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Neuroprotective effect of progesterone on acute phase changes induced by partial global cerebral ischaemia in mice

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Abstract

The possible neuroprotective effect of progesterone, a steroid hormone, on acute phase changes in a mouse model of cerebral ischaemia induced by bilateral common carotid artery occlusion (BCAO) was studied. A total of 72 male mice were included in the study. The BCAO model was used to induce partial global cerebral ischaemia. Morphological assessment included measurement of infarct size and brain oedema. Post-ischaemic seizure susceptibility was assessed using a subconvulsive dose of pentylenetetrazole (30 mg kg^{-1} i.p.). Biochemical estimations included tumour necrosis factor α (TNF-α) levels and enzyme parameters such as lipid peroxidation, superoxide dismutase, catalase and glutathione peroxidase, and protein estimation. BCAO induced a significant infarct size and oedema in the saline-treated control group, along with an increase in oxidative stress, indicated by increased lipid peroxidation and decreased levels of antioxidants such as superoxide dismutase, catalase and glutathione peroxidase. Progesterone (15 mg kg^{-1} i.p.) administration showed a neuroprotective effect by significantly reducing the cerebral infarct size as compared with the control group. Post-ischaemic seizure susceptibility was also reduced as the number of positive responders decreased. Brain oedema subsided, but not significantly. Progesterone significantly reduced TNF-α levels compared with the ischaemia group. Progesterone improved levels of all the antioxidants, indicating activity against oxidative stress induced by BCAO. The results demonstrate the neuroprotective effect of progesterone against ischaemic insult, suggesting a role for the steroid as a neuroprotective agent.

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Introduction

Partial global cerebral ischaemia (ischaemia of the forebrain and sub-cortical tissue) is characterized by rapid onset of neurological injury due to interruption of blood flow to the brain (Baker et al 1998; Easton et al 1998). The interruption of blood flow to the tissues in brain leads to various pathophysiological conditions such as neuronal cell death. Global cerebral ischaemia results in neuronal death, irrespective of post-ischaemic reperfusion (Neumar 2000). Reperfusion after cerebral ischaemia further adds to the complications of stroke by releasing various mediators such as proinflammatory cytokines and free radical generation, thus increasing the oxidative stress to the brain and ultimately leading to neuronal cell death. Post-ischaemic seizures are a well-known occurrence as ischaemic injury decreases the seizure threshold, making the tissues more susceptible to even subconvulsive doses of established convulsants. Seizures can occur soon after the onset of ischaemia or can be delayed (Osvaldo & Goldstein 2004). Experimental studies in laboratory animals suggest that repeated seizure-like activity in the setting of cerebral ischaemia significantly increases infarct size and can impair functional recovery, an effect that can be ameliorated by the administration of certain neuroprotective agents (Williams & Tortella 2000, 2002; Williams et al 2001).

Sudden shock to the brain disrupts the NMDA, glutamate, cholinergic, acetylcholine and $GABA_A$ receptor systems. Neurosteroids, such as progesterone, progestin or a progestin metabolite, can stop microglia from releasing harmful free radicals, modulate the effects of glutamate, stimulate myelin production, and potentiate GABA transmission. The mechanism

by which progesterone provides protection against ischaemic brain injury may be related to its potentiating effect on GABA inhibition and attenuation of excitatory amino acid responsiveness, amplifying adenosine's inhibitory action on cerebral cortical neuronal activity, reducing brain oedema, and acting as a free radical scavenger. All the studies related to the evaluation of progesterone as a neuroprotective agent in cerebral ischaemic conditions have been done with occlusion of the middle cerebral artery in animal models (Jiang et al 1996; Chen et al 1999). In the present study, the evaluation of the effectiveness of progesterone in ischaemia was done using a different model of cerebral ischaemia. We investigated the effects of in-vivo administration of progesterone on ischaemia and reperfusion-induced cerebral injury, together with its role in ischaemia-induced seizure susceptibility and behavioural and biochemical changes in a model of partial global cerebral ischaemia in mice.

Materials and methods

Animals and treatment

Individually caged male Swiss/albiro mice, 20–30 g, were maintained under standard laboratory conditions (12-h light–dark cycle). A total of 72 mice were included in the study. The mice had free access to food and water. Animals were fed with a standard chow diet. The institutional animal ethics committee approved the study and all procedures were carried out according to the Committee for the Purpose of Control and Supervision of Experiments on Animals guidelines for animal experimentation in India. Progesterone was obtained from Ind Swift Laboratories (Chandigarh, India). Progesterone was administered intraperitoneally (i.p.) dissolved in 10% dimethylsulfoxide.

Animals were divided into three groups. Group 1 (sham-operated group; a total of 24 mice, comprising four subgroups of n=6): mice were subjected to the surgical procedure but the arteries were not occluded. After 10 min, the animals were sutured and allowed to recover. After 72 h, each subgroup of mice was subjected to assessment of different parameters as described below. Group 2 (surgery; saline-treated group; a total of 24 mice, comprising four subgroups of n=6): mice were subjected to the surgical procedure followed by clamping of bilateral common carotid arteries with aneurysm clips for 10 min to produce partial global cerebral ischaemia. Subsequently, the carotids were unclamped and cerebral reperfusion was allowed for 72 h. Normal saline (0.2mL) was given i.p. 30 min before ischaemia (Day 1) and three more doses at 24-h intervals on Day 2, Day 3 and Day 4. After 72 h, each subgroup of mice was subjected to assessment of different parameters as described below. Group 3 (progesterone-treated group; a total of 24 mice, comprising four subgroups of n=6): mice surgically operated as described for Group 2. The mice were treated with 4 doses of progesterone (15 mg kg^{-1} i.p.). The dosing intervals were: 30 min before producing cerebral ischaemia (Day 1); 24 h after the first dose (Day 2); 48 h after the first dose (Day 3); and 72 h after the first dose (Day 4). After 72 h (i.e. on Day 4, 1 h after the last dose), each subgroup of mice were subjected to assessment of different parameters as described below.

Induction of cerebral ischaemia

Mice were anaesthetized with chloral hydrate (400 mg kg^{-1} i.p.). A midline incision was made in the region between the neck and sternum, and the trachea was exposed. Both the right and left common carotid arteries were located lateral to the sternocleidomastoid, freed from the surrounding tissues and the vagus nerve was separated. Cerebral ischaemia was induced by clamping both the arteries with aneurysm clips (Traystman 2003). After 10 min of cerebral ischaemia, the clips were removed from both arteries to allow the reflow of blood through the carotid arteries. The incision was sutured in layers with surgical sutures (Homi & Mixco 2003). The sutured area was cleaned with 70% ethanol and sprayed with antiseptic powder. After completion of the surgical procedure, the animals were shifted individually to their home cage and were allowed to recover. While performing the surgical procedure, the body temperature was maintained at 37°C by a heated infrared lamp. All surgical instruments used in the surgical procedure were sterilized before use.

Measurement of cerebral infarct size

Animals were killed under ether anaesthesia and the brain removed. The brain was kept overnight at −4°C. Frozen brain was sliced into uniform sections, 1 mm in thickness. The slices were immersed in 1% triphenyltetrazolinium chloride at 37°C in 0.25 M phosphate buffer (pH 8.5) for 20 min; tissue sections were dipped in a 10% formaldehyde solution for 5 min (Himori et al 1990). Triphenyltetrazolinium chloride is converted to red formazone pigment by NAD and lactate dehydrogenase and therefore stained the viable cells deep red. The infarcted cells have lost the enzyme and cofactor and remain unstained (dull yellow). The brain slices were placed on a glass plate. A transparent plastic grid with 100 squares cm^{-2} was placed over it. The number of squares falling over the non-stained dull yellow area and the total number of squares covered by each brain slice were counted. The infarcted area was expressed as a percentage of total brain volume.

Measurement of cerebral oedema

Animals were killed by decapitation under ether anaesthesia. The brains were removed and weighed immediately to yield the wet weight. The brain water content, an indicator of brain oedema, was measured by the wet/dry method (Dempsey et al 2000).

Seizure susceptibility

Seizure susceptibility was assessed by giving pentylenetetrazole i.p. at the subconvulsive dose of 30 mg kg^{-1} after 72 h of reperfusion. Seizures were recorded according to the following scale (0–6 score) (Giorgi et al 1991): 0, no response; 1, ear and facial twitching; 2, 1–20 myoclonic body jerks in 10 min; 3, more than 20 body jerks in 10 min; 4, clonic forelimb convulsions; 5, generalized clonic convulsions with rearing and falling down episodes; 6, generalized convulsions with tonic extension episodes.

Behavioural assessments

The following behavioural assessments were carried out after 72 h of cerebral ischaemia.

Short-term memory
Short-term memory was evaluated using the elevated plus maze (Vogel & Vogel 2002) at 72 h following cerebral ischaemia. Transfer latency time (TLT) measured on the plus maze on Day 1 and Day 2 served as an index of learning or acquisition, whereas TLT on Day 3 served as an index of retrieval or memory. Utmost care was taken not to change the relative location of the plus maze with respect to any object serving as a visual clue in the laboratory.

Hole-board test
The hole-board test comprises an open field with holes on the bottom into which animals can poke their noses (Vogel & Vogel 2002). Mice were placed on the hole-board and tested for 5 min before and after being subjected to cerebral ischaemia. The number of counts for nose poking was calculated to evaluate the exploratory behaviour of the animal.

Rota rod test
The rota rod test (Vogel & Vogel 2002) was used to evaluate fore- and hindlimb motor coordination. Animals were prescreened based on their ability to remain on the revolving rod for 1 min.

Biochemical parameters

Brain protein assay
Mouse brain protein assay was done according to the method of Lowry et al (1951).

Lipid peroxidation assay
Tissue lipid peroxidation was evaluated by measurement of thiobarbituric acid reactive substances. Malondialdehyde has been identified as the product of lipid peroxidation, which reacts with thiobarbituric acid to give red light absorbancy at 535 nm (Ohkawa et al 1979).

Estimation of superoxide dismutase (SOD)
The assay for the estimation of SOD is based on the principle of the inhibitory effect of SOD on reduction of nitro blue tetrazolium dye by superoxide anions generated by the photooxidation of hydroxylamine hydrochloride ($NH_2OH.HCl$) (Kono 1978).

Estimation of catalase
Catalase activity was measured according to Luck (1963). The reaction mixture contained 3 mL of 0.66 M phosphate buffer (pH 7.0) and 1.25×10^{-2} M H_2O_2 in the sample cuvette. The reference cuvette contained 3 mL of 0.66 M phosphate buffer (pH 7.0). The reaction was started by adding brain tissue homogenates to the sample and reference cuvettes. The rate of elimination of hydrogen peroxide by catalase was measured by recording the time (in s) required for a 0.05 decline of absorbance at 240 nm. Catalase activity (in international units) was calculated by the following formula and was expressed in terms of nmol H_2O_2 consumed min^{-1} (mg of protein)$^{-1}$ (Luck 1963):

$$\text{IU} = ((17/\text{time (s) for 0.050 absorbance change}) \times (1/\text{weight of homogenate})) \times 13$$

Estimation of glutathione peroxidase (GPx)
GPx was estimated using brain tissue homogenate according to the method of Paglia & Valentine (1967). Samples of brain tissue homogenate diluted with 50 mM phosphate buffer (pH 7.4) were added to a reaction mixture comprising 5 mM EDTA, 0.01 mL of 1.125 M sodium azide, 0.1 mL of 0.15 M GSH, 2.4 units of glutathione reductase (10 μL) and 0.1 mL of 8.4 mM NADPH to make a final volume of 2.9 mL. The reaction mixture was incubated at 22°C for 10 min. The reference cuvette contained 100 μL of distilled water instead of brain tissue homogenate. Following addition of 0.1 mL of 2.2 mM hydrogen peroxide solution, the decrease in absorbance at 340 nm was recorded for 3–4 min. The concentration of the enzyme was calculated in terms of NADPH consumed min^{-1} (mg homogenate)$^{-1}$ using an extinction coefficient of 6.22 mM^{-1} cm^{-1}.

Estimation of brain TNF-α
TNF-α was estimated using an enzyme-linked immunosorbent assay kit (Diaclone, France) according to the manufacturer's instructions. A monoclonal antibody specific for mice TNF-α was coated on to the well of the microtitre strip provided. Samples including serially diluted standards of known concentrations of TNF-α (provided in the kit) and the test sample of TNF-α (obtained from the supernatant of the brain homogenate after centrifuging at 1000 *g* for 10 min at −4°C) were pipetted into these wells in a volume of 100 μL. The blank was created with 100 μL of standard diluents. The microwell strip was covered with a plate cover and incubated for 2 h at room temperature (25°C). Each well was washed 3 times with 0.3 mL of washing solution. To each well, 50 μL of diluted biotinylated anti-mTNF-α was added. Strips were incubated for 1 h at room temperature. The wells were washed. To each well 100 μL of diluted streptavidin-HP solution was added. Strips were incubated for 30 min at room temperature. The wells were washed again. To each well, 100 μL of ready-to-use tetramethylbenzidine substrate solution was added and incubated in the dark for 30 min at room temperature. The enzyme–substrate reaction was stopped with 100 μL of H_2SO_4. Readings were taken immediately thereafter at 450 nm as the primary wavelength using an enzyme-linked immunosorbent assay reader. The concentration of TNF-α was determined by extrapolating the optical density value onto the standard curve obtained from the known concentration of TNF-α provided in the kit. The TNF-α level was expressed in terms of pg mL^{-1} of brain supernatant.

Statistical analysis

All data are expressed as mean ± s.e.m. Inter-group comparisons were made using analysis of variance followed by post-hoc Tukey's test. Data for seizure susceptibility were analysed using the Kruskal–Wallis test followed by post-hoc Bonferroni's test. A P value less than 0.05 was considered significant.

Results

Effect of progesterone on brain water content

The brain water content in the sham-operated mice 72 h after surgery was 74.23±0.36% (Table 1). Cerebral ischaemia significantly increased the brain water content ($P<0.01$). Treatment with progesterone changed the brain water content and brain oedema was reduced compared with the saline-treated control group, but the difference was not statistically significant.

Post-ischaemic seizure susceptibility and effect of progesterone on seizure score

Table 2 shows the effect of progesterone on seizure susceptibility assessed by giving pentylenetetrazole (subconvulsive dose of 30 mg kg^{-1} i.p.) after 72 h of ischaemia. As shown in Table 2, the number of positive responders increased quite significantly in the saline-treated control group compared with the sham-operated group. The mean seizure score was also increased ($P<0.01$) compared with the sham-operated group, showing high seizure susceptibility after ischaemia. Administration of progesterone caused a significant reduction in the mean seizure score ($P<0.02$) and also a reduction in the number of positive responders.

Effect of ischaemic insult and progesterone on exploratory behaviour in mice

The number of head dips in the saline-treated control group decreased (24.83±2.14) as compared with the sham-operated group (34.00±2.93), indicating less exploratory behaviour in mice after ischaemia. Treatment with progesterone had no significant effect on the number of head dips.

Table 1 Effect of progesterone on brain water content

Group	Brain water content (%)
Group 1 (sham-operated)	74.23±0.36
Group 2 (surgery; saline-treated)	76.28±0.51*
Group 3 (progesterone-treated)	76.10±0.38

Data are expressed as mean±s.e.m. *$P<0.01$, significantly different compared with Group 1.

Table 2 Effect of progesterone on positive responders and post-ischaemic seizure susceptibility after pentylenetetrazole administration

Group	% Positive responders, score >3	Seizure score (mean±s.e.m.)
Group 1 (sham-operated)	0%	0.5±0.34
Group 2 (surgery; saline-treated)	66.67%	3.5±0.87*
Group 3 (progesterone-treated)	16.67%	0.67±0.49$^{\#}$

Data are expressed as mean±s.e.m. *$P<0.01$, significantly different compared with Group 1; $^{\#}P<0.02$, significantly different compared with Group 2 (Kruskal–Wallis test followed by post-hoc Bonferroni's test).

Effect of progesterone on TLT

The TLT data are summarized in Table 3. The acquisition phase denotes the TLT after 3 days of training the animals. With training, TLT was reduced from the cut-off TLT (i.e. 90 s) in all groups. The retention phase denotes the TLT at different time intervals with subsequent dosing of responsive drugs. At 24 h after ischaemia and 1 h after the second dose of drug, there was no significant difference in TLT in the sham-operated group. Even after 48 h and 72 h, TLT was not different compared with the acquisition phase in the sham-operated group. Comparisons of the acquisition phase in different groups also showed no significant difference in TLT. After surgery and bilateral common carotid artery occlusion (BCAO), TLT was significantly increased to 90 s at all the time intervals ($P<0.05$). Comparing the groups, TLT was increased in the saline-treated control group as compared with sham-operated group, indicating memory impairment as a result of BCAO. Treatment with progesterone at 24 h significantly reduced the TLT compared with the surgery group ($P<0.001$); treatment with progesterone at 72 h showed an even more significant reduction in TLT ($P<0.0001$).

Evaluation of the anxiolytic effect of progesterone

In the saline-treated control group, mice made slightly more entries into the closed arms and spent more time in the closed arms compared with the sham-operated group, indicating increased anxiety associated with ischaemia (Table 4). Administration of progesterone (15 mg kg^{-1} i.p.) had a significant effect, with increased time spent in the open arms compared with the sham-operated and normal saline-treated control groups ($P<0.05$) and fewer entries into the closed arms.

Effect of progesterone on cerebral ischaemia induced antioxidant activity

The antioxidant parameters are given in Table 5. After ischaemic insult, lipid peroxidation increased significantly compared with the sham-operated group ($P<0.001$); it was significantly attenuated with progesterone treatment ($P<0.001$). Further, antioxidant activity was decreased with ischaemia as shown by the low levels of SOD, GPx and catalase compared with the sham-operated group ($P<0.001$). Treatment with progesterone raised the antioxidant enzyme levels, indicating improved antioxidant enzyme activity against cerebral ischaemia and reperfusion induced oxidative stress (Table 5).

Effect of progesterone on cerebral infarct size, muscle coordination and TNF-α values after cerebral ischaemia and reperfusion

The mean infarct size in the saline-treated control was 87.65±1.95%, which was significant compared with the sham-operated group ($P<0.001$). Treatment with progesterone showed a reduction in cerebral infarct size ($P<0.001$) (Table 6).

Table 3 Effect of progesterone on the transfer latency time in mice

Group	Acquisition phase (s)	Retention phase 24 h (s)	Retention phase 48 h (s)	Retention phase 72 h (s)
Group 1 (sham-operated)	21.25 ± 2.17	26.50 ± 1.32	28.50 ± 2.96	26.00 ± 3.24
Group 2 (surgery; saline-treated)	20.75 ± 5.02	90.00 ± 0.00*#	90.00 ± 0.00*#	90.00 ± 0.00*#
Group 3 (progesterone-treated)	30.25 ± 10.58	38.50 ± 12.02†	19.75 ± 2.81†	8.50 ± 0.29*¶

Transfer latency data are expressed as mean ± s.e.m. Retention phases followed 24, 48 and 72 h after the acquisition phase. Ischaemic surgery was done immediately after the acquisition phase. Progesterone was given 30 min before ischaemia and 24, 48 and 72 h after ischaemia. After 1 h of dosing, the retention phase was noted at each level. *$P < 0.05$, significantly different compared with the acquisition phase; #$P < 0.01$, significantly different compared with Group 1; †$P < 0.001$, significantly different compared with Group 2; ¶$P < 0.0001$, significantly different compared with Group 2 at 72 h.

Table 4 Behaviour of mice in the elevated plus maze and effect of progesterone on anxiety

Group	Entries into open arms	Entries into closed arms	Total number of entries	Time spent in open arms (s)	Time spent in closed arms (s)
Group 1 (sham-operated)	2.5 ± 0.67	3.5 ± 0.92	6.0 ± 1.51	99.83 ± 38.07	200.17 ± 38.07
Group 2 (surgery; saline-treated)	3.33 ± 1.12	4.67 ± 1.33	8.0 ± 2.39	90.50 ± 24.93	209.5 ± 24.93
Group 3 (progesterone-treated)	4.33 ± 0.72	2.17 ± 0.60	6.5 ± 1.18	244.33 ± 15.49*#	55.67 ± 15.49*#

Data are expressed as mean ± s.e.m. *$P < 0.05$, significantly different compared with Group 1; #$P < 0.05$, significantly different compared with Group 2.

Table 5 Mean values of lipid peroxidation (LPO), superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase levels

Group	LPO levels (nmol mL^{-1})	SOD levels (units (mg protein)$^{-1}$)	GPx levels (units (mg protein)$^{-1}$)	Catalase levels (units (mg protein)$^{-1}$)
Group 1 (sham-operated)	1.53 ± 0.18	3.76 ± 0.39	8.99 ± 0.64	3.13 ± 0.30
Group 2 (surgery; saline-treated)	9.55 ± 0.67*	1.59 ± 0.17*	3.29 ± 0.22*	0.79 ± 0.12*
Group 3 (progesterone-treated)	4.41 ± 0.34#	6.16 ± 0.23#	10.56 ± 0.81#	2.79 ± 0.29#

Data expressed as mean ± s.e.m. *$P < 0.001$, significantly different compared with Group 1; #$P < 0.001$, significantly different compared with Group 2.

Table 6 Effect of progesterone on cerebral infarct size, muscle coordination and TNF-α values after cerebral ischaemia and reperfusion

Group	Percentage infarct size	Time spent on rota rod (s)	TNF-α values (pg mL^{-1})
Group 1 (sham-operated)	26.86 ± 2.91	112.17 ± 6.1	40.67 ±6.38
Group 2 (surgery; saline-treated)	87.65 ± 1.95*	23.17 ± 5.1*	241.67 ± 22.46*
Group 3 (progesterone-treated)	38.79 ± 2.41#	91.17 ± 16.9#	115.50 ± 14.55#

Data expressed as mean ± s.e.m. *$P < 0.001$, significantly different compared with Group 1; #$P < 0.01$, significantly different compared with Group 2.

When subjected to the rota rod test after 72 h of ischaemia, animals in the saline-treated control group showed a reduction ($P < 0.001$) in the time spent on the rota rod compared with the sham-operated group, indicating impaired motor coordination following ischaemia. Comparing the treatment group with the saline-treated control group, progesterone reduced the impairment in muscle coordination, with an increase ($P < 0.01$) in the time spent on the rota rod (Table 6).

Upon partial global cerebral ischaemia, TNF-α activity increased significantly compared with the sham-operated group ($P < 0.001$) (Table 6). Acute treatment with progesterone decreased the TNF-α level significantly, suggesting vascular protection against cerebral ischaemia ($P < 0.001$) (Table 6).

Discussion

The partial global cerebral ischaemia and reperfusion model is reported to mimic the clinical situation of cerebral ischaemia (Alonso de Lecinana et al 2000). Global cerebral ischaemia results in neuronal death irrespective of post-ischaemic reperfusion (Neumar 2000). Global cerebral ischaemia of short duration followed by reperfusion was used in the present study. The BCAO model is used to produce partial global cerebral ischaemia for the study of various parameters. Progesterone provides acute neuroprotection in the transient middle cerebral artery occlusion model in rats and in the

global ischaemia model in cats. Various studies have shown that progesterone and its metabolites (e.g. allopregnanolone and medroxyprogesterone) alone or in combination with oestrogens has neuroprotective properties such as a reduction of infarct size following middle cerebral artery occlusion, suppression of inflammatory responses and induced NOS expression after ischaemic insult (Cervantes et al 2002; Gibson et al 2005; Littleton-Kearney et al 2005; Sayeed et al 2006). This is the first study of its kind in which the BCAO model has been used in mice; earlier studies were done using the middle cerebral artery occlusion model in rats. The results may therefore vary compared with previous studies because of species variation.

BCAO increased brain infarct size compared with the sham-operated group. Administration of progesterone ($15\,mg\,kg^{-1}$ i.p.) decreased the infarct size significantly. There was an increase in the susceptibility to seizures and the number of positive responders increased significantly after cerebral ischaemia. Administration of progesterone decreased the seizure score to near baseline levels, showing an increased threshold for seizures after pentylenetetrazole administration (subconvulsive dose of $30\,mg\,kg^{-1}$ i.p.). Similarly, another study reported that neurosteroids share an important site of action, tonic inhibition mediated by δ-subunit-containing $GABA_A$ receptors (Maguire et al 2005). The study indicated that periodic alterations in specific $GABA_A$ receptor subunits occur during the oestrous cycle in mice, causing cyclic changes of tonic inhibition in hippocampal neurons. In late dioestrus (high-progesterone phase), enhanced expression of δ-subunit-containing $GABA_A$ receptors increases tonic inhibition, and a reduced neuronal excitability is reflected by diminished seizure susceptibility and anxiety (Maguire et al 2005).

Progesterone also decreased the brain water content but the reduction was not significant compared with the increased brain oedema after cerebral ischaemia and reperfusion injury. Short-term memory impairment was observed in the surgery group, with a cut-off TLT of 90 s. Administration of progesterone improved the short-term memory, with a decrease in TLT compared with the surgery group at all time intervals. At 72 h, TLT was reduced to a significant level, indicating a memory enhancement effect by progesterone. Progesterone ($15\,mg\,kg^{-1}$ i.p.) reduced the impaired motor coordination quite significantly and improved grip strength. Although progesterone increased the number of dips as compared with the surgery group, we could not draw firm conclusions about the effect of progesterone on exploratory behaviour at present. Progesterone increased the time spent in the open arms and decreased the number of entries into the closed arms, suggesting an anxiolytic property. During ischaemia, and especially during reperfusion, free radicals may be produced to such an extent that the endogenous antioxidant systems are overwhelmed. Free radicals are demonstrated to promote lipid peroxidation. In the present study, there was a significant increase in lipid peroxidation and a significant decrease in all the other antioxidant enzymes. Treatment with progesterone decreased the lipid peroxidation levels and increased antioxidant enzymes such as SOD, GPx and catalase. The improvement in antioxidant enzyme levels to significant values suggests antioxidant and free radical scavenger activity of progesterone against cerebral ischaemia and reperfusion induced oxidative stress. During ischaemia, a lot of mediators are released in a progressive manner, such as O_2 radicals, endothelin-1 and TNF-α, which lead to various vascular complications (Fagan et al 2004). Treatment with progesterone reduced the levels of TNF-α, combating the vascular complications caused during the acute phase of ischaemic stroke.

In conclusion, the present study confirmed the neuroprotective effect of progesterone in cerebral ischaemia and reperfusion induced by BCAO in mice. Further studies are needed to explore these findings in the clinical setting.

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