HUMAN UMBILICAL CORD BLOOD CELLS OR ESTROGEN MAY BE BENEFICIAL IN TREATING HEATSTROKE

Sheng-Hsien Chen[1,2,3], Kuo-Feng Huang[2], Mao-Tsun Lin[4], Fong-Ming Chang[1]*

[1]Institute of Clinical Medicine, College of Medicine, National Cheng Kung University, Departments of [2]Obstetrics and Gynecology, and [4]Medical Research, Chi Mei Medical Center, Tainan, and [3]Department of Medicine, Taipei Medical University, Taipei, Taiwan.

SUMMARY

This current review summarized animal models of heatstroke experimentation that promote our current knowledge of therapeutic effects on cerebrovascular dysfunction, coagulopathy, and/or systemic inflammation with human umbilical cord blood cells (HUCBCs) or estrogen in the setting of heatstroke. Accumulating evidences have demonstrated that HUCBCs provide a promising new therapeutic method against neurodegenerative diseases, such as stroke, traumatic brain injury, and spinal cord injury as well as blood disease. More recently, we have also demonstrated that post- or pretreatment by HUCBCs may resuscitate heatstroke rats with by reducing circulatory shock, and cerebral nitric oxide overload and ischemic injury. Moreover, $CD34^+$ cells sorted from HUCBCs may improve survival by attenuating inflammatory, coagulopathy, and multiorgan dysfunction during experimental heatstroke. Many researchers indicated pro- (e.g. tumor necrosis factor-α [TNF-α]) and anti-inflammatory (e.g. interleukin-10 [IL-10]) cytokines in the peripheral blood stream correlate with severity of circulatory shock, cerebral ischemia and hypoxia, and neuronal damage occurring in heatstroke. It has been shown that intravenous administration of $CD34^+$ cells can secrete therapeutic molecules, such as neurotrophic factors, and attenuate systemic inflammatory reactions by decreasing serum TNF-α but increasing IL-10 during heatstroke. Another line of evidence has suggested that estrogen influences the severity of injury associated with cerebrovascular shock. Recently, we also successfully demonstrated estrogen resuscitated heatstroke rats by ameliorating systemic inflammation. Conclusively, HUCBCs or estrogen may be employed as a beneficial therapeutic strategy in prevention and repair of cerebrovascular dysfunction, coagulopathy, and/or systemic inflammation during heatstroke. [*Taiwanese J Obstet Gynecol* 2007;46(1):15–25]

Key Words: $CD34^+$ cell, cytokine, estrogen, heatstroke, human umbilical cord blood cell, inflammation

Animal Heatstroke Models Execute the Empirical Triad Employed for the Diagnosis of Conventional Human Heatstroke

Heatstroke is a life-threatening disease defined as a hyperpyrexia (> 40°C) and multiple organ (in particular, the central nervous system [CNS]) dysfunction or failure. The CNS dysfunction includes delirium, convulsion, or coma [1]. Neurologic injury has been ascribed to metabolic disorders, edema of the brain, or cerebral ischemia and damage [2,3]. Other organ dysfunctions follow severe heatstroke, including hypotension, disseminated intravascular coagulopathy (DIC), hepatic failure, hyperventilation, pulmonary edema, renal failure, rhabdomyolysis, metabolic acidosis, and systemic inflammation as shown in Table 1, [1,4–7]. DIC is indicated by increased prothrombin time, activated partial thromboplastin time, and D-dimer, and decreased platelet count and protein C. Biochemical markers indicate cellular ischemia and injury/dysfunction: plasma

**Correspondence to:* Dr Fong-Ming Chang, Institute of Clinical Medicine, College of Medicine, National Cheng Kung University, Tainan 710, Taiwan.
E-mail: fchang@mail.ncku.edu.tw
Accepted: August 16, 2006

Table 1. Symptoms and signs during heatstroke in both humans and rats

Symptoms and signs	Humans*	Rats†
1. Hyperpyrexia (↑core temperature)	+	+
2. Multiple organ dysfunction and failure:	+	+
Hypotension (↓mean arterial pressure)	+	+
ECG alterations	+	+
Hyperventilation (↓blood PCO_2)	+	+
Pulmonary edema	+	+
Hepatic failure (↑glutamic oxaloacetic transaminase, ↑glutamic pyruvic transaminase, ↑alkaline phosphatase)	+	+
Renal failure (↑blood urea nitrogen, ↑creatinine)	+	+
Rhabdomyolysis (↑blood K^+)	+	+
Disseminated intravascular coagulopathy (DIC: ↑prothrombin time, ↑activated partial thromboplastin time, ↑D-dimer, ↓platelet count, ↓protein C)	+	+
Metabolic acidosis (↑blood lactate, ↓blood pH)	+	+
Systemic inflammation (↑pro-inflammatory cytokines)	+	+
Cerebral ischemia, injury, and dysfunction (ischemia: ↑glutamate, ↑intracranial pressure, ↓cerebral perfusion pressure, ↑lactate/pyruvate; damage: ↑glycerol; dysfunction: delirium, convulsion, or coma)	+	+

**Information from [1,5–7]; †information from [2,4,8–14]. DIC = disseminated intravascular coagulopathy; + = presence; ↑ = increased; ↓ = decreased.*

levels of blood urea nitrogen (BUN), creatinine, glutamic oxaloacetic transaminase (SGOT), glutamic pyruvic transaminase (SGPT), and alkaline phosphatase (Alp), and cerebral levels of glycerol, glutamate, lactate/pyruvate ratio, dihydroxy benzoic acid, lipid peroxidation, oxidized-form glutathione, reduced-form glutathione, dopamine, and serotonin are all raised during heatstroke. Intracranial pressure (ICP) is also elevated during heatstroke. In contrast, the values of mean arterial pressure (MAP), cerebral perfusion pressure (CPP = MAP–ICP), and cerebral levels of local cerebral catalase, glutathione peroxidase, and glutathione reductase activities are all significantly decreased during heatstroke.

In addition, it is well known that the lactate/pyruvate ratio is a marker of cell ischemia, whereas glycerol is a marker of how severely cells are affected by ongoing pathology [8–10]. Excessive accumulation of glutamate has been shown in ischemic brain tissue [11–13]. Indeed, when the animals are exposed to hot environment, cerebral ischemia and hypoxia, which occurred during heatstroke, were associated with an increased production of glycerol, lactate/pyruvate ratio, glutamate, and inducible nitric oxide synthase (iNOS)-dependent NO in the brain [14–16]. In addition, PO_2 in rat brain was decreased after the onset of heatstroke. Thus, it appears that excessive accumulation of glycerol, glutamate, lactate/pyruvate ratio, and iNOS-dependent NO in the brain may be secondary to cerebral ischemia, hypoxia, and/or injury in the rat.

The empirical triad used for the diagnosis of classic human heatstroke includes hyperpyrexia, CNS disorders, and a history of heat stress [1]. Basing on this triad, the anesthetized rat [4,17,18], the unanesthetized rat [19–21], the unanesthetized rabbit [2], the unanesthetized mouse [22–24], the anesthetized mouse [25], and the anesthetized baboon [26] all displayed a uniform response and reacted similarly to humans with heatstroke. Table 1 summarizes signs and symptoms of heatstroke in both humans and rats. It can be derived from the table that the anesthetized rat model can nearly resemble the full spectrum of signs and symptoms occurring during heatstroke in humans. For this reason, cerebrovascular dysfunction is an attractive target for therapy in heatstroke.

Resuscitation from Experimental Heatstroke by Transplantation of Human Umbilical Cord Blood Cells

Neural transplantation has been used to study and promote the regenerative potential of the brain after an ischemic insult. Fetal neural stem cells can attenuate experimental brain injury and improve outcome in stroke victims [27,28]. However, transplantation of embryonic grafts is plagued with logistical and ethical considerations. Thus, it is reasonable to seek alternative sources or an equivalent of stem cells. Stem cells

have been isolated from various tissues in animals and humans, including adult bone marrow, cord blood, and even adult brain [29–35].

HUCBCs are rich in hematopoietic stem cells ($CD34^+$ cells) [36]. Two percent of the HUCBCs are stem cells capable of reconstituting blood lineages. These HUCBCs have been used to reconstitute bone marrow and blood cell lineages in children with malignant and nonmalignant diseases [37]. HUCBCs is assumed to exhibit a neuroprotective effect on the ischemic brain by multipotent stem cells derived from the nervous system with the capacity to regenerate and give rise to cells belonging to all three cell lineages in the nervous system: neurons, oligodendrocytes, and astrocytes [46]. These cells could be induced to express neural proteins [38–40]. When the HUCBCs were administered via the tail vein, surviving HUCBCs were identified in the cortex and striatum of the injured hemisphere [41–43]. The behavioral dysfunctions produced by stroke [42–44], traumatic brain injury [45], and spinal cord injury [46] were significantly improved by intravenous delivery of HUCBCs. Among these cells, only 2% expressed neuronal markers, and 6% expressed glial fibrillary acidic protein. These results indicated that there is a signal that can attract the HUCBCs toward the site of ischemia and damage and stimulate development of neural markers. However, it is not known whether administration of HUCBCs attenuates circulatory shock and cerebral ischemia during heatstroke.

More recently, we have also demonstrated that HUCBCs therapy may resuscitate rats with heatstroke by reducing circulatory shock, and cerebral nitric oxide overload and ischemic injury; central delivery of HUCBCs seems superior to systemic delivery of HUCBCs in resuscitating rats with heatstroke [15]. Our results, in part, are consistent with previous findings concerning the efficacy of transplanting HUCBCs as a treatment for conventional stroke. It has been shown that intravenous delivery of either whole bone marrow or umbilical cord blood [42,45,47] can produce behavioral recovery.

Furthermore, previous results have revealed that microvascular disturbances, including cerebral ischemia, breakdown of the blood–brain barrier permeability, and formation of cerebral vasogenic edema, occur during heatstroke in rodents [2,48]. Therefore, the current results indicated that after the onset of heatstroke, disruption of the blood–brain barrier may facilitate selective entry of HUCBCs into the ischemic sites within the brain [15]. Apparently, intracerebral administration had some advantages in reaching the target sites in the brain more easily than with the intravenous route of injection. This can explain why intracerebroventricular delivery is superior to intravenous delivery in resuscitation from heatstroke. As demonstrated in our study, the prolongation of survival among rats with HUCBCs transplants was found to be related to maintenance of appropriate levels of both MAP and CPP, as well as reduction in both intracranial hypertension and cerebral neuronal damage exhibited during the onset of heatstroke. The maintenance of cerebral blood flow and PO_2 in animals treated with HUCBCs might be brought about by higher CPP resulting from lower ICP (due to reduction in cerebral edema and cerebrovascular congestion) and higher MAP during the development of heatstroke, as demonstrated by our previous results [15].

Our previous results showed that the heatstroke-induced arterial hypotension, cerebral ischemia, and inducible nitric oxidase overexpression and NO overproduction in rat brain can be suppressed by pretreatment with aminoguanide (an inducible nitric oxidase inhibitor) [14]. In the following study, we also demonstrated that HUCBCs transplantation attenuated arterial hypotension, cerebral ischemia, and NO production in rat brain during heatstroke. These findings together suggest that HUCBCs transplantation may maintain appropriate levels of systemic and cerebral circulation during heatstroke by reducing cytokines production as well as inhibiting inducible nitric oxidase-dependent NO production in the brain.

It has been shown that HUCBCs could be induced to express neural proteins [38], class III β-tubulin, glial fibrillary acidic protein, Galc (a marker of oligodendrocytes) [39,40], and neurofilament microtubule-associated protein 2 [49]. When these cells were transplanted into the neonatal subventricular zone, some of them differentiated into neuronal and glial phenotypes within the neurogenic region [50]. In addition, there was an increase in glial-derived neurotrophic factor levels following HUCBCs transplantation after stroke [51]. It is likely that HUCBCs, rather than adult peripheral blood mononucleocytes (PBMCs), may release any mediators (i.e. cytokines, NO, and endothelial or other neurotrophic factors), reduce hypotension, decrease intracranial hypertension, and confer neuronal protection during heatstroke.

Owing to their greater availability, feeble immunogenicity, and lower risk of mediating viral transmission, HUCBCs have come out as an alternative to bone marrow [52]. Moreover, HUCBCs can mediate therapeutic effects in several animal models of neurologic diseases, including stroke [42,43,45,46]. Our studies involving rats indicate that HUCBCs could potentially be an excellent source of cells for the treatment of heatstroke, because they are widely available and have been used clinically. However, additional studies in experimental models are needed.

Infusion of HUCBCs Protect Against Cerebral Ischemia and Damage During Heatstroke in the Rat

Intravenously delivered HUCBCs found in the brain 4 weeks after middle cerebral artery occlusion in the rat were excessively localized to the ischemic hemispheres [44]. As above-mentioned, intravenously delivered HUCBCs had been shown to improve both morphologic and functional recovery of heat-stroked rats [15]. However, to our knowledge, there is no evidence about the preventive effects of HUCBCs administered before the initiation of cerebral ischemia and injury. To extend these findings, we examined both the morphologic and functional alterations in the presence of HUCBCs or PBMCs 24 hours before initiation of heatstroke. Here, we successfully demonstrated that HUCBCs pretreatment could be a good choice for preventing heatstroke occurrence.

As compared with those of vehicle-pretreated or PBMC-pretreated heatstroke rats, the values for both the latency (interval between initiation of heatstress and onset of heatstroke) and survival time (interval of onset of heatstroke to death) were significantly greater in those of HUCBC-pretreated heatstroke rats (79–81 minutes for latency and 118–138 minutes for survival time). Furthermore, we also demonstrated 12 minutes after the termination of heat exposure in the PBMC-treated group, all the MAP, CBF, brain PO_2, and heart rate values were significantly lower than those of the normothermic controls ($p < 0.05$). On the other hand, the values of Tbr (brain temperature), Tco (colonic temperature), and levels of glutamate, glycerol, lactate/pyruvate ratio, and NO in the extracellular fluids of the striatum in the PBMC-treated group were significantly higher 12 minutes after the termination of 68 minutes heat exposure than in those of the normothermic controls. Heatstroke-induced arterial hypotension, cerebral ischemia and hypoxia, and increased levels of glutamate, glycerol, lactate/pyruvate ratio, and NO in the extracellular levels of striatum were significantly attenuated by pretreatment with HUCBCs 24 hours before the initiation of heat exposure.

In our previous results, we also illustrated after the onset of heatstroke, animals pretreated with PBMCs displayed higher values of striatal neuronal damage score and iNOS immunoreactivity compared with those of normothermic controls. In addition, histopathologic verification revealed that heatstroke caused cell body shrinkage, pyknosis of the nucleus, loss of Nissl substance, disappearance of the nucleolus, and overexpression of iNOS (inducible NO synthetase) in the striatum of the PBMC-pretreated rats. However, with the HUCBCs pretreatment neuroprotection was ensured. And delivered HUCBCs were localized to brain as determined by immunohistochemistry and polymerase chain reaction during heatstroke.

Pretreatment [53] or post-treatment [15] with HUCBCs significantly attenuates the arterial hypotension, cerebral ischemia and hypoxia, and increased levels of ischemia and damage markers in the brain during heatstroke. These findings demonstrate that HUCBCs are effective for prevention and repair of circulatory shock and ischemic damage in the brain during heatstroke by reducing iNOS-dependent NO formation in the brain. However, treatment with PBMC fails to produce any significant protection. We attribute the discrepancy between PBMCs and HUCBCs treatments to the lack of pleuropotential of PBMCs. The results we obtained have further shown that HUCBCs have an ability to prevent circulatory shock and brain ischemic damage during the heatstroke and, moreover, HUCBCs administered right after the onset of heatstroke is still effective for improving circulatory shock and ischemic damage in the brain.

Intravenously delivered HUCBCs found in the brain after the onset of heatstroke were localized to the ischemic hemisphere. Moreover, immunofluorescent localization of the HUCBCs by human nuclei detection suggested that their numbers were large and widely distributed to different brain structures. The intravenously injected HUCBCs may be following homing signals that attract them to the injured site [42,54], in particular the hyperthermic brain. In addition, intravenous administration of HUCBCs can secrete therapeutic molecules, such as neurotrophic factors, which when exogenously administered by themselves are neuroprotective in stroke models; the surviving cells, when administered intravenously, were identified in the injured hemisphere [55,56]. In our previous results, the HUCBCs grafts probably promote neuroprotection against heatstroke-induced neuronal damage via the inhibition of iNOS/NO based on the decrease of those concentration and N-methyl-D-aspartate in brain [53,57]. Indeed, the notion that stem cells exert an inherent neuroprotective effect was introduced by Ourednik et al [58] and Teng et al [59] and has gained great traction. Moreover, HUCBCs pretreatment can maintain an appropriate level of MAP during heatstroke by reducing overproduction of cytokines as well as depression of baroreceptor sensitivity. From these results, the possible involvement of neurotrophic factors secreted by HUCBCs cannot be completely eliminated. Although the surviving HUCBCs were identified in different brain structures after the onset of heatstroke, the CNS availability of grafted cells is not a prerequisite for acute neuroprotection provided that therapeutic molecules secreted by these cells could cross the

blood–brain barrier [55]. In addition, there may be some beneficial ingredients in the HUCBCs preparation, but it remains to be determined whether this must be delivered with intact cells [60].

Finally, it should be accentuated that it is still somewhat difficult to conceive whether a cellular pretreatment therapy would have practical application except the most extreme and risky circumstances. Clinical application for this indication may be considered until mechanisms and parameters for beneficial outcomes are better defined.

$CD34^+$ Cells may Improve Survival During Experimental Heatstroke by Ameliorating Inflammatory, Coagulatory, and Multiorgan Dysfunction

The above-mentioned issues demonstrated that HUCBCs are effective for prevention and repair of cerebrovascular dysfunction during heatstroke. However, the role of $CD34^+$ cells contained within HUCBCs in protecting against heatstroke-induced multiorgan dysfunction and failure should be explored.

In fact, after the onset of heatstroke, ischemic injury is noted to occur in different brain structures, including striatum, hypothalamus, cortex, and thalamus [61,62]. In our previous study, the striatum is chosen as a representative region for the measurement of blood flow, PO_2, cellular ischemia and injury markers, and neuronal damage scores. In our following study, the isolation of $CD34^+$ hematopoietic progenitor cells is performed by positive selection of $CD34^+$ expressing cells. Mononuclear cells from human umbilical cord blood are obtained by density gradient centrifugation over Ficoll Pague [63]. With the $CD34^+$ Progenitor Cells Isolation Kit, hematopoietic progenitor cells, present at a frequency of about 2% in cord blood, can be rapidly and efficiently enriched to a purity of about 98%. Intravenously delivered $CD34^+$ progenitor cells found in the brain after the onset of heatstroke were localized to all ischemic structures including cortex, hypothalamus, hippocampus, striatum, cerebellum, and brain stem [61,62]. Immunofluorescent localization of the $CD34^+$ cells by human nuclei detection indicated that their numbers were large and widely distributed to all the brain structures studied also. The intravenously administered $CD34^+$ cells may be following homing signals that attract them to the ischemic or damaged structures [42,54,64], in particular the hyperthermic brain, which had similar results with the pretreated HUCBCs study.

In addition, our unpublished data successfully demonstrated that resuscitation with intravenous doses of $CD34^+$, but not $CD34^-$ cells, immediately at the onset of heatstroke significantly and dose-dependently improve survival during heatstroke. As mentioned before, cerebrovascular dysfunction, hypercoagulable state (or DIC), and systemic inflammation may contribute to the multiple organ dysfunction and death. Intravenously delivered $CD34^+$ cells significantly attenuated all the heatstroke-induced reactions during heatstroke, when administered immediately at the onset of heatstroke. In fact, it has been demonstrated that systemic administration of human cord blood-derived $CD34^+$ cells to immunocompromised mice subjected to stroke 48 hours earlier induces neovascularization in the ischemic zone and provides a favorable environment for neuronal regeneration [65]. However, our results provided the first evidence that the tissue ischemia and injury that occurred during heatstroke can be repaired immediately by systemic administration of $CD34^+$ cells in the rat.

It has been shown that heatstroke causes overproduction of TNF-α and IL-1 in both the CNS and the peripheral blood stream; this is associated with arterial hypotension, cerebral ischemia and neuronal damage, and high mortality rate [66–68]. Activated inflammation has also been shown to be related to the severity of acute heart failure [69], septic shock [70], and circulatory shock [67]. The administration of an IL-1 receptor antagonist or glucocorticoids immediately after the start of heatstroke is able to attenuate circulatory shock and cerebral ischemia and injury and to improve survival during heatstroke. Our unpublished results further demonstrated that treatment with $CD34^+$ cells causes attenuation of arterial hypotension, intracranial hypertension, and cerebral ischemia and damage during heatstroke by reducing overproduction of TNF-α in the serum. On the other hand, our findings further demonstrated that, after the onset of heatstroke in rats, treatment with $CD34^+$ cells causes a significant increase in the serum level of IL-10 accompanied by a reduction of the above-mentioned heatstroke reactions. It is believed that IL-10 has important anti-inflammatory and immunosuppressive properties through attenuation of TNF-α and other proinflammatory cytokines [71]. Putting these observations together, it seems that $CD34^+$ cells therapy may ameliorate arterial hypotension and cerebral ischemia and damage by increasing IL-10 but suppressing TNF-α production. It is generally believed that both anti- and pro-inflammatory reactions are normal components of the same immune response, which correlatively battle infection while preventing immune pathology [72].

In the meanwhile, our accumulated evidences showed that $CD34^+$ cells therapy may have attenuated the heatstroke-induced cerebral ischemia and injury by reducing iNOS-dependent NO and hydroxyl radical

production in the CNS. Recent studies also indicated that the expression of several neurotrophic factors (including glial cell-derived neurotrophic factor [GDNF]) in the brain was influenced by ischemia [73–75]. A more recent report further demonstrated that mesenchymal stem cells that produce neurotrophic factors reduced ischemic damage in the rat middle cerebral artery occlusion model [76]. Similarly, our unpublished results showed that $CD34^+$ cells inducing endogenous GDNF expression attenuated cerebral ischemia and injury in the rat heatstroke model. Further studies are required to verify whether the cerebral ischemia and injury during heatstroke can be suppressed by exogenous administration of GDNF.

In conclusion, from the above-unpublished data, we successfully demonstrated that $CD34^+$ cells sorted from HUCBCs cause attenuation of cerebrovascular dysfunction, hypercoagulable state, and activated inflammation during experimental heatstroke.

Resuscitation from Experimental Heatstroke by Estrogen Therapy

In the past, a large number of studies demonstrated that estradiol influences cognition, the incidence and progression of Alzheimer's disease, and the severity of injury associated with cerebrovascular stroke [77–79]. These researchers indicated that estrogen replacement therapy lowers the risk or the severity of neurodegenerative decline associated with cerebrovascular stroke. Since estradiol influences numerous aspects of CNS function, understanding the cellular and molecular mechanisms that underlie possible protective actions is essential in preventing the deleterious consequences of a prolonged hypoestrogenic state and improving the women's health. Animal models have been advanced to examine estrogen's ability to protect against brain injury. Designs have been developed to mimic cerebral ischemia that occurs with stroke by blocking of one or more cerebral arteries to produce a reproducible infarct in the brain. These experimental manipulations supply forceful proof that estradiol is a neuroprotective factor. Furthermore, estrogen replacement in ovariectomized (OVX) adult female rats significantly restores protection of the brain to a level similar to that observed in intact animals [80–82]. An ovarian factor involved in the protection was suggested by the finding that ovariectomy eliminates the endogenous protective effect observed in female rats following cerebral ischemia. Additionally, serum estradiol levels have been shown to correlate inversely with ischemic stroke damage in female rats [83].

In addition, estrogen appears to display immunoprotective effects on macrophage functions potentially by downregulating anti-inflammatory cytokine release [84]. Estrogen-induced changes in T cell cytokine production have also been demonstrated in experimental models of cell-mediated acute inflammatory disease, such as stroke. Furthermore, estrogen treatment of interferon-γ (IFN-γ) deficient mice resulted in a significant reduction in disease severity. Estrogen-treated mice had lower levels of cytokine and chemokine production and had reduced levels of chemokine receptor-positive cells in the CNS [85]. Although estrogen has been shown to effect multiple cellular components of the immune system in ways that are not at first glance consistent, as in the case of Th1 and Th2 phenotype. Descriptions for these differences may reside in the diversions of the immune mechanisms involved in these models. Additionally, through understanding how estrogen can produce various results in different systems, one should consider the general biology of estrogens and how it would be expected to interact with the immune system. A fatal aspect of estrogen's biology is its ability to support cell proliferation and survival. In correspondence with this biology, estrogens appear to augment B cell survival and T cell proliferation, though not in every system studied [86].

Kim et al indicated that estrogen may act to show preference for T cells toward a Th2 phenotype. Support for estrogen enhancement of Th2 responses, specifically, is provided by studies in experimental autoimmune encephalomyelitis (EAE) models. The amelioration of EAE by estrogen was strongly correlated with enhanced *in vitro* IL-10 production by specific T cells [87]. IL-10 is a pleiotropic cytokine with a broad spectrum of biologic effects on lymphoid and myeloid cells. One of the known functions of IL-10 is its ability to inhibit the production of pro-inflammatory mediators, including IFN-γ, TNF-α, IL-6, IL-1, granulocyte-macrophage colony stimulating factor, and the generation of NO by lipopolysaccharide-activated monocytes/macrophages. Nevertheless, the molecular mechanisms underlying the anti-inflammatory effects of these cytokines remain unknown [88,89].

According to several studies and our previous results, both morbidity and mortality occurred in heatstroke may be related to endotoxemia and the release of IL-1 [90,91]. The cerebral ischemia associated with heatstroke can be attenuated by pretreatment of animals with an IL-1 receptor antagonist; here, the heatstroke rodents produced much more proinflammatory cytokines such as IL-1, IL-6, TNF-α, and IFN-γ [61,91,92]. Indeed, we successfully demonstrated that estrogen replacement may improve survival during heatstroke

by ameliorating inflammatory responses and cardiovascular dysfunction [93]. In the study, we measured the cardiovascular responses of normal adult male and female rats with or without estrogen replacement during heatstroke. In addition, we determined the effects of estrogen replacement with premarin (United States Pharmacopeia) 1 mg/kg intravenously [93], immediately after the onset of heatstroke, on cardiovascular responses in surgically or chemically (leuprolide 100 μg/kg/day was administered subcutaneously beginning 4 weeks before heat stress) [95] OVX rats on production of TNF-α [96] and IL-10 [97]. Humans with exertional heatstroke were found to have a high level of IL-10 in the serum [98]. However, in the rats with nonexertional heatstroke, the serum levels of IL-10 were undetectable [98]. After the onset of heatstroke in rats, treatment with premarin produces a significant increase in the serum level of IL-10 accompanied by a reduction of heatstroke reactions. This implies that premarin may ameliorate cerebral ischemia and damage by increasing IL-10 but decreasing TNF-α production.

As compared with the estrus female rats, the OVX rats, the leuprolide treated rats, and male rats all had lower levels of plasma estradiol and lower survival time values during heatstroke [93]. However, after an intravenous dose of premarin, both the plasma estradiol and survival time values were significantly increased. Compared with the normothermic controls, the vehicle-treated male and OVX rats all displayed higher levels of serum TNF-α, prothrombin time, activated partial thromboplastin time, D-dimer, BUN, creatinine, SGOT, SGPT, and Alp, as well as cerebral levels of glycerol, glutamate, and lactate/pyruvate ratio, and decreased platelet counts and protein C levels. These heatstroke-related reactions could be suppressed by premarin therapy. In particular, the serum levels of IL-10 in these groups were significantly elevated by premarin during heatstroke [93]. The heatstroke-induced hyperpyrexia, arterial hypotension, intracranial hypertension, cerebral hypoperfusion, hypoxia, and ischemia, renal and hepatic failure, hypercoagulable state, and systemic inflammation were all significantly attenuated by premarin therapy in OVX rats. Therefore, estrogen replacement may improve survival during heatstroke by reducing inflammatory responses, hypercoagulable state, and cerebrovascular dysfunction.

These results are in part consistent with previous investigations. For example, it has been shown that estrogen administration provides tissue protection from middle cerebral artery occlusion by increasing cerebral blood flow [99]. The female proestrus rats have normalized organ function at 24 hours after trauma-hemorrhage, whereas male and female estrus (low serum estrogen levels compared as female proestrus rats) animals have shown a marked depression in cardiovascular function [100]. The results indicate that the maintenance of cardiac functions after severe blood loss is associated with high levels of estradiol at the start of the experiment. It has also been shown that estrogen produces a significant increase in blood flow not only to reproductive tissues but also to skin, thyroid gland, pancreas, spleen [101,102], coronary vasculature [94], and brain [64].

Possible Mechanisms Put Forth by HUCBCs or CD34$^+$ Cells or Estrogen as a New Therapeutic Strategy in Heatstroke

The figure shows a scheme depicting events between the exposure of rodents to a hot environment and death from heatstroke, with known or proposed interrelationships

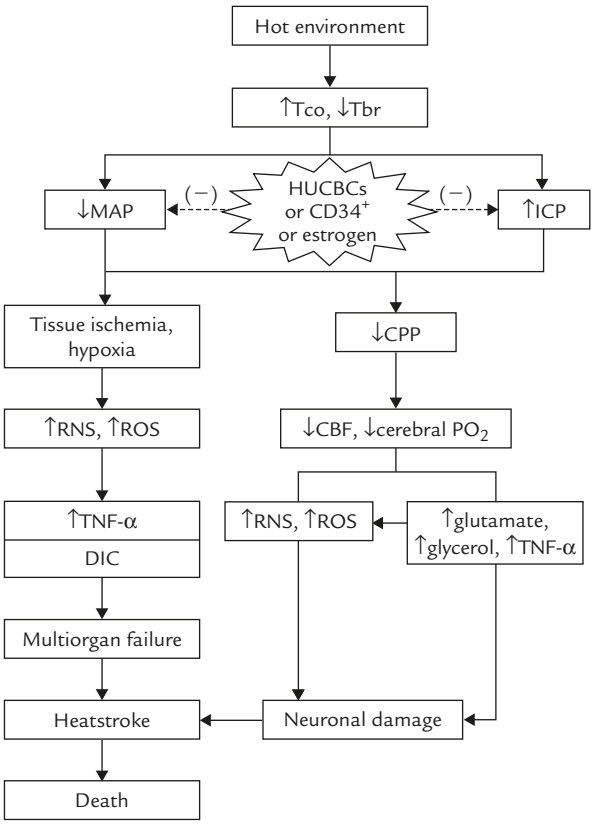

Figure. Proposed scheme of the interacting sequence of events occurring from the beginning of heat exposure to heatstroke occurrence. Arrows indicate increased (↑) or decreased (↓). Tco = core temperature; Tbr = brain temperature; MAP = mean arterial pressure; ICP = intracranial pressure; CPP = cerebral perfusion pressure; RNS = radical nitrogen species; ROS = radical oxygen species; CBF = cerebral blood flow; TNF-α = tumor necrosis factor-α; DIC = disseminated intravascular coagulopathy; (−) = reversal.

Table 2. Effects of various treatment agents on heatstroke-induced responses

Treatment regimens*	Heatstroke-induced responses								
	Hypotension	Hyperpyrexia	DIC	Systemic inflammation	Hepatic failure	Renal failure	Cerebral ischemia	Rhabdomyolysis	Metabolic acidosis
HUCBCs (post-treatment)	●	●	?	?	?	?	●	?	?
HUCBCs (pretreatment)	●	●	?	?	?	?	●	?	?
$CD34^+$ cells	●	●	●	●	●	●	●	X	●
Estrogen	●	X	?	●	?	?	●	?	?

**Adopted at the time point of onset of heatstroke. HUCBCs = human umbilical cord blood cells; DIC = disseminated intravascular coagulopathy. ● = reversal; X = no effect; ? = undetermined.*

from the published and unpublished data. After the onset of heatstroke, animals exhibit cutaneous vasodilation, splanchnic vasoconstriction, hyperpyrexia, hypotension, and intracranial hypertension. In the CNS, cerebral ischemia (caused by both hypotension and intracranial hypertension) leads to neuronal damage due to both the cessation of blood flow leading to oxygen and nutrient deprivation and the initiation of a cascade as secondary mechanisms. This neurotoxic cascade involves overloading of reactive oxygen and nitrogen species, glutamate, dopamine, and serotonin, and cerebral inflammation and activated coagulation. Similarly, in the periphery, tissue ischemia in the splanchnic organs (resulting from shifting blood to the skin) leads to multiple organ failure and dysfunction due to both the systemic inflammation and activated coagulation. Therefore, measures, which are able to attenuate the hypotension, hyperpyrexia, hypercoagulable state, systemic inflammation, cerebral ischemia, and metabolic acidosis, can be used to limit multiple organ dysfunction or failure in heatstroke as shown in Table 2. For example, heatstroke-induced hypotension, intracranial hypertension, cerebral hypoperfusion and hypoxia, hypercoagulable state, systemic inflammation, and tissue ischemia and injury in multiple organs can be improved by HUCBCs or $CD34^+$ cells. However, in the absence of these agents, these heatstroke reactions can still be improved by premarin adopted immediately after the onset of heatstroke. Before initiation of heat stress, prior manipulations with HUCBCs were also found to be able to protect against heatstroke syndromes. In fact, treatment combinations of $CD34^+$ cells and estrogen seem to have enhanced effects during heatstroke due to similar therapeutic mechanisms over the systemic inflammatory reactions between these regimens (Chen and colleagues, unpublished data).

Acknowledgments

This work was supported by the grants from Chi Mei Medical Center, Tainan, Taiwan; CMFHR9309, CMFHR9416, and CMFHR9501 and presented by Sheng-Hsien Chen, MD, in part, at "The 2nd International Meeting on Thermal Physiology and Pharmacology of Temperature Regulation, Phoenix, Arizona, USA, 3–6 March 2006" as a winner of Young Scientist Award.

References

1. Bouchama A, Knochel JP. Heat stroke. *N Engl J Med* 2002; 346:1978–88.

2. Shih CJ, Lin MT, Tsai SH. Experimental study on the pathogenesis of heat stroke. *J Neurosurg* 1984;60:1246–52.
3. Chen SH, Niu KC, Lin MT. Cerebrovascular dysfunction is an attractive target for therapy in heat stroke. *Clin Exp Pharmacol Physiol* 2006;33:663–72.
4. Lee-Chiong TL Jr, Stitt JT. Heatstroke and other heat-related illnesses. The maladies of summer. *Postgrad Med* 1995;98: 26–8, 31–3, 36.
5. Hassanein T, Razack A, Gavaler JS, Van Thiel DH. Heatstroke: its clinical and pathological presentation, with particular attention to the liver. *Am J Gastroenterol* 1992;87:1382–9.
6. Parsons LR. Surviving the hot zone. *Emerg Med Serv* 1993; 22:42–6.
7. Hsu SF, Niu KC, Lin CL, Lin MT. Brain cooling causes attenuation of cerebral oxidative stress, systemic inflammation, activated coagulation, and tissue ischemia/injury during heatstroke. *Shock* 2006;26:210–20.
8. Hillered L, Persson L. Neurochemical monitoring of the acutely injured human brain. *Scand J Clin Lab Invest Suppl* 1999;229:9–18.
9. Hillered L, Persson L, Ponten U, Ungerstedt U. Neurometabolic monitoring of he ischemic human brain using microdialysis. *Acta Neurochir* 1990;102:991–7.
10. Hillered L, Valtysson J, Enblad P, Person L. Interstitial glycerol as a marker for membrane phospholipid degradation in the acutely injured human brain. *J Neurol Neurosurg Psychiatry* 1998;64:486–94.
11. Nilsson OG, Saveland H, Boris-Moller F, Brandt L, Wieloch T. Increased levels of glutamate in patients with subarachnoid haemorrhage as measured by intracerebral microdialysis. *Acta Neurochir Suppl* 1996;667:45–7.
12. Persson L, Hillered L. Chemical monitoring of neurosurgical intensive care patients using intracerebral microdialysis. *J Neurosurg* 1992;76:72–80.
13. Ungerstedt U. Microdialysis—a new technique for monitoring local tissue events in the clinic. *Acta Anaesthesiol Scand Suppl* 1997;110:123. [Abstract]
14. Chang CP, Lee CC, Chen SH, Lin MT. Aminoguanidine protects against intracranial hypertension and cerebral ischemic injury in experimental heatstroke. *J Pharmacol Sci* 2004;95: 56–64.
15. Chen SH, Chang FM, Tsai YC, Huang KF, Lin MT. Resuscitation from experimental heatstroke by transplantation of human umbilical cord blood cells. *Crit Care Med* 2005;33:1377–83.
16. Kuo JR, Lin CL, Chio CC, Wang JJ, Lin MT. Effects of hypertonic (3%) saline in rats with circulatory shock and cerebral ischemia after heatstroke. *Intensive Care Med* 2003;29: 1567–73.
17. Lee JJ, Lin MT, Wang NL, Lin CL, Chang CK. Platonin, a cyanine photosensitizing dye, causes attenuation of circulatory shock, hypercoagulable state, and tissue ischemia during heat stroke. *Shock* 2005;24:577–82.
18. Chou YT, Lai ST, Lee CC, Lin MT. Hypothermia attenuates circulatory shock and cerebral ischemia in experimental heatstroke. *Shock* 2003;19:388–93.
19. Sharma HS, Alm P. Nitric oxide synthase inhibitors influence dynorphin A (1-17) immunoreactivity in the rat brain following hyperthermia. *Amino Acids* 2002;23: 242–59.
20. Sharma HS, Westman J, Cervos-Navarro J, Dey PK, Nyberg F. Opioid receptor antagonists attenuate heat stress-induced reduction in cerebral blood flow, increased blood–brain barrier permeability, vasogenic edema and cell changes in the rat. *Ann N Y Acad Sci* 1997;813:559–71.
21. Sharma HS, Westman J, Alm P, et al. Involvement of nitric oxide in the pathophysiology of acute heat stress in the rat. Influence of a new antioxidant compound H-290/51. *Ann N Y Acad Sci* 1997;813:581–90.
22. Chatterjee S, Premachandran S, Sharma D, Bagewadikar RS, Poduval TB. Therapeutic treatment with L-arginine rescues mice from heat stroke-induced death: physiological and molecular mechanisms. *Shock* 2005;24:341–7.
23. Leon LR, DuBose DA, Mason CW. Heat stress induces a biphasic thermoregulatory response in mice. *Am J Physiol Regul Integr Com Physiol* 2005;288:197–204.
24. Leon LR, Blaha MD, DuBose DA. Time course of cytokine, corticosterone, and tissue injury responses in mice during heat strain recovery. *J Appl Physiol* 2006;100:1400–9.
25. Lee WC, Wen HC, Chang CP, Chen MY, Lin MT. Heat shock protein 72 overexpression protects against hyperthermia, circulatory shock, and cerebral ischemia during heatstroke. *J Appl Physiol* 2006;100:2073–82.
26. Bouchama A, Roberts G, Al Mohanna F, et al. Inflammatory, hemostatic, and clinical changes in a baboon experimental model for heatstroke. *J Appl Physiol* 2005; 98:697–705.
27. Kordower JH, Freeman TB, Snow BJ, et al. Neuropathological evidence of graft survival and striatal reinnervation afer the transplantation of fetal mesencephalic tissue in a patient with Parkinson's disease. *N Engl J Med* 1995;332: 1118–24.
28. Jansen EM, Solberg L, Underhill S, et al. Transplantation of fetal neocortex ameliorates sensorimotor and locomotor deficits following neonatal ischemic-hypoxic brain injury in rats. *Exp Neurol* 1997;147:487–97.
29. Prockop DJ. Marrow stromal cells as stemal cells as stem cells for nonhematopoietic tissues. *Science* 1997;147:487–97.
30. Pittenger MF, Mackay AM, Beck SC, et al. Multilineage potential of adult human mesenchymal stem cells. *Science* 1999;284:143–7.
31. Bianco P, Gehron Robey P. Marrow stromal stem cells. *J Clin Invest* 2000;105:1663–8.
32. Lu L, Shen RN, Broxmeyer HE. Stem cells from bone marrow, umbilical cord blood and peripheral blood for clinical application: current status and future application. *Crit Rev Oncol Hematol* 1996;22:61–78.
33. Kelly P, Kurtxberg J, Vichinsky E, Lubin B. Umbilical cord blood stem cells: application for the treatment of patients with hemoglobinnopathies. *J Pediatr* 1997;130:695–703.
34. Erices A, Conget P, Minguell JJ. Mesenchymal progenitor cells in human umbilical cord blood. *Br J Haematol* 2000; 109:235–42.
35. Gage FH. Mammalian neural stem cells. *Science* 2000;287: 1433–8.
36. Nieda M, Nicol A, Denning-Kendall P, et al. Endothelial cell precursors are normal components of human umbilical cord blood. *Br J Haematol* 1997;98:775–7.
37. Sirchia G, Rebulla P. Placental/umbilical cord blood transplantation. *Hematologia* 1999;84:738–47.

38. Sanchez-Ramos JR, Song S, Kamath SG, et al. Expression of neural markers in human umbilical cord blood. *Exp Neurol* 2001;171:109–15.
39. Bicknese AR, Goodwin HS, Quinn CO, et al. Human umbilical cord blood cells can be induced to express markers for neurons and glia. *Cell Transplant* 2002;11:261–4.
40. Buzanska L, Machaj EK, Zablocka B, et al. Human cord blood—derived cells attain neuronal and glial features *in vitro*. *J Cell Sci* 2002;115:2131–8.
41. Zigova T, Song S, Willing AE, et al. Human umbilical cord blood cells express neural antigens after transplantation into the developing rat brain. *Cell Transplant* 2002;11:265–74.
42. Chen J, Sanberg PR, Li Y, et al. Intravenous administration human umbilical cord blood reduces behavioral deficits after stroke in rats. *Stroke* 2001;32:2682–8.
43. Willing AE, Vendrame M, Mallery J, et al. Mobilized peripheral blood cells administered intravenously produce functional recovery in stroke. *Cell Transplant* 2003;12:449–54.
44. Vendrame M, Cassady J, Newcomb J, et al. Infusion of human umbilical cord blood cells in a rat model of stroke dose-dependently rescues behavioral deficits and reduces infarct volume. *Stroke* 2004;35:2390–5.
45. Lu D, Sanberg PR, Mahmood A. Intravenous administration of human umbilical cord blood reduces neurological deficit in the rat after traumatic brain injury. *Cell Transplant* 2002;11:275–81.
46. Saporta S, Kim JJ, Willing AE, et al. Human umbilical cord blood stem cells infusion in spinal cord injury: engraftment and beneficial influence on behavior. *J Hematother Stem Cell Res* 2003;12:271–8.
47. Li Y, Chopp M, Chen J, et al. Intrastriatal transplantation of bone marrow nonhematopoietic cells improves functional recovery after stroke in adult mice. *J Cereb Blood Flow Metab* 2000;20:1311–9.
48. Sharma HS, Westman J, Nyberg F. Pathophysiology of brain edema and cell changes following hyperthermic brain injury. *Prog Brain Res* 1998;115:351–412.
49. Ha Y, Choi DH, Yeon DS, et al. Neural phenotype expression cultured human cord blood cells *in vitro. Neuroreport* 2001; 12:3523–7.
50. Zigova T, Song S, Willing AE, et al. Human umbilical cord blood cells express neural antigens after transplantation into the developing rat brain. *Cell Transplant* 2002;11:265–74.
51. Lobel DA, Hadman M, Sanberg PR, et al. Early intracerebral and intra-arterial transplantation of human umbilical cord blood cells into an ischemic rat brain model. *Exp Neurol* 2003;181:97.
52. Wagner JE, Broxmeyer HE, Byrd RL, et al. Transplantation of umbilical cord blood after myeloablative therapy: analysis of engraftment. *Blood* 1992;79:1874–81.
53. Chen SH, Chang FM, Tsai YC, et al. Infusion of human umbilical cord blood cells protect against cerebral ischemia and damage during heat stroke in the rat. *Exp Neurol* 2006; 199:67–76.
54. Newman M, Willing A, Cassady CJ, et al. *In vitro* migration and phenotype identification of human umbilical cord blood (HUCB) cells to stroke brain. *Exp Neurol* 2003;181:84–112. (Abstract)
55. Borlongan CV, Hadman M, Sanberg CD, Sanberg PR. Central nervous system entry of peripherally injected umbilical cord blood cells is not required for neuroprotection in stroke. *Stroke* 2004;35:2385–9.
56. Wen YS, Huang MS, Lin MT, Lee CH. Hypothermic retrograde jugular perfusion reduces brain damage in rats with heatstroke. *Crit Care Med* 2003;31:2641–5.
57. Wang JL, Ke DS, Lin MT. Heat shock pretreatment may protect against heatstroke-induced circulatory shock and cerebral ischemia by reducing oxidative stress and energy depletion. *Shock* 2004;23:161–7.
58. Ourednik J, Ourednik V, Lynch WP, Schachner M, Snyder EY. Neural stem cells display an inherent mechanism for rescuing dysfunctional neurons. *Nat Biotech* 2002;20:1103–10.
59. Teng YD, Lavik EB, Qu X, et al. Functional recovery following traumatic spinal cord injury mediated by a unique polymer scaffold seeded with neural stem cells. *Proc Natl Acad Sci USA* 2002;99:3024–9.
60. Wu X, Kochanek PM. Should we add stem cells to the code cart in resuscitation of heatstroke? *Crit Care Med* 2005;33: 1458–9.
61. Kao TY, Chio CC, Lin MT. Hypothalamic dopamine release and local cerebral blood flow during onset of heatstroke in rats. *Stroke* 1994;25:2483–7.
62. Kao TY, Lin MT. Brain serotonin depletion attenuates heatstroke-induced cerebral ischemia and cell death in rats. *J Appl Physiol* 1996;80:680–4.
63. Ye Z, Gee AP, Bowers WE, et al. *In vitro* expansion and characterization of dendritic cells derived from human bone marrow CD34+ cells. *Bone Marrow Transplant* 1996;18:997–1008.
64. Chen J, Li Y, Wang L, et al. Therapeutic benefit of intravenous administration of bone marrow stromal cells after cerebral ischemia in rats. *Stroke* 2001;32:1005–11.
65. Taguchi A, Soma T, Tanaka H, et al. Administration of $CD34^+$ cells after stroke enhances neurogenesis via angiogenesis in a mouse model. *J Clin Invest* 2004;114:330–8.
66. Hung CH, Chang NC, Cheng BC, Lin MT. Progressive exercise preconditioning protects against circulatory shock during experimental heatstroke. *Shock* 2005;23:426–33.
67. Lin MT, Liu HH, Yang YL. Involvement of interleukin-1 receptor mechanism in development of arterial hypotension in rat heat stroke. *Am J Physiol* 1997;273:H2072–7.
68. Liu CC, Chien CH, Lin MT. Glucocorticoids reduce interleukin-1 concentration and result in neuroprotective effects in rat heatstroke. *J Physiol* 2000;527:333–43.
69. Nakamura T, Suzuki S, Ushiyama C, Shimada N, Koide H. Effect of phosphodiesterase III inhibitor on plasma concentrations of endothelin-1 and tumour necrosis factor in patients with acute heart failure. *Acta Cardiol* 2002;57:19–21.
70. Schirmer WJ, Schirmer JM, Fry DE. Recombinant human tumor necrosis factor produces hemodynamic changes characteristic of sepsis and endotoxemia. *Arch Surg* 1989;124: 445–8.
71. Oberholzer A, Oberholzer C, Moldawer LL. Cytokine signaling—regulation of the immune response in normal and critically ill states. *Crit Care Med* 2000;28:N3–12.
72. Moore KW, de Waal Malefyt R, Coffman RL, O'Garra A. Interleukin-10 and the interleukin-10 receptor. *Annu Rev Immunol* 2001;19:683–765.
73. Abe K, Hayashi T. Expression of the glial cell line-derived neurotrophic factor gene in rat brain after transient MCA occlusion. *Brain Res* 1997;776:230–4.

74. Kokaia Z, Zhao Q, Kokaia M, et al. Regulation of brain-derived neurotrophic factor gene expression after transient middle cerebral artery occlusion with and without brain damage. *Exp Neurol* 1995;136:73–88.
75. Lin TN, Wang PY, Chi SI, Kuo JS. Differential regulation of ciliary neurotrophic factor (CNTF) and CNTF receptor alpha (CNTFR alpha) expression following focal cerebral ischemia. *Brain Res Mol Brain Res* 1998;55:71–80.
76. Kurozumi K, Nakamura K, Tamiya T, et al. Mesenchymal stem cells that produce neurotrophic factors reduce ischemic damage in the rat middle cerebral artery occlusion model. *Mol Ther* 2005;11:96–104.
77. Sherwin BB. Hormones, mood, and cognitive functioning in postmenopausal women. *Obstet Gynecol* 1996;87: 20S–6.
78. Sherwin BB. Estrogen effects on cognition in menopausal women. *Neurology* 1997;48:S21–6.
79. Wise PM, Dubal DB, Wilson ME, Rau SW, Liu Y. Estradiol: a trophic and protective factor in the adult brain. *Front Neuroendocrinol* 2000. [In press]
80. Dubal DB, Kashon ML, Pettigrew LC, et al. Estradiol protects against ischemic injury. *J Cereb Blood Flow Metab* 1998;18:1253–8.
81. Zhang Y-Q, Shi J, Rajakumar G, Day AL, Simpkins JW. Effects of gender and estradiol treatment on focal brain ischemia. *Brain Res* 1998;784:321–4.
82. Alkayed NJ, Harukuni I, Kimes AS, et al. Gender-linked brain injury in experimental stroke. *Stroke* 1998;29:159–66.
83. Angele MK, Knoferl MW, Schwacha MG, et al. Sex steroids regulate pro- and anti-inflammatory cytokine release by macrophages after trauma-hemorrhage. *Am J Physiol* 1999; 277:C35–42.
84. Ito A, Bebo BF Jr, Matejuk A, et al. Estrogen treatment down-regulates TNF-alpha production and reduces the severity of experimental autoimmune encephalomyelitis in cytokine knockout mice. *J Immunol* 2001;167:542–52.
85. Lang TJ. Estrogen as an immunomodulator. *Clin Immunol* 2004;113:224–30.
86. Kim S, Liva SM, Dalal MA, Verity MA, Voskuhl RR. Estriol ameliorates autoimmune demyelinating disease: implications for multiple sclerosis. *Neurology* 1999;12:1230–8.
87. Howard M, O'Garra A. Biological properties of interleukin 10. *Immunol Today* 1992;13:198–200.
88. Moore KW, O'Garra A, de Waal Malefyt R, Vieira P, Mosmann TR. Interleukin 10. *Annu Rev Immunol* 1993;11: 165–90.
89. Bouchama A, Parhar RS, el-Yazigi A, Sheth K, al-Sedairy S. Endotoxemia and release of tumor necrosis factor and interleukin 1 alpha in acute heatstroke. *J Appl Physiol* 1991; 70:2640–4.
90. Bouchama A, al-Sedairy S, Siddiqui S, Shail E, Rezeig M. Elevated pyrogenic cytokines in heatstroke. *Chest* 1993; 104:1498–502.
91. Lin MT, Kao TY, Jin YT, Chen CF. Interleukin-1 receptor antagonist attenuates the heat stroke-induced neuronal damage by reducing the cerebral ischemia in rats. *Brain Res Bull* 1995;37:595–8.
92. Lin MT. Heatstroke-induced cerebral ischemia and neuronal damage. Involvement of cytokines and monoamines. *Ann N Y Acad Sci* 1997;813:572–80.
93. Chen SH, Chang FM, Niu KC, Lin MYS, Lin MT. Resuscitation from experimental heatstroke by estrogen therapy. *Crit Care Med* 2006;34:1113–8.
94. Toung TJ, Traystman RJ, Hurn PD. Estrogen-mediated neuroprotection after experimental stroke in male rats. *Stroke* 1998;39:1666–70.
95. Jett EA, Lerner MR, Lightfoot SA, et al. Prevention of rat mammary carcinoma utilizing leuprolide as an equivalent to oophorectomy. *Breast Cancer Res Treat* 1999;58:131–6.
96. Liu CC, Ke D, Chen ZC, Lin MT. Hydroxyethyl starch produces attenuation of circulatory shock and cerebral ischemia during heatstroke. *Shock* 2004;22:288–94.
97. Bouchama A, Hammami MM, Al Shail E, De Vol E. Differential effects of *in vitro* and *in vivo* hyperthermia on the production of interleukin-10. *Intensive Care Med* 2000;26: 1646–51.
98. Lu KC, Wang JY, Lin SH, Chu P, Lin YF. Role of circulating cytokines and chemokines in exertional heatstroke. *Crit Care Med* 2004;32:399–403.
99. McCullough LD, Alkayed NJ, Traystman RJ, et al. Post-ischemic estrogen reduces hypoperfusion and secondary ischemia after experimental stroke. *Stroke* 2001;32:796–802.
100. Jarrar D, Wang P, Cioffi WG, et al. The female reproductive cycle is an important variable in the response to trauma-hemorrhage. *Am J Physiol Heart Cric Physiol* 2000;279:H1015–21.
101. Rosenfeld CR, Killam AP, Battaglia FC, et al. Effect of estradiol-17, on the magnitude and distribution of uterine blood flow in nonpregnant, oophorectomized ewes. *Pediatr Res* 1973;7:139–48.
102. Magness RR, Phernetton TM, Zheng J. Systemic and uterine blood flow distribution during prolonged infusion of 17beta-estradiol. *Am J Physiol* 1998;275:H731–43.