

Therapeutic efficacy of tadalafil and erythropoietin in experimental spinal cord injury

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ABSTRACT

BACKGROUND: This experimental study was an investigation of the efficacy of erythropoietin and tadalafil in rats with induced spinal cord injury (SCI).

METHODS: Thirty-five Sprague Dawley rats were distributed into 5 groups. First group was used for normal biochemical values. Spinal cord injury was induced in 4 remaining groups with clip compression technique after laminectomy process to T10 vertebra. Second group was designated solvent group and received 1 cc physiological serum after injury. Third group was medicated with intraperitoneal 2000 u/kg single dose erythropoietin after injury. Orogastic 2 mg/kg single dose tadalafil was administered to fourth group after injury. Fifth group did not receive any treatment and was used for biochemical values with injury. All subjects were sacrificed 48 hours after application. Malondialdehyde (MDA) and total antioxidant capacity (TAOC) values were evaluated using blood and tissue samples.

RESULTS: Lowest serum and tissue MDA values were found in group with erythropoietin intake. While highest serum TAOC values of all groups were seen in tadalafil group, highest tissue TAOC values were observed in group given erythropoietin.

CONCLUSION: It was concluded that by decreasing oxidative stress, tadalafil and erythropoietin can inhibit secondary damage in SCI.

Keywords: Erythropoietin; neuroprotection; spinal cord injury; tadalafil.

INTRODUCTION

Though no longer thought to be hopeless, spinal cord injury (SCI) still cannot be treated efficiently with modern medicine. Therefore, it creates physical, emotional, and economic burdens for both the individual and society.^[1] Allen's 2-step injury model encouraged related studies and additional laboratory studies are ongoing, but efficient treatment for SCI has not

yet been found. In recent years, additional therapeutic effects of erythropoietin (EPO) and tadalafil have been studied aside from its routine clinical use, and promising results have been published. Presently described is research regarding effects of both agents on reducing secondary damage in cases of SCI, and discussion based on data available in the literature.

MATERIALS AND METHODS

This experimental study was conducted at the Medical and Surgical Experimental Research Center (MESERC) with the approval of the university ethics committee for animal experimentation. Total of 35 female Sprague Dawley rats weighing between 200 and 250 g were randomly divided into 5 groups. General anesthesia for the subjects was provided with intraperitoneal injection of 60 mg/kg ketamine hydrochloride (Ketalar; Pfizer, Inc., NY, NY, USA) and 12 mg/kg xylazine (Rompun, Bayer AG, Leverkusen, Germany) mixture. T10 laminectomy was performed to expose spinal cord. Clip com-

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Ulus Travma Acil Cerrahi Derg
2016;22(6):521-525
doi: 10.5505/tjtes.2016.

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TJTES

pression technique was then used to achieve experimental SCI in all groups except first group, which served as control group. Aneurysm clip providing closing force of 1.43 N (Yasargil FE 740 K; Aesculap AG, Tuttlingen, Germany) was applied to the spinal cord, and macroscopic SCI was observed (Fig. 1). Second group was designated solvent group (injury+solvent) and was given 1 cc physiological serum. Single dose 2000 u/kg EPO (Eprex 2000; Cilag AG, Schaffhausen, Switzerland) (treatment 1) was administered intraperitoneally to third group after clip application to the spinal cord. Orogastric 2 mg/kg single dose tadalafil (Cialis; Eli Lilly and Co., Indianapolis, IN, USA) (treatment 2) was given to fourth group. Fifth group received no treatment after SCI (injury group). Subjects were sacrificed using intracardiac exsanguination technique 48 hours post surgery. Serum and tissue malondialdehyde (MDA) and total antioxidant capacity (TAOC) values were measured using blood and tissue samples.

Biochemical Measures

Blood samples were centrifuged for 10 minutes at 3000 rpm to obtain serum for MDA and TAOC studies. Spinal cord segments 1 cm in length, which included injured cord area, were harvested as samples. Blood and tissue samples were transferred immediately to deep freezer for preservation at -80°C until biochemical testing was conducted. Measurement of lipid peroxide levels was performed using method described by Ohkawa et al., which consists of spectrophotometric measurement of color at 532 nm created by reaction of tiobarburtic acid with MDA in acid environment. Protein levels were expressed as nmol/mg in tissue and as nmol/mL in plasma. [2] TAOC was measured using Total Antioxidant Status kit (Mega Tıp San. Tic. Ltd. Şti., Gaziantep, Turkey) in automatic biochemical analyzer and results obtained were expressed as nmol Trolox Eq/L. [3]



Figure 1. Clip application to the spinal cord.

Statistical Method

SPSS (Statistical Package for Social Sciences) software version 15.0 (SPSS, Inc., Chicago, IL, USA) was used to evaluate study findings. Results were presented as mean±standard deviation. One way analysis of variance was applied and least significant difference test was used for group comparisons. Confidence interval of 95% and value of p<0.05 were accepted as significance thresholds.

RESULTS

Serum and tissue MDA and TAOC levels were examined in every group. Post-hoc evaluation for in-group comparison revealed serum TAOC levels were highest after tadalafil medication (p<0.0001). Minimum tissue TAOC level was seen in injury group and maximum was demonstrated in EPO group (p<0.0001). Distribution of mean TAOC values can be seen in Figure 2. Serum and tissue TAOC levels are summarized in Table 1.

Statistical evaluation indicated maximum serum MDA values were found in injury group, decreased with tadalafil treatment, and were lowest after EPO treatment (p<0.05). Tissue MDA values were highest maximum in injury group, and low-

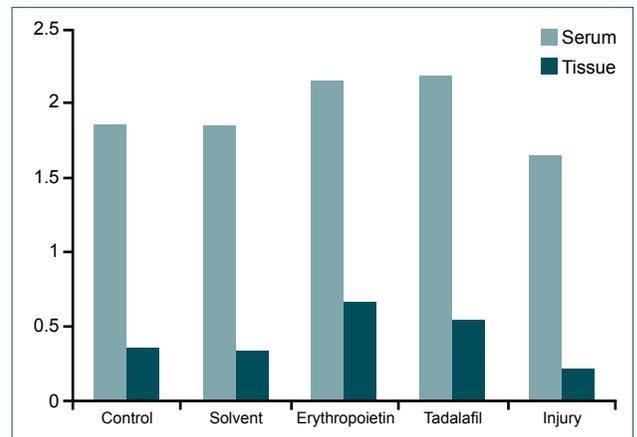


Figure 2. Serum and tissue TAOC levels.

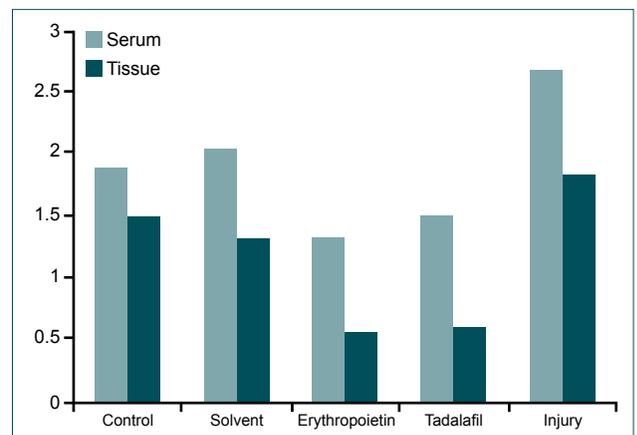


Figure 3. Serum and tissue MDA levels.

Table 1. TAOC levels by group

| | TAOC levels of serum | Statistical values |
|-----------------------|----------------------|--------------------|
| | Mean±SD | |
| Control | 1.85±0.35 | F=162.03 |
| Solvent | 1.83±0.52 | p=0.000 |
| Erythropoietin | 2.14±0.64 | |
| Tadalafil | 2.17±0.33 | |
| Injury | 1.64±0.24 | |
| TAOC levels of tissue | | |
| | Mean±SD | |
| Control | 0.36±0.44 | F=130.16 |
| Solvent | 0.33±0.02 | p=0.000 |
| Erythropoietin | 0.67±0.06 | |
| Tadalafil | 0.54±0.01 | |
| Injury | 0.22±0.03 | |

TAOC: Total Anti-Oxidant Capacity; SD: Standard deviation.

Table 2. MDA levels by group

| | MDA levels of serum | Statistical values |
|----------------------|---------------------|--------------------|
| | Mean±SD | |
| Control | 1.87±0.43 | F=2.68 |
| Solvent | 2.04±1.05 | p=0.05 |
| Erythropoietin | 1.32±0.70 | |
| Tadalafil | 1.50±1.16 | |
| Injury | 2.68±0.70 | |
| MDA levels of tissue | | |
| | Mean±SD | |
| Control | 1.49±0.62 | F=6.28 |
| Solvent | 1.31±0.73 | p=0.001 |
| Erythropoietin | 0.55±0.24 | |
| Tadalafil | 0.58±0.59 | |
| Injury | 1.83±0.65 | |

MDA: Malondialdehyde; SD: Standard deviation.

est in EPO group (p<0.05). Distribution of serum and tissue MDA mean values is provided in Figure 3. Serum and tissue levels of MDA are summarized in Table 2.

DISCUSSION

EPO is a glycoprotein hormone. It was first isolated from urine of anemic patients.^[4] Essentially, it participates in proliferation and differentiation of erythroid precursor cells.

Therefore, routine clinical use includes anemia treatment for primary patients with renal tumors and chronic renal insufficiency. EPO is produced in the kidneys^[5] and its secretion increases in response to hypoxia, reaching 50 times normal physiological values in blood.^[6]

Neuroprotective effects of EPO have been reported in recent years in addition to previously known clinical effects.^[7,8] In experimental studies done in field of SCI, EPO has been reported to be extremely effective at enhancing neurological recovery.^[9-12] In clinical studies, EPO has been demonstrated to be spinal as well as cranial neuroprotective agent. Ehrenreich et al. associated high dose intravenous EPO treatment with improvement in clinical outcome in study evaluating clinical results in cases of acute stroke at the end of first month.^[13] There are also other studies in the literature supporting neuroprotective impact of EPO in acute stroke cases.^[14]

This neuroprotective quality of EPO on central and peripheral nervous systems has been reported to occur through various mechanisms. EPO has been claimed to promote axonal regeneration to increase neoangiogenesis, to inhibit apoptosis, to have anti-inflammatory effects, to have anti-ischemic effects, to decrease microglial infiltration, to inhibit scar formation, and thereby may contribute to neurological improvement.^[15-19] Nevertheless, the exact mechanism of neuroprotection of EPO is still not certain. Even if key determinant to EPO secretion is hypoxia, EPO receptors are more sensitive to pro-inflammatory cytokines such as tumor necrosis factor-1 and interleukin-1 beta than hypoxia.^[20,21] Weak stimulation of EPO receptors as result of hypoxia, unlike stimulation of EPO, makes us think that there is a not-yet discovered mechanism for neuroprotective role of EPO.

Tadalafil augments cyclic guanosine monophosphate (cGMP) concentration by inhibiting phosphodiesterase 5 enzyme (PED5).^[22] CGMP is a strong mediator of nitric oxide pathway. In sum, tadalafil inhibits PED5 enzyme and as a consequence, increasing nitric oxide concentration causes vasodilatation.

Tadalafil is used for routine treatment of erectile dysfunction. Clinical studies supporting use for pulmonary hypertension and heart disease have also been published.^[23,24] In recent years, alongside routine clinical use, neuroprotective effect has been subject of much study.

Neuroprotective effect of tadalafil is reported to be especially efficient in brain ischemia.^[25,26] Similar efficiency has been reported in decreasing oxidative stress related to SCI.^[27] Neuroprotection mechanism of tadalafil is not yet known; neurogenesis, synaptic plasticity, and physiological modulation of neurotransmitters caused by cGMP have all been proposed.^[28-31] Most likely, tadalafil plays role in neuroprotection through effect on cGMP.

In the present study, neuroprotective effects of EPO and

tadalafil on SCI were analyzed. MDA and TAOC values, which reflect lipid peroxidation, were measured using blood and tissue samples. Most efficient decrease of serum and tissue MDA values was found in EPO-treated group. Neuroprotective effect of EPO use in SCI by decreasing MDA values is consistent with existing literature. Antioxidant effect of tadalafil in SCI was observed in finding of maximum serum TAOC values in tadalafil-treated group, which is also as previously stated in the literature. In general, all of present study data confirm what is found in the literature; EPO and tadalafil have an effect on reducing secondary damage in SCI cases. However, additional clinical and experimental studies of neuroprotective abilities of these agents are needed.

Conflict of interest: None declared.

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DENEYSEL ÇALIŞMA - ÖZET

Deneysel spinal kord travmasında eritropoetin ve tadalafilin terapötik etkinliği

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AMAÇ: Bu çalışma, spinal kord travması (SCI) oluşturulan sıçanlarda eritropoetin ve tadalafilin etkinliğini araştırmak için yapıldı.

GEREÇ VE YÖNTEM: Sprague-Dawley cinsi 35 adet sıçan beş gruba ayrıldı. Spinal kord travması T10 vertebraya laminektomi işleminden sonra klip kompresyon yöntemi uygulanarak gerçekleştirildi. Birinci grupta travma yapılmadı ve normal biyokimyasal değerler için kullanıldı. İkinci grup çözücü grubu idi ve travma sonrası 1 cc serum fizyolojik verildi. Üçüncü grup travma sonrasında tek doz 2000 ü/kg intraperitoneal yol ile eritropoetin verildi. Dördüncü gruba ise travma sonrasında tek doz 2 mg/kg oragastrik yol ile tadalafil verildi. Beşinci gruba herhangi bir tedavi verici işlem yapılmadan sadece travma uygulandı ve travmadaki biyokimyasal değerlerin tespiti için kullanıldı. Tüm denekler cerrahi işlemden 48 saat sonra desakrafiliye edildi. Kan ve doku örneklerinden malondialdehid (MDA) ve toplam antioksidan kapasite (TAOK) değerleri ölçüldü.

BULGULAR: Eritropoetin verilen grupta diğer tüm gruplara göre en düşük serum ve doku MDA değerlerine ulaşılmıştır. Tüm gruplara göre serum TAOK değerleri en yüksek tadalafil verilen grupta bulunmuş iken doku TAOK değerleri en yüksek eritropoetin verilen grupta tespit edilmiştir.

TARTIŞMA: Tadalafil ve eritropoetin doku oksidatif stresi azaltarak spinal kord travmasında ikincil hasarlanmayı engelleyebileceği sonucuna varıldı.

Anahtar sözcükler: Eritropoetin; nöroproteksiyon; spinal kord hasarı; tadalafil.

Ulus Travma Acil Cerrahi Derg 2016;22(6):521–525 doi: 10.5505/tjtes.2016.37571