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Neuroprotective Effects of Erythropoietin in Patients with Severe Closed Brain Injury

Şiddetli Kapalı Beyin Hasarı Hastalarında Eritropoietinin Nöroprotektif Etkileri

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ABSTRACT

AIM: Our research was focused on the neuroprotective function of erythropoietin (Epo) in patients with severe closed traumatic brain injury (TBI).

MATERIAL and METHODS: Our model examined the influence of the outcome and neurological recovery in 42 adults with TBI who were admitted to ICU within 6 hours of their injury and were recruited into a randomized controlled study of two groups; only the patients of the intervention group received 10,000 i.u. of Epo for 7 consecutive days. A prognostic model based on CRASH II injury model and outcome was measured by survival and Glasgow Outcome Scale-Extended version (GOS-E) score at 6 months post-injury.

RESULTS: Six patients (18.7%) died during the first two weeks; 4 of the control group and 2 of the intervention group. A mortality rate of 22.2% and 8.3% for the control and intervention group respectively was observed. A lower rate of good outcome (GOS-E score > 4) at 6 months was mentioned among patients of the control group.

CONCLUSION: The study provides evidence of lower mortality and better neurological outcome for the patients who received Epo increasing the possibility that Epo therapy could be used in clinical practice, limiting neuronal damage induced by TBI.

KEYWORDS: Brain, Injury, Erythropoietin, Mortality, Outcome

ÖZ

AMAÇ: Araştırmamız, şiddetli kapalı travmatik beyin yaralanması (TBY) olan hastalarda eritropoietinin (Epo) nöroprotektif işlevi üzerine odaklanmıştı.

YÖNTEM ve GEREÇLER: Modelimiz, yaralanmadan sonra 6 saat içinde yoğun bakıma gelen ve iki grup halinde randomize kontrollü bir çalışmaya kaydedilen 42 yetişkinde sonuç ve nörolojik iyileşme üzerine etkiyi inceledi; sadece girişim grubundaki hastalara arka arkaya 7 gün boyunca 10.000 i.u. Epo uygulandı. Yaralanmadan 6 ay sonra CRASH II yaralanma modeli temelinde prognostik bir model kullanıldı ve sonuç sağkalım ve Glasgow Sonuç Ölçeği-Genişletilmiş versiyon (GOS-E) puanıyla ölçüldü.

BULGULAR: İlk iki haftada kontrol grubunda 4 ve girişim grubunda 2 hasta olarak altı hasta (%18,7) öldü. Kontrol ve girişim grupları için sırasıyla %22,2 ve %8,3 mortalite oranı gözlendi. Kontrol grubunda 6 ayda daha düşük bir iyi sonuç oranı (GOS-E puanı > 4) görüldü.

SONUÇ: Çalışma, Epo alan hastalarda daha düşük mortalite ve daha iyi nörolojik sonuç bulguları vermiştir ve böylece Epo tedavisinin klinik uygulamada kullanılıp TBY nedeniyle oluşan nörolojik hasarı sınırlaması olasılığını ortaya koymuştur.

ANAHTAR SÖZCÜKLER: Beyin, Yaralanma, Eritropoietin, Mortalite, Sonuç

INTRODUCTION

Traumatic brain injury (TBI) is the term referred to brain injury caused by external trauma. Brain damage represents a major health problem worldwide due to the fact that can cause death or persistent disability (24). Except of a potentially catastrophic debilitating medical emergency, brain injuries remain the main cause of combat casualties, with an estimated 15-25% of all injuries sustained in 20th century conflicts (6).

The Medical Research Council (MRC) CRASH (Corticosteroid Randomisation After Significant Head Injury) trial includes patients within 6 hours of the injury, uses standardized definitions of variables, and achieves almost complete follow-up at 6 months. It is the largest clinical trial conducted in patients with TBI and presents an opportunity for the development of a prognostic model (9).

Erythropoietin (Epo), a member of the type I cytokine superfamily, is a 165 aminoacid (~30 kDa) glycoprotein that stimulates erythroid progenitors within the bone marrow to mature into erythrocytes. Although peripherally administered recombinant human Epo (rhEpo) has shown to

penetrate the blood-brain barrier and reduce brain injury (4, 17), its potential neuroprotective efficacy in an *in vivo* model of experimental TBI has been rarely investigated (4, 26, 30, 31). In addition, there are numerous studies regarding the widespread efficacy of rhEpo in injury models of spinal cord (14, 18) and subarachnoid hemorrhage (15, 17, 33) promoting the role of Epo as an essential mediator of protection in the central nervous system (CNS).

In the presented study, we tried to investigate the role of Epo following severe closed TBI. Specifically, the aim of this research was to evaluate the effectiveness of Epo administration on neurological impairment, to determine if Epo reduces secondary brain injuries and helps patients make a better recovery after TBI. Additionally, the current research tried to develop and validate practical prognostic model for death at 14 days and death or severe disability 6 months after patients' injuries.

Study Aims

The aims of the present study were to compare 6-month survival and functional outcome in patients with severe closed TBI, monitored prospectively within the first 6 hours of their injury. Patients were enrolled in the study as soon as they were transferred to the Intensive Care Unit (ICU) and Epo administration was started immediately within the first 6 hours after the patients' injury. In the current study, it was hypothesized that the administration of Epo would improve the neurological outcome, increase the survival, and minimize disability due to a severe closed TBI. The hypothesis was based on animal studies in rat models of TBI which supported that the administration of rhEpo protects neurons (25), reduces cerebral edema, and promotes the recovery of motor deficits (3).

All human studies were approved by the appropriate ethics committees of the two hospitals included in this study. The studies were performed according to the ethical standards determined in the 1964 Declaration of Helsinki. All the persons included in the studies gave their informed consent.

MATERIAL and METHODS

Study Design

Two ICU departments participated in this study; the ICU of the 401 General Army Hospital of Athens and the Athens University School of Nursing at KAT General Hospital, which stand for Center of Assessing Trauma.

The basic model included three predictors: age, GCS, and pupil reactivity. The patients were selected for the study if, at any stage during pre-hospital care, they had sustained blunt head trauma, a GCS of less than 9, or hypotension (systolic blood pressure <100mmHg); were aged >18 years, and were admitted to the ICU within 6 hours of their injury. Patients with multisystem trauma were also included. On the other hand, patients with penetrating trauma, pregnant, subjects, those with a serious pre-morbid disease or peripheral edema, and patients who had cardiac arrest or on sinus rhythm were excluded from the study. Table I summarizes the inclusion and exclusion criteria.

The prospectively collected data were based on patients' vital signs, GCS, injury severity scores and laboratory data during their admission and furthermore all the significant events after their admission. The primary outcome was the functional outcome, as measured using the Glasgow Outcome Scale-Extended version (GOS-E), measured at 6 months post-injury. The GOS-E is based on the simpler Glasgow Outcome Scale (GOS) but has been shown to be more sensitive to change in mild to moderate TBI (29).

Administration of the GOS-E comprises information about consciousness, independence in activities in daily life, work, social activities, family and friends, and return to normal life. It is a well validated measure of disability shown to be predictive of patient need for supervision and assistance after TBI. Scores based on responses are categorized from 1 to 8 (starting from 1): dead, vegetative state, lower severe disability, upper severe disability, lower moderate disability, upper moderate disability, lower good recovery and upper good recovery (the last represents the number 8 in the scale). GOS-E has been observed to have an important relationship with measures of the injury's severity and other rating scales establishing TBI patients' disability. GOS-E was administered to the injured patient at 6 months post-injury. Secondary outcomes included survival during hospitalization and to 3 and 6 months after initial brain injury. Other measures included the initial intracranial pressure (ICP), duration of ICP elevation, worst oxygenation expressed as lowest PaO_2/FiO_2 ratio, the duration of inotropic support, and mechanical ventilation.

Outcomes

Death of a patient was recorded during hospitalization or 14 days after the randomization. An unfavorable outcome at the 6-month period was defined with the GOS. GOS represents a scale that comprises five categories including the following: a. death, b. persistent vegetative state (unable to interact with the environment; unresponsive), c. severe disability (able to follow commands; unable to live independently), d. moderate disability (able to live independently; unable to return to work or school), and e. good recovery (able to return to work or school). For the purpose of this study, we differentiated outcomes into favorable (moderate disability or good recovery) and unfavorable (death, vegetative state, and severe disability) (Table II).

Prognostic Model Based on CRASH II Injury Prognostic Model

This prognostic model may be used on the estimation of mortality (during the first 14 days) and severe disability (during the first 6 months) in patients with TBI. These predictions are based on the average outcome in adult patients with TBI (GCS of 9 or less) within 6 hours of their injury, and can only support clinical judgment without replacing it.

Statistical Analysis

Frequencies and percentages or means and standard deviations were used where appropriate to describe patient characteristics for the entire group and for survivors and non survivors. *Student's t* tests were used to compare survivors and non survivors when appropriate ($p < 0.001$).

Patients

The study cohort consisted of 42 patients. The sample was separated into a control group of 18 patients and an intervention group of 24 patients. Epo administration started within 6 hours from the time of the initial injury since available preclinical data of experimental stroke suggest that Epo was much less effective if it was administered later than 6 hours after the initial injury (4). Therefore, studies concerning rhEpo administration in the setting of critical illness should begin before or immediately after admission to the ICU, in a time period of 6 hours after the injury (8).

Since no scientific evidence-based data exist in the international medical references regarding treatment with Epo for neuroprotection in TBI, we arbitrary treated our trial group patients with Epo 10,000 i.u. for 7 consecutive days and then the administration of Epo was stopped.

Table I: Inclusion and Exclusion Study Criteria

Inclusion Criteria
Age ≥ 18 to ≤ 65 years
Admission time < 24 hours since primary traumatic injury
Expectation of hospitalization ≥ 48 hours
Hemoglobin < 12 g/dL
Written informed consent from legal surrogate
Exclusion Criteria
GCS = 3 and fixed dilated pupils
History of deep venous thrombosis, pneumonic embolism or other thromboembolic event
A chronic hypercoagulable disorder, including known malignancy
Treatment with Epo in the last 30 days
Inability to give first dose of study drug within 24 hours of primary injury
Pregnancy or lactation or within 3 months post partum
Uncontrolled hypertension (systolic blood pressure of > 200 mm Hg or diastolic blood pressure of > 110 mm Hg)
Acute myocardial infarct
Expected to die imminently (< 24 hours)
Inability to perform lower limb ultrasound
Known sensitivity to mammalian cell derived products
Hypersensitivity to the active substance or to any of the additives
Pure red cell aplasia (PRCA)
Receiving hematinic agents (e.g. cyclosporin, Fe sulphate)
End-stage renal failure (receives chronic dialysis)
Severe pre-existing physical or mental disability or severe co-morbidity that may interfere with the assessment of outcome
Spinal cord injury
Treatment with any investigational drug within 30 days before enrollment

Table II: Primary and Secondary Outcome Measures

Primary Outcome Measures
Combined proportion of unfavorable neurological outcomes at 6 months: severe disability (defined as GOS-E scores 2-4) or death (GOS-E score 1)
Secondary Outcome Measures
Probability of an equal or greater GOS-E level at 6 months compared to the probability of a lesser GOS-E level, using a proportional odds model
Proportion of surviving patients with unfavorable neurological outcome (GOS-E 2-4) at 6 months
Mortality at 6 months

RESULTS

A total number of 42 patients were included in the study. Table III shows the characteristics of these patients. Most of the patients were men (88.8% in the control group and 95.8% in the intervention group). This can be justified by the fact that most of the patients in the presented study were motorcyclists or drivers of a vehicle, in contrast with women patients that were either pedestrians or co-drivers. More than half (62%) of participants in the study were included during the first 2 hours of the injury. Road traffic crashes were the most common cause of injury (83%). The mean age of the control group was 46.5±4.5 vs 29.4±1.3 of the intervention group.

The APACHE II score resulted as mean 18.6±1.1 in the control group and 21.4±0.35 for the intervention group. All patients had a GCS of less than 9 when intubated and, specifically the score was 7.2 ± 0.8 vs 4.4±0.2, regarding the control and the intervention group respectively.

All of the participants underwent computed tomography (CT) while a small percentage of 6% were scheduled for magnetic resonance imaging (MRI). A total number of 6 (18.7%) out of 42 patients died during the first two weeks; 4 of them from the control group and 2 of them from the intervention group. That means a mortality rate of 22.2% and 8.3% for the control and intervention group respectively; observation with a statistically significant difference ($p < 0.001$).

Due to the fact that hypotension was present in almost all patients, vasoconstrictors/inotropes, and specifically noradrenaline were added to the treatment. More specifically, 10 out of 18 patients in the control group vs. 21 out of 24 patients in the intervention group were treated additionally with noradrenaline for maintaining an optimum mean arterial blood pressure.

Furthermore, 10 (55%) patients from the control group suffered other severe injuries (major bone fractures, pneumothorax), as well as 15 (62.5%) of the patients in the intervention group, causing a non significant statistical difference concerning other major injuries that may contribute to the poor survival of some of the patients in the trial.

ICP was monitored in each of the two groups, resulting in high ICP in 7/18 patients in the control group and 3/24 patients in the intervention group. Decompressive craniotomy was performed in one patient in the control group as it was considered necessary by the neurosurgical team. Furthermore, surgery was considered inevitable in 8 patients of the control group and 3 patients of the intervention group.

The patients' total stay in the ICU was 35.9±11.6 days for the control group and 25.5±3.3 for the intervention group ($p = 0.263$). In addition, GOS resulted $p = 0.018$ and the GOS-E resulted $p = 0.003$ between the two groups of the study. No side effects or complications such as hypertension, thromboembolic episodes, and raised blood levels of urea or creatinine appeared from the use of Epo in the trial group during the study.

DISCUSSION

Severe TBI occurs more commonly in males (male to female ratio 4:1) and remains a major cause of death and disability. The most common cause of TBI is motor vehicle accidents (27). Furthermore, mortality rates following severe TBI remain one of the leading causes of death in the western societies. Severe TBI also represents a heavy social and economical burden (39).

At the time of collision, focal contusion and diffuse axonal injury occur because of acceleration and rotational forces, while secondary brain injury due to hypoxia, hypotension or elevated ICP is associated with worse neurological outcome (1). At the cellular level, the mechanism of brain injury causes disruption of the neuronal cytoskeleton and leads to irreversible division of the axon within 12 hours from the accident. Only some of the compounds that have been demonstrated to be neuroprotective in preclinical models have entered clinical development while some have been studied in controlled efficacy trials. For example, progesterone seems to afford protection in the immediate

Table III: General Characteristics of Study Patients

	Control group	Intervention group	P value*
Number of patients (n)	18	24	
Males (n)	16	23	
Age (years)	46.5±4.5	29.4±1.3	0.003
APACHE II	18.6±1.1	21.4±0.35	0.001
GCS intubation	7.2±0.8	4.4±0.2	0.002
Length of ICU stay (days)	35.9±11.6	25.5±3.3	0.263
GOS	3±0.3	4.4±0.3	0.018
GOS-E	4.2±0.6	6.6±0.5	0.003
CRASH II prediction at 14 days	46.3±7.3	18.5±4.5	0.007
Actual outcome-death	4/18 (22.2%)	2/24 (8.3%)	

**P value for comparison between the two groups.*

post-injury period due to its anti-inflammatory properties (12), while estrogen appears to preserve the autoregulatory function, have an antioxidant effect, reduce A beta production and neurotoxicity, reduce excitotoxicity, and increase the expression of the antiapoptotic factor bcl-2 and the activation of mitogen-activated protein kinase pathways (32).

The actions of Epo in TBI patients are not certain yet. For example, vascular effects of Epo may play some role in preserving cerebral blood flow after brain injury. Although this could be beneficial, it is probably not the major neuroprotective mechanism of Epo. The Epo-mediated neuroprotection mechanisms include direct neurotrophic (5) and antioxidant effects (13), reduction of nitric oxide-mediated injury (23), susceptibility to glutamate toxicity (21), and inflammation (35), induction of anti-apoptotic factors (39), neuroprotection by improving blood flow to the injured tissue (16), and protective effects on the glia (38). Early rhEpo also spares spatial memory and hippocampal CA1 neurons (23), while Epo-induced erythropoiesis may protect by enhancing utilization of the damaging free iron liberated by hypoxia-ischemia (28). It has been reported that both an increase and decrease in nitric oxide (NO) and NO's activity have been observed after Epo administration. *In vitro* studies with prolonged (several days) exposure to Epo increases NO's activity in endothelial cells (10), while similar *in vitro* studies suggest that acute administration of Epo can stimulate NO release (2). On the other hand, chronic administration of Epo to rats causes significant hypertension, and increases the production of NO (41). Additionally, short-term effects of Epo administration in humans suggest that Epo impairs endothelial function (36).

In the present study, we treated our trial group patients with Epo 10,000 i.u. for 7 consecutive days due to the fact that no scientific evidence-based data exist in the international literature regarding treatment with Epo for neuroprotection in TBI. It has been reported that the range of the effective neuroprotective dose of rhEpo (1,000 - 30,000 U/kg) is well above the range used to treat anaemia (≤ 500 U/Kg) (19). The timing and quantity of rhEpo was measured in cerebrospinal fluid after an intravenous dose of 5,000 U/kg in fetal sheep; thus it was demonstrated that only a small fraction (< 2%) of peak circulating rhEpo crossed the blood-brain barrier, peaking 3 hours after the injection (20). A recent study, in which rhEpo was directly measured in brain tissue taken from rats, confirmed that systemic rhEpo is only detected in the brain after the administration of high doses (34). It has been reported that intravenous administration of Epo (5,000 U/kg) to normal rats increased the concentration of Epo in cerebrospinal fluid within 30 minutes (4). Another study compared the neuroprotective dosing regimens of Epo and reported that either 3 injections of 5,000 U/kg or a single injection of 30,000 U/kg were optimal (22). Furthermore, the high-dose rhEpo treatment was found to be of benefit with no excess in adverse outcomes when evaluated in adults with middle cerebral artery stroke (10). The high doses required for neuroprotection has prompted concerns about potential unwanted adverse consequences of rhEpo. In addition, Ulusal et al. (37) investigated the dose-dependent *in vivo* effects of rhEpo on structural and morphological alterations in vasospastic arteries in an experimental rat model after aneurysmal subarachnoid hemorrhage. The authors reported that the wall thicknesses in vasospastic arteries were augmented and vessel lumen areas were diminished in EPO-administered groups when they compared the control group with the two rhEpo treatment groups. They also detected no significant difference related to the dosage of rhEpo; even intraperitoneal administration of 250 IU/kg and 500 IU/kg morphologically improved the vasospasm.

In the present study, a mortality rate of 22.2% and 8.3% for the control and intervention group respectively was found during the first 14 days of hospitalization. This observation has a statistically significant difference, indicating that the patients who received Epo up to 6 hours after TBI had a better prognosis than the patients who did not. In addition, non significant statistical differences concerning other major injuries (major bone fractures, pneumothorax) that might contribute to the poor survival of some of the patients were observed. Finally, the patients' total stay in the ICU was 35.9±11.6 days for the control group and 25.5±3.3 for the intervention group (p = 0.263), while GOS resulted p = 0.018 and the GOS-E resulted p = 0.003 between the control and the intervention group; statistically significant differences showing that all of these variables were prognostic factors for poor outcome.

Furthermore, older age predicted poor prognosis while low GCS did not predict poor prognosis. GCS did not show a clear linear relation with mortality. We were surprised that mortality in the intervention group patients with lower GCS up to 4 was lower than in control group patients with a GCS up to 7. Logical argument for this observation include increasing age in control group which was associated with worse outcomes, the changes in brain plasticity due to age, or the early use of Epo in the management of the patients of the intervention group.

What is known about the systemic effects of the early administration of Epo? Neuroprotective agents have the greatest effect when administered either before or very soon following injury. An experimental study in mice has proved the reduction of contusion volume when Epo was administered within 6 hours after injury, but not with administration at 9 hours after injury (4). Furthermore, another clinical trial has supported the significant improvement in outcome of ischemic stroke patients administered rhEpo intravenously within 8 hours of the onset of their symptoms (10). In a mouse model of TBI, the Epo was administered 1 hour and 24 hours after injury and the authors demonstrated that the improvement in neurological recovery was accompanied by reduced activation of glial cells (18). However, it is more than obvious that additional clarification of the optimal time window for Epo neuroprotection in TBI is needed.

It has been reported that long-term rhEpo treatment in the adult population is associated with hypertension, seizures,

thrombotic events, polycythemia, and red cell aplasia (7). A U.S. Food and Drug Administration (FDA) warning for the increased risk of thrombotic events and death caused by all erythropoietic agents was released for adult patients with chronic kidney failure or cancer who receive rhEpo at higher than recommended doses (11). No side effects or complications such as hypertension, thromboembolic episodes, and raised blood levels of urea or creatinine appeared from the use of Epo in our study.

Strengths and Limitations of the Study

Our study's strengths are the use of a well described cohort of patients, prospective and standardized collection of data on prognostic factors, no loss to follow-up, and the use of a validated outcome measure at a specific time after TBI. To the best of our knowledge, only a few prognostic models have been developed for patients with closed TBI who received Epo during their stay in the ICU.

However, several limitations of the presented study should be considered. The sample size was relatively small; this fact limits the statistic power and does not guarantee accurate and valid predictions. Furthermore, this study was conducted at two single centers and is therefore not validated in another cohort. In addition, the fact that the data come from a clinical trial could have limited external validity. For example, patients were collected within 6 hours of TBI and we cannot estimate the patients evaluated beyond this time. Finally, our models were developed with data from a clinical study, and validated only in patients from one country.

More research is therefore needed to obtain reliable data from these settings and further prospective validation is needed to strengthen this model. On the other hand, future and further research is needed to evaluate the doses and the time of Epo administration that reduces secondary brain injury and helps patients make a better recovery after TBI.

CONCLUSIONS

The existence of considerable experimental evidence and the supportive clinical evidence from clinical trials suggest a possible beneficial effect of exogenous Epo therapy in severe closed TBI. Although many recent studies support these treatments, clinicians should be conservative towards applying this treatment outside clinical trials due to essential concerns about their efficacy and safety. Nevertheless the high morbidity and mortality associated with severe TBI demands the urgent investigation of the safety and efficacy of these treatments.

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