Original Investigation

The Effects of Minocycline on the Hippocampus in Lithium-Pilocarpine Induced Status Epilepticus in Rat: Relations with Microglial/Astrocytic Activation and Serum S100B Level

Erkut Baha BULDUK[1], Gokhan KURT[1], Sureyya BARUN[2], Onder AYDEMIR[3], Murat KIZILTAS[2], Murat OKTEM[4], Turan TURHAN[5], Pergin ATILLA[6], Sevda MUFTUOGLU[6]

[1]Gazi University, Faculty of Medicine, Department of Neurosurgery, Ankara, Turkey
[2]Gazi University, Faculty of Medicine, Department of Pharmacology, Ankara, Turkey
[3]Gazi University, Faculty of Medicine, Department of Public Health, Ankara, Turkey
[4]Duzen Laboratories Group, Department of Biochemistry, Ankara, Turkey
[5]Ankara Numune Education and Research Hospital, Department of Biochemistry, Ankara, Turkey
[6]Hacettepe University Medical School, Department of Histology, Ankara, Turkey

ABSTRACT

AIM: To investigate possible correlations between serum S100B levels and microglial/astrocytic activation in status epilepticus (SE) in lithium–pilocarpine-exposed rat hippocampi and whether serum S100B levels linearly reflect neuroinflammation. Additionally, to assess the effects of minocycline (M), an inhibitor of neuroinflammation.

MATERIAL and METHODS: Rats were divided into 4 groups (6/group), namely, control (C), sham, SE, and SE+M. Animals were exposed to lithium–pilocarpine to induce SE in the SE and SE+M groups. Cardiac blood was collected to measure S100B levels, and coronal brain sections including the hippocampus were prepared to examine microglial/astrocytic activation and to evaluate neuroinflammation at day 7 of SE.

RESULTS: Serum S100B levels, OX42 (+) microglia in CA1, and GFAP (+) astrocytes in both CA1 and dentate gyrus (DG) were higher in the SE+M group than in the C group. Most importantly, highly positive correlations were found between S100B levels and microglial activation in CA1, apart from astrocytic activation in CA1 and DG. Unexpectedly, microglial activation in CA1 and astrocytic activation in DG were also enhanced in the SE+M group compared with the C group. Moreover, M administration reversed the neuronal loss observed in DG during SE.

CONCLUSION: These results suggest that serum S100B is a candidate biomarker for monitoring neuroinflammation and that it may also help predict diagnosis and prognosis.

KEYWORDS: S100B, Astrocyte, Microglia, Minocycline, Seizure

■ **INTRODUCTION**

Epilepsy is a chronic neurological disorder characterized by recurrent seizures resulting from uncontrollable neural excitation in the brain (28), and status epilepticus (SE) is associated with an increased risk of developing epilepsy (25,26). Although seizures and epilepsy are primarily considered neuronal diseases, studies have shown that pathogenesis may be associated with non-neuronal components (e.g., neuroinflammation) (23,27).

Neuroinflammation appears to be one of the etiological factors that promote epileptogenesis (57). Contrastingly, seizure activity induced by various methods can cause inflammation

Corresponding author: Sureyya BARUN
E-mail: barun@gazi.edu.tr

in the brain through the transcription of inflammatory genes (14,56). A previous study has shown that the frequency of seizures is positively correlated with the levels of inflammation in the affected tissue in epileptic patients (47). Moreover, anti-inflammatory treatments lead to anticonvulsant effects in patients with epileptic seizures who are refractory to classical antiepileptic drugs (58). Neuroinflammation is also implicated as a causative factor in neurodegeneration resulting from seizures (2), and activation of astrocytes and microglia is associated with neuroinflammatory processes (60). Thus, it has been suggested that neuroinflammation is not merely an epiphenomenon but that it may actively contribute to the pathophysiology of epilepsy.

S100B is a member of the multigenic family of Ca2+-binding proteins of the EF-hand type and is prominently expressed in astroglial cells (24). Its action in the brain is both as an intracellular regulator and extracellular signal. Intracellular S100B acts as a stimulator of cell proliferation and migration and inhibits apoptosis. The activation of astrocytes may contribute to repair after brain damage (16), and astrocytes constitutively release S100B into the extracellular space, which then acts on neurons, astrocytes, and microglia via its primary receptor, namely, receptor for advanced glycation end products (15,44).

As an extracellular factor, low concentrations (nM) of S100B promote neuronal survival, whereas higher concentrations (μM) result in harmful events, including brain inflammation and neuronal apoptosis (16). Microglial cells are activated by S100B under pathological conditions and release cytokines (1). Thus, serum S100B levels are elevated in central nervous system (CNS) pathologies, including trauma, stroke, inflammatory diseases, psychiatric conditions, and seizures (35,65); conversely, normal serum S100B levels reliably predict the absence of significant CNS injury (3).

Minocycline (M) is a second-generation tetracycline antibiotic that readily crosses the blood–brain barrier (BBB) and exhibits anti-inflammatory, antiapoptotic, and neuroprotective effects independent of its antimicrobial action (40,67). Therefore, it has been used to evaluate the inhibition of inflammation under various CNS pathologies (48,68,69).

It is known that neuroinflammatory processes contribute to seizure pathophysiology and that S100B levels increase due to epileptic seizures (29,35,49). As S100B may enter the bloodstream, S100B has been proposed as a biomarker for brain damage (32). However, it remains unknown whether S100B levels linearly reflect neuroinflammatory processes, including microglial/astrocytic activation and neuronal loss, after seizures. If true, S100B levels can be used for predicting the following: 1) contribution of neuroinflammation to pathophysiology, 2) efficiency of treatment, and 3) prognosis. Further, if indeed there is a correlation between these parameters, S100B may be useful as a biomarker. To date, any potential associations among neuroinflammation, neuronal loss, and serum S100B levels have not been investigated in SE. Given the above, we hypothesized that S100B, an astrocytic protein, elevated as a result of various neurological insults, is a biomarker of neuroinflammation after

lithium–pilocarpin-induced SE and that its level will reduce as a result of M treatment.

Thus, we aimed to assess any possible correlations among microglial/astrocytic activation, neuronal loss, and serum S100B levels in a rat model of SE. Additionally, the anti-inflammatory effect of M was used to observe any change in these parameters. Microglial/astrocytic activation was investigated by analyzing glial fibrillary acidic protein (GFAP) and OX 42 expressions, respectively (22,62). Further, brain tissue S100B expression was not assessed because its expression is not considered a marker of microglial/astrocytic activation.

■ MATERIAL and METHODS

Animals

In total, 24 male Sprague Dawley rats weighing 200–250 g, were used. The animals were housed in a facility with a 12-hour artificial light–dark cycle and allowed free access to a standard diet and tap water. All experiments were conducted according to the ethical standards in Directive 86/609/EEC. This study was approved by the Gazi University Ethics Committee for Animals (2012/45-03).

Experimental Protocol

Animals were divided into 4 groups:

1) Control group (C),

2) Sham group (S),

3) SE group,

4) M+SE group.

Each group included 6 animals. In the C group, all rats received an identical volume of saline (0.9% NaCl, i.p.) as treated animals, instead of the chemicals. Animals in the S group were treated identically to those in the SE group except for pilocarpine administration. The animals received lithium, methylscopolamine, and diazepam. However, saline was administered instead of pilocarpine.

Induction of SE using Lithium–Pilocarpine

Rats were pretreated with lithium chloride (127 mg/kg, i.p.) before pilocarpine injections (30 mg/kg, i.p.) as described previously (30). Seizures were graded according to the Racine score (46). Continuous limbic seizures corresponding to classes 4-5 were defined as SE. In this study, rats exhibited seizures in a few minutes following the initial pilocarpine injection, which corresponded to classes 1-3 in the SE and SE+M groups (46). Therefore, pilocarpine was reinjected at 30 minutes following the first injection to induce SE, and all rats administered the second pilocarpine injection remained in SE until termination with diazepam. To the best of our knowledge, this is the first experimental protocol for lithium–pilocarpine-induced SE. Because it has been previously shown that SE lasting for 30–60 minutes is sufficient to induce hippocampal damage (9), diazepam was administered (10 mg/kg, i.p.) to terminate the seizures 1 hour after the first pilocarpine injec-

tion. If the first dose of diazepam was not effective, injections (5 mg/kg, i.p.) were repeated till the seizures stopped or for a maximum of 3 times. Additionally, methylscopolamine (1 mg/kg, i.p.) was administered 30 minutes before the first pilocarpine injection to reduce peripheral cholinergic side effects of pilocarpine (e.g., salivation, diarrhea, body tremors, and chromodacryorrhea). All rats exposed to lithium–pilocarpine were fed water-soaked food until they were able to eat normal, dry food pellets. Rats were also administered 0.9% saline (10 mL/per animal) subcutaneously to restore volume loss. All the experiments were conducted at the same time and in the morning. As pre-insult delivery of M, followed by post-insult administration, exhibits the greatest improvement after brain injury, we also employed this strategy (50). In the SE+M group, the first M injection (40 mg/kg, i.p.) was administered 1 hour before the lithium chloride injection; this was repeated for 7 days. Considering that astrocytic and neuroplastic changes occur immediately following pilocarpine-induced seizures, animals were sacrificed after 7 days of SE, and the brains were removed to analyze astroglial and microglial damage (50,51). Intracardiac blood was collected from rats for estimating serum S100B levels before removing the brain.

Histological Examination

All animals were anesthetized using thiopental (30 mg/kg, i.p.) and were sacrificed. Brains were removed and put on ice. The area including the hippocampus was removed from the rest of the brain by coronal dissections, both in the front and back. After fixation in 10% buffered formaldehyde for 72 hours, the hippocampus tissue was routinely processed for parafinization, 5-µm-thick coronal serial sections cut on a microtome, and every 1 in 5 sections (1,14,28,40,60) stained with hematoxylin–eosin (HE). GFAP immunohistochemistry was performed on 2 consecutive sections (16,24,25,27,35,47,56,65,68,69) for assessing astrocyte activation, whereas OX-42 immunohistochemistry was performed on the next 2 consecutive sections (2,3,15,23,44,48,49,57,58,67) for estimating microglial cell activation. GFAP immunoreactive cells and OX-42 immunoreactive cells were evaluated both in the dentate gyrus (DG) and CA1 regions in each section. Immunoreactive cells in 3 areas in each section were counted independently by 2 histologists blinded to the identity of the samples. The mean values for both were taken for each section, and the mean values for each antibody were calculated in each experimental group. Pycnotic neurons were also counted by the same method in the HE-stained sections for each experimental group.

Assessment of Serum S100B Levels

Intracardiac blood samples (3 ml) were collected from rats and centrifuged at 2000 rpm for 10 minutes; serum was collected and stored at −80°C until analysis. Commercially available ELISA kits were used according to the manufacturer's protocols (SunRed Biotechnology Lot No. 201502).

Chemicals

Lithium chloride, methylscopolamine, pilocarpine, thiopental, and diazepam were purchased from Sigma-Aldrich (Steinheim, Germany).

Statistical Analyses

Data were evaluated by Statistical Package for Social Sciences (SPSS) for Windows 15.0 (SPSS Inc, Chicago, IL). Descriptive statistics are presented as median (minimum–maximum). The Kruskal–Wallis test was used to compare more than 3 groups, and the post-hoc Bonferroni correction was used to estimate statistical significance. Statistical significance level for Type-I error was set at 5% ($p<0.05$). Correlations were evaluated using the Spearman correlation coefficient.

■ RESULTS

Experimental observations of adult rats subjected to lithium–pilocarpine-induced SE. Classes 1–3 seizures were observed in all 24 rats within a few minutes after the first pilocarpine injection, and classes 4-5 seizures, which correspond to SE, were observed in all rats following the second pilocarpine injection; these intensive seizures lasted 30 minutes until termination with diazepam (46).

Most previously published studies indicate that most rats experience SE recurrence in the 90–120-min period following SE termination using antiseizure drugs (4). Contrastingly, we found that diazepam injection terminates seizures in all animals with no recurrence during the 90–120-min period. However, it must be noted that the animals were not monitored by electroencephalography (EEG) but were only observed during the experiments. Further and favorably, other studies report that lithium–pilocarpine-induced SE for 60 min does not lead to epilepsy during a subsequent 6-month period (5). Scopolamine can inhibit SE when administered before pilocarpine but is not effective after SE has been established (7). Because methylscopolamine cannot cross the BBB, it is used to reduce the peripheral muscarinic adverse effects of pilocarpine (8). However, methylscopolamine may have crossed the BBB due to greater permeability after neuroinflammation, thereby possibly contributing to the inhibition of recurrence in our study (10-12). Lastly, it is also possible that this effect is specific to this model.

Of the 6 rats in the SE+M group, 2 died, yielding a mortality rate of 36%. Therefore, experiments were repeated with 2 animals and the data combined to maintain 6 animals per group. No deaths were observed in the other groups.

Serum S100B Levels

Significant differences in serum S100B levels were found among the experimental groups ($p=0.008$), and comparisons between 2 groups showed that SE and M+SE groups had higher S100B levels than the C group ($p=0.008$ and $p=0.002$, respectively; Figure 1).

Immunohistochemical Evaluation

Evaluation of OX 42(+) microglia in CA1

There was a significant difference in OX 42(+) microglia in CA1 in the experimental groups ($p=0.012$) and OX 42(+) microglia were significantly higher in the SE and SE+M groups than the C and S groups ($p=0.004$, $p=0.008$, $p=0.002$, and $p=0.008$, respectively; Figure 2).

Evaluation of OX 42(+) microglia in DG

There was no statistically significant difference in cell numbers among the groups (p=0.277; Figure 3).

Evaluation of GFAP (+) astrocytes in CA1

Significant differences in GFAP (+) astrocytes in CA1 were found among the experimental groups (p=0.012). GFAP (+) astrocyte cell numbers in CA1 were significantly higher in the SE+M group than the C and S groups (p=0.008 and p=0.003, respectively; Figure 4).

Evaluation of GFAP (+) astrocytes in DG

GFAP (+) astrocyte numbers in DG were significantly different

among the experimental groups (p=0.007), with higher cell numbers in the SE and SE+M groups than the C and S groups (p=0.001, p=0.004, p=0.005, and p=0.008, respectively; Figure 5).

Correlation between S100B levels and OX 42(+) microglia and GFAP (+) astroglia in the experimental groups

There was no correlation between these parameters in the C group.

In the S group, a negative correlation was found between the S100B level and GFAP (+) astroglia in CA1 in SE (r=−0.90) (Figure 6).
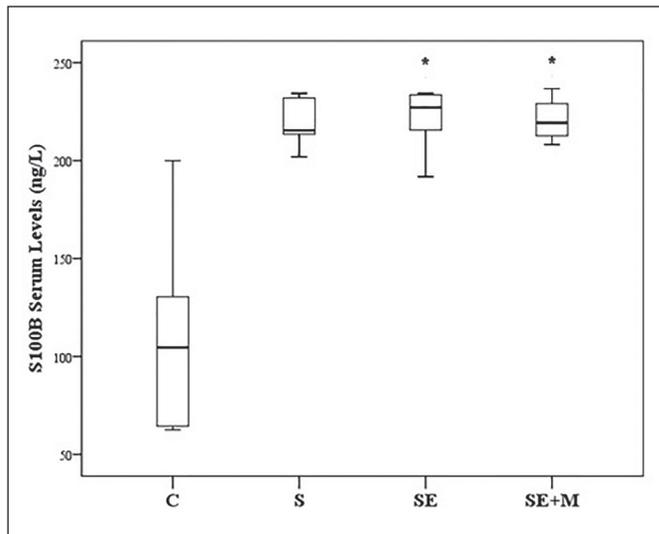

Figure 1: S100B levels in the experimental groups. Data are the median (range) of 6 experiments. *p<0.05 compared with C.
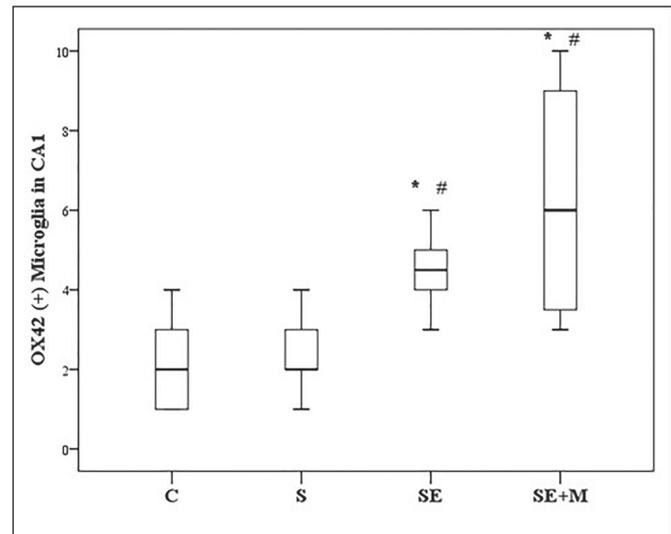

Figure 2: OX 42(+) microglia in CA1 in the experimental groups. Data are the median (range) of 6 experiments. *p<0.05 compared with C; #p<0.05 compared with S.

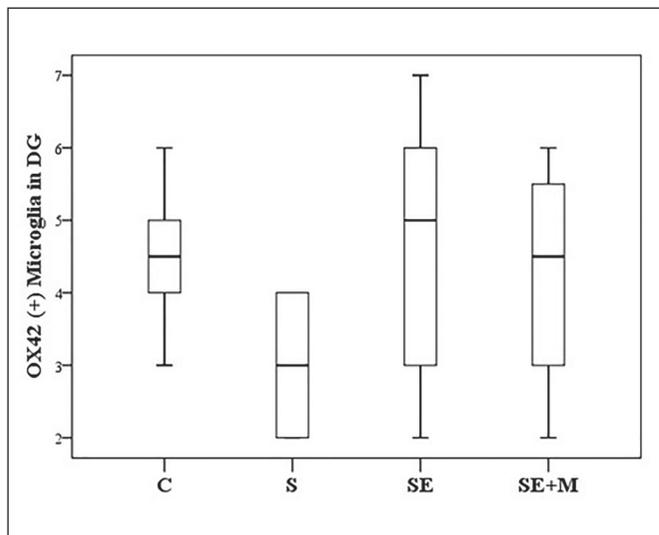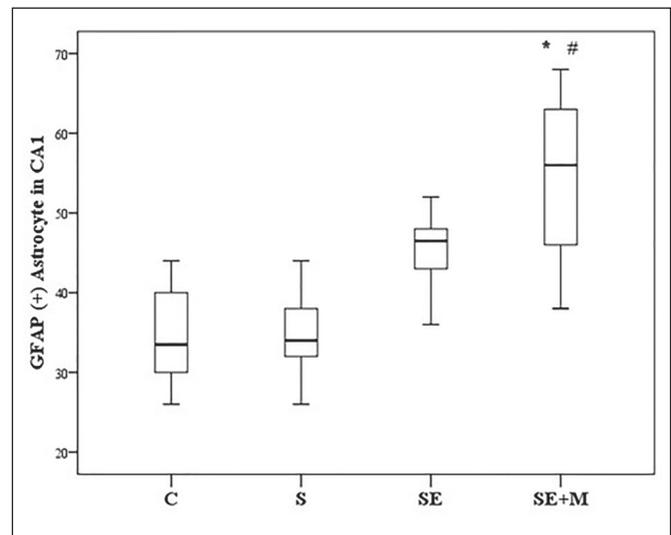**Figure 3:** OX 42(+) microglia in DG in the experimental groups. Data are the median (range) of 6 experiments.

Figure 4: GFAP (+) astrocytes in CA1 in the experimental groups. Data are the median (range) of six experiments. *p<0.05 compared with C; #p<0.05 compared with S.

In the SE group, the following results were obtained:

1) GFAP (+) astrocytes in CA1 and DG showed a significant positive correlation (r=0.94; Figure 7)

2) a positive correlation was found between S100B levels and OX 42(+) microglia in CA1 (r=0.82; Figure 8).

No correlation was observed between S100B levels and OX 42(+) microglia. S100B levels were positively correlated with GFAP (+) astrocytes in CA1 and DG (r=0.94 and r=0.83, respectively; Figures 9, 10).

No correlation between the parameters tested was found in the M+SE group (Table I).

Histological Evaluation

Group C

Microscopic examination showed normal morphology of DG and CA1. GFAP (+) astroglia and weak OX 42(+) immunoreactivity were also detected in DG and CA1 (Figures 11A, B; 12A, B, respectively).

Groups S and SE

Histological appearances are not presented for these groups. Data are given in Figures 2-5.

Group M+SE

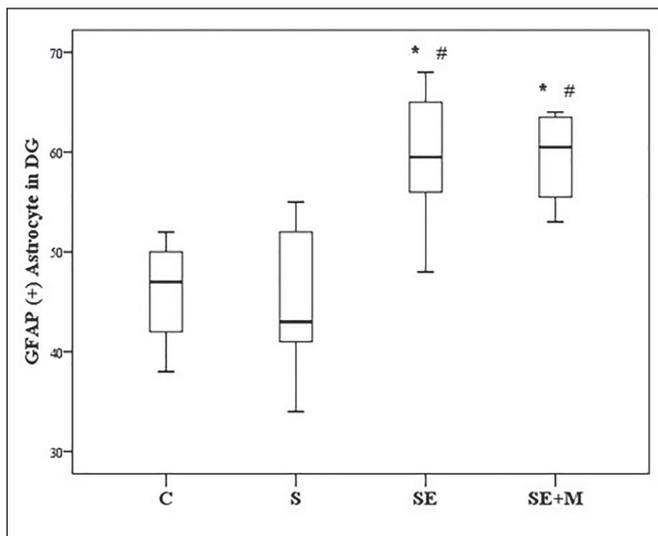GFAP (+) astroglia were prominent in DG and CA1 in this group

Figure 5: GFAP (+) astrocytes in DG in the experimental groups. Data are the median (range) of 6 experiments. *p<0.05 compared with C; #p<0.05 compared with S.
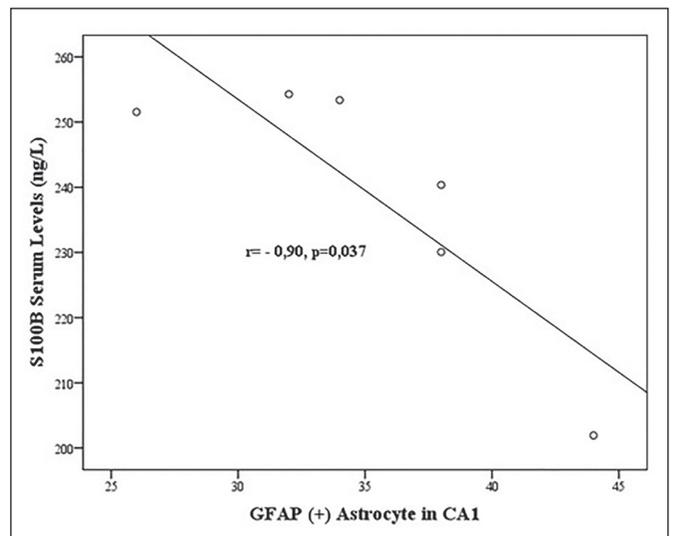

Figure 6: Negative correlation between S100B levels and GFAP (+) astrocytes in CA1 in the S group.
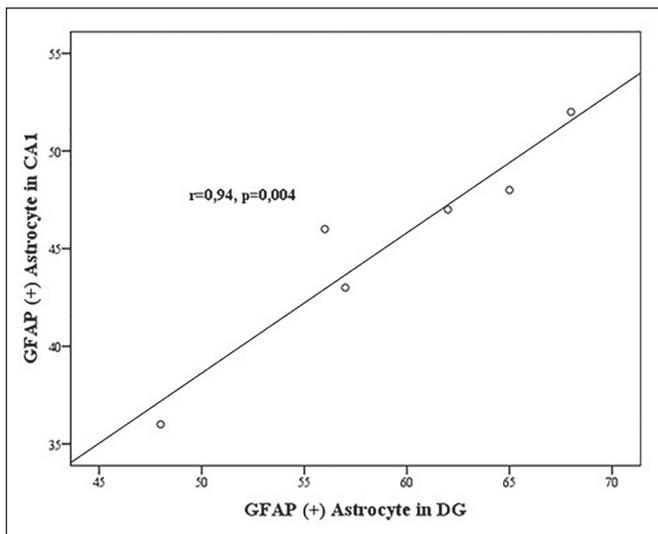

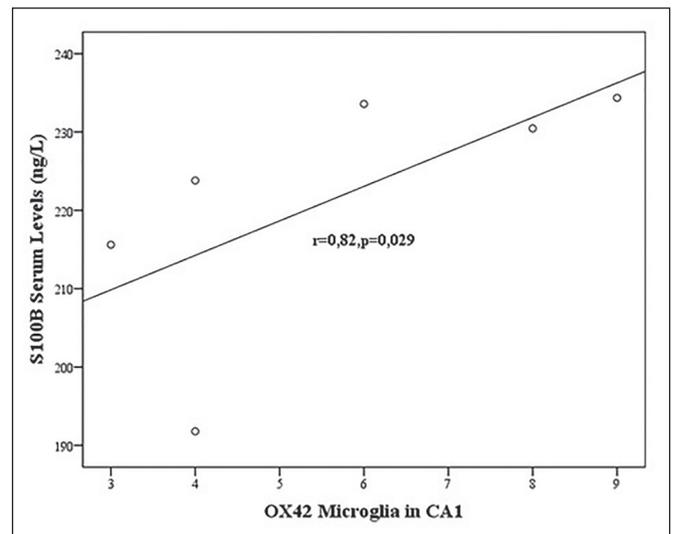**Figure 7:** Positive correlation between GFAP(+) astrocytes in DG and CA1 in the SE group.

Figure 8: Positive correlation between S100B levels and OX 42(+) microglia in CA1 in the SE group.

Table I: Investigation of the Correlation Between S100B Level and Microglial/Astrocytic Activation in SE+M Group

SE + M		GFAP (+) DG	GFAP (+) CA1	OX42 (+) DG	OX42 (+) CA1	S100B
GFAP (+) DG	r	1.000				
	p	-----				
GFAP (+) CA1	r	-0.200	1.000			
	p	0.800	-----			
OX42 (+) DG	r	0.001	-0.400	1.000		
	p	0.998	0.600	-----		
OX42 (+) CA1	r	-0.001	0.200	0.001	1.000	
	p	0.998	0.800	0.998	-----	
S100B	r	0.400	-0.800	-0.200	-0.400	1.000
	p	0.600	0.200	0.800	0.600	-----

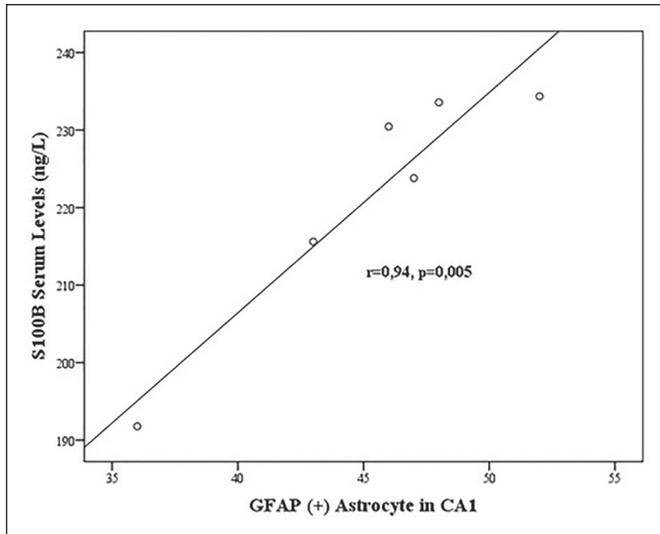*r: Spearman correlation coefficient.*

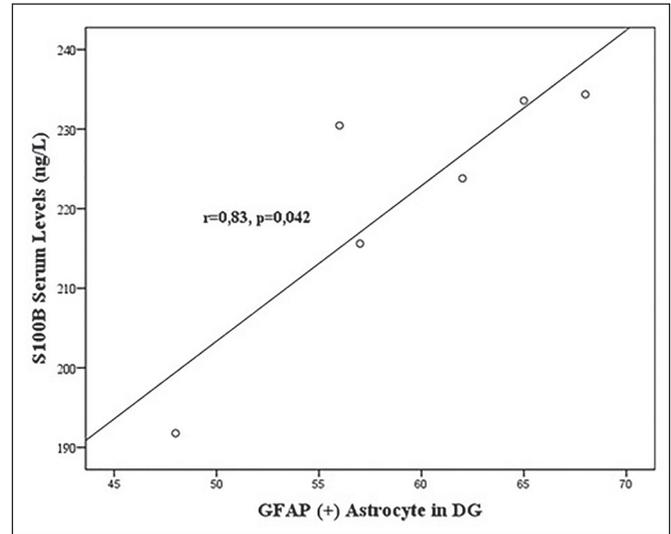**Figure 9:** Positive correlation between S100B levels and GFAP(+) astrocytes in CA1 in the SE group.

Figure 10: Positive correlation between S100B levels and GFAP (+) astrocytes in DG in the SE group.
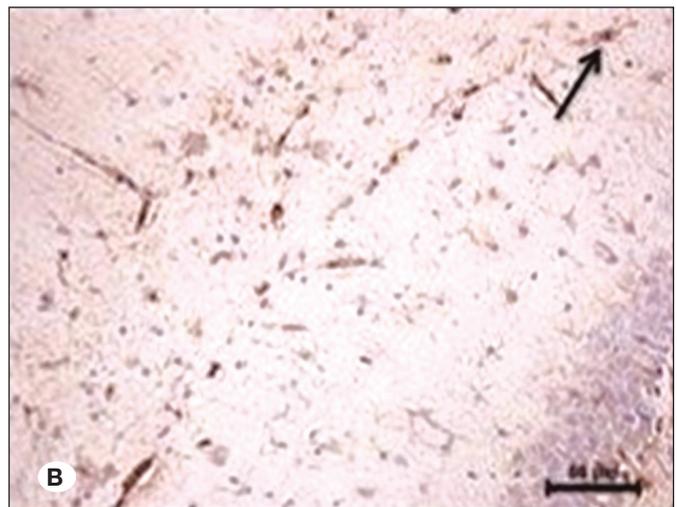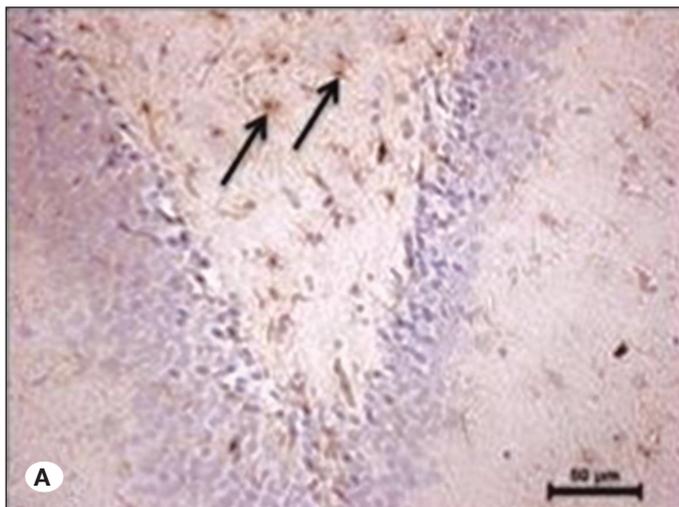

Figure 11: GFAP (+) astrocytes in DG **(A)**, and CA1 **(B)** in the C group (HRP/ABC-hematoxylin–eosin staining; **A:** ×200, **B:** ×200).

Figure 12: OX 42(+) microglia in DG **(A)**, and CA1 **(B)** in the C group (HRP/ABC-hematoxylin–eosin staining; **A:** ×200, **B:** ×200).

Figure 13: GFAP (+) astrocytes in DG **(A)**, and CA1 **(B)** in the M+SE group (HRP/ABC-hematoxylin–eosin staining; **A:** ×200, **B:** ×200).
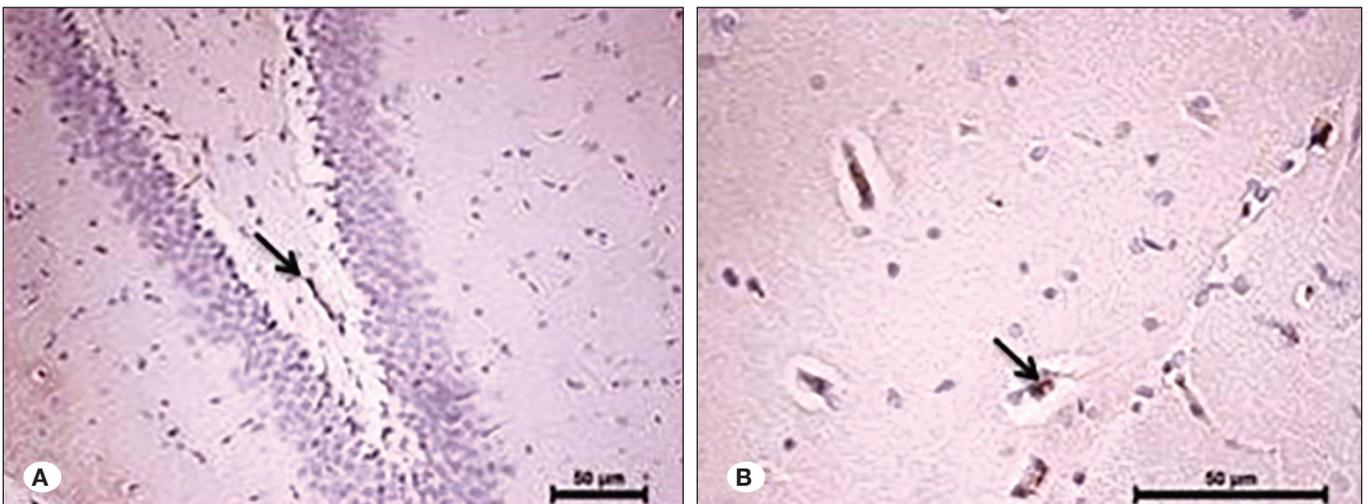

Figure 14: OX42 (+) microglia in DG **(A)**, and CA1 **(B)** in the M+SE group.

Table II: Pycnotic Neurons in DG and CA1 in Experimental Groups

	n	DG	p*	CA1	p*
		Median (range)		Median (range)	
C	6	55 (34-66)		4.5 (3-9)	
S	6	71 (52-98)	0.001ᵃ	8 (4-11)	0.127
SE	6	129 (110-165)		8.5 (5-13)	
SE+M	6	54 (48-62)		8.5 (6-11)	

Kruskal-Wallis Test
ᵃ *Significant difference between SE and C, and SE and SE+M.*

(Figures 13A, B). Additionally, OX 42 (+) microglial cells were observed in both DG and CA1 (Figure 14A, B).

Pycnotic neurons were counted, and data are presented in Table II.

Necrotic neurons were found to be positively correlated with OX 42(+) microglia (r=0.58) in CA1 in SE.

■ DISCUSSION

S100B is expressed predominantly in astrocytes and plays a critical role in neuroinflammation (10), and reactive astroglia overexpress GFAP and secrete S100B after seizures (23,27,45). Additionally, S100B stimulates microglial activation and leads to the release of interleukin-6 and interleukin 1α (52,63). Further, neuroinflammation is crucial in neuronal loss in the CNS. The activation of Toll-like receptors (TLRs) expressed in the microglia causes injury to neurons (33). Research and clinical data have indicated that seizures are associated with elevated S100B protein levels in both serum and cerebrospinal fluid (CSF) (35,37). However, it remains unknown whether serum S100B levels linearly reflect neuroinflammatory changes and neuronal loss in seizures.

We showed that serum S100B levels are significantly higher after lithium–pilocarpine-induced SE, and other studies have shown that S100B levels are elevated in CSF in SE in an identical animal model (13). Moreover, S100B levels in serum and CSF are increasingly being used as biomarkers of brain damage and neurodegenerative diseases to determine the extent of neuronal damage (65). Thus, in agreement with other published results, the elevated S100B levels in SE observed in our study also appear to originate from the activation of astrocytes, which was in turn due to seizure-induced excitotoxicity (12,38,49). S100B is released from astrocytes through the activation of the metabotrophic glutamate receptor 3 (49). In line with this view, it has been found that mGlu2 and 3 receptor expressions are increased in the hippocampi of patients with temporal lobe epilepsy (55).

GFAP (+) astrocytes in DG after SE were significantly higher than those of controls. However, astrocytic activation was not different in CA1 between the SE and C groups. Nevertheless, a highly positive correlation was observed between GFAP (+) astrocytes in both DG and CA1 (r=0.94) in SE. Taken together, these results suggest that GFAP (+) astrocytes in DG and CA1

increase in tandem, but astrocyte activation in CA1 did not reach statistical significance.

To the best of our knowledge, this is the first report on possible correlations between serum S100B levels and microglial/astrocytic activation in CA1 and DG. The major findings are that S100B levels are positively correlated with GFAP (+) astrocyte numbers both in DG and CA1 (r=0.83 and r=0.94, respectively), suggesting that serum S100B levels probably linearly reflect astrocyte activation both in DG and CA1.

OX42 (+) microglial cells in DG were not different among the 4 experimental groups, implying that DG is not markedly involved in microglial activation. Further supporting this result is the observation that there is no correlation between OX 42 (+) microglia in DG and S100B levels.

In CA1, OX 42(+) microglia were significantly higher in the SE group than the C group. Furthermore, OX 42 (+) microglia were positively correlated with significantly high levels of S100B (r=0.82), and previous studies have also reported similar results (39,53). Consistent with this result, other reports have demonstrated that S100B released from activated astrocytes stimulates microglial activation (52,63).

Taken together, it appears that serum S100B levels linearly reflect microglial activation in CA1 and astrocytic activation in both CA1 and DG. These results are important for predicting the degree of neuroinflammatory processes by measuring serum S100B levels. Additionally, astrocytes may release cytotoxic factors and glial cell line-derived neurotrophic factor in the presence of activated microglia (7). Therefore, our data also provide important insights regarding neuronal loss. Necrotic neurons were found to be positively correlated with OX 42(+) microglia in CA1 in SE (r=0.58), indicating a prominent cytotoxic effect of microglia in CA1. Previous studies have suggested that neuroinflammation is a driving force for neurodegeneration (21). The activation of TLRs expressed on microglia leads to neuronal injury (33). Additionally, the synergistic effects of S100B with interferon gamma in stimulating nitric oxide secretion in microglia may have also contributed to the observed neurotoxicity in CA1 in SE (1,43).

Another aspect that required consideration in this study was that microglial/astrocytic activation are ongoing processes on day 7 following SE induction. Likewise, the activation of

microglia has been shown to continue for at least 3–5 days following pilocarpine-induced SE (51). Therefore, parallel elevation in S100B levels is an expected result in our study.

In the M-treated group, S100B levels were higher than those of the C group. Unexpectedly, M also led to an increase in OX 42(+) microglia in CA1 and GFAP (+) astrocytes, both in DG and CA1, compared with the C group. These results do not concur with those of previous studies, which demonstrate an anti-inflammatory effect of M (8,20,42,61). However, there was no correlation between S100B levels and microglial/astrocytic activation in both DG and CA1 in rats exposed to SE in the presence of M. Taken together, these results imply that M does not attenuate SE-induced microglial/astrocytic activation. It is possible that this lack of correlation between these parameters in M-treated rats is due to the limited inflammatory capacity of the tissue. Moreover, neuroinflammation leads to greater BBB permeability (34). Therefore, another explanation for the observed lack of correlation may be that the inhibitory action of M on the increased permeability of BBB obscured any positive correlation between S100B levels and microglial/astrocytic activation in the hippocampus. In support of this hypothesis, other studies have shown that M can alleviate BBB permeability loss through various mechanisms (19,41,64) and that serum and brain S100B levels are poorly correlated, with serum levels dependent primarily on the integrity of BBB and not on the level of S100B in the brain (31).

Herein, M reversed neuronal necrosis observed in CA1 in SE. It has been reported that both astrocytic and microglial activation precedes neuronal damage (30). Thus, it is feasible to expect that M, a microglia inhibitor, would attenuate the harmful effects of seizures such as neuronal necrosis. However, M reversed the neuronal necrosis observed in DG in SE but without a demonstrable anti-inflammatory effect, probably implying that the neuroprotective effect of M is not anti-inflammatory–related. In fact, the anti-inflammatory effects were not clearly observable in our study. In line with our results, but apart from its anti-inflammatory actions, M has previously been shown to have neuroprotective properties and could reduce neuronal necrosis (5,18,20,66). Additionally, it was suggested that it is possible for M to prevent an increase in inflammatory cytokines without affecting their inactivate state (8). Other neuroprotective mechanisms may include antiapoptotic protein upregulation and oxidative stress counteraction (6,59).

The S group, although not statistically different from the C group, showed an increase in S100B levels. Additionally, a negative correlation was found between S100B levels and GFAP (+) astrocytes. This effect may have been due to the inhibitory effect of lithium on microglial activation (17). Furthermore, scopoloamine reduces the toxicity of glutamate receptor agonists, like kainate and domoic acid (36,54). Therefore, it is unclear if these results can be reproduced in other experimental SE models. Thus, future studies need to obtain additional information to evaluate this experimental animal model.

■ CONCLUSION

SE led to an increase in S100B levels and caused the persistent activation of microglia and astrocytes. These findings show that S100B levels are highly and positively correlated with OX42 (+) microglia in CA1 and GFAP (+) astrocytes in both CA1 and DG. Additionally, SE-induced neuronal necrosis in DG could be reversed by M without an accompanying anti-inflammatory effect. S100B levels were also found to be higher in M-administered animals than in controls. Nevertheless, there was no correlation between S100B levels and microglial/astrocytic activation. Neuroinflammation and the search for biomarkers are currently the focus of epileptogenesis research. The translational aspect of this study is that the S100B protein may be a candidate biomarker for evaluating the relationship between neuroinflammation and epileptogenesis. Predicting neuroinflammatory alterations in the presence or absence of drug treatment, or during preoperative or postoperative periods of epilepsy surgery, will enhance our understanding of the pathophysiology of epilepsy and will also help in designing new treatment approaches. Additionally, biomarkers may be useful as cost-effective tools for screening potential antiepileptogenic drugs.

■ ACKNOWLEDGMENT

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