neurons and not microglia. Minocycline attenuated TNF-α by approximately 50% but did not totally ablate its production. That minocycline decreased brain TNF-α levels suggests that it may represent a therapeutic adjunct to hypothermia in CA neuroprotection.

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Introduction

Deep hypothermic circulatory arrest (DHCA) has been used to create a bloodless field in cardiac surgery, enabling repair of congenital anomalies of the heart or acquired diseases of the aorta. Deep hypothermia is also used experimentally as an emergency treatment for prolonged cardiac arrest (CA) with delayed resuscitation via cardiopulmonary bypass (CPB) in both small and large animal models.[1–4] The underlying mechanisms of hypothermic preservation are not yet fully understood.

Neurocognitive disabilities are a well-recognized complication of hypothermic circulatory arrest. We and others have reported that prolonged CA produces neuronal death and microglial proliferation and activation that are only partially mitigated by hypothermia.[5] The exposure to the artificial materials of the CPB circuit has also been reported to induce neuroinflammation.[6,7]

Cytokines are pleiotropic proteins produced by multiple central nervous system (CNS) cells that participate in an orchestrated reaction to an insult. Traditionally, they have been considered pro- or anti-inflammatory, depending on their main course of action. However, individual cytokines including tumor necrosis factor alpha (TNF-α), a principal mediator of neuroinflammation in the brain, could play a dual role depending on the intensity of the insult or the period of recovery.[8–10]

The source of cytokines, including TNF-α, in CNS injuries remains controversial. Most studies suggest that microglia are the major source of cytokines.[11–13] Other glial cells[14–17] and neurons[18] are also capable of cytokine production, depending on the type of insult.[19] The timing of the cytokine assessment could also be an important factor, since the sources of cytokines can vary over time.

TNF-α is one of the major cytokines released by microglia. TNF-α can induce apoptosis and necroptosis in neural tissue and increase inflammation. TNF-α mRNA expression is up-regulated in cerebral ischemia,[20] with microglia purportedly acting as its major source.[21] It has previously been shown that TNF-α expression in the hippocampus is attenuated by mild hypothermia following hypoxic insult.[22] In addition, we recently reported a decrease in microglial activation in the hippocampus following prolonged CA when treated with deep hypothermia compared to moderate hypothermia, with improved neurologic outcome.[5]

There are well known regional differences in damage after CA or DHCA. The role of TNF-α or other cytokines in various brain regions has not yet been fully explored. Traditionally, studies focused on selectively vulnerable regions, namely hippocampal CA1 region or cerebellar Purkinje neurons. There is an emerging interest in striatum that also shows selective vulnerability in global brain ischemia.[23]

Therapies to attenuate TNF-α production – antibody based therapies may be promising in the setting of conditions such as traumatic brain injury (TBI) where blood-brain barrier (BBB) is injured, but there are challenges in the setting of CA and DHCA, where BBB is often intact or minimally affected.

Minocycline is a widely used antibiotic that readily passes BBB.[24] Minocycline demonstrated anti-inflammatory and anti-apoptotic properties in several models of neurologic injury.[25–30] The primary effect of minocycline is probably inhibition of activation of microglia[30] although a number of direct neuroprotective effects have also been shown in neurons.[31,32] Minocycline thus could have potential value in CA — for example — as an adjunct to hypothermia.[33,34]

In this study, we hypothesized that after rapid lethal hemorrhage and prolonged hypothermic CA 1) TNF-α will be increased in brain, 2) minocycline will attenuate TNF-α production, and 3) microglia will be the major source of TNF-α. Naïve rats and rats subjected to CPB were used as controls. In order to assess possible regional differences, four distinct brain regions were studied.

Methods

The study was approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh. We used our previously established model of rapid hemorrhage followed by prolonged hypothermic CA resuscitated with CPB[5] (Figure 1) that has been described in detail before.

In brief, adult male Sprague-Dawley rats were anesthetized with isoflurane, intubated and mechanically ventilated with FiO2 0.5. The left femoral artery and vein were cannulated for blood pressure monitoring and blood sampling. The right femoral artery and right jugular vein were cannulated for CPB resuscitation. Rectal and tympanic temperatures were monitored. Baseline blood samples were obtained, and hemodynamic values were recorded. Heparin sodium (200 units) was administered to prevent clotting.

After instrumentation, intubated rats were weaned to spontaneous ventilation with FiO2 0.3 and isoflurane 2%. Rapid exsanguination (12.5 ml of blood over 5 min) was performed via the internal jugular catheter. The shed blood was collected at room-temperature and used immediately for priming of the CPB circuit. After the rapid exsanguination phase, CA was ensured with intravenous administration of 9 mg of esmolol (0.9 ml) and 0.2 mEq of potassium chloride (0.1 ml). After 5 min of CA, hypothermia was induced with 270 ml of room-temperature flush solution (Plasma-Lyte A, Baxter) administered via the right femoral artery catheter at 50 ml/min. The flush was drained from the jugular vein catheter.

After 20 min of CA, resuscitation was started with CPB primed with the shed blood. After 5 min reperfusion, minocycline (90 mg/kg) or same volume of vehicle (PBS) were given according to randomization. Heating and cooling were achieved by warming the returned blood from CPB, heated blanket and overhead lamp. Blood samples for biochemistry and hematology were obtained every 15 min during CPB resuscitation and then every hour to guide treatment during the intensive care unit (ICU) phase. The samples were processed immediately using a point-of-care blood analyzer (Stat Profile, Nova Biomedical; Waltham, MA).

Arterial blood gas management followed alpha-stat principles. pH and electrolyte values outside of the normal range were corrected during CPB and ICU phases by adjustments in ventilation and/or administration of sodium bicarbonate, calcium chloride. Additional blood obtained from an isoflurane-anesthetized donor rat was used to maintain hematocrit > 25%.

The blood was withdrawn immediately prior to the experiment, stored in a syringe at room temperature and transfused over 1 h during the CPB-resuscitation phase. CPB support was gradually discontinued after 60 min. Mechanical ventilation with an FiO_2 of 1.0 was continued while maintaining normocapnia. Rats scheduled for sacrifice at 24 h were weaned from mechanical ventilation, decannulated, extubated and placed in a cage with supplemental O2 at 1 L/min, resulting in FiO2 ~ 0.3. At 6 h or 24 h after resuscitation, rats were deeply anesthetized with isoflurane, intubated, mechanically ventilated and perfused with heparinized ice-cold normal saline. The rats were then decapitated, brains removed and dissected into four regions of interest: cortex (CTX), striatum (STRI), hippocampus (HIP) and cerebellum (CEREB). Each of these regions is well known to develop selectively vulnerable neuronal death. The samples were snap-frozen in liquid nitrogen and then stored at −70 °C freezer until further processing.

Since others indicated that exposure to artificial materials of an experimental CPB circuit used in our study induces systemic and cerebral inflammation,[6,7] we included a control group that was subjected to the same duration (60 min) of CPB but without preceding hemorrhage or CA. The temperature profile of this group was designed to mirror the CA group. We have also studied sham animals subjected to the same cannulation and anesthesia, without ischemic insult or CPB. This group was kept normothermic.

Rats were randomly assigned to four groups, n=6 per group: 1) sham rats (sham); 2) CPB controls (CPB); 3) CA group (CA); 4) CA treated with minocycline (CA+M).

Additional rats in group CA sacrificed at 6 h were used for immunocytochemistry (n=6) or at 24 h for ELISA TNF-α (n=6).

In additional experiments, we have screened the possibility of invasion of macrophages into the brain after hypothermic CA. Micrometer sized paramagnetic iron oxide particles (MPIO, 9 mg Fe/kg) were injected intravenously in shams (negative control), hypothermic CA or traumatic brain injury (TBI, positive control). Migration of these macrophages into the brain after 24 h was tracked ex-vivo using 11.7 T magnetic resonance imaging.

Assessment of TNF-α levels

Each tissue region was homogenized in 5× volume of 1X PBS. Homogenates were centrifuged at 14000g for 30 min and supernatant was retained. Protein concentration was measured using the BCA assay (Pierce). TNF-α was measured by enzyme-linked immunosorbent assay (ELISA TNF-α kit, R&D Systems) according to manufacturer's instructions. The final concentrations were then adjusted for the protein content in the sample.

Immunocytochemistry

Samples were mounted in OTC media and 10 μm sections were cut and mounted on Superfrost slides (Fisher). Tissue sections were fixed for 10 min in acetone, rinsed with 1× TBST and blocked in 3% Normal Horse Serum for 30 min at room temperature. Slides were washed in 1× TBST and incubated overnight at 4 °C in primary antibody (TNF-α 1:40, R&D Systems, NeuN 1:100, Millipore). The next day, sections were rinsed 3 times with 1× TBST and incubated for 1 hour at room temperature in secondary antibody (donkey anti-goat Alexa 488 for TNF-α, horse anti-rabbit Alex 594 for NeuN, both at 1:400 dilution). Sections were rinsed, coverslipped with aqueous mounting media and visualized for co-localization. For Iba-1 staining, the adjacent 10um sections were used, but fixed in 4% paraformaldehyde for 10 minutes. The same procedure was used above with a 1:250 dilution for Iba-1 (Wako Chemicals) and a goat anti-rabbit Alexa 594 as the secondary antibody.

Statistical analysis

Repeated measures analysis of variance (ANOVA) was used to compare heart rate, mean arterial pressure and tympanic and rectal temperatures between groups. One-way ANOVA was used to compare physiologic and biochemical data and TNF-α levels between groups or regions. Post-hoc Tukey's test was used. A p value < 0.05 was considered statistically significant.

Results

All rats survived the insult until the scheduled timepoint of sacrifice.

There were no differences in physiologic or biochemical data between groups at baseline. Physiologic parameters (heart rate, blood pressure) and temperature profiles during resuscitation are shown in Figs. 2 and 3. Heart rate was significantly higher in shams vs. other groups ($p<0.01$) (Fig. 2, top panel). Mean arterial pressure profiles were similar in CA and CA+M groups, with significant differences vs. sham ($p<0.01$) or CPB ($p<0.05$) groups, respectively (Fig. 2, bottom panel). Tympanic and rectal temperature profiles were also similar in both CA groups, but differed vs. shams or CPB control group (Fig. 3).

Prolonged CA resulted in marked physiologic and biochemical disturbances, including extremely low pH <7.0, BE exceeding −20, and increased lactate up to 6 mmol/L. These changes were gradually improved during resuscitation and were largely ameliorated by the end of the ICU phase. Minor changes were also observed in the CPB group. (Table 1)

Shams and CPB controls had no detectable TNF-α in any brain region. CA markedly increased brain TNF-α in all regions compared to shams or CPB controls. Striatum showed an early specific increase in TNF-α levels after CA. The levels measured in the striatum were 10-fold higher than in the hippocampus or other brain regions. Minocycline decreased TNF-α levels in CTX, HIP and STRI by ~ 50% but did not totally ablate TNF-α increase. (Fig. 4) At 24 h, the levels of TNF-α were undetectable across all regions (data not shown).

TNF-α showed selective co-localization with NeuN, identifying neurons. (Fig. 5) The morphological type and cell density of TNF-α positive cells did not correspond with microglia (Figure 6).

No difference in MPIO-labeled macrophage invasion between shams and rats subjected to hypothermic CA was appreciated, while rats subjected to TBI showed massive macrophage infiltration (Supplemental File 1).

Discussion

In our model of prolonged DHCA, we report 1) a marked increase of TNF-α across multiple brain regions, and 2) a unique finding of an early selective increase in TNF-α levels in the striatum, 10-fold higher than other brain regions studied. Using immunocytochemistry, we identified that the increased TNF-α colocalized with neurons. Minocycline was able to attenuate the increase in most regions, but did not fully mitigate TNF-α production.

The severe hemodynamic and physiologic derangements resulting from the insult are consistent with our previous reports, documenting a good reproducibility of the model. Importantly, there were no differences in any parameters between CA and CA+M groups, respectively, speaking against a major hemodynamic or physiologic effect of minocycline that could independently affect regional brain TNF-α production after prolonged hypothermic CA.

TNF-α

Multiple pro- and anti-inflammatory cytokines were identified and studied in experimental models of brain ischemia and in clinical settings.[35] In our study, we focused on TNF-α as an important cytokine mediating neuronal injury. Our choice of TNF-α as a footprint for neurologic damage is supported by a body of evidence suggesting that TNF-α is linked to worse neurologic outcome, although some reports ascribed beneficial effects to TNF-α.[36] Neutralization of TNF-α after focal brain ischemia models in rats led to decreased infarct volume and cerebral edema.[37] In transgenic mice overexpressing TNF-α, ischemia led to a five-fold increase in TNF-α in the border zone approximating the ischemic penumbra and the infarct core, and to a worse neurologic outcome, including larger infarct volume and increased neuronal apoptosis.[38] In contrast, others reported no differences between wild type TNF-α mice and TNF-α gene-deficient mice in hippocampal neuronal damage after global brain ischemia.[39] This is in line with our findings that TNF-α may not be the key mediator in hippocampus but could play a major role in other brain regions.

The effect of TNF-α can be also dependent on the level of its expression. In the nigrostriatal dopaminergic circuit of adult mice, chronic low expression of TNF-α was neuroprotective, while high expression of TNF-α produced neurodegenerative effects associated with gliosis and inflammatory infiltration.[40]

The finding of TNF-α co-localization with neurons in striatum is surprising but does have support in the literature. Under physiological situations, neurons are the only CNS cells expressing TNF-α.[41] After incomplete brain ischemia in rats, early increase of of TNF-α mRNA was observed in the cortex as early as at 3 h, peaking at 12 h. TNF-α protein was found associated with neurons in the ischemic cortex at 6 h, before neutrophil or macrophage infiltration. Additionally, scattered TNF-α immunoreactive neurofilaments were observed in the striatum at 12–24 h. No colocalization with the astrocytes was seen. Unfortunately, microglia were not studied.[18] Early colocalization of TNF-α in neurons in that study agrees with our current work in striatum after DHCA.

After focal cerebral ischemia, TNF-α was found in the neural processes, and to a moderate extent also in neuronal soma and glia. Many of TNF-α expressing neurons were in close proximity to activated microglial cells, which exhibited a high level of TNF-α immunoreactivity.[42]

Yasuda et al. studied neuronal degeneration, gliosis and temporospatial cytokine profile in the hippocampus in a global brain ischemia model. Extensive hippocampal CA1 cell loss was accompanied by reactive gliosis. An early isolated peak of TNF-α at 6 h was seen. After a period of low TNF-α levels, a second period of four-fold higher levels stretched from day 7 to 21. The source of TNF-α were not identified.[43] Given the similarity with our model, it is possible that the early release of TNF-α may have originated from a separate source, possibly neurons, while delayed TNF-α increase coincided with increased microglial proliferation and activation.

Regionality

Hippocampus and especially its CA1 area are considered the most vulnerable region to ischemic insults. Moreover, recent findings showed that important regional differences in response of individual brain regions exist. This may require individual, region-specific treatment. Our model is characterized with extensive neuronal degeneration associated with microglial activation and proliferation. At one week after the insult, there were ~ 30% Fluoro-Jade B positive neurons in the hippocampus, and ~38% in striatum, respectively. CA triggered an increase of TNF-α in all regions that may have biological relevance, but they were dwarfed by the marked increase in striatum early after DHCA.

Saito et al. previously reported increased TNF-α levels in both hippocampus and striatum early after global ischemia model in gerbils, with levels in hippocampus higher than in striatum.[44] Several prior reports identified striatum as a selectively vulnerable region. In a neonatal piglet model of DHCA, the ratio of pro- vs. anti-apoptotic proteins was the least favorable in striatum, compared to hippocampus or cortex.[45] It has also been reported that striatum can react to remote injury, e.g. in hippocampus, by elaborating cytokines.[46]

Hypothermia

Our model used moderate intra-arrest hypothermia, followed by a prolonged mild hypothermia as an integral part of the current clinical post-resuscitative paradigm. Experimentally, hypothermia effectively ameliorated neuronal degeneration after global and focal brain ischemia in multiple models.[47,48]

The exact mechanisms by which hypothermia exerts its effects are not yet fully understood. Effects of hypothermia on brain cytokine production after ischemic insults have not been studied systematically. The effect of hypothermia itself was not explored in our study since both groups subjected to CA followed an identical hypothermic protocol.

Our current results indicate that minocycline attenuates TNF-α which is to the greatest extent increased in the striatum. This region was not previously studied in our model. We cannot rule out that benefits previously seen with minocycline or deeper hypothermia were stemming from effects exerted outside hippocampus.

Minocycline

We chose minocycline as a treatment based on previous reports of its beneficial effects in multiple models, including global and focal brain ischemia, TBI, spinal cord injury and intracerebral hemorrhage.[25] Both motor and neurocognitive behavior were improved by treatment with minocycline, even when the initiation of treatment was delayed for several hours after the insult.[49] We used high-dose intravenous administration of minocycline to ensure adequate brain tissue levels.[50]

Minocycline is considered to target primarily microglia, but effects on other CNS cells including oligodendrocytes,[51] astrocytes[52] or neurons[31,32,49] have been described. Most importantly, minocycline attenuated cell death induced by oxygen-glucose deprivation in neuronal culture (without microglia) to the same extent as TNF-αantibody, supporting a possible direct effect of minocycline on neurons.[53]

Studies in brain ischemia models showed beneficial effects of minocycline on behavioral outcome in juvenile rats,[26,54,55] but only a limited effect in adult rats.[56] One study reported a positive effect of minocycline but not hypothermia after focal brain ischemia in adult rats.[33] These effects were suggested to be mediated by microglia,[26] or independent of microglia.[55] Several cytokines were altered by minocycline treatment in the developing rats, but no effect on TNF-α was seen.[54] This argues for a different pattern of neuroinflammatory response to CA, depending on the developmental stage of the animal. These findings along with our current results identifying neurons as the likely source of early TNF-α may explain the relative lack of a definitive effect of minocycline in our prior study using an identical model.

Sanchez Mejia et al. showed that minocycline was able to ameliorate activation of caspases and neuronal death early after TBI. Of note, caspase-1 and -3 were also localized specifically in neurons.[30] It should be noted that the aforementioned studies usually used acute minocycline treatment, usually limited to three days. Prolonged administration of minocycline over 4 weeks resulted in improved neurobehavioral deficits and reduced microgliosis.[57]

Control groups

We included a control group subjected to CPB without CA to explore the effects of CPB in our model that incorporated CPB as an integral part of the resuscitation efforts. Jungwirth et al. previously described increased cerebral TNF-α mRNA expression 4 h after CPB vs. shams.[58] In our study, we were unable to detect TNF-α protein 6 h after CPB in any of the regions. It is acknowledged that increased mRNA expression does not need to be translated into an increased protein production, or the production could be delayed. Our CPB control group used hypothermia (25–34 °C) to mirror the hypothermic phase of CPB-resuscitation in the CA groups, contrasting normothermia maintained in the study by Jungwirth et al. This could have ameliorated TNF-α induction in brain.

Our study has certain limitations. We were unable to include a normothermic control group that would eliminate the protective effects of hypothermia that could have blunted the effect of minocycline. We have demonstrated in the model development feasibility study that normothermic controls are unable to survive this insult.[1] This is certainly not surprising given that we are modeling a 20 min CA insult that can only be survived with hypothermia. We explored two early timepoints after resuscitation. It is possible that earlier or later phases of reperfusion could produce different results, and other CNS cells may be involved at different stages.

The use of FiO_2 1.0 after resuscitation from CA is controversial but may aggravate injury.[59–61] In addition, the impact of of hyperoxia post CA on cytokines in brain remains unclear. Systemic cytokine levels were increased with hyperoxia after experimental CPB in a rat[62] but brain tissue levels were not studied.

The role of TNF-α identified early after CA in neurons in our study is not yet elucidated. Future studies aimed at selective attenuation of the TNF-α surge in the striatum may help to identify whether TNF-α is linked with cell death by apoptosis and necroptosis or cell survival cascades in a cause-and-effect relationship. Based on our findings its role in striatal neuronal death deserves to be explored.

In conclusion, we report unique regional differences in early TNF-α production after prolonged hypothermic CA in rats resuscitated with CPB, identifying striatum as a region with the highest TNF-α levels. Surprisingly, TNF-α co-localized with neurons. Minocycline attenuated the TNF-α increase in most brain regions. Our data suggest that if neuroinflammatory pathways contribute to neuronal death early after CA, region specific differences in these pathways could play important roles in mediating selective vulnerabilities.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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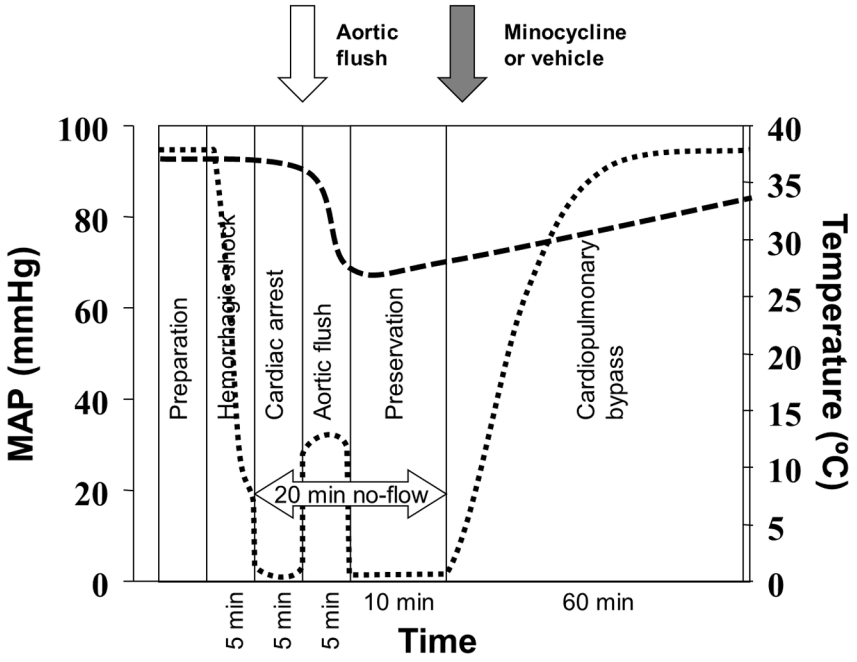

Figure 1.
Schematic representation of the experiment. Dotted line represents mean arterial pressure (left axis) and the dashedline represents tympanic temperature of the rats subjected to cardiac arrest.

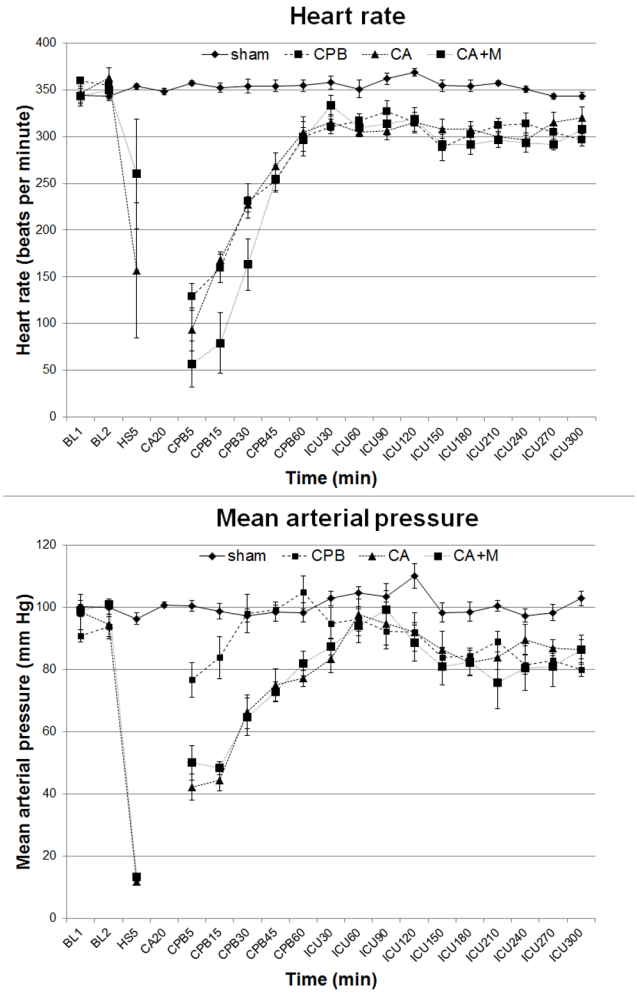

Figure 2.

Heart rate (top panel) and mean arterial pressure (bottom panel) after prolonged hypothermic CA. Heart rate: $p<0.01$sham vs. other groups. Mean arterial pressure: $p<0.01$ sham vs. CA or CA+M groups; $p<0.05$ CPB vs. CA or CA+M groups. BL, baseline; HS, end of hemorrhagic shock; CA, cardiac arrest; CPB, cardiopulmonary bypass; ICU, intensive care.

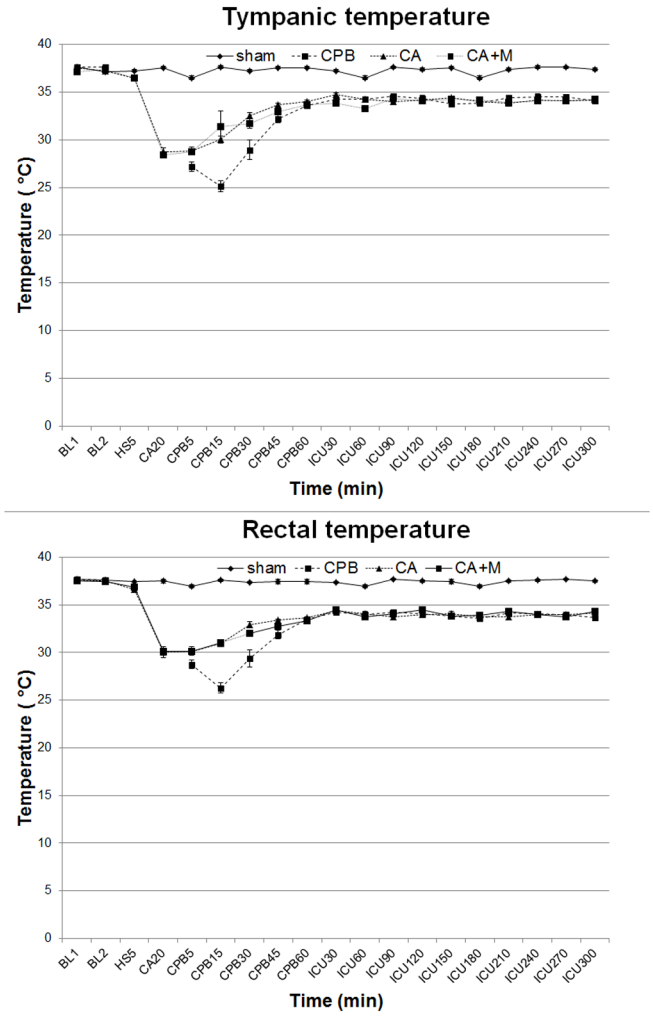

Figure 3.

Tympanic (Tty, top) and rectal (Trec, bottom) temperatures during CA. Tty: $p<0.01$ sham vs. other groups; CA vs. CPB. $p<0.05$ CPB vs. CA+M. Trec: $p<0.01$ CA or CA+M vs. other groups, and sham vs. CPB. CA, cardiac arrest.

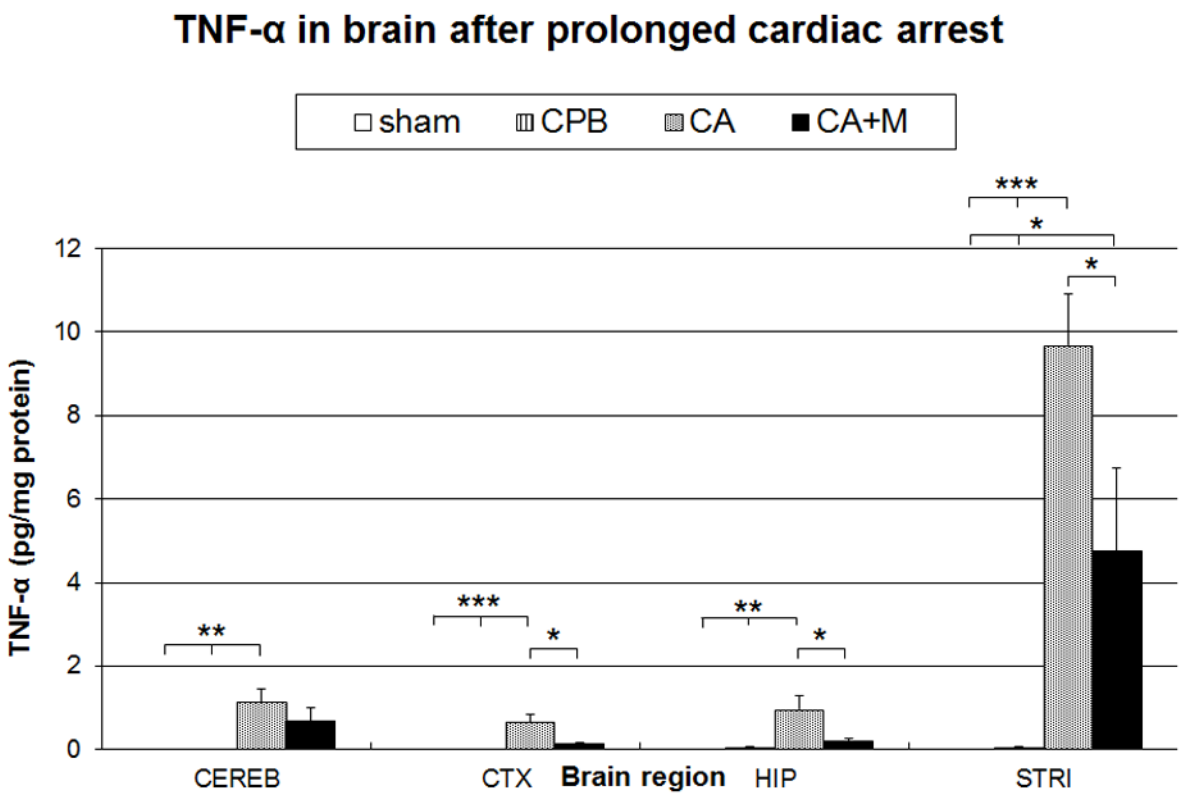

Figure 4.

TNF-α protein levels in individual brain regions at 6 hours after prolonged cardiac arrest. A unique increase of TNF-α is observed in the striatum. Minocycline attenuated the TNF-α increase in selected brain regions.* $p<0.05$; ** $p<0.01$; *** $p<0.001$

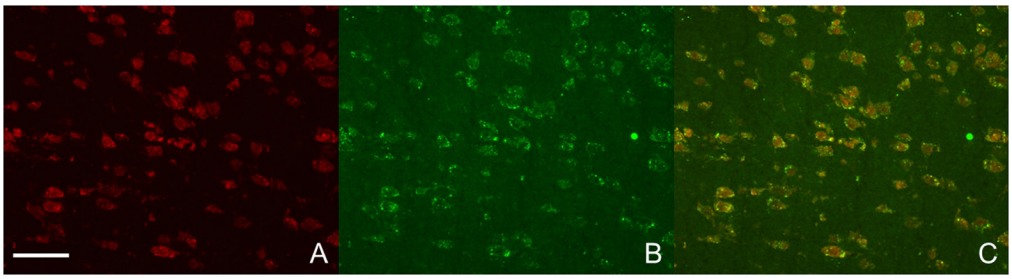

Figure 5.

Prolonged 20 min cardiac arrest resulted in a massive TNF-α expression in the striatum (A, NeuN staining visualizing neurons; B, TNF-α staining; C, merge A&B). The TNF-α-expressing cells co-localize with neurons (striatum, 20x). The marker in panel A represents 40 μm.

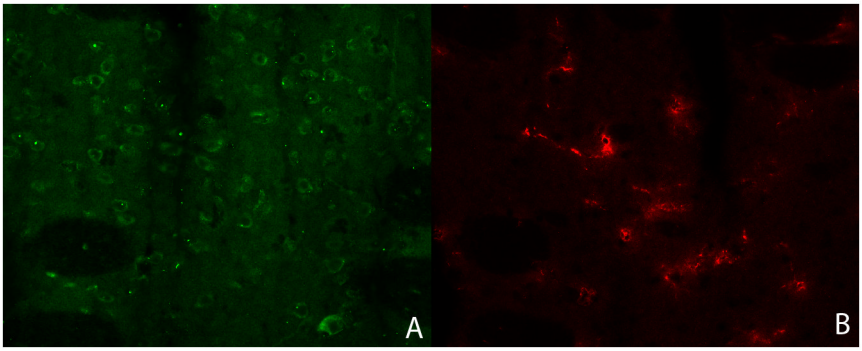

Figure 6.
TNF-alpha positive cells (green, panel A) and microglia (red, panel B) in striatum show different morphology and cell density, further supporting our findings that microglia are an unlikely to be a major source of the TNF-alpha production at 6 h.

Table 1

Biochemical and hematological values after prolonged cardiac arrest.

		BL	CPB5	ICU15	ICU120	ICU300
pHa	**sham**	7.39±0.03	7.39±0.05^{abc}	7.39±0.01^{f}	7.40±0.04	7.37±0.03
	CPB	7.41±0.06	7.19±0.17^{bcd}	7.26±0.06^{eg}	7.32±0.06^{g}	7.33±0.06
	CA	7.41±0.02	6.95±0.09^{ef}	7.36±0.06^{a}	7.41±0.06^{a}	7.37±0.06
	CA+M	7.37±0.05	6.93±0.10^{ef}	7.34±0.07	7.41±0.07	7.32±0.07
paO2	**sham**	259±65	384±69	403±25	364±105	420±61
	CPB	304±64	460±77	338±64	396±130	434±44
	CA	224±44	451±73	323±74	394±62	414±70
	CA+M	256±80	482±55	370±63	356±78	414±53
paCO2	**sham**	39±5	41±2^{bc}	40±5	37±5	41±3
	CPB	37±7	48±8^{bc}	44±9	38±2	38±4
	CA	39±5	31±5^{ef}	43±7	40±7	42±9
	CA+M	46±9	28±4^{ef}	43±5	38±7	36±8
BE	**sham**	−0.5±1.7	0.1±1.4^{bcf}	−0.2±2.3^{f}	−1.6±1.6	−1.3±1.7
	CPB	−0.8±1.0	−5.9±1.3^{bce}	−7.0±2.5^{ebh}	−6.0±3.0^{gh}	−5.0±2.8
	CA	−0.3±1.8	−22.9±2.7^{ef}	−2.0±2.4^{f}	0.9±5.3^{a}	−1.0±4.6
	CA+M	1.8±1.0	−24.2±2.3^{ef}	−2.6±2.4	0.5±3.1^{a}	−5.6±6.1
Lactate	**sham**	1.2±0.5	1.4±0.6^{bc}	1.5±0.6^{bc}	1.8±0.3^{gh}	2.2±0.4^{c}
	CPB	1.9±0.1	2.5±0.6^{bc}	4.8±2.4^{h}	4.7±1.7	3.1±0.8^{h}
	CA	1.2±0.6	6.6±1.0^{ef}	6.9±2.9^{e}	5.3±3.2^{d}	4.6±2.4
	CA+M	1.0±0.4	5.4±1.6^{ef}	8.6±2.0^{ae}	5.6±1.2^{d}	7.3±3.4^{ae}
Hct	**sham**	40±4	42±3^{bcf}	40±4^{bcf}	37±3	40±5
	CPB	41±2	26±3^{eh}	27±3^{e}	32±2	33±2
	CA	40±5	25±3^{e}	28±3^{e}	36±5	36±4

		BL	CPB5	ICU15	ICU120	ICU300
	CA+M	37±3	20±4[ae]	30±3[e]	37±4	35±7
Glucose	**sham**	256±62	266±53	273±61	243±77[bc]	230±78[agh]
	CPB	340±63[h]	292±52	358±55[b]	167±48	128±26[d]
	CA	233±61	223±59	200±74[f]	117±29[e]	121±48[d]
	CA+M	212±51[a]	302±59	265±53	96±36[e]	134±59[d]

BL = baseline, CPB5 = 5 min after start of CPB, ICU15 = 15 min after weaning from CPB, ICU120= 2 h after weaning from CPB, ICU300 = at 6 h resuscitation time.

[a] p<0.05 vs. CPB;

[b] p<0.01 vs. CA;

[c] p<0.01 vs. CA+M;

[d] p<0.05 vs. sham;

[e] p<0.01 vs. sham;

[f] p<0.01 vs. CPB;

[g] p<0.05 vs. CA;

[h] p<0.05 vs. CA+M.