Molecular Psychiatry (2001) 6, 445–449

www.nature.com/mp

ORIGINAL RESEARCH ARTICLE

Increased S100B blood levels in unmedicated and treated schizophrenic patients are correlated with negative symptomatology

M Rothermundt[1], U Missler[2], V Arolt[1], M Peters[1], J Leadbeater[3], M Wiesmann[2], S Rudolf[4], KP Wandinger[5] and H Kirchner[4]

[1]*Department of Psychiatry, University of Muenster School of Medicine, Albert-Schweitzer-Str 11, D-48129 Muenster, Germany;* [2]*Department of Neuroradiology, Medical University of Luebeck, Ratzeburger Allee 160, D-23538 Luebeck, Germany;* [3]*Psychiatric Hospital, Friedrich-Ebert-Str, D-23774 Heiligenhafen, Germany;* [4]*Institute of Immunology and Transfusion Medicine, Medical University of Luebeck, Ratzeburger Allee 160, D-23538 Luebeck, Germany;* [5]*Department of Neurology, Charite Campus Mitte, NWFZ 2680, R 04 023, Schumannstr 20/21, D-10117 Berlin, Germany*

Keywords: nerve tissue protein S100; schizophrenia; antipsychotic agents; negative symptomatology; psychiatric status

S100B, a calcium-binding protein produced by astroglial cells, is a marker of astroglial cellular integrity. It has been shown to be increased in acute brain damage and neurodegeneration. A recent study showed increased S100B levels in medicated acutely psychotic patients with schizophrenia. The study presented here included 26 drug-free patients with acute schizophrenia and 26 matched healthy controls. S100B blood concentrations were determined using a quantitative immunoassay upon admission and after 6 weeks of neuroleptic treatment. The PANSS was used to investigate psychopathology. Unmedicated schizophrenic patients showed significantly increased S100B levels compared to matched healthy controls. After 6 weeks of treatment, 11 patients showed normal S100B levels while in 15 patients the levels remained increased. These patients showed significantly higher PANSS negative scores upon admission and after 6 weeks of treatment. Schizophrenic patients display a loss of astroglial integrity which is not caused by neuroleptic medication. Continuously increased S100B levels are associated with negative symptomatology. *Molecular Psychiatry* (2001) **6,** 445–449.

There is continuing discussion on the neurodegenerative mechanisms which contribute to the etiopathogenesis of schizophrenia. Support for this hypothesis comes from postmortem studies and especially from recent volumetric MRI studies indicating that ventricular enlargement and hemispheric volumetric reductions may have a progressive component in patients with schizophrenia (for a review see DeLisi[1]). Changes in brain structure and volume appear to arise from a reduction of neuritic processes (such as dendrites and synapses) rather than from a loss of neuronal or glial cell bodies (for a review see McGlashan & Hoffman[2]). A missing link for this hypothesis is a biochemical marker for cellular integrity in the brain that can be measured during acute psychotic episodes of schizophrenic patients. S100B has the potential to serve as such a marker.

The term S100 comprises a heterogeneous family of acidic calcium-binding proteins of which the two proteins S100A1 and S100B are considered to be the most relevant members regarding neurological disease.[3] S100B predominates in the brain. S100A1 and S100B form dimeric proteins with a molecular weight of 21 kDA, which have previously been named S100a (S100A1–S100B), S100b (S100B–S100B), and S100a0 (S100A–S100A).[4] S100B is synthesized mainly by astrocytes and evolves paracrine and autocrine effects on neurons and glia.[5] In adult brains it plays a role in neuronal plasticity and long-term potentiation. Those effects appear to be mediated primarily through binding to key synaptic proteins and inhibition of their phosphorylation. S100B in particular has an important role in regulating the protein kinase C phosphorylation of GAP-43, a growth-associated protein which is involved in axonal growth and synaptogenesis during development, synaptic remodeling and long-term potentiation. S100B interacts with and stabilizes microtubule-associated proteins (MAPs), such as tau and MAP-2. Increased S100B and MAP-2 promote dendritic maturation and induce a loss of dendrites.[6] Lower concentrations of extracellular S100B act on glial and neuronal cells as a growth differentiating factor, while higher concentrations induce apoptosis.[7] Structural damage to glial cells causes leakage of S100B protein into the extracellular compartment and cerebrospinal fluid; it also causes it to enter the bloodstream.[4]

Increased CSF and plasma levels of S100B have been detected after traumatic brain injury, toxic or ischemic brain damage, and in multiple sclerosis.[8–17] S-100B is also elevated in several kinds of dementia, especially Alzheimer's disease.[5] Our group showed increased S-100B plasma levels in medicated patients suffering from schizophrenia,[18] whereas Gattaz and colleagues reported decreased S100B concentrations in medicated patients with schizophrenia.[19]

In the present study we were able to investigate unmedicated or even drug-naive acutely psychotic

Table 1 PANSS psychopathology scores of initially unmedicated patients with an acute exacerbation of paranoid schizophrenia upon admission and after 6 weeks of neuroleptic medication

	Unmedicated patients	*SD*	*After 6 weeks of neuroleptic treatment*	*SD*	*P*
Mean PANSS **total** score	86.7	17.9	66.7	17.8	0.0001
Mean PANSS **positive subscale** score	25.0	6.3	16.0	6.7	0.0001
Mean PANSS **negative subscale** score	19.5	8.3	17.3	7.9	0.294
Mean PANSS **general psychopathology subscale** score	42.1	9.6	35.0	12.6	0.003

schizophrenic patients. The patients were reinvestigated after 6 weeks of neuroleptic treatment.

The mean PANSS (Positive and Negative Syndrome Scale) total score upon admission was 86.7 ± 17.9, ranging from 55 to 127. After 6 weeks of neuroleptic treatment the mean PANSS score was 66.7 ± 17.8 (range: 36–97), indicating a significant improvement of psychopathology ($Z = -3.89$, $P \leq 0.0001$). The positive and general psychopathology subscales showed a significant decrease after 6 weeks of treatment ($Z = -4.25$, $P \leq 0.0001$; $Z = -3.02$, $P = 0.003$, respectively), while the negative subscale showed no significant change ($Z = -1.05$, $P = 0.29$, Table 1).

Upon admission, the S100B plasma level in schizophrenic patients (mean 0.098 $\mu g\,l^{-1}$, SD 0.076) was significantly higher compared to the matched healthy controls (mean 0.034 $\mu g\,l^{-1}$, SD 0.0175, $Z = -3.28$, $P = 0.001$, Figure 1). After 6 weeks of treatment (mean 0.052 $\mu g\,l^{-1}$, SD 0.033 $\mu g\,l^{-1}$) the level of significance was no longer reached ($Z = -1.92$, $P = 0.056$). There was a significant positive correlation between the negative subscale score and the S100B concentration after 6 weeks (rho = 0.493, $P = 0.02$). Furthermore, intraindividual differences between negative subscale scores upon admission and after treatment were significantly correlated with the S100B concentration after 6 weeks, indicating that little change or even deterioration of the negative symptomatology was associated with high S100B levels (rho = –0.7, $P \leq 0.0001$).

Upon admission, 15 patients showed S100B levels that were higher than the mean levels of the healthy controls plus two standard deviations (>0.069 $\mu g\,l^{-1}$). After 6 weeks of treatment these patients had significantly higher PANSS negative scores than the patients with lower S100B upon admission ($Z = -2.39$, $P = 0.016$, Figure 2), while upon admission the PANSS negative scores did not differ between the groups ($Z = -1.56$, $P = 0.12$). PANSS total scores did not differ between groups either upon admission ($Z = -1.38$,

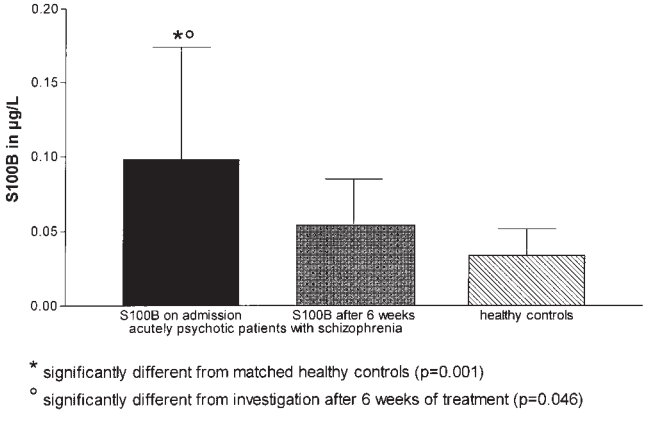

Figure 1 S100B blood levels (mean plus SD) in acutely psychotic unmedicated schizophrenic patients and after 6 weeks of treatment compared with healthy controls.

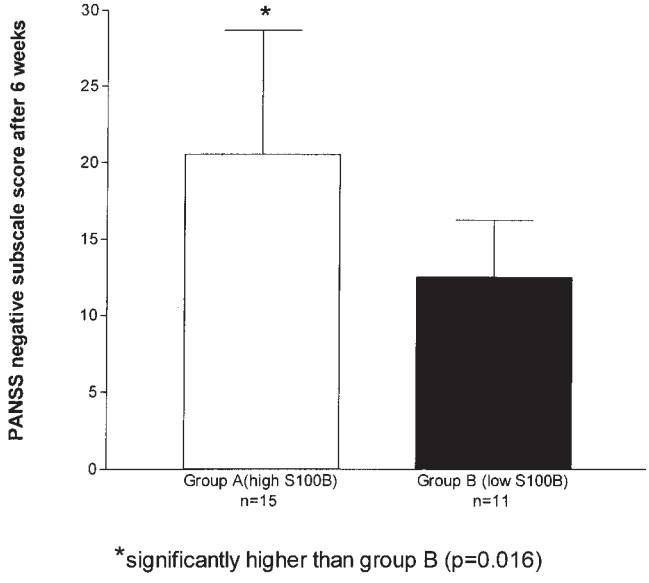

Figure 2 PANSS negative subscale scores (mean plus SD) of schizophrenic patients after 6 weeks of treatment. Patients were grouped according to their S100B blood levels upon admission. Group A had S100B levels higher than the mean plus two standard deviations of matched healthy controls (mean: 0.034 $\mu g\,l^{-1}$; SD: 0.0175 $\mu g\,l^{-1}$; mean plus 2 SD: 0.069 $\mu g\,l^{-1}$) while group B showed S100B levels lower than 0.069 $\mu g\,l^{-1}$. After 6 weeks of treatment, patients with high S100B levels upon admission (group A) demonstrated significantly higher PANSS negative scores than patients with low S100B levels upon admission (group B).

$P = 0.18$) or after 6 weeks of treatment ($Z = -0.48$, $P = 0.65$).

After 6 weeks of treatment, seven patients still had S100B levels above 0.069 $\mu g\ l^{-1}$. These patients demonstrated significantly less improvement in the PANSS total ($Z = -2.51$, $P = 0.011$) and the negative subscale scores ($Z = -2.55$, $P = 0.010$) after treatment compared to patients with lower S100B levels after 6 weeks of treatment (Figure 3).

No differences in S100B levels were detected between the group of patients treated with typical and the group treated with atypical neuroleptic drugs ($Z = -0.029$, $P = 0.98$). At both time points of investigations the S100B levels of drug-naive patients did not differ from the levels of patients with a history of neuroleptic medication (admission: $Z = -0.318$, $P = 0.75$; after 6 weeks: $Z = -0.349$; $P = 0.73$). There was no significant correlation between S100B and the dose of haloperidol equivalents after 6 weeks of treatment (rho = –0.04, $P = 0.85$). No sex-related differences were observed ($Z = -0.18$, $P = 0.89$). There were no significant correlations between S100B and age (rho = –0.13, $P = 0.54$), onset of illness (rho = –0.21, $P = 0.30$), or duration of illness (rho = 0.12, $P = 0.57$).

To our knowledge, this is the first study investigating S100B serum levels in unmedicated schizophrenic patients; it also includes a follow-up investigation after 6 weeks of neuroleptic treatment. We were able to show that S100B plasma levels are increased in unmedicated acutely psychotic schizophrenic patients as previously shown by Wiesmann *et al*[18] for medicated patients. Increased S100B plasma levels in schizophrenic patients are obviously not an artifact caused by neuroleptic medication. However, these results contrast with the findings of Gattaz *et al*[19] who reported decreased S100B plasma levels in schizophrenic outpatients, mostly treated with clozapine. Differences in the investigated patient samples could account for these discrepancies. The fact that the sample of Gattaz *et al*[19] included schizophrenic outpatients predominantly treated with clozapine allows the assumption that those patients were investigated in a residual, remitted or at least in a not highly acute stage of disease, whereas our sample consisted of acutely psychotic inpatients suffering from an acute episode of paranoid schizophrenia. After 6 weeks of neuroleptic treatment, the S100B levels in the total group of patients in our study did not significantly differ from healthy controls anymore. S100B plasma levels may serve as a marker for the acute stage of schizophrenic psychosis and might normalize or even decrease in a chronic stage of disease, as reported by Gattaz *et al*.[19]

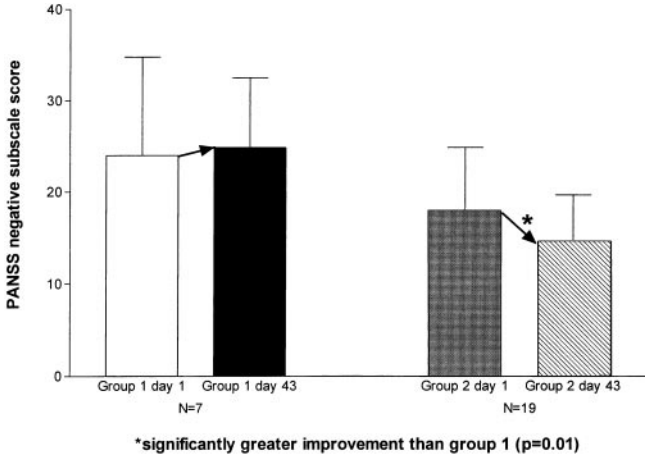

Figure 3 PANSS negative subscale scores of schizophrenic patients upon admission (day 1, unmedicated) and after 6 weeks of treatment (day 43). Patients were grouped according to their S100B blood levels after 6 weeks of treatment. Group 1 had S100B levels higher than the mean plus two standard deviations of matched healthy controls (mean: 0.034 $\mu g\ l^{-1}$, SD: 0.0175 $\mu g\ l^{-1}$; mean plus 2 SD: 0.069 $\mu g\ l^{-1}$), while group 2 had S100B levels lower than 0.069 $\mu g\ l^{-1}$. Patients with high S100B blood levels upon admission (group 1) showed no change in the PANSS negative score during treatment, while patients with low S100B (group 2) showed a significant improvement in negative symptomatology.

The negative dimension of the schizophrenic symptomatology is correlated with S100B plasma levels. Patients with initially high S100B plasma levels showed significantly higher PANSS negative scores after 6 weeks of treatment than patients who upon admission showed S100B levels closer to normal. Also, patients with high S100B levels after 6 weeks of treatment showed no change in the PANSS negative subscale scores, while those patients whose S100B levels returned to normal experienced a significant improvement in negative psychopathology. These findings allow the interpretation that amongst patients suffering from more severe negative symptoms, astroglial integrity is affected to a greater degree not only in an acute stage of the disease but also after 6 weeks of treatment. A loss of astroglial intactness appears to be associated with the development of negative symptoms rather than positive or general psychopathology symptoms. A restoration of astroglial integrity seems to be associated with improvement of negative symptoms.

In schizophrenia, severe negative symptoms are a main cause for an unfavorable clinical outcome leading to invalidity. Besides affective and social impairment, cognitive deficits are major factors in determining adverse outcome. Findings reporting a possible influence of cellular integrity (as indicated by S100B plasma levels) on cognitive function are therefore of special interest in this context. In neuropsychological testing, minor head injury patients with elevated S100 showed disrupted reaction time, attention and speed of information processing.[20] In head injury or stroke patients, a higher plasma concentration of S100 predicted an unfavorable clinical outcome compared to patients with lower S100 levels.[12–14] Transgenic mice with an overproduction of S100B showed significant learning deficits and behavior similar to mice with manifestations of hippocampal dysfunction.[6,21]

Increased S100B plasma levels in acute schizophrenia indicate that there might be a loss of cellular integrity in the brain in that stage of the disease, and

they appear to be associated with a more severe negative psychopathology and the persistence of these symptoms. These findings seem to support the hypothesis of Lieberman *et al*,[22] stating that there might be a progressive degenerative component in the early phase of the disease and that poorer outcome is associated with an increase in ventricular volume over time. Future research should focus on the investigation of additional biochemical parameters, MRI findings and cognitive functions in patients with increased S100B.

Materials and methods

After written informed consent was acquired, plasma samples were taken by venipuncture from 26 in patients (10 males, 16 females, aged 20–59 years, mean age 37.0 ± 12.9 years) suffering from an acute episode of paranoid type schizophrenia (DSM-IV 295.3) and 26 age- and sex-matched healthy controls (aged 20–59 years, mean age 37.0 ± 12.9 years), who were recruited at the blood donation service of the University of Luebeck. Patients and controls were diagnosed independently by two psychiatrists according to DSM-IV criteria. No patients had received any psychotropic medication for at least 6 months prior to examination, seven patients were even drug-naive. The PANSS was used to evaluate psychopathology. All patients were examined twice by the same rater: upon admission (unmedicated), and after 6 weeks of neuroleptic treatment. Fourteen patients received typical (four bromperidole, three fluphenazine, three benperidole, three haloperidole, one flupenthixole), and 12 received atypical neuroleptic drugs (four clozapin, three olanzapine, five risperidone). The mean duration of illness was 9.96 ± 10.35 years, ranging from 0 to 39 years. The controls had no lifetime history of any psychiatric disorder. Systemic diseases (neoplasms, autoimmune diseases, infectious diseases, neurological, and cardiovascular diseases), brain injury, co-morbid psychiatric diagnosis (eg dementia) and substance abuse were excluded by taking a detailed history, reviewing charts, and performing a physical examination of patients and healthy controls.

Blood samples were centrifuged within 4 h, aliquoted, and frozen at –80°C until analysis. S-100B concentrations were determined by an immunofluorometric sandwich assay using a monoclonal anti-S-100B antibody on the solid phase and a polyclonal rabbit anti-S100 antibody for detection, as described previously.[12] The assay's lower detection limit for S-100 is 0.015 $\mu g\,l^{-1}$. The assay is specific for S-100B which predominates in the brain.[23] The intraassay (within-run) imprecision (CVs) is 3.2% at 0.51 $\mu g\,l^{-1}$, 2.1% at 5.97 $\mu g\,l^{-1}$, and 2.3% at 11.4 $\mu g\,l^{-1}$. The total imprecision (between-day, CVs) is 11.5% at 0.45 $\mu g\,l^{-1}$, 7.9% at 4.79 $\mu g\,l^{-1}$, and 7.8% at 15.45 $\mu g\,l^{-1}$. Analytical recovery ranged between 90 and 101%.

Due to the non-Gaussian distribution of our data, non-parametric tests were employed for statistical evaluation. The Wilcoxon Matched-Pairs Signed-Ranks Test, the Mann–Whitney U-Test, and the Spearman Correlation Coefficient were used as provided by the SPSS 9.0 program.

Acknowledgements

The authors thank Julian P Keogh for critically reviewing the text.

References

1 DeLisi LE. Defining the course of brain structural change and plasticity in schizophrenia. *Psychiatry Res* 1999; **92**: 1–9.
2 McGlashan TH, Hoffman RE. Schizophrenia as a disorder of developmentally reduced synaptic connectivity. *Arch Gen Psychiatry* 2000; **57**: 637–648.
3 Heizman CW, Cox JA. New perspectives on S100 proteins: a multifunctional Ca^{2+}, Zn^{2+} and Cu^{2+} binding protein family. *BioMetals* 1998; **11**: 383–397.
4 Beaudeux JL, Dequen L, Foglietti MJ. Pathophysiologic aspects of S-100beta protein: a new biological marker of brain pathology. *Ann Biol Clin* 1999; **57**: 261–272.
5 Griffin WST, Stanley LC, Ling C, White L, MacLeod V, Perrot LJ *et al*. Brain interleukin 1 and S-100 immunoreactivity are elevated in Down syndrome and Alzheimer disease. *Proc Natl Acad Sci* 1998; **86**: 7611–7615.
6 Whitaker-Azmitia P, Wingate M, Borella A, Gerlai R, Roder J, Azmitia EC. Transgenic mice overexpressing the neurotrophic factor S-100β show neuronal cytoskeletal and behavioral signs of altered aging processes: implications for Alzheimer's disease and Down's syndrome. *Brain Res* 1997; **776**: 51–60.
7 Fano G, Biocca S, Fulle S, Mariggio MA, Belia S, Calissano P. The S-100: a protein family in search of a function. *Prog Neurobiol* 1995; **46**: 71–82.
8 Missler U, Wiesmann M. Measurement of S-100 protein in human blood and cerebrospinal fluid: analytic method and preliminary clinical results. *Eur J Clin Chem Clin Biochem* 1995; **33**: 743–748.
9 Büttner T, Weyers S, Sprengelmeyer T, Postert R, Kuhn W. S-100 protein: serum marker of focal brain damage after ischemic territorial MCA infarction. *Stroke* 1997; **28**: 1961–1965.
10 Griffin WST, Yeralan O, Sheng JG, Boop FA, Mrak RE, Rovnaghi CR *et al*. Overexpression of the neurotrophic cytokine S100b in human temporal lobe epilepsy. *J Neurochem* 1995; **65**: 228–233.
11 Lamers KJB, van Engelen BGM, Gabreels FJM, Hommes OR, Borm GF, Wevers RA. Cerebrospinal neuron-specific enolase, S-100 and myelin basic protein in neurological disorders. *Acta Neurol Scand* 1995; **92**: 247–251.
12 Missler U, Wiesmann M, Friedrich C, Kaps M. S-100 protein and neuron-specific enolase concentrations in blood as indicators of infarction and prognosis in acute ischemic stroke. *Stroke* 1997; **28**: 1956–1960.
13 Raabe A, Grolms C, Keller M, Döhnert J, Sorge O, Seifert V. Correlation of computed tomography findings and serum brain damage markers following severe head injury. *Acta Neurochir* 1998; **140**: 787–792.
14 Rothoerl RD, Woertgen C, Holzschuh M, Metz C, Brawanski A. Rapid evaluation of S-100 serum levels. Case report and comparison to previous results. *Brain Inj* 1999; **13**: 387–391.
15 Sindic CJM, Chalon MP, Cambiaso CL, Laterre EC, Masson PL. Assessment of damage to the central nervous system by determination of S-100 protein in the cerebrospinal fluid. *J Neurol Neurosurg Psychiatry* 1982; **45**: 1130–1135.
16 Vinesi P, Geloso MC, Michetti F. S-100 proteins in trimethyltin-induced neurodegeneration in the rat hippocampus. An immunochemical and immunocytochemical study. *Mol Chem Neuropathol* 1997; **32**: 129–141.
17 Wiesmann M, Missler U, Hagenström H, Gottmann D. S-100 protein plasma levels after aneurysmal subarachnoid haemorrhage. *Acta Neurochir* 1997; **139**: 1155–1160.
18 Wiesmann M, Wandinger KP, Missler U, Eckhoff D, Rothermundt M, Arolt V *et al*. Elevated plasma levels of S-100b protein in schizophrenic patients. *Biol Psychiatry* 1999; **45**: 1508–1511.
19 Gattaz WF, Lara DR, Elkis H, Portela LV, Goncalves CA, Tort AB

et al. Decreased S100B protein in schizophrenia: preliminary evidence. *Schizophr Res* 2000; **43**: 91–95.
20 Waterloo K, Ingebrigtsen T, Romner B. Neuropsychological function in patients with increased serum levels of protein S-100 after minor head injury. *Acta Neurochir* 1997; **139**: 26–31.
21 Gerlai R, Roder J. Abnormal exploratory behavior in transgenic mice carrying multiple copies of the human gene for S100 beta. *J Psychiatry Neurosci* 1995; **20**: 105–112.
22 Lieberman JA. Pathophysiologic mechanisms in the pathogenesis and clinical course of schizophrenia. *J Clin Psychiatry* 1999; **60**: S9–S12.
23 Donato R. Perspectives in S-100 protein biology. *Cell Calcium* 1991; **12**: 713–726.

Correspondence: Dr M Rothermundt, Department of Psychiatry, University of Muenster, Albert-Schweitzer-Str 11, D-48129 Muenster, Germany. E-mail: rothermu@uni-muenster.de
Received 20 November 2000; revised 22 January 2001; accepted 25 January 2001