Biochemical and Histopathological Effects of Catechin on Experimental Peripheral Nerve Injuries

Deneysel Periferik Sinir Hasarında Kateşin Etkisinin Biyokimyasal ve Histopatolojik Olarak İncelenmesi

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ABSTRACT

AIM: Catechin is a type of polyphenol, along with epicatechin, epigallocatechin, and epigallocatechin-gallate (EGCG). This study aims to investigate the effect of EGCG, a major metabolite of catechin, which is the principle bioactive compound in green tea, on rats with peripheral nerve injury.

MATERIAL and METHODS: A total of 74 rats were divided into six groups, namely the control, the trauma, the normal saline, a 25mg/kg EGCG, a 50mg/kg EGCG and a daily consumption group (10mg/kg EGCG was given intraperitoneally for 14 days before the trauma). Except the first group, the other groups underwent a 1-minute sciatic nerve compression by clip with 50gr/cm2 pressure. Nerve samples were obtained at 28 day after trauma for the biochemical and histopathological analysis.

RESULTS: Our study showed that the Daily consumption, 25mg/kg EGCG and 50mg/kg EGCG groups demonstrated statistically significant decreased lipid peroxidation levels and particularly daily consumption, and the 25mg/kg EGCG group showed a favourable reduction of degeneration and edema histologically.

CONCLUSION: This study shows that Catechin and its derivatives have a protective effect on peripheral nerve injury.

KEYWORDS: Axonal degeneration, Epigallocatechin-gallate, Electron microscope, Light microscope, Lipid peroxidation, Peripheral nerve injury

ÖZ

AMAÇ: Kateşinler polifenol grubunden olup başlıca kateşin, epikateşin, epigallocateşin ve epigallocateşingallat (EGKG) gibi alt grup maddeler içermektedir. Çalışmada kateşinin temel metaboliti olan EGKG’ in, periferik sinir hasarı yapılmış sıçanlardaki etkisinin gösterilmesi amaçlanmıştır.

YÖNTEM ve GERECLERLER: Toplam 74 Albino-Wistar cinsi rat kontrol, travma, normal saflar ve 25mg/kg, 50mg/kg EGCG ve günlük tüketim (travma öncesi 14 gün boyunca intraperitoneal 10 mg/kg EGKG verildi) EGKG grupları olmak üzere 6 gruba ayrıldı. İlk gruba hariç diğer gruplarda siyatik sinir örnekleri alınarak biyokimyasal ve histopatolojik olarak incelendi.

BULGULAR: Çalışmamızda günlük tüketim EGKG, 25mg/kg EGKG, 50mg/kg EGCG gruplarında istatistiksel olarak anlamlı düzeyde lipid peroksidasyonunun azaldığı ve özellikle travma-günümüz tüketim EGKG, travma+25 mg/kg EGKG gruplarında histolojik olarak da dejenerasyon ve edemin olumlu yönde azaldığı saptanmıştır.

SONUÇ: Bu çalışma kateşin ve türevlerinin periferik sinir hasarı gibi nöronal hasarlı durumlarında koruyucu etkisinin olduğu gösterilmiştir.

ANAHTAR SÖZCÜLER: Askornal dejenerasyon, Epigallocateşingallat, Elektron mikroskobu, Işık mikroskobu, Lipid peroksidasyon, Periferik sinir hasarı

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INTRODUCTION

Peripheral nerve injury causes loss in the labor force, economic loss and psychological problems. It is a topic of much interest, due to lack of available treatment options. The severity of a nerve compression lesion depends on several factors, including compression pressure, length of pressure, and the area which the pressure affects. Neuronal tissue damage continues after the compression is resolved (25). Compression injury to the nerve induces Wallerian degeneration and axon regeneration, whereas after central nervous system (CNS) injury axons fail to regenerate. These findings were first described by Ramon Y. Cajal under light microscope examinations in 1928 (33). Similarly, the process has also been shown by electron microscopic studies. The post-traumatic nerve tissue changes, observed by electron microscope, include intracytoplasmic edema, and changes in the nucleus, mitochondria, axon and myelin sheath (24).

Similar to the blood–brain barrier in the CNS, there is a blood–nerve barrier in the peripheral nervous system. The blood–nerve barrier regulates the endoneurial microenvironment. Many studies have examined the effect of local experimental compression and intraneural microcirculation, and concluded that 20 – 30 mm Hg external pressure induces a blockage of venous blood flow in the epineurium. Pressure of 80 mm Hg leads to complete cessation of intraneural blood flow (19).

Injury to a peripheral nerve triggers an initiation of a response that incorporates a sequence of biochemical alterations. Severe injury can lead to neuronal edema, more intense neutrophil infiltration, and apoptosis. Increased neutrophil infiltration, myeloperoxidase activity, and the level of tissue malondialdehyde (MDA) lead to an increased level of lipid peroxidation (15). Lipid peroxidation is a toxic process and a self-propagating chain-reaction (6, 15). Lipid peroxidation can impair membrane function directly and damage cell components indirectly. A marked increase in lipid peroxidation is observed after injury. Hall and Braugler reported that lipid peroxidation increases to a peak level at 1, 24 and 48 hours after spinal cord trauma (17).

The tea plant, Camellia sinensis, is a member of the Theaceae family (4, 14, 37). Tea leaves contain polyphenol and polyphenol oxidase enzymes. The main component of green tea extract polyphenol is called catechins. Black tea contains 250 mg/L of catechin, compared to green tea which contains 420 mg/L (40). Approximately 25-35% of the dry weight of tea leaves is catechins. A single cup of green tea contains 100-200 milligrams of catechin (39, 41). The anticancer, antitumor, antimutagenic, chemopreventive, antiproliferative, antiinflammatory, antioxidant, anti diabetic, antiallergic, antihypertensive, antiplatelet, antiobesity, hypcholesterolemic, and neuroprotective effects of catechins have been shown in various in vivo and in vitro studies (36).

The flavonoids contain a double bond and eight isomers. The main catechin group consists of eight polyphenolic flavonoid-type compounds, namely, catechin (C), epicatechin (EC), gallo-


**Standard Preparation**

**Anesthesia and Surgical procedure:** The rats, which were left hungry for one night, were weighed, and anaesthetized with Xylocaine (Rompun®, 2% solution, Bayer, Istanbul, Turkey) 10mg/kg and Ketamine Hydrochloride (Ketalar®, 5% solution, Parke Davis-EWL, Eczacibasi, Levent, Istanbul, Turkey) 50 mg/kg intraperitoneally prior to surgery (42). Rats were placed in the prone position, and the sciatic nerve, going under the gluteus maximus muscle, was explored through a longitudinal skin incision, at the level of the greater trochanter, proximal to the right lower extremity, and dissected by avoiding tractional damage. Neural injury was produced by compression of the sciatic nerve of each rat for 1 min using an aneurysm clip (Yasargil FE 693 temporary aneurysm clip, Aesculap) with a closing pressure of 50 g/cm² for axonotmesis nerve injury. Then the incision was closed anatomically. Four weeks later, all rats were anesthesized, and specimens of the damaged sciatic nerves, including 0.5 cm proximally and distally nerve segments, were collected from the prior incision sites. At the end of the study period the rats were sacrificed by administering phenobarbital.

**Biochemical examination:** The lipid peroxidation value was calculated for each rats in terms of nanomoles per gram of tissue.

**Examination by light and electron microscope:** Tissue samples were minced into 1 mm-3 pieces, and fixed in 0.1 M phosphate buffer (pH 7.4) containing 2.5% glutaraldehyde for 2 hours. The samples were washed 3 times with buffer, and 1% osmium tetroxide was used for post fixation. The fixed tissues were dehydrated in alcohol. Finally, the tissues were treated with propylene oxide and then mounted as tissue blocks using the Araldite CY212 kit. The tissues were then polymerized at 56°C in an incubator for 48 hours, and the cured block was trimmed and made into semi-thin sections and stained in Toluidine Blue solution to be examined with a light microscope. Thin-sections, acquired from marked areas, were stained by uranyl acetate and lead citrate, and observed with Carl Zeiss EVO LS 10+ ED transmission electron microscope (TEM), and were illustrated by appropriate magnifications.

Histopathological specimens were examined by light microscopy, and the changes in myelinated fibers were assessed by histological scoring (Table I). 12 or more microscopic fields were selected at random from sciatic nerve of each rat and degenerated axons were counted according a protocol which starts from the first right corner of the rectangular field to the last left corner. All samples were evaluated by 2 independent histopathologists blind to the present study.

**RESULTS**

**Biochemical Results**

However, there was a significant difference between group 2 and 3 as well as groups 4, 5 and 6 (p<0.05). In other words, EGCG administered in daily consumption and in therapeutic doses decreased the level of lipid peroxidation in a statistically significant manner (Figure 1).

**Histological Results**

**Light microscopy results:**

The samples were harvested from rats in all groups at traumatized area. In Group 1, Schwann cells, and myelinated and non-myelinated nerve fibers were evaluated. Duplication and undulation of the myelin sheath, axonal degenerations (axon withdrawal) and nerve fibers with severe degeneration were assessed.

<table>
<thead>
<tr>
<th>Histology Score</th>
<th>Grade</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>Normal</td>
</tr>
<tr>
<td>1</td>
<td>Duplication of the myelin sheath</td>
</tr>
<tr>
<td>2</td>
<td>Undulation of the myelin sheath</td>
</tr>
<tr>
<td>3</td>
<td>Axonal degenerations (axon withdrawal)</td>
</tr>
<tr>
<td>4</td>
<td>Nerve fibers with severe degeneration</td>
</tr>
</tbody>
</table>

**Table I: Histological Grading Score of Nerve Injury**

**Figure 1:** Multiple Comparison of Lipid Peroxidation Values between the groups Trauma, trauma + saline > control, 10mg/kg, 25 mg/kg, 50 mg/kg respectively.
withdrawal of myelinated nerve fibers, Schwann cell hypertrophy, and widespread tissue edema were detected in Group 2 (Figure 2A, B). Group 3 had similar results to group 2.

In trauma groups was seen that qualitatively, satic nerve injury consisted of Wallerian degeneration characterized by variable degree of degeneration in great myelinated axons and endoneural edema. Group 4 and Group 6 showed withdrawal of myelin sheath, duplication of myelin sheath, and undulation especially in great myelinated nerve fibers, as in the trauma group. Although these groups also had Schwann cell hypertrophy, edema in myelinated nerve fibers was not observed in these groups. Group 5 showed decreased duplication in myelinated nerve fibers, undulated appearance and Schwann cell hypertrophy, which were observed in the trauma group (Group 2) (Figure 3A, B).

Light microscopy observations showed that based on the distribution of groups related to histopathological changes of myelinated fibers, multiple comparison procedure indicated a statistically significant decrease in myelin sheath separation, myelin sheath undulation and axonal degeneration in Group 4 and Group 6 compared to Group 2 and Group 3 (p<0.05).

In Group 5, intensive degeneration in myelin and axons were found to be significantly higher compared to other groups (p<0.05).

**Figure 2:**

A) Sciatic nerve semi-thin light microscopy sections of normal structured myelinated nerve fibers (4) as well as hypertrophic Schwann cells (â), myelinated sheath duplication (¡) and undulation (é), withdrawal of axonal structures on some myelinated fibers (8), and generalized edema throughout the tissue (+) is seen (Toluidine Blue x400).

B) Figure of the same group showing normal structured myelinated nerve fibers (4), duplication in myelinated sheath (¡) and undulation (é), withdrawal of axonal structures on some myelinated fibers (8), and edema of nerve fibers (+) is seen (Toluidine Blue x400).

**Figure 3:**

A) The sciatic nerve semi-thin light microscopy sections in the myelinated nerve fibers showing a decrease of the duplication (ª) and undulated appearance (ê) but the myelin sheath is sheltering the axon in some areas (u) and forming blebs ('') through axoplasm (Toluidine Blue x400).

B) Semi-thin sections of the same group showing the normal observed structure of myelinated nerve fibers (4), myelinated sheath duplication (¡), undulated appearance (ê) and intense degeneration (u) of axonal structures (Toluidine Blue x400).

fibers with small diameter, whereas great myelinated nerve fibers showed myelin sheath separation, axon withdrawal, and swelling and cristolysis in the mitochondria. The cells were hypertrophic. Myelinated nerve fibers were in normal structure through the tissue. Edema, which was observed in trauma groups, was not detected in endoneurium (Figure 6A, B).

Finally, application of the appropriate doses of catechin was observed to suppress endoneurial edema and increase of collagen fibers and wallerian degenerations (Table III) caused by trauma. Although catechin was observed to reverse undulation and myelin duplication in myelinated nerve fibers, it was considered insufficient to prevent prominent degeneration in myelinated nerve fibers with great diameter.

Table III: Distribution of Wallerian Degenerations Measurement in All Groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>The Number of Axons with Wallerian Degenerations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (control group)</td>
<td>7</td>
</tr>
<tr>
<td>Group 2 (trauma group)</td>
<td>21</td>
</tr>
<tr>
<td>Group 3 (saline group)</td>
<td>25</td>
</tr>
<tr>
<td>Group 4 (25 mg/kg EGCG group)</td>
<td>32</td>
</tr>
<tr>
<td>Group 5 (50 mg/kg EGCG group)</td>
<td>26</td>
</tr>
<tr>
<td>Group 6 (daily consumption group (10 mg/kg EGCG)</td>
<td>26</td>
</tr>
</tbody>
</table>

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Injury to peripheral nerves leads to microvascular damage, which can cause to endoneural edema and promote the increase of endoneural fluid pressure. The removal of compression lead to the resumption of blood flow into nerve cells which not only provide nutrients, including oxygen, but also increases the formation of free oxygen radicals and lipid peroxidation, and this process is called reperfusion injury (24). The central and peripheral nervous systems are rich in antioxidants have potentials to counter the tissue-damaging effect of the inflammatory response. In our study, EGCG, a polyphenol with antioxidant properties, possessed substantial antioxidant activity measured by several biochemical assays in a rat sciatic nerve damage model at daily consumption dose (10mg/kg) and treatment doses (25mg/kg and 50mg/kg), but dense degeneration was detected particularly in axon and myelin sheath with the treatment dose of 50mg/kg.

DISCUSSION

If the compression pressure on peripheral nerves is higher than capillary perfusion pressure, oxidative stress-induced cell impairment develops after ischemia (9, 24). Antioxidants have potentials to counter the tissue-damaging effect of the inflammatory response. In our study, EGCG, a polyphenol with antioxidant properties, possessed substantial antioxidant activity measured by several biochemical assays in a rat sciatic nerve damage model at daily consumption dose (10mg/kg) and treatment doses (25mg/kg and 50mg/kg), but dense degeneration was detected particularly in axon and myelin sheath with the treatment dose of 50mg/kg.

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myelin, a substance rich in lipids. Release of oxygen-free radicals activated by ischemic-reperfusion injury triggers lipid peroxidation and accelerates tissue damage (34). The products of lipid peroxidation can further damage membrane proteins, including membrane-bound receptors and enzymes by increasing membrane permeability for ions (37). Damage to cell membranes causes ion imbalances, allows extracellular calcium to enter the cell, and leads to edema and necrosis.

Free radicals induce oxidative stress, which is balanced by the body's endogenous enzymatic and nonenzymatic antioxidant systems. The most efficient enzymatic antioxidants involve glutathione peroxidase, catalase and superoxide dismutase, whereas nonenzymatic antioxidants include vitamins and minerals. The reaction involves the catechin losing a hydrogen atom to a reactive free radical. As chain-breaking antioxidants, tea catechins are thought to interrupt deleterious oxidation reactions (12). Similarly, catechins chelate metal ions such as copper and iron to form inactive complexes and prevent the generation of potentially damaging free radicals (3). Also, catechins have been found to inhibit lipooxygenase and cyclooxygenase activity, enzymes which are capable of increasing oxidative stress or damage in some tissues (18, 21).

Catechin appeared to limit neuronal damage. It was shown that 6-hydroxydopamine-induced nuclear factor-kappaB activation and cell death was attenuated by tea extracts in neuronal cultures (23). EGCG, helps to prevent ethanol-associated apoptosis in fetal rhombencephalic neurons, and presents anti-apoptotic effect on liver (22, 32). Sutherland et al. found in their study that catechins' actions of attenuating oxidative stress and the inflammatory response may account for their confirmed neuroprotective capabilities following cerebral ischemia. It was found that EGCG had a dose-dependent response with 25 and 50 mg/kg eliciting significant neuroprotection (36). Rahman et al. reported that EGCG might be an appropriate intervention for the treatment of acute cerebral ischemia (32). Chaturvedi and colleagues said that black tea extract might slow progression of Parkinson disease, a chronic, progressive degenerative disorder (5). Bastianetto et al. showed the protective effect of catechin derivatives in black tea on amyloid-dependent neurotoxicity (2).

Experimental research with mice has shown that EGCG causes severe hepatic necrosis and 67% mortality when given daily at 50mg/kg ip. In fact, clinical preparations of tea extracts have also exhibited hepatotoxic effect (39). In contrast, there is evidence that purified green tea extracts in vivo are hepatoprotective. These results suggest that the route and method of administration may determine whether catechins induce hepatotoxicity or have hepatoprotective effects (39).

In the present study, comparison of biochemical values in tissue samples demonstrated that lipid peroxidation levels in treatment and protection groups, namely Groups 4, 5 and 6, were lower at a statistically significant level than that of Group 2 and 3, but were close to the values of Group 1. EGCG attenuates secondary ischemic damage that follows peripheral nerve injury as it was shown by Sutherland et al. that EGCG also reduces neurodegeneration associated with cerebral ischemic process.

Detailed histopathological examination including myelin sheath separation, ondulation of the myelin sheath, axonal degeneration, and intensive degeneration in myelins and axons were performed. Particularly these values in Group 4 and 6 were found to be lower as compared to that of Group 2 and 3. Intensive degeneration in myelins and axons detected in the treatment group, Group 5, indicated to increased degeneration. As a matter of fact, Sutherland et al. reported that the effective dose of EGCG for ischemia protection and early treatment of cerebral ischemia was 25-50 mg/kg, however, since EGCG given at this dose needs to cross the blood-brain barrier to be effective, lower doses of EGCG may also have both therapeutic and toxic effects in a dose-dependent manner, in well vascularized tissues such as peripheral nerves.

**CONCLUSION**

Our study showed that EGCG, might be effective in the prevention and treatment of secondary damage related to peripheral nerve injury in daily consumption and low-dose treatment groups. Nevertheless it might induce degenerative process particularly when given at a high dose which necessitates dose dependent further investigations. Despite the absence of concrete correlation of histological recovery and neurological findings, the present study demonstrated that EGCG might have inhibitory effects on neurodegeneration and potentially neuroprotective which might be a critical guide to future studies in this field.

**REFERENCES**