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Neuromuscular Dysfunction in Experimental Sepsis and Glutamine

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Background: Electrophysiological studies show that critical illness polyneuromyopathy appears in the early stage of sepsis before the manifestation of clinical findings. The metabolic response observed during sepsis causes glutamine to become a relative essential amino acid.

Aims: We aimed to assess the changes in neuromuscular transmission in the early stage of sepsis after glutamine supplementation.

Study Design: Animal experimentation.

Methods: Twenty male Sprague-Dawley rats were randomized into two groups. Rats in both groups were given normal feeding for one week. In the study group, 1 g/kg/day glutamine was added to normal feeding by feeding tube for one week. Cecal ligation and perforation (CLP) surgery was performed at the end of one week. Before and 24 hours after CLP, compound muscle action potentials were recorded from the gastrocnemius muscle.

Results: Latency measurements before and 24 hours after CLP were 0.68±0.05 ms and 0.80±0.09 ms in the control group and 0.69±0.07 ms and 0.73±0.07 ms in the study group ($p<0.05$).

Conclusion: Since enteral glutamine prevented compound muscle action potentials (CMAP) latency prolongation in the early phase of sepsis, it was concluded that enteral glutamine replacement might be promising in the prevention of neuromuscular dysfunction in sepsis; however, further studies are required.

Keywords: Experimental, glutamine, neuromuscular, sepsis

Neuromuscular dysfunction (NMD) observed in intensive care unit patients leads to difficulty in "weaning" from mechanical ventilation and is associated with high rates of mortality and morbidity. Myopathic changes termed critical illness myopathy (CIM) also occur in some cases in addition to critical illness polyneuropathy (CIP) which is considered to be a neurological component of sepsis and characterized by demyelination and axonal degeneration (1). The frequency of these neuromuscular disorders, which are known as critical illness polyneuromyopathy (CIPNM) in systemic inflammatory response syndrome (SIRS) and sepsis patients, ranges from 50-70%. CIPNM is clinically diagnosed only in the late stages of the disease. However, electrophysiological studies show that the findings of CIPNM appear in the early stage of sepsis before the manifestation of clinical findings (2,3). Decreased amplitude, latency prolongation and a decline in nerve conduction velocity are the electrophysiological findings that call attention in the early phase of sepsis (2,3).

Unavoidable catabolic stress seen in sepsis is associated with high levels of oxidative stress reduction in protein mass and antioxidant capacity, intestinal immune barrier dysfunction and immunodeficiency. Amino acids which are stored and released from the skeletal muscles when required, in particular glutamine, which is synthesized and stored in the skeletal muscle, are depleted during the synthesis of acute phase proteins (C-reactive protein, alpha-1-acid glycoprotein, fibrinogen etc.) and gluconeogenesis.

A reduction is seen in muscle mass, muscle strength and muscle activity due to erosion in the skeletal muscles as well as the depletion of the amino acids (4,5). Electromyographic studies and muscle biopsies demonstrate that myosinolysis occurs along with necrosis and muscle atrophy in type II fibers (6-8).

This study was presented as an oral preseantation at the 34th Congress of the European Society of Clinical Nutrition and Metabolism (ESPEN 2012), 8-11 September 2012, Barcelona, Spain.

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Glutamine, which is synthesized in the skeletal muscle via the enzyme glutamine synthetase, is the most abundant non-essential amino acid found in the body and stored within the skeletal muscles. The metabolic response observed during sepsis causes glutamine to become a relatively essential amino acid (9). While skeletal muscles decrease in sepsis, depletion and requirement of glutamine increase (9,10). In addition, glutamine levels in skeletal muscle reduce.

The decreased glutamine level in the striated muscles causes intracellular shrinkage and dryness in the tissues of the striated muscles, causing changes in anabolic and catabolic behaviors of the cell and activation of Adenosine monophosphate-activated protein kinase (AMPK) (11,12).

It has been highlighted with greater emphasis in recent years that metabolic and especially nutritional factors need to be investigated with regard to the development and prevention of NMD in the sepsis associated with increased protein catabolism and loss of striated muscle mass.

Therefore, in our study, our aim was to assess changes in the neuromuscular transmission that develop in the early stage of sepsis with enteral glutamine replacement by using electrophysiological recordings.

MATERIALS AND METHODS

The study was initiated after obtaining approval from the Animal Ethics Committee of Ege University School of Medicine.

Before and during the study, all of the rats were housed in cages in an acclimatized room at standard room temperature by allowing free access to water and standard chow with 12 hr light/dark cycles.

During the procedures of electrophysiological recordings and orogastric tube (feeding tube) insertion, rats were anesthetized using intraperitoneal (IP) injection of Ketamine 100 mg/kg and IP injection of Xylazine 10 mg/kg.

The study was performed on 20 adult Wistar rats: 10 male and 10 female rats, each weighing approximately 250 g rats were randomly divided into 2 equal groups.

Groups

Group I (n=10): Sepsis group (cecal ligation and puncture (CLP) surgery + normal feeding) Rats in group 1 were given normal feeding and CLP surgery was performed under anesthesia at the end of week one.

Group II (n=10): Sepsis+glutamine group (CLP surgery + normal feeding + 1 g/kg/day enteral glutamine); (Glutamine Resource, Nestle)

To maximize glutamine stores prior to performing the CLP procedure, rats in group 2 were given 1g/kg/day enteral glutamine by orogastric tube (feeding tube) for one week in addition to normal feeding and then CLP surgery was performed under anesthesia.

Constitution of sepsis model

Sepsis was induced by the CLP model which was performed beforehand in different researches (13,14). After the administration of anesthesia under septic conditions, a 2 cm midline incision was made. After laparotomy, the cecum was ligated with a 3.0 silk suture at its base and perforated once with a 22-gauge needle. The cecum was gently squeezed to extrude a small amount of feces from the perforation site. Then, the cecum was returned to the peritoneal cavity and the laparotomy incision was closed with 3.0 silk sutures. During the procedure, all of the rats were resuscitated using an IP injection of 10 mL/kg of normal saline.

Electrophysiological recordings

Electrophysiological recordings of the rats under anesthesia were obtained from the left sciatic nerve stimulated supramaximally at the notch (intensity 10 V. duration 0.1 ms. frequency 1 Hz) (Stimulator: SS2L Electrode and BSLSTMA Trigger BIOPAC System. Inc.; Santa Barbara, USA). HSTM01 superficial disc electrodes (BIOPAC System Inc., Santa Barbara, USA) were used for electrical stimulation. Compound muscle action potential (CMAP) of the gastrocnemius muscle was monitored (Figure 1-4).

Compound Muscle Action Potentials are accepted motor and sensory components of peripheral nerves. It is used as an electrodiagnostic test in animals, where amplitude, latency and duration are evaluated (2,3,15-18).

Latency, amplitude and total duration parameters of CMAP were calculated using the Biopac Student Lab Pro software (BIOPAC Data MP35 Acquisition System; BIOPAC Systems Inc; Santa Barbara, USA) and displaying them in the digital environment. The mean value of five CMAP waves calculated for each rat was taken (Figure 1-4).

Changes in CMAP values (latency, amplitude and duration) within each group before and after laparotomy at the end of the study were evaluated in the Department of Biostatistics and Medical Informatics in Ege University School of Medicine. “Analysis of variance” was used for repeated measures, the “t-test” was used for the comparison of two groups with independent baseline values, and the “paired t-test” was used for the evaluation of change from the baseline values in each of the groups. A value of $p<0.05$ was considered to be statistically significant.

PASW Statistics 18 (formerly SPSS Statistics, SPSS Inc.; Rm 1804, 18/F, Westlands Centre, Westlands Road, Quarry Bay, Hong Kong) was used.

RESULTS

Two rats died at the end of the first 24 hours in both groups.

There was no statistically significant difference in the weights of the rats in all groups.

(Observed power=54.1%).

While preoperative CMAP latency was 0.68±0.05 ms in the sepsis group, it was found to be 0.80±0.09*ms at the end of 24 hours after surgery, and the difference was statistically significant ($p<0.05$) (Table 1, Figure 1, 2).

While preoperative CMAP latency was 0.69±0.07 ms in the glutamine group, it was found to be 0.73±0.07 ms at the end of 24 hours after surgery, and the difference was not statistically significant (Table 1, Figure 3, 4).

While preoperative CMAP amplitude was 10.05±1.05 mV in the sepsis group, it was found to be 9.89±0.97 mV at the end of 24 hours after surgery and the difference was not statistically significant (Table 1, Figure 1, 2).

While preoperative CMAP amplitude was 10.22±0.90 mV in the glutamine group, it was found to be 10.26±0.77 mV at the end of 24 hours after surgery, and the difference was not statistically significant (Table 1, Figure 3, 4).

While preoperative CMAP duration was 8.40±1.53 ms in the sepsis group, it was found to be 8.38±0.86 ms at the end of 24 hours after surgery, and the difference was not statistically significant (Table 1, Figure 1, 2).

While preoperative CMAP duration was 9.24±0.59 ms in the glutamine group, it was found to be 9.43±0.50 ms at the end of 24 hours after surgery, and the difference was not statistically significant (Table 1, Figure 3, 4).

During the measurements performed at the end of 24 hours, as expected in Group I, it was observed that CMAP latency prolongation was statistically significant, whereas there was no statistically significant difference in Group II in which enteral glutamine replacement was performed (Table 1, Figure 1-4).

TABLE 1. Measurements of compound gastrocnemius muscle action potentials

	Latency (ms)		Amplitude (mV)		Duration (ms)	
	0^{th} hour (n=10)	24^{th} hour (n=8)	0^{th} hour (n=10)	24^{th} hour (n=8)	0^{th} hour (n=10)	24^{th} hour (n=8)
Group I	0.68±0.05	0.80±0.09*	10.05±1.05	9.89±0.97	8.40±1.53	8.38±0.86
Group II	0.69±0.07	0.73±0.07	10.22±0.90	10.26±0.77	9.24±0.59	9.43±0.50

All values are mean±SD for each group.
*p<0.05 (compared to baseline value).
ms: millisecond; mV: milivolt

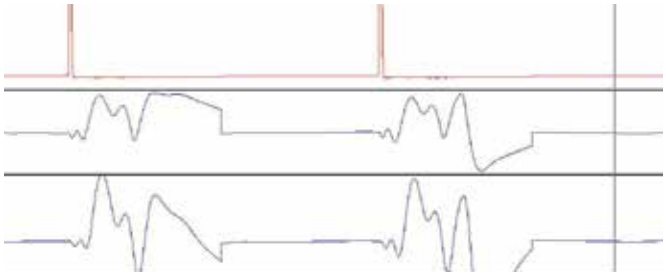

FIG. 1. A sample of compound muscle action potential recorded 24 hours before sepsis in Group I

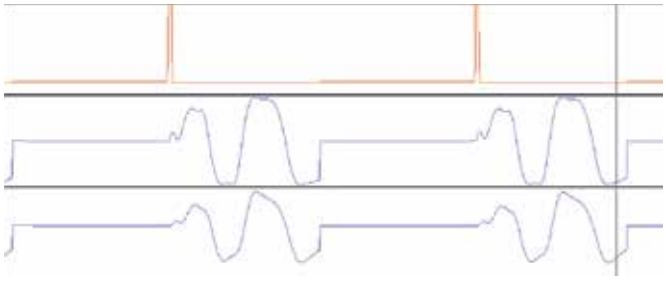

FIG. 2. A sample of compound muscle action potential recorded 24 hours after sepsis in Group I

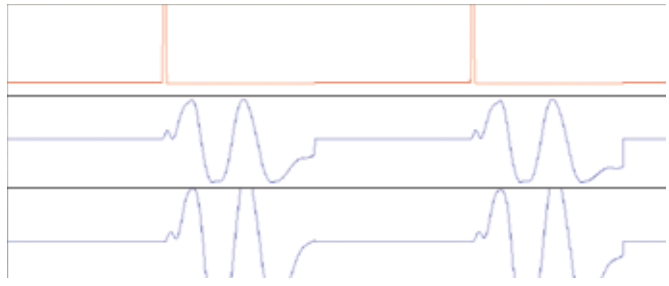

FIG. 3. A sample of compound muscle action potential recorded 24 hours before sepsis in Group II

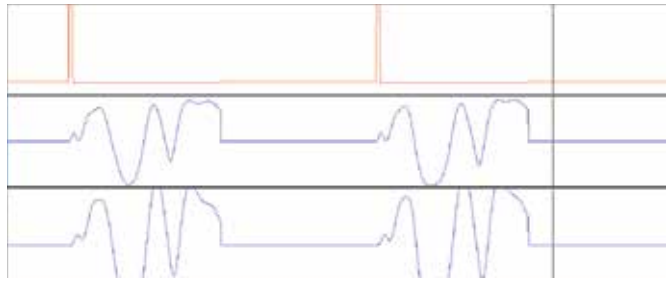

FIG. 4. A sample of compound muscle action potential recorded 24 hours after sepsis in Group II

DISCUSSION

Critical illness polyneuropathy resulting from disturbances in microcirculation and auto-regulation of peripheral sensory/motor nerves in a background of SIRS and sepsis is associated with primary axonal neuropathy and is considered to be an important cause of neuromuscular dysfunction seen in sepsis (6,19-21). Cytokines released during sepsis also cause an increase in capillary permeability due to a histamine-like effect (20,21). The resulting endoneural edema leads to hypoxia and energy deficit by deteriorating diffusion in the intercapillary space since the axonal transportation of structural proteins is highly energy-dependent. This energy deficit induces primary axonal degeneration of the distal nerves (20). Bolton et al. (21) suggested that the release of tumor necrosis factor (TNF), arachidonic acid, and metabolites of histamine, which are considered to be the principal factors responsible for systemic effects of sepsis and SIRS, as well as complement activation cellular adhesion systems, proinflammatory cytokines and free oxygen radicals might lead to primary axonal degeneration. Also, muscular dysfunction most often accompanies neuropathic changes seen in the sepsis. It is suggested that myopathic changes described as CIM and considered an important cause of muscle weakness develop due to denervation secondary to polyneuropathy and primarily reduction in striated muscle mass and muscle fibers (6).

Although CIP and CIM, which are considered to be major causes of muscle weakness in sepsis, can be found independently from each other, they are mostly seen together; this picture is termed CIPNM (1,6,22,23).

Neuromuscular dysfunctions described as CIPNM considered to be developing in an inflammatory and catabolic background are frequently accompanied by a disruption of the balance between the protein synthesis rate and the protein degradation rate, decreased mitochondrial efficiency, decreased neuronal dysfunction in central/peripheral nervous system, inflammation, inactivation, and sleep patterns (12).

Approximately 17% of the total body protein is lost in the patients due to catabolic response developing in sepsis. Sepsis-induced protein loss basically indicates erosion in the skeletal muscles (24,25).

Along with the increase in protein degradation in sepsis after unavoidable catabolism, protein synthesis also decreases due to the reduction in amino acid concentration. The amount of protein synthesis becomes limited with the use of decreased amino acids. Amino acid concentration, particularly glutamine, decreases in the skeletal muscles and plasma (26). The amount of intracellular amino acyl-tRNA transferase enzyme, which is responsible for protein synthesis of amino acids in ribosomes, is decreased in sepsis (25).

Degeneration of the skeletal muscles due to unavoidable protein loss causes a reduction in muscle mass, strength and activity (4,5). Neuromuscular dysfunction becomes inevitable after a reduction in striated muscle mass.

It is suggested that the reduction in myofibers and sarcoplasmic proteins constituting fast-twitch fibers in striated muscles due to protein catabolism in sepsis is accompanied by myofiber atrophy, myosinolysis, muscle fiber necrosis and atrophic findings in contractile myosin filaments (7,27).

Axonal degeneration that develops in sensorial and motor nerves manifests itself as the prolongation of the latency observable in electrophysiological studies. On the other hand, CIM, another condition causing neuromuscular dysfunction and contributing to muscle weakness by accompanying CIP in sepsis, can only be diagnosed histologically. However, the decrease observed in CMAP amplitude is associated with prolongation of CMAP duration during electrophysiological recordings performed in the early stage of sepsis, and calls attention to muscle fiber membrane in the pathophysiology of CIPNM (20). It has been suggested that the decrease in CMAP amplitude and prolongation in CMAP duration can develop secondary to the dysfunction of energy-dependent sodium-potassium pumps in the muscle (12). This energy change observed in muscle fibers seems to be similar to hibernation. In animals, during hibernation period, a decrease in striated muscle tension occurs at a rate of 23%. Changes in intracellular amino acid metabolism, evident in glutamine, and a reduction in striated muscle mass are suggested to be similar to sepsis. Shrinkage occurring in the striated muscle cells resulting from dehydration due to decreased intramuscular glutamine concentration is suggested to cause changes in catabolic/anabolic behaviors in the striated muscle cells. Increased catabolism and a decline in intramuscular glutamine concentration lead striated muscle cells to decrease. Glutamine deficiency also has negative qualitative effects. Decreased intracellular glutamine concentration in the striated muscle causes cellular decrease and shrinkage. In the studies performed, cellular glutamine concentration is suggested to be a triggering factor in the response of catabolic/anabolic behavior models of the cells (11,12,28).

It has been reported that decreased intracellular glutamine concentration is a necessary prerequisite for AMPK, which triggers the cellular energy program of intracellular glutamine and protein metabolism in the catabolic state (12). The available findings indicate that the decreased striated muscle and plasma glutamine levels in sepsis may be a remarkable reason for the development of neuromuscular dysfunction, and glutamine replacement in sepsis may also have positive effects regarding the prevention of neuromuscular dysfunction in addition to accepted favorable contributions as an immuno-nutrient.

Striated muscles are considered to be the major tissue for glutamine homeostasis, which relatively becomes an essential amino acid in the sepsis. Since 80% of the total body amino acid pool is in the striated muscles, when protein catabolism increases in sepsis, glutamine is released from the skeletal muscles elementarily. While glutamine consumption/requirement increases, an unavoidable decrease in glutamine level and mass of the striated muscle is observed (10,29).

Following endotoxin injection, it is observed that amino acid concentrations in the muscle and plasma decrease rapidly. It was shown that the release of glutamine from the muscle into plasma also increased simultaneously with the decrease in the plasma level of glutamine (10). Despite the rate of glutamine release from the muscle into plasma increasing during sepsis, the decrease in the plasma levels of glutamine cannot be prevented. Although the release of glutamine from the muscle tissue into the plasma increases, the basic reason for the decrease in plasma concentrations of glutamine is considered to be the increase of glutamine utilization in splanchnic tissues. After major surgical interventions in sepsis, it was demonstrated that glutamine uptake in the splanchnic tissues increased by up to 50-100% (10).

Glutamine is the most abundant non-essential amino acid found in the tissue and plasma (27,29-31). It also serves as an important precursor for biosynthetic events. It increases gluconeogenesis and protein synthesis. It plays an important role as a carrier of nitrogen and carbon. Glutamine inhibits protein degradation and is an important precursor for glutathione synthesis (32-34). Due to the increased glutamine requirement in sepsis, the non-essential amino acid glutamine relatively becomes an essential amino acid. In sepsis, glutamine that is mobilized from the striated muscles cannot meet the requirements and exogenous glutamine replacement is needed (9).

In addition, it was also reported that 11-40 g/day enteral glutamine supplementation in critically ill patients decreased morbidity and mortality (35). Similarly, parenteral glutamine supplementation at the dose of 0.3-0.5 g/kg/day decreased hospital stay, infectious complications and MOF (36).

With acute and chronic replacement of enteral glutamine, it was shown that plasma levels of glutamine were maximal in the acute phase and especially in the first 30 and 45 minutes, but during the long-term replacement, the amount of glutamine stored in the liver and particularly in the gastrocnemius muscle increased without any change in plasma levels of glutamine. However, the extent to which this increased level of glutamine in the gastrocnemius muscle as a result of chronic glutamine replacement could affect the neuromuscular transmission disorder developing in the early stage of sepsis was not studied and there was no experimental model investigating how this change was reflected in the neuromuscular transmission disorders.

In our before experimental sepsis model, we showed that CMAP latency prolongation and decreased CMAP amplitude were the earliest electrophysiological findings and that they appeared in the first 24 hours of sepsis. (2) Similarly, as supported by our study findings, latency prolongation in the sepsis group seems to be statistically different (Table 1, Figure 1, 2).

Also in malignancies, glutamine stores are depleted similar to in sepsis due to metabolic stress and increased cell proliferation. In addition to known positive features of glutamine, experimental and clinical studies performed in malignant diseases with decreased blood and tissue levels of glutamine demonstrate that glutamine has a neuroprotective feature. It is suggested that glutamine exerts this effect with upregulation in Nerve Growth Factor (NGF) nRHA (37,38). Peripheral neuropathies that develop in patients with malignant disease treated with neurotoxic chemotherapeutic drugs were shown to deteriorate in parallel with the decrease in the serum level of NGF (37,39). Glutamine is also known to be protective against cardiac toxicity and neurotoxicity caused by chemotherapeutic agents. Glutamine particularly prevents peripheral neuropathy developing in the patients with metastatic breast cancer who receive stem cell transplantation and high-dose paclitaxel (37,40,41). Furthermore, metabolites arising from glutamine metabolism were reported to prevent polyneuropathies induced by chemotherapeutic agents (42). Similar to the prevention of peripheral neuropathies developing due to chemotherapeutic agents by glutamine, the absence of increase in CMAP latency of the group in which glutamine replacement was performed is striking in our study.

In the sepsis group in which glutamine replacement was performed in our study, the absence of an increase in the replacement of a specific product, especially glutamine stored in the striated muscles, can provide favorable effects on neuromuscular transmission disorders seen in sepsis.

Recent studies have indicated that exogenous glutamine supplementation increased plasma glutamine levels but failed to prevent protein degradation (43,44).

In addition, there is also evidence that glutamine supplementation might increase mortality. Glutamine is used by lymphocytes as a nitrogen and carbon precursor. It is suggested that glutamine supplementation increased lymphocyte proliferation and cytokine production in sepsis. Thus, in surgical, trauma and bone marrow transplantation patients, in addition to its favorable effects, glutamine might increase inflammatory response in sepsis and MOF patients. Severe inflammatory stimulation may stimulate proliferation of immune cells and increase glutamine requirement. Supplemented glutamine may also increase glutamine degradation by increasing lymphocyte proliferation and cytokine production (44-46).

In their study published in 2013, Heyland et al. (47) and colleagues reported that glutamine significantly increased 28-day mortality. Then, glutamine supplementation for intensive care patients was criticized, especially in liver and kidney dysfunction. Today, timing of supplementation, dosage and relation with organ failure are under discussion. In Heyland's study, glutamine was used as enterally and parenterally at high doses. Besides this high dose, in the glutamine group with increased mortality, there was an increased number of organ failure patients, which might influence mortality rate together with the timing of initiation (47,48).

Therefore, the complexity of the immune system and unpredictable interaction with glutamine should be taken into account during patient selection.

In contrast to those studies discussing glutamine supplementation in critically ill patients and sepsis, there are a limited number of experimental studies on glutamine supplementation in sepsis-induced polyneuromyopathy. In an experimental sepsis model published in 2005 it was reported that parenteral glutamine supplementation did not have any favorable impact on diaphragmatic function but had positive effects on biochemical and histopathologic parameters. Similar to our study model, they used CMAP to evaluate neuromuscular function, but in this study glutamine supplementation was parenteral and CMAP was measured from diaphragmatic muscles instead of M.gastrocnemius (49).

In our study model, we did not induce severe sepsis and glutamine was given only enterally. The degree of cecal puncture in the CLP model is closely related with the resulting endotoxemia and mortality. Sepsis severity and mortality increase with increasing puncture size. In our sepsis model, we used 22 G intracath for cecal puncture, which might lead to less severe sepsis with a lower mortality rate.

It could be concluded that it was necessary to avoid high doses of parenteral glutamine supplementation in MOF and shock patients, especially in the early period of critical illness. However, there are limited data on glutamine supplementation in sepsis-induced CIPNM. Therefore, in terms of our study results, glutamine supplementation might favorably influence neuromuscular conduction if patients are selected carefully. More detailed studies are needed in this field of investigation.

CONCLUSION

During the measurements performed at the end of 24 hours, it was observed that CMAP latency prolongation in the untreated sepsis group (Group I) was statistically significant, but there was no statistically significant difference in Group II in which enteral glutamine replacement was performed.

It is therefore observed that the use of glutamine prevents CMAP latency prolongation in the early stage of sepsis.

It was concluded that enteral glutamine replacement may be promising in the prevention of NMD in sepsis, but further studies are required.

Ethics Committee Approval: The study was initiated after obtaining approval from the Animal Ethics Committee of Ege University School of Medicine (Date: 28.07.2009, Number: 2009-77).

Informed Consent: N/A.

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