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Original Article

S100B brain expression and plasma concentrations in a preeclampsia rat model

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ABSTRACT

Objective: To assess brain damage using the neuroinflammation marker S100B in a preeclampsia rat model.
Methods: Non-pregnant and pregnant rats were infused with saline or low-dose-endotoxin on day 14 of pregnancy. S100B expression in the brain (immunohistochemistry) and S100B plasma concentrations (ELISA) were studied.
Results: No differences in S100B expression in brain tissue were observed between the four groups. Pregnant endotoxin treated animals did not show increased levels of plasma S100B levels as compared with control pregnant rats, while significantly higher plasma S100B levels were found in non-pregnant endotoxin versus pregnant endotoxin infused rats.
Conclusion: Pregnancy nor experimental preeclampsia, alter S100B in rat brain, or in plasma. Increased plasma S100B in non-pregnant endotoxin-treated rats may indicate brain injury in these rats, whereas pregnancy might be protective.

1. Introduction

Pre-eclampsia is responsible for the world's largest maternal mortality rates, mostly due to acute cerebral complications such as eclampsia (posterior reversible encephalopathy syndrome (PRES)), and intracerebral hemorrhage [1]. PRES is thought to be caused by a failure of the brain's autoregulatory response to increases in blood pressure in conjunction with endothelial cell dysfunction. It is associated with a loss of integrity of the blood–brain barrier; inflammatory cells and fluid can penetrate the brain and cause edema and cell death. Interruption of this delicate balance between capillary and cellular perfusion pressures may lead to the neurological complications of preeclampsia [1].

S100B is an acidic calcium-binding protein, mainly expressed by astrocytes, oligodendrocytes and Schwann cells [2]. Experimental and clinical studies have shown S100B to be involved in neuroinflammation [3]. Because approximately 95% of S100B is located in the CNS, the results of several studies, both human and animal, have suggested that an increase in S100B levels in blood as well as increased expression in brain tissue could be a potential marker of neuronal injury indicating astrocytic death, reactive gliosis and/or blood–brain barrier dysfunction [4–7].

Neuroinflammation is a key component of various central nervous system (CNS) diseases, promoting both reparation and damage of neural tissue [3]. The use of neuroinflammatory biomarkers (such as S100B) in hypertensive disease in pregnancy may represent a novel avenue for early diagnosis, and eventually prevention, of cerebrovascular complications in preeclamptic women who present with neurological signs and symptoms, but current data are limited [5,8].

To assess the possible relationship between preeclampsia and neuronal brain damage, we investigated the brain

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injury marker S100B in brain tissue and plasma in a rat model for mild preeclampsia.

2. Materials and methods

2.1. Animals

Female Wistar outbred rats (200–220 g) were housed in standard conditions, in a temperature- and light-controlled room (12 h light–dark cycle), with free access to water and food. Vaginal smears were taken daily, and rats were rendered pregnant by housing them on proestrus with fertile male Wistar rats for one night. When spermatozoa were detected in the smear the next day, this day was designated day 0 of pregnancy. A permanent canula was inserted in the right jugular vein in all rats under 2% isoflurane/oxygen anesthesia according to standard methods [9]. The canula allows stress-free infusions.

2.2. Experimental design

The ultra-low-dose endotoxin rat model for preeclampsia was used to simulate a preeclamptic state [10]. This model is characterized by hypertension and proteinuria in the last week of pregnancy. Endotoxin, derived from *Escherichia coli* (*E. coli*, 0.55:B5, Whittaker MA Bioproducts, Walkerville, MD) was dissolved at a dose of 1.0 μg/kg body weight in 2 ml of pyrogen free saline solution. Endotoxin (or saline for control) was infused via the jugular vein canula with an infusion rate of 2.0 ml/h for the duration of 1 h. Pregnant rats were infused at day 14 of pregnancy, non-pregnant rats 6 days prior to sacrifice.

The rats were divided into four groups: (1) non-pregnant rats infused with saline (non-pregnant controls, $n = 5$); (2) non-pregnant rats infused with low-dose endotoxin ($n = 7$); (3) pregnant rats infused with saline (pregnant controls, $n = 7$); and (4) pregnant rats infused with low-dose endotoxin ($n = 7$).

Because we were not able to obtain blood samples in all rats, the study population with regard to the ELISA consisted of: (1) non-pregnant rats infused with saline ($n = 5$); (2) non-pregnant rats infused with low-dose endotoxin ($n = 5$); (3) pregnant rats infused with saline ($n = 7$); and (4) pregnant rats infused with low-dose endotoxin ($n = 6$).

2.3. Preparation of brain tissue

At day 20 of pregnancy or 6 days after infusion in non-pregnant rats, rats were decapitated under isoflurane/oxygen anesthesia. Immediately after decapitation, trunk blood of the rat was collected in heparin tubes. Blood samples were centrifuged (562*g*, 10 min), blood plasma collected and centrifuged again (1083*g*, 10 min). Plasma was stored at −80 °C until analysis.

The brain was quickly removed and divided into forebrain, cerebellum and brain stem. Brain tissue was fixed in 4% paraformaldehyde for 24 h and, until embedding, stored in 70% ethanol. Before embedding in paraffin wax, tissue dehydrated by submitting to ethanol (96% and 100%) and finally xylol. Of the forebrain, cerebellum and brain stem, sections of 5 μm were cut and mounted on silane coated glass slides (Starforst adhesive grün, Knittel Gläser, Braunschweig, Germany).

2.4. Immunohistochemical staining of S100B in brain tissue

For immunohistochemical analysis, paraffin embedded sections were dewaxed in xylene and rehydrated in ethanol. S100B antigen was retrieved by heating sections in a trisaminomethane/ethylenediaminetetraacetic acid (Tris/EDTA) buffer (10 mM Tris/1 mM EDTA, pH 9.0) in a microwave oven (400 W) for 15 min. Sections were cooled in Tris/EDTA for 30 min and then washed in phosphate buffered saline (PBS) for 5 min. After incubation with normal rabbit serum (Dako, Glostrup, Denmark, 1:10, 30 min, room temperature (RT)), the sections were incubated with the primary antibody (polyclonal rabbit anti-S100B (Dako, Glostrup, Denmark), 1:200, 60 min, RT). Endogenous peroxidase activity was blocked using 0.075% hydrogen peroxide (H_2O_2) in PBS (30 min, RT). After incubation with a secondary antibody (swine anti-rabbit horseradish peroxidase labeled (Dako, Glostrup, Denmark, 1:100, 30 min, RT)), the staining was finished by performing an 3-amino-9-ethyl carbazole (AEC)-staining (10 min, RT). Sections were counterstained with haematoxylin.

Of each section of the forebrain, cerebellum and brain stem, 10 comparable fields were photographed with a computerized microscope (Leica DFC420C, Wetzlar, Germany) at a 400× magnification. Each photograph covers 0.06097 mm^2. Of each section, the fields are comparable with fields in other sections with regard to anatomical location and estimated total number of cells. The hippocampus, a part of the forebrain, was also photographed. Because the hippocampus is a small structure, only five fields were photographed to avoid overlap. The hippocampus was not present in all sections: sections obtained in the lateral part of the forebrain did not contain hippocampal tissue. Hippocampal tissue was not present in one non-pregnant rat infused with saline, two non-pregnant rats infused with low-dose endotoxin, two pregnant rats infused with saline and two pregnant rats infused with low-dose endotoxin. Of each photograph, the total number of S100B-positive cells was scored blindly by two examiners. The average of the two examiners per photograph was calculated. The number of positive cells of all photographs of each section was added up, and expressed as number of positive cells/mm^2. The fields photographed were compared with fields in the same brain region from other rats for differences in distribution of S100B-positive cells and intensity of the S100B-staining.

2.5. ELISA to determine S100B concentrations in plasma

To determine the S100B concentration in rat plasma, we used a commercial available enzyme-linked immunosorbent assay (ELISA) kit (Biovendor, Heidelberg, Germany). All the procedures were performed according to the manufacturer's protocol. All samples were measured in duplicates.

2.6. Statistical analysis

Statistical analysis was performed using SPSS 16.0 (SPSS Inc., Chicago, USA). All results were expressed as mean ± standard error of mean (SEM). The effect of pregnancy or endotoxin was evaluated using Two-way ANOVA and post hoc Bonferroni. $P < 0.05$ was considered significant.

3. Results

3.1. S100B expression in brain tissue

To examine the expression of the S100B protein in brain tissue, we performed immunohistochemical staining of sections of the forebrain, cerebellum and brain stem.

Figs. 1–4 demonstrate representative microphotographs of the forebrain, cerebellum, brain stem and hippocampus, immunostained for the S100B protein.

In the forebrain, the S100B protein seems to be mainly localized in the glial cells, but some neurons also contain S100B. In the forebrain, no major differences between the four groups of rats with regard to the intensity of the S100B-staining were observed (Fig. 1). Moreover, in all groups, the S100B-positive cells were diffusely distributed throughout the sections, and not grouped.

In Fig. 2, representative microphotographs of the cerebellum are shown. In all four groups, S100B-positive glial cells are mainly localized in the layer between the granule cell layer (A) and the molecular layer (B) of the cerebellum, which is called the Purkinje cell layer (C). The Purkinje cells are negative for the S100B protein. No major differences were observed with regard to the intensity of the S100B-staining in the cerebellum between the different groups.

Fig. 3 shows representative microphotographs of the brain stem. In these photographs, nerve tracts (D) can be observed. S100B-positive cells in the brain stem are mainly glial cells. With regard to the intensity of the staining of the sections of the brain stem, no major differences between the four groups could be observed. No difference in distribution of S100B-positive cells was observed.

Representative microphotographs of the hippocampus are demonstrated in Fig. 4. The dentate gyrus (E) is shown in these photographs. Both neurons and glial cells contain S100B. No major differences between the four groups with regard to the intensity of the S100B-staining in the hippocampus could be observed. There were no differences with regard to the distribution of the S100B-positive cells between the four different groups in the hippocampus.

Table 1 demonstrates the number of S100B positive cells per mm^2 in the different brain regions. No significant differences were detected in the number of S100B positive cells in the different brain regions between the non-pregnant controls and the pregnant controls, nor between the pregnant controls and the pregnant endotoxin group. Differences in non-pregnant endotoxin versus pregnant endotoxin and non-pregnant controls versus non-pregnant endotoxin were not observed either.

3.2. S100B concentration in plasma

No significant differences were observed between the non-pregnant controls and the pregnant controls, nor

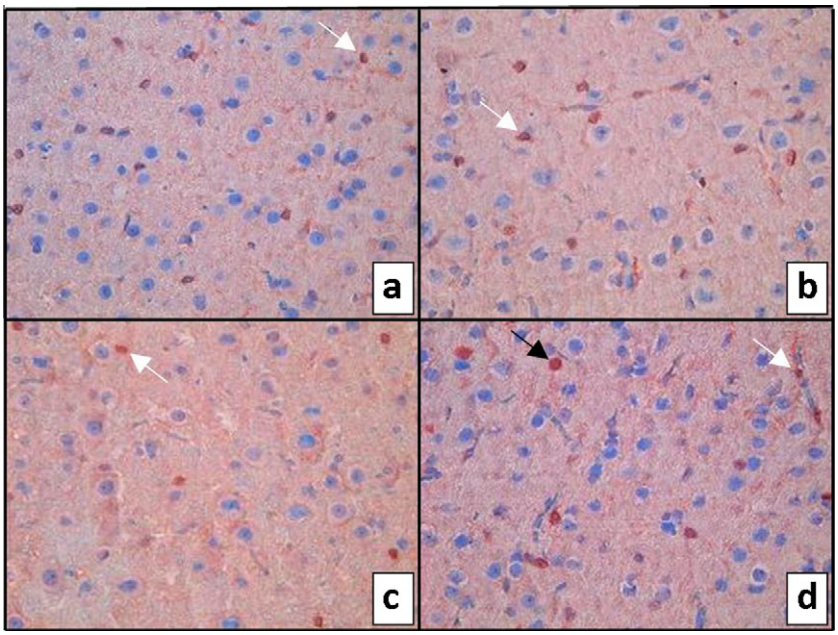

Fig. 1. Representative microphotographs of the forebrain immunostained for S100B and counterstained with haematoxylin of non-pregnant and pregnant rats infused with saline or endotoxin. (a) Non-pregnant rat infused with saline, (b) pregnant rat infused with saline, (c) non-pregnant rat infused with endotoxin and (d) pregnant rat infused with endotoxin. In the forebrain, the S100B protein seems to be mainly localized in the glial cells (white arrow), but some neurons (black arrow) also contain S100B. S100B-positive cells are indicated by arrows: white arrow, glial cell; black arrow, neuronal cell. Magnification: 400×. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

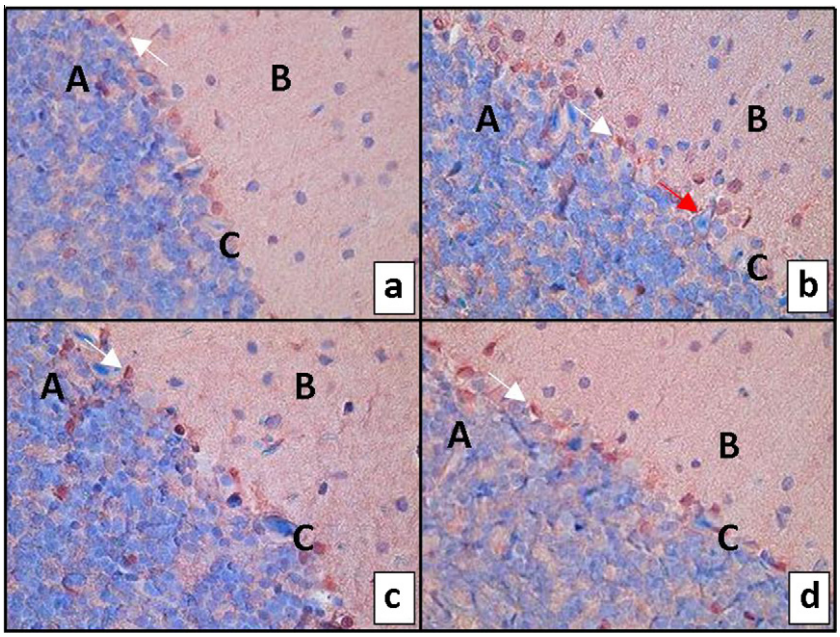

Fig. 2. Representative microphotographs of the cerebellum immunostained for S100B and counterstained with haematoxylin of non-pregnant and pregnant rats infused with saline or endotoxin. (a) Non-pregnant rat infused with saline, (b) pregnant rat infused with saline, (c) non-pregnant rat infused with endotoxin and (d) pregnant rat infused with endotoxin. Different types of layers can be observed in these microphotographs: the granule cell layer (A) and the molecular layer (B) of the cerebellum. Between these two layers a layer of Purkinje cells (C) can be observed. S00B positive glial cells (white arrows) are mainly localized in the Purkinje cell layer; Purkinje cells (red arrow) are negative for S100B. Magnification: 400×. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

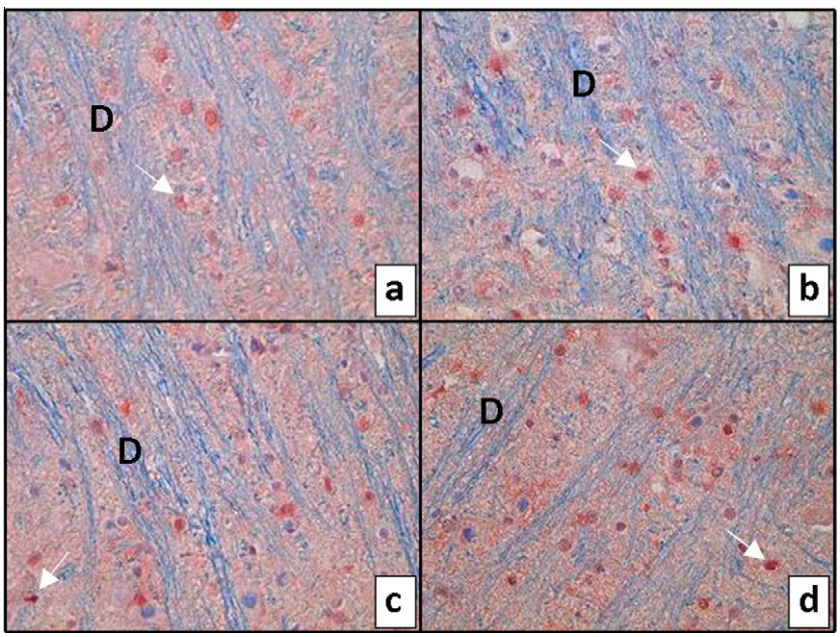

Fig. 3. Representative microphotographs of the brain stem immunostained for S100B and counterstained with haematoxylin of non-pregnant and pregnant rats infused with saline or endotoxin. (a) Non-pregnant rat infused with saline, (b) pregnant rat infused with saline, (c) non-pregnant rat infused with endotoxin and (d) pregnant rat infused with endotoxin. In the brain stem, nerve tracts can be observed (D), surrounded by glial cells. S100B-positive glial cells are indicated by white arrows. Magnification: 400×. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

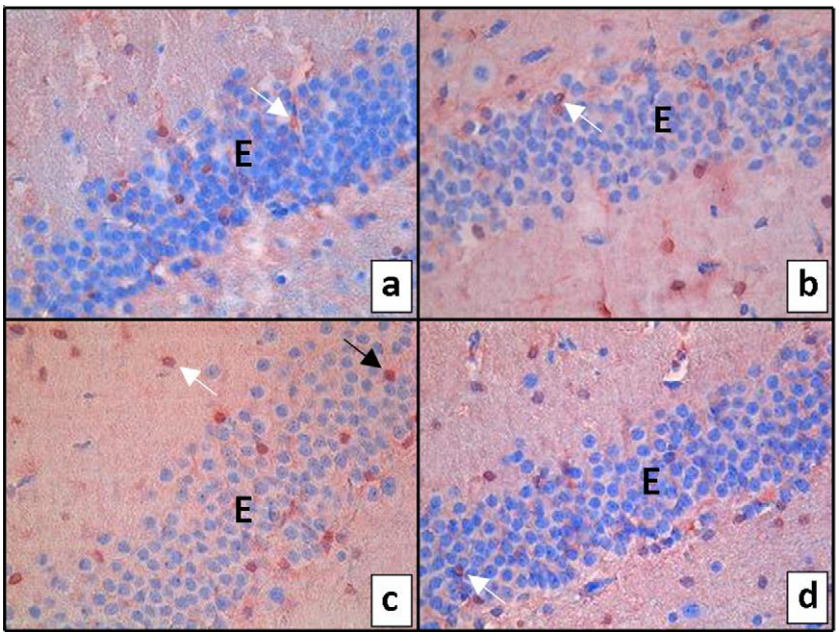

Fig. 4. Representative microphotographs of hippocampus immunostained for S100B and counterstained with haematoxylin of non-pregnant and pregnant rats infused with saline or endotoxin. (a) Non-pregnant rat infused with saline, (b) pregnant rat infused with saline, (c) non-pregnant rat infused with endotoxin and (d) pregnant rat infused with endotoxin. The dentate gyrus is shown in these photographs (E). The dentate gyrus is surrounded by both glial cells and neurons. S100B-positive cells are indicated by arrows: white arrow, glial cell; black arrow, neuronal cell. Magnification: 400×. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Number of S100B positive cells per mm^2 in the forebrain, cerebellum, brain stem and hippocampus in different groups of rats.

Group	Brain regions (mean ± SEM)			
	Forebrain	Cerebellum	Brain stem	Hippocampus
Non-pregnant saline	152.8 ± 11.6 (n = 5)	182.9 ± 12.5 (n = 5)	242.9 ± 46.1 (n = 5)	192.9 ± 20.0 (n = 4)
Non-pregnant endotoxin	151.1 ± 15.4 (n = 7)	220.2 ± 17.1 (n = 7)	300.8 ± 51.9 (n = 7)	224.1 ± 20.4 (n = 5)
Pregnant saline	165.6 ± 13.5 (n = 7)	238.2 ± 20.0 (n = 7)	260.9 ± 21.3 (n = 7)	221.6 ± 8.5 (n = 5)
Pregnant endotoxin	170.2 ± 12.7 (n = 7)	191.3 ± 12.2 (n = 7)	273.4 ± 35.7 (n = 7)	211.6 ± 8.7 (n = 5)

between the pregnant controls and the pregnant endotoxin group (Fig. 5). In the non-pregnant endotoxin group, the S100B concentration in plasma was significantly higher when compared with the pregnant endotoxin group ($P < 0.05$).

4. Discussion

This study demonstrates the expression of the S100B protein in different brain regions and plasma S100B concentrations in a preeclampsia rat model. No differences in S100B brain expression nor plasma concentrations were observed between pregnant and non-pregnant controls. Pregnancy per se, thus, does not influence the expression of the S100B protein in the rat brain nor S100B plasma concentrations. Moreover, no differences in S100B plasma concentrations nor S100B brain expression were detected between the pregnant controls and pregnant rats with experimental preeclampsia. Thus, in this mild preeclampsia rat model, we were unable to demonstrate evidence of brain injury. However, the S100B concentration in plasma was significantly higher in the non-pregnant endotoxin treated rats, when compared with the pregnant endotoxin treated rats (preeclampsia rat model). Pregnancy might

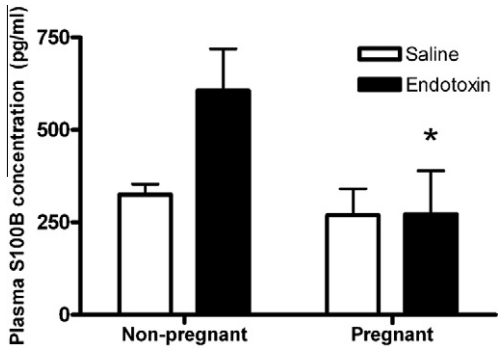

Fig. 5. Mean (±SEM) of S100B concentrations in plasma of non-pregnant (left set of bars) and pregnant rats (right set of bars) after infusion of saline (open bars) or endotoxin (black bars). *$P < 0.05$ vs non-pregnant rats infused with endotoxin, Two-way ANOVA ($P = 0.0472$) followed by post hoc Bonferroni.

have a protective effect on brain tissue after endotoxin infusion in rats.

In this study, we used the ultra-low-dose endotoxin preeclampsia rat model, which has been used for several years in preeclampsia research [10–14]. The histopathological and clinical events in this model simulate the predominant features of mild human preeclampsia such as a significant increase in blood pressure and in urinary albumin excretion [10]. The fact that we did not find evidence of brain injury in this model, may suggest that this mild preeclampsia rat model is not severe enough to induce cerebrovascular pathology. This may be in line with the fact that pregnant women with severe preeclampsia are more at risk for developing cerebrovascular pathology (PRES) when compared with women with mild preeclampsia [1]. Therefore, it is possible that S100B plasma concentrations and brain expression are increased in animal models for severe preeclampsia, when compared with animal models for mild preeclampsia.

To our surprise, we observed increased concentrations of S100B in the plasma of non-pregnant endotoxin treated animals. In contrast, we did not find increased expression of S100B in brain tissue of these animals. Our finding of increased plasma S100B is also in contrast to previous studies, in which we observed no effect of endotoxin in non-pregnant rats on various parameters [10–12]. It may, however, be in line with several studies reporting neuroinflammation induced by endotoxin in rats [15,16]. The dose of endotoxin used in our study, however, is much lower and given intravenously rather then intraperitoneally or via intracerebral injection. The high S100B plasma concentrations in the non-pregnant endotoxin treated rats might thus be caused by endotoxin-induced neuroinflammation.

Little is known about S100B levels and their significance in the peripheral blood, and cerebrospinal fluid in human (pre) eclampsia. Schmidt et al. studied serum S100B concentrations in women with several forms and severity of hypertensive disease in pregnancy [5]. This small study included 50 women. Of these women, 10 had experienced eclampsia, and 18 women had preeclampsia. No significant difference in serum S100B concentration could be demonstrated between the preeclamptic group and the normotensive group but serum S100B was significantly higher in eclampsia. Unfortunately, hardly any clinical characteristics of the study population with preeclampsia were provided, and therefore, no conclusions can be drawn with regard to the clinical relevance and utility of S100B serum levels. Whether the increased levels of plasma S100B only arise from the CNS in eclampsia remains to be established, since in pregnancy, S100B may also be found in fetal tissue. Tskitishvili et al. found the percentage of positively stained amniotic epithelial cells increased in preeclampsia compared with healthy pregnant controls, while in the amniotic fluid the S100B concentration was significantly higher in patients with preeclampsia, when compared with the healthy controls [8].

Although little is known about S100B in preeclampsia, it has the potential to become an important clinical biomarker of central nervous system injury and neuroinflammation in pregnancy and preeclampsia [17]. In various central nervous system disorder, such as subarachnoid hemorrhage, ischemic stroke and traumatic injury, the level of the S100B protein in peripheral blood samples is elevated [17]. The expression of the S100B protein in brain tissue and S100B concentrations in peripheral blood have also been investigated in several animal models for brain injury. S100B expression in the stroke-prone hypertension rat model was increased compared with healthy, normotensive rats [6]. Hippocampal S100B expression was increased in rats exposed to chronic cerebral hypoperfusion when compared with control rats [18]. Lipcsey et al. demonstrated increased expression of the S100B protein in the pig brain, mainly in the astrocytes, in septic encephalopathy [19]. Tanaka et al. demonstrated significant increases in serum S100B levels in a rat with cerebral hemorrhage when compared with rats without cerebral hemorrhage [20]. These non-pregnant animal models demonstrate that brain injury (e.g. stroke and hypoperfusion) can cause a higher S100B expression in rat brain tissue and higher S100B concentrations in peripheral blood. Whether also in pregnancy S100B will be affected by brain injury, remains to be established.

Concluding, this study demonstrates that pregnancy nor mild preeclampsia influenced the expression of the S100B protein in brain tissue and S100B plasma concentrations in the endotoxin rat model. Taking into account that the pathophysiology of PRES in (pre) eclampsia involves breakthrough of the blood–brain barrier and, eventually, ischemic damage to cells in the central nervous system, it is feasible that an increase in the S100B protein could be observed in preeclamptic women with impending PRES. To further elucidate the clinical utility of brain neuroinflammatory biomarkers animal studies with models for severe preeclampsia should be performed.

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