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In vivo evidence that erythropoietin has a neuroprotective effect during subarachnoid hemorrhage

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Abstract

To ascertain in vivo whether recombinant human erythropoietin has a neuroprotective effect on the cortex during subarachnoid hemorrhage, 56 rabbits were divided into the following groups: Group 1 control sham operated plus placebo ($n = 14$; saline solution — NaCl 0.9%); Group 2 control sham operated plus recombinant human erythropoietin ($n = 14$); Group 3 subarachnoid hemorrhage plus placebo ($n = 14$); Group 4 subarachnoid hemorrhage plus recombinant human erythropoietin ($n = 14$; intraperitoneal administration of recombinant human erythropoietin immediately after inducing subarachnoid hemorrhage). In none of the Groups 1 and 2 animals was subarachnoid hemorrhage induced. In Group 3 rabbits, an increase in locomotor activity (open field apparatus) was observed 24, 48 and 72 h after surgery, and the mortality rate was 42.9% within 72 h after surgery, and, no increase in locomotor activity was observed in Group 4 rabbits, which survived for at least 72 h. Our findings suggest that recombinant human erythropoietin may be of benefit in the treatment of subarachnoid hemorrhage.

Keywords: Erythropoietin; Subarachnoid hemorrhage; Brain

1. Introduction

Erythropoietin is a key hormone in the regulation of red blood cell production, and recombinant human erythropoietin has been used in the treatment of uremic anemia. However, in humans recombinant human erythropoietin therapy often causes arterial hypertension. Several authors confirm the importance of the non-erythropoietic effects of erythropoietin, which affects vascular resistance (Buemi et al., 1999).

Recent studies, moreover, have shown that there is a close link between erythropoietin and the brain. Juul et al. (1999) have demonstrated that erythropoietin plays an important role in neurodevelopment and in brain homeostasis in the elderly, and have also demonstrated that its receptor is present in the developing human brain as early as 5 weeks after conception.

In the brain, capillary endothelial cells express two forms of erythropoietin mRNA receptor (Yamaji et al., 1996). Moreover, erythropoietin mRNA has been found in biopsies from the human hippocampus, amygdala and temporal cortex, erythropoietin mRNA expression can be increased in the mouse brain by inducing anemia and in isolated primary astrocytes on exposure to low oxygen levels (Marti et al., 1996).

Studies on rats with permanent occlusion of the middle cerebral artery have shown that recombinant human erythropoietin prevents neuronal death from glutamate or ischemic damage and cortical infarction (Morishita et al., 1997; Sadamoto et al., 1998).

Bernaudin et al. suggest that the mechanism underlying this effect is endogenous and suggest that continous ery-

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Table 1
Effects of recombinant human erythropoietin (1000 UI/kg i.p.) on survival of animals subjected to subarachnoid hemorrhage followed for 72 h. Recombinant human erythropoietin was given immediately after subarachnoid hemorrhage

Treatment	Number of animals	Survivors	(%) Survivors
Control + placebo	14	14	100
Control + rHuEpo	14	14	100
SAH + placebo	14	8[a]	57.1
SAH + rHuEpo	14	14	100

[a] $p < 0.05$ vs. subarachnoid hemorrhage + recombinant human erythropoietin.

thropoietin formation takes place during the active evolution of a focal cerebral vascular lesion and that the erythropoietin/erythropoietin receptor system may be involved in neuroprotection and restructuring (such angiogenesis and gliosis) after ischemia. Previous studies demonstrated that rodent models of subarachnoid hemorrhage are associated with the induction of persistent neurological and behavioral deficits (Germanò et al., 1994).

Our aim was to evaluate in vivo whether erythropoietin has a neuroprotective effect following subarachnoid hemorrhage in rabbits. To this end, we evaluated locomotor activity and mortality rate following its administration.

2. Materials and methods

Forty-two adult New Zealand albino rabbits of both sexes (21 M, 21 F; weight 2.0–2.5 kg) were divided into groups: Group 1 ($n = 14$) sham operated plus placebo (saline solution — NaCl 0.9%); Group 2 ($n = 14$) sham operated plus recombinant human erythropoietin; Group 3 ($n = 14$) induced subarachnoid hemorrhage, gives the same placebo as that given to Group 1 animals; Group 4 ($n = 14$), subarachnoid hemorrhage was induced and recombinant human erythropoietin administered intraperitoneally.

The intraperitoneal (i.p.) route was used for drug administration, treatment being started immediately after induction of subarachnoid hemorrhage, with the daily administration of the vehicle NaCl 0.9% (1 ml/kg body weight) or recombinant human erythropoietin, at a dosage (1000 UI/kg body weight) found to be effective (Sadamoto et al., 1998).

We used the techniques described by Takahashi et al., (1993)to induce subarachnoid hemorrhage. The animals were anaesthetized with an intramuscular injection of combined ketamine (40 mg/kg) and xylazine (8 mg/kg). All experimental protocols were approved by the Ethics Committee of the University of Messina.

2.1. Induction of subarachnoid hemorrhage

In Groups 2 and 3 animals, the central ear artery was cannulated to obtain 5 ml of autologous arterial blood. A 23-gauge butterfly needle was inserted percutaneously into the cisterna magna. One milliliter of liquor was extracted for erythropoietin assay and 3 ml of autologous blood was injected over a period of 10 s. The animals were then positioned with their heads down for 20 min to facilitate the settling of blood in the basal cisterns. The tests were always performed between 10:00 and 12:00 a.m. The animals, which were monitored closely for respiratory distress, were immediately placed on a ventilator if necessary. They had free access to food and water over the next 72 h, and were observed closely for adequate food intake and for any neurological deficits.

2.2. Locomotor activity

After 24, 48 and 72 h, an open field test was performed on all groups. The rabbits were placed in an open field apparatus; the number of squares crossed and the number of rearings in 6 min were determined. We considered results only from subarachnoid hemorrhage animals, animals that survived for 72 h .

All the animals were killed at 72 h, and 2 ml of liquor was obtained for erythropoeitin assay. The animals were then evaluated for any incomplete subarachnoid clots or inadequate perfusion .

Statistical analysis was done with a Statistical Product for Social Science release 7.5 statistics software package (Statistical Product for Social Science, IL, USA) and a one-way ANOVA (analysis of variance) to evaluate any differences between means in the four groups, a value of $p < 0.05$ being considered significant. For data on survival, statistical analysis was done with Fischer's exact probability test.

3. Results

A thick subarachnoid clot was observed over the basal surface of the brainstem in each animal with subarachnoid hemorrhage. In both experiments, Group 2 and 3 animals were found to have substantial corrugation of the internal elastic lamina of the basilar arteries.

Table 2
Effects of recombinant human erythropoietin (1000 UI/kg i.p.) on locomotor activity of animals surviving after subarachnoid hemorrhage followed by 24, 48 and 72 h (squares/6 min)

	24 h	48 h	72 h
Control + placebo	74 ± 5	71 ± 4	70 ± 5
Control + rHuEpo	75 ± 5	70 ± 4	70 ± 5
SAH + placebo	105 ± 11[a]	95 ± 8[a]	94 ± 8[a]
SAH + rHuEpo	77 ± 7[b]	75 ± 5[b]	71 ± 5[b]

[a] $p < 0.01$ vs. control + placebo.
[b] $p < 0.01$ vs. subarachnoid hemorrhage + placebo.

Table 3
Time course of cerebrospinal erythropoietin (mU/ml) in four rabbits groups

	Basal	After 72 h
Control + placebo	2.1 ± 0.2	1.9 ± 0.29
Control + rHuEpo	1.95 ± 0.3	2.0 ± 0.36
SAH + placebo	1.8 ± 0.2	1.72 ± 0.16
SAH + rHuEpo	1.91 ± 0.3	2.3 ± 0.2

All animals in Groups 1, 2 and 4 survived for at least 72 h, while 42.9% of Group 3 rabbits died within this time. The mortality rate was reduced by recombinant human erythropoietin (1000 UI/kg i.p.) as shown in Table 1

In Group 3 rabbits, an increase in locomotor activity was observed 72 h after surgery. No increase in locomotor activity was observed in Group 4 rabbits (treated with recombinant human erythropoietin — for details see methods, 1000 UI/kg i.p.) (Table 2).

Group 2 and 3 animals had increased erythropoietin liquor concentrations, although not to a statistically significant extent (Table 3)

4. Discussion

Recent studies have demonstrated that there is a close link between erythropoietin and the brain. Recombinant erythropoietin has protective and curative effects in several types of microcirculatory dysfunction following, for example, brain ischemia or post ischemic reperfusion of the splanchnic territory and other pathological conditions leading to shock (Buemi et al., 1993; Masahiro et al., 1998). Our findings show that recombinant human erythropoietin administered immediately after subarachnoid hemorrhage has a beneficial effect on the survival and on the motor activity of treated rabbits compared with those given a placebo. The changes observed were associated with an increase in the concentrations of erythropoietin in the brain, although not to a statistically significant extent. However, it has been well demonstrated that low concentrations of intracerebral erythropoietin can have a protective effect on the brain. Sakanaka et al. (1998) have shown that in mongolian gerbils, low concentrations of erythropoietin (2.5 units/day) prevent ischemia in a dose-dependent manner, reduce learning disability and save hippocampal CA1 neurons from lethal ischemic damage. However, at a dose of 50 or 500 units/day the drug failed to ameliorate ischemic neuronal damage.

The mechanisms underlying the beneficial effect of recombinant human erythropoietin administration in brain damage are still unclear. Probably recombinant human erythropoietin has a direct effect on the vascular wall. Cerebral vasospasms following subarachnoid haemorrhage is a serious clinical condition with an unfavorable prognosis. Recent clinical and experimental studies suggest that mechanical, chemical and neurogenic factors are responsible for the occurrence of cerebral vasospams following subarachnoid hemorrhage (Egemen et al., 1988). In vitro, recombinant human erythropoietin can restore the contractile response to phenylephrine (Buemi et al., 1999). This may depend on the ionophoric effect of recombinant human erythropoietin on calcium and ions in the smooth muscle fiber cells (Buemi et al., 1991). Moreover, as subarachnoid hemorrhage induces nitric oxide formation, recombinant human erythropoietin may exert a neuroprotective action by reducing the nitric oxide-mediated formation of free radicals or by antagonizing their toxicity (Squadrito et al., 1999).

The beneficial effects of recombinant human erythropoietin observed following its administration immediately after subarachnoid hemorrhage may, however, be due to other factors: (Marti et al., 1997) demonstrated in man that recombinant human erythropoietin does not cross the intact hematoencephalic barrier. Yet, if administered i.v., the hormone can increase pO_2 and reduce pCO_2 levels in the liquor and, as observed by us (Buemi et al., 2000), can modify the pH and Na^+ content of the liquor itself.

Since it is well known that local cerebral changes in the acid–base balance may interfere with neuronal communication and neurotransmission, the above effects of recombinant human erythropoietin may contribute to the neuroprotective effect of this drug in transient cerebral ischemia (Velisek et al., 1998).

In conclusion, recombinant human erythropoietin administered immediately after the induction of subarachnoid hemorrhage is followed by a reduction in mortality rate and has a beneficial effect on the rabbit brain. Further research is required to identify and define the mechanism underlying its action as a neuroprotective agent and the optimal dosing regime required to achieve a beneficial effect. Nonetheless, our findings provide the first indication that recombinant erythropoietin may be useful in the treatment of subarachnoid hemorrhage.

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