Chapter

Technologically Processed Highly Diluted Antibodies to S100 Protein in the Treatment of Neurotic Disorders: The Review

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Abstract

Neurotic disorders (NDs) are among the most common mental diseases leading to a decrease in the quality of life, lack of socialization, and increased mortality. The diagnosis and treatment of all types of NDs are challenging. In the light of the ongoing search for an effective and safe therapeutic strategy influencing certain aspects of ND pathogenesis, technologically processed highly diluted antibodies to S100 protein (TP Abs to S100) seem to be a promising treatment option for patients with NDs. TP Abs to S100 possess stress-protective, anxiolytic, antidepressant, antiamnestic, and neuroprotective activities. In the current review, we describe the mechanisms of action and pharmacological effects of TP Abs to S100 demonstrated in nonclinical (preclinical) and clinical studies. Based on the data, we tried to evaluate the future prospects of the TP Abs to S100 as the drug of choice for ND treatment.

Keywords: neurotic disorder, anxiety, anxiolytic therapy, S100 protein, somatoform disorder

1. Introduction

Neurotic disorders (NDs) are among the most common mental diseases leading to a decrease in the quality of life, lack of socialization, and increased mortality [1]. Around 20–40% of primary care outpatients are diagnosed with NDs according to International Disease Classification (ICD)-10 or Diagnostic and Statistical Manual of Mental Disorders (DSM) V criteria [2].

ICD-10 classification of the NDs F40–F48 includes phobic anxiety disorders (F40), other anxiety disorders (AD, F41), obsessive-compulsive disorder (OCD, F42), reaction to severe stress, adjustment disorders (F43), dissociative and conversion disorders (F44), somatoform disorders (SD, F45), and other nonpsychotic mental disorders (F48). In the DSM V, the same disorders are classified as Anxiety

Disorders, Obsessive-Compulsive and Related Disorders, Trauma- and Stressor-Related Disorders, Dissociative Disorders, and Somatic Symptom Disorder [3, 4].

Phobias are present in 1.3–5.7% of all NDs [5]. Anxiety symptoms are thought to occur in every 14th person during the lifetime [6]. The prevalence of SD is 20–25%, but at least one medically unexplained symptom is found in 40–49% of patients [7, 8]. Around 10% of all psychiatric patients have dissociative disorder [9, 10]. A lifetime prevalence of OCD is 2.3%, and the rate of adjustment disorder is 1–2% [5].

The overlaps between AD, phobias, and SD were shown and considered a result of similarity of pathogenesis, which involves disturbances in hypothalamic-pituitary-adrenal axis (HPAA), cytokine levels, and changes in the state of receptors in the nervous system [11–15]. Continued and prolonged stress may disturb the HPAA to such an extent that the negative feedback mechanisms (glucocorticoid negative feedback, in particular) are disrupted, and the adaptive responses of the HPAA may then become maladaptive. Enhanced proinflammatory cytokine production and overactivation of the sympathetic nervous system contribute to a state of chronic low-grade inflammation.

NDs have a great social impact. A British survey (1993) reported that 8.3% of 10,000 responders had ND limiting their daily activities and 3.4% experienced severe "disabling" NDs, associated with a higher chance of being unemployed [16]. The cost of AD treatment in the European Union was approximately 41 billion € in 2004 and 66 billion € in 2010 [17, 18]. Taking into consideration the prediction of the growing influence of mental health problems on the economic output by 2030 [19], we expect the increasing burden of NDs.

The diagnosis and treatment of all types of NDs are challenging. More than 20% of AD patients are undertreated and continue to suffer from symptoms [11]. A study by Wang revealed a 2–3-year delay in the diagnosis of NDs [20]. Around 40–66% of SD cases are underdiagnosed in primary care [21]. The first line of treatment for most of NDs is selective serotonin reuptake inhibitors (SSRIs). Nevertheless, their efficacy and safety are still under consideration. The high placebo effect was shown in randomized controlled studies of SSRI in the treatment of phobic disorder, OCD, and generalized anxiety disorder (GAD) [22]. There are only 40–60% of responders to first-line therapy among OCD patients [23]. In the Cochrane review by Kleinstaeuber et al., low-quality evidence for the efficacy of new generation antidepressants in SD was obtained [24]. Adverse events such as insomnia, nausea, sexual dysfunction, and withdrawal are common for SSRI. Negative drug interactions are also limiting their use in patients receiving therapy for somatic diseases. Other antidepressant drugs such as tricyclic antidepressants (TCA) have been shown to be effective for the treatment of some NDs in several trials, although the Cochrane review did not reveal any significant differences in the comparison of tricyclic antidepressants (TCA) and other medications in SD [24]. The safety profile of TCA is more unfavorable than SSRI. The use of benzodiazepines in ADs is limited due to the sedation, myorelaxant effect, and negative impact on cognition they provoke in long-term use. Among nonpharmacological treatments, only cognitive behavioral therapy was shown to be effective with greater results in combination with medication [22].

In the light of the ongoing search for an effective and safe therapeutic strategy influencing certain aspects of ND pathogenesis, technologically processed highly diluted antibodies to the brain specific S100 protein (TP Abs to S100) seem to be a perspective substance for treatment.

In the central nervous system (CNS), the brain-specific S100 protein is synthesized mainly by astrocytes and then transported to neurons where it is involved in numerous processes. In particular, it was shown that S100 affects the

differentiation and survival of neurons, the growth of dendrites, the integrity of cytoskeleton, and energy metabolism [25].

Increased level of S100 is considered a marker of blood brain barrier failure. S100 serum levels are elevated after stroke, subarachnoid hemorrhage, and brain trauma and correlate positively with patient outcome. However, the brain-specific S100 protein may be secreted peripherally, and its elevated serum levels are also found in heart diseases and infections. High serum levels of the brain-specific S100 protein are also found in patients with schizophrenia, depressive/bipolar disorders, and obesity, but which cells are the sources of S-100 protein in these conditions is unknown [25, 26].

A number of nonclinical studies of TP Abs to S100 efficacy, safety, and mechanisms of action using the commonly applied experimental *in vivo* and *in vitro* models preceded clinical investigation. While studying the drug's primary and secondary pharmacodynamics, it was shown that TP Abs to S100 exert stress-protective [27], anxiolytic [28–33], antidepressant [30, 31, 34], antiamnestic [35–37], and neuroprotective [38, 39] activities.

Target identification and mechanism-of-action studies revealed that the drug recruits serotonin-, dopamine-, GABA-, noradrenaline-, and glutamatergic systems [29, 30, 40–42] and thereby might be considered a player in various neurotransmitter-mediated processes. Moreover, TP Abs to S100 influence $sigma_1$ receptor [41] that in turn modulates the activity of almost all neurotransmitter systems and thereby possesses a spectrum of psychotropic activities [43, 44].

Data on the TP Abs to 100 mechanisms of action and identified pharmacodynamics of the drug are consistent with the literature data on the relationship between influencing certain neurotransmitter systems (their receptors) and observing subsequent psychotropic effects. For example, it is known that benzodiazepines mediate their anxiolytic activity and sedation via GABAA receptors [45, 46]. GABAB receptor agonists are known to attenuate the behavioral deficit-restoring effect of antidepressants [47, 48]. Ligands of 5-HT1A, 5-HT1B, 5-HT1F, 5-HT2a, 5-HT2B, 5-HT2C, and 5-HT3 receptors were shown to regulate aggression, anxiety, learning, addiction, locomotion, memory, mood, and so on [49]. Ligands of the glycine site of the NMDA receptor exhibit anxiolytic and antidepressant properties and impact memory-related processes [50–53, 80]. D3 receptor deficiency can result in chronic depression and anxiety [54]. $Sigma_1$ receptor ligands have a whole spectrum of psychotropic effects due to their modulating effect on all major neurotransmitter systems [43, 44], which also are in line with TP Abs to S100 mechanism of action.

More than 2000 patients with GAD (F41.1), SD (F45), adjustment disorders (AjDs) (F43.2), neurasthenia (F48.0), and anxiety accompanying somatic diseases (cardiovascular and gastrointestinal disorders) took part in phase III, IV, and post-marketing clinical trials (CTs) of TP Abs to S100, including two double-blind placebo-controlled randomized CTs and nine open-label comparative randomized CTs [55–57]. TP Abs to S100 were shown to be as effective as clonazepam 0.5–1 mg/day, bromdihydrochlorphenylbenzodiazepine 1.5 mg/day (for 7 days), and tofisopam 100 mg/day but causing less adverse events (AEs) [58–60].

The evidence on the safety of TP Abs to S100 was obtained in clinical and nonclinical trials. In CTs, TP Abs to S100 exerted less AEs typical for other anti-anxiety medications such as daytime sleepiness and muscle relaxation. No cases of withdrawal symptoms, addiction to TP Abs to S100, or negative drug interactions have been registered up-to-date. In nonclinical trials, no myorelaxant and toxic effects were observed.

In the current review, we describe the mechanisms of action and pharmacological effects of TP Abs to S100 demonstrated in nonclinical (preclinical) and clinical

studies. Based on the data, we attempt to evaluate the future perspectives of the TP Abs to S100 as the drug of choice for ND treatment.

2. Preclinical trials of technologically processed highly diluted antibodies to S100 protein

2.1 Pharmacodynamics

2.1.1 Biological activity

2.1.1.1 Antistress activity of TP Abs to S100

Antistress activity of TP Abs to S100 was studied using three approaches.

2.1.1.1.1 Effect on somato-vegetative manifestations of stress

Negative emotions arising from stress caused by the anticipation of pain or other negative expectations (in particular, on the eve of surgical operations, educational tests, important meetings, etc.) are accompanied by anxiety and fear. Concurrently, a cascade of somato-vegetative manifestations of stress is initiated [61].

Modeling of a conditioned emotional reflex to unescapable electric pain stimulation was performed on outbred white male rats weighing 220–280 g [27]. This was followed by monitoring of animal behavior in a stressful situation (repeated placement in an experimental 'dangerous' camera) as well as emotional responses when stress was intensified by an additional negative provocation (approaching an unfamiliar object to the animal's head). Antistress activities of TP Abs to S100 and diazepam ('classical' benzodiazepine tranquilizer, positive control) were estimated by administering drugs one day after development of the conditioned reflex.

Rats in the control group (hereinafter, animals that received distilled water as a placebo) when they were subsequently placed in a "dangerous" chamber responded by freezing (45%) or actively trying to escape the chamber (35%) (**Table 1A**). Only 20% of rats showed calm behavior. At the same time, somato-vegetative manifestations of stress were observed in animals (especially with a passive reaction): increased frequency of breathing, urination, defecation, and squeaking. Both TP Abs to S100 and diazepam caused a decrease in the number of rats with a passive and active response to stress, as well as significantly (three times) increased the number of animals with a calm orientation-exploratory activity. Somato-vegetative manifestations of stress also dissipated in both groups.

The emotional reaction of anxiety and anxiety associated with the expectation of pain in a "dangerous" chamber was significantly enhanced when using additional provocation—bringing an unfamiliar object to the head of the animal. This was manifested as an increase in the number of rats with active (up to 40%) and passive (up to 55%) behavior and a decrease in the number of animals with calm behavior (down to 5%) (**Table 1B**). Respiratory symptoms, squeaking, frequency of defecation, and urination also increased. Both drugs (TP Abs to S100 and diazepam) reduced the severity of stress induced by expectation of pain. TP Abs to S100 reduced both the number of animals with a spontaneous active and passive reaction by 20%, while diazepam reduced the number of animals with active attempts to escape the chamber (by 35%) more than the number of animals with freezing (only by 10%). The same trend continued with additional negative provocation, which may be the result of the sedative activity of diazepam, which TP Abs to S100 do not have.

Parameter	[A] Stress induced by anticipation of pain			[B] Stress induced by anticipation of pain with additional negative stimulation		
	Control	Diazepam	TP Abs to S100	Control	Diazepam	TP Abs to S100
Percent of animals with:						
Passive behavior (freezing)	45	35	**25***	55	45	**15***
Active behavior with attempts to get out of the chamber	35	5*	**15***	40	5*	**20***
Calm exploratory behavior	20	60*	**60***	5	50*	**65***
Freezing in response to provocation	*n/a*	*n/a*	*n/a*	55	45	**15***
Aggression in response to provocation	*n/a*	*n/a*	*n/a*	40	15	**10***
Hurried breathing	55	35	**25***	75	40	**35***
Frequency (%) of:						
Squeaking	25	5*	**5***	45	20*	**25***
Fecal boluses	50	25*	**25***	65	25*	**45**
Urinations	35	25	**25**	60	25*	**25***

Note: animals were intragastrically administered distilled water (2.5 ml/kg, control), TP Abs to S100 (2.5 ml/kg), or diazepam (1 mg/kg) at a single dose 30 minutes prior to testing. n/a, not applicable; TP Abs to S100, technologically processed highly diluted antibodies to S100 protein.
** $p < 0.05$ versus corresponding control.*
Bold entries were made to emphasize the results in TP Abs to S100 group.

Table 1.
TP Abs to S100 antistress activity in a model of a conditioned emotional reflex to unescapable electric pain.

2.1.1.1.2 Effect on c-Fos protein expression

It is known that immediate-early response *c-fos* gene expression in the hypothalamic paraventricular nucleus is one of the primary biological markers of stress [62]. The effectiveness of stress-protective compounds can be assessed by their ability to suppress *c-fos* expression in the brain.

The study was conducted on male Wistar rats weighing 250–280 g [63], classified as active or passive (stress-resistant or predisposed to stress, respectively) in the open field (OF) test [64]. The OF test is widely used to study the behavior of rats [65]: animals are placed in the center of the OF arena and the horizontal and vertical activity, the number of entries into the center zone, as well as the number of acts of defecation and urination (emotionality) is recorded.

Rats were administered TP Abs to S100 or imipramine (antidepressant drug that modulates *c-fos* expression) and then subjected to 1-hour immobilization with simultaneous electrocutaneous irritation. Immunohistochemical detection of c-Fos protein in the parvocellular neurons of the paraventricular nucleus of the hypothalamus was performed in samples obtained 90 min after the procedure, at the peak of the protein expression [62].

In response to stress, c-Fos protein level significantly increased (vs. intact animals) in both active and passive animals (20–25 fold), and in the latter, this increase was more pronounced (**Figure 1**). TP Abs to S100 and imipramine demonstrated equally and pronounced antistress activity in passive animals: 1.2- and 1.5-fold decrease in the number of Fos-positive cells was observed, respectively.

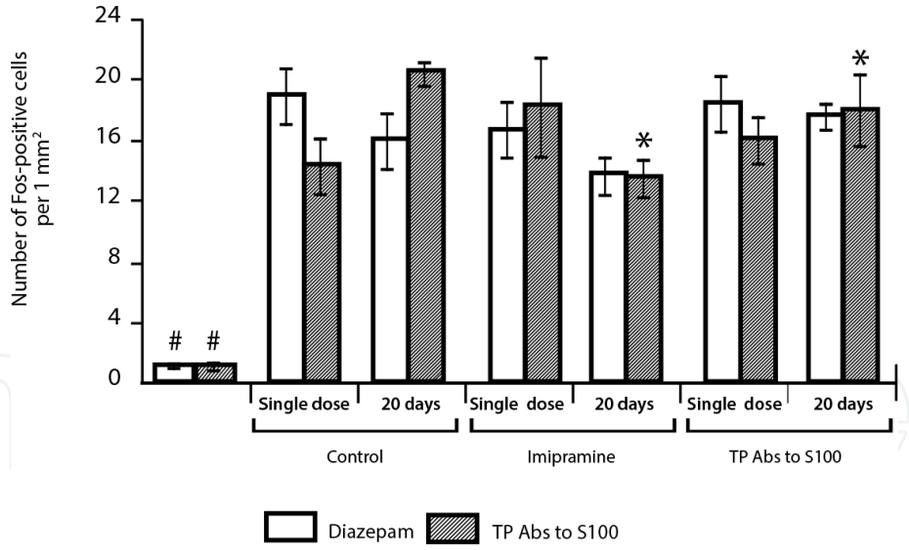

Figure 1.
TP Abs to S100 effect on c-Fos protein expression (stress marker) in the rat hypothalamic paraventricular nucleus after 1-hour immobilization with simultaneous electrocutaneous irritation. Note: animals were intragastrically administered distilled water (2.5 ml/kg, control), TP Abs to S100 (2.5 ml/kg) or imipramine (12 mg/kg) at a single dose or for 20 days preceding stress exposure. Data are expressed as M ± SD. $^{*}p < 0.05$ ($^{\#}p < 0.001$) versus corresponding control. TP Abs to S100, technologically processed highly diluted antibodies to S100 protein.

2.1.1.1.3 Effect on gastric ulcers after an immobilization stress

Another important biological marker of stress is development of ulcers in the gastric mucosa. For example, it is known that immobilization stress is accompanied by severe gastric ulceration [66].

The study of antistress activity of TP Abs to S100 was carried out on male Wistar rats weighing 250–280 g, classified as active or passive in the OF test [67]. Animals were administered TP Abs to S100 at a dose of 2.5 ml/kg for 5 consecutive days or placebo. On the 6th day, half of the rats from each group were immobilized by fixing their paws on a special platform for 1 h, and then the number of animals with ulcers and total number of ulcers formed in the stomach was counted.

TP Abs to S100 decreased by 33.4% the number of animals with ulcers in the group of passive (but not active) rats, which complements the previously obtained results on the higher efficacy of the drug in passive, highly sensitive to stress animals.

TP Abs to S100 also reduced the total number of ulcers in both groups by more than 50%. Again, in control passive animals, there were 1.3 times more ulcers than in control active ones. However, after TP Abs to S100 administration, there was no such difference.

2.1.1.2 Anxiolytic activity of TP Abs to S100

The studies were carried out on outbred white male rats weighing 230–250 g [31] using the most widely validated tests (the Vogel conflict test, the elevated plus maze test, and the OF test) [65]. The activity of TP Abs to S100 was compared to diazepam.

The conflict situation in the *Vogel test* was created by exposing animals to opposing behavioral tendencies: motivation to drink and fear, when every attempt to drink was punished by an electric shock. This lead to a significant reduction in water consumption. Drugs with anxiolytic properties alter behavior and cause an increase in drinking.

To study the activity of TP Abs to S100, depending on the individual reaction to stress, animals were grouped into highly (stress-resistant) and low active (predisposed to stress) in the forced swim test with water wheel (Nomura test), in which stress is modeled, and asthenia and depressive behavior are evaluated. Then, animals were treated with TP Abs to S100 or diazepam, and the Vogel conflict test was performed.

Anxiolytic effect of TP Abs to S100 was not inferior to that of diazepam: the number of punished water intakes in highly active groups increased by 27.4 and 28.7%, respectively (**Figure 2**). Meanwhile, in low-activity animals characterized by a predisposition to asthenia and depressive behavior [64], TP Abs to S100 efficacy was superior to diazepam (2.8 and 2 times vs. control, respectively). The data obtained indicate that in addition to the anxiolytic activity TP Abs to S100 have an antiasthenia activating effect, which distinguishes them from diazepam that induces both anxiolytic and sedative effects.

The *elevated plus maze test* is based on the fear of heights and open spaces: animals are placed on the central platform of the maze and the latent period before the first entry into the open arms, the number of full and incomplete entries and the duration of stay in them, as well as the number of head dips below the level of the open arms is recorded.

It was established that TP Abs to S100 and diazepam had a similar anxiolytic effect in this test: both drugs increased the number of entries into the open arms (1.9 and 2.3 times, respectively), the time spent in the open arms (5.4 and 7 times), as well as the number of head dips (5 and 9 times) versus control animals (**Table 2**).

In the *OF test*, the antianxiety activity of TP Abs to S100 and diazepam was demonstrated by the fact that rats began to go to the center of the field, which was not observed in the control group (**Table 3**). However, unlike diazepam, which reduced the horizontal activity of animals by 1.5 times, TP Abs to S100 did not change this parameter and, therefore, did not have a sedative effect.

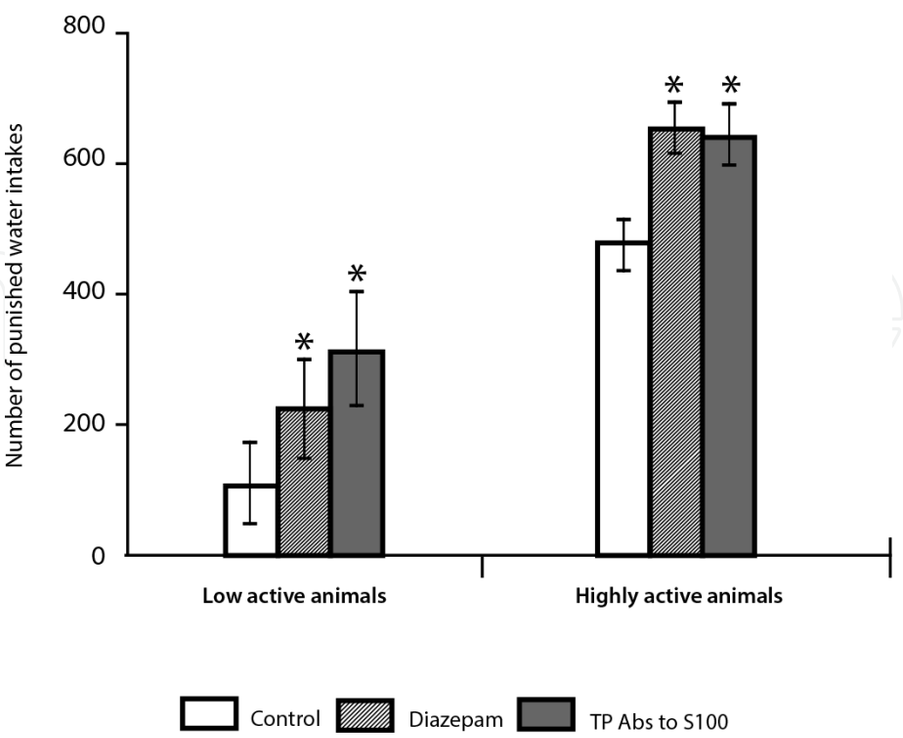

Figure 2.
*TP Abs to S100 demonstrate anxiolytic activity in the Vogel conflict test. Note: animals were intragastrically administered distilled water (2.5 ml/kg, control), TP Abs to S100 (2.5 ml/kg) or diazepam (2 mg/kg) at a single dose 30 minutes prior to testing. Data are expressed as M ± SD. *p < 0.05 versus corresponding control. TP Abs to S100, technologically processed highly diluted antibodies to S100 protein.*

Parameter group	Number of entries into open arms	Number of entries into enclosed arms	Time spent in open arms, sec	Number of head dips
Control	1.1 ± 0.55	2.8 ± 0.65	12.1 ± 8.15	0.5 ± 0.43
Diazepam	2.6 ± 0.80*	1.5 ± 0.8	85.3 ± 38.5*	4.5 ± 1.12*
TP Abs to S100	**2.1 ± 0.42***	**2.3 ± 0.48**	**65.4±27.5***	**2.4 ± 0.95***

Note: animals were intragastrically administered distilled water (2.5 ml/kg, control), TP Abs to S100 (2.5 ml/kg), or diazepam (2 mg/kg) at a single dose 30 minutes prior to testing. Data are expressed as M ± SD. TP Abs to S100, technologically processed highly diluted antibodies to S100 protein.
** $p < 0.05$ versus control.*
Bold entries were made to emphasize the results in TP Abs to S100 group.

Table 2.
TP Abs to S100 anxiolytic activity in the elevated plus maze test.

Parameter group	Number of entries into the arena center	Horizontal activity	Vertical activity	Exploratory activity
Control	0 ± 0	18.2 ± 2.4	8.2 ± 3.3	11.1 ± 3.1
Diazepam	1.8 ± 0.9*	12.5 ± 1.8*	6.2 ± 1.4	8.7 ± 1.5
TP Abs to S100	**2.4 ± 0.7***	**15.8 ± 2.1**	**5.8 ± 2.6**	**8.9 ± 1.6**

Note: animals were intragastrically administered distilled water (2.5 ml/kg, control), TP Abs to S100 (2.5 ml/kg), or diazepam (2 mg/kg) at a single dose 30 minutes prior to testing. Data are expressed as M ± SD. TP Abs to S100, technologically processed highly diluted antibodies to S100 protein.
** $p < 0.05$ versus control.*
Bold entries were made to emphasize the results in TP Abs to S100 group.

Table 3.
TP Abs to S100 anxiolytic activity in the open field test.

2.1.1.3 Antiaggressive activity of TP Abs to S100

Anxiety disorders are often accompanied by covert or overt aggression. The antiaggressive activity of TP Abs to S100 was studied in the tests of motivated and unmotivated aggression on outbred adult white male rats weighing 200–250 g in comparison with diazepam [68].

In the *test of unmotivated aggression caused by inescapable shock*, the threshold of aggressive response of a pair of animals placed on a grid floor was determined by increasing the stimulating current. Animals manifested shock-elicited aggression when they assumed upright "boxing" posture and tried to bite and strike each other with front and hind paws.

TP Abs to S100 and diazepam after a single dose and course administration exerted antiaggressive activity: single TP Abs to S100 administration increased the threshold of aggressive response by 23.1%, and after a 4-day administration—by 31.3% compared with the control, while diazepam increased this threshold by 26.3 and 34.9%, respectively (**Figure 3**).

The *test of motivated aggression* is based on the study of the intensity of the aggressive reaction elicited in a pair of rats trying to escape electric shock. Rats were individually taught to avoid pain caused by electric irritation of the paws on a safe bench installed in the center of the chamber. Then, they were placed in pairs in the chamber, and their behavior was observed for 2 min. Control animals began to fight for a safety on the bench, which had a capacity to tightly fit both animals. The criterion for the effectiveness of substances with antiaggressive action in this test was the duration of joint avoidance of pain exposure.

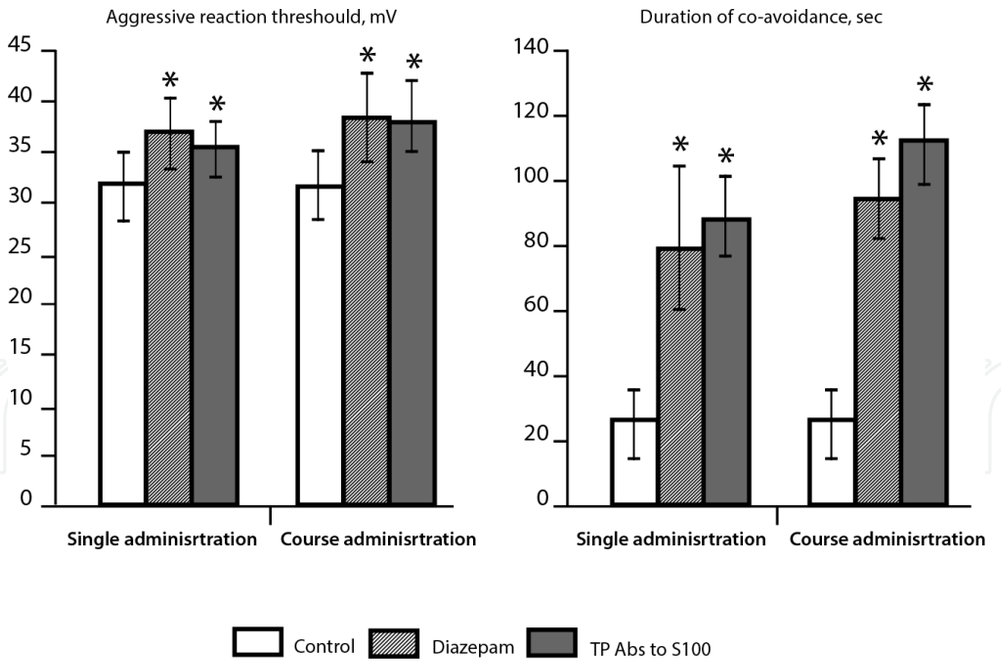

Figure 3.
*TP Abs to S100 and diazepam effects on rat's aggressive reaction parameters in the tests of motivated and unmotivated aggression. Note: animals were intragastrically administered distilled water (2.5 ml/kg, control), TP Abs to S100 (2.5 ml/kg) or diazepam (2 mg/kg) at a single dose or for 5 days (2 times per day) prior to testing. Data are expressed as M ± SD. *p < 0.05 versus corresponding control. TP Abs to S100, technologically processed highly diluted antibodies to S100 protein.*

TP Abs to S100 and diazepam had a pronounced antiaggressive effect, increasing the duration of joint avoidance: with a single dose, respectively, 3.4 and 3.1 times, and with a course—3.8 and 3.3 times (**Figure 3**).

2.1.1.4 Other psychotropic and neurotropic activities of TP Abs to S100

Along with the above-described activities (stress-protective and anxiolytic), TP Abs to S100 were shown to exert:

- *antidepressant* effect in Porsolt's and Nomura's forced swimming tests [30, 31, 34];
- *antiamnestic* and *neuroprotective* effects in the models of ischemic and hemorrhagic stroke [35–39], multiple sclerosis [69], Alzheimer's disease [36], attention deficit hyperactivity disorder [37], and *in vitro* glucose and oxygen deprivation [70].

2.2 Mechanisms of action of TP Abs to S100

TP Abs to S100 belong to a novel class of drugs that are produced from various antibodies (drug substances) using a single technological platform. This technology allows to obtain active pharmaceutical ingredients that, while retaining antibody specificity (targeting), exert a modulating effect on the target and its biological activity [71–73]. As the endogenous target of TP Abs to S100 is the brain-specific protein S100 that can influence functional activity of GABA-, serotonin-, dopamine-, noradrenaline-, and glutamatergic systems and sigma$_1$ receptors [74–78], these CNS elements had been studied while screening TP Abs to S100 mechanisms of action (**Figure 7**). For this purpose, various *in vivo* and *in vitro* approaches have been used (including the *in vitro* assessment of receptor's functional activity providing the validated protocols existed).

2.2.1 GABA-ergic system involvement in TP Abs to S100 mechanisms of action

2.2.1.1 GABA-A-ergic system

To assess the role of this system in the implementation of TP Abs to S100 anxiolytic effect, GABA-A receptors were selectively blocked, and the behavior of animals was evaluated in the Vogel conflict test [29].

The study was performed on outbred white male rats weighing 230–250 g. Before testing, animals were administered TP Abs to S100 or diazepam. For blockade of the GABA-A receptors and the chloride channel of the GABA-benzodiazepine receptor complex, bicuculline and picrotoxin, respectively, were administered simultaneously with the tested drugs.

With blockade of the GABA-A receptor, a 1.8-fold decrease in the anticonflict effect of TP Abs to S100 was observed, and a 2-fold decrease with diazepam; with blockade of the chlorine channel—1.6 and 2.4-fold decrease, respectively (**Figure 4**). The data obtained indicate the involvement of the abovementioned subunits of the GABA-benzodiazepine-chloride ionophore receptor complex in the implementation of the anxiolytic effect of TP Abs to S100.

2.2.1.2 GABA-B-ergic system

In this experiment, GABA-B receptors were selectively stimulated or blocked and anxiolytic or antidepressant effects of TP Abs to S100, diazepam and amitriptyline were evaluated in the Vogel conflict test and the Nomura test [40].

Outbred white male rats weighing 200–250 g were pretreated with baclofen, a selective agonist of GABA-B receptors, or phaclofen, an antagonist of GABA-B receptors. Then, the animals were administered test drugs, and their effect was evaluated.

In the Vogel conflict test, baclofen reduced the anxiolytic effect of TP Abs to S100 by 2.2-fold and did not affect the effect of diazepam. Phaclofen increased the anxiolytic effect of TP Abs to S100 by 1.4-fold (**Figure 5**). Moreover, as expected, none of the ligands influenced the effect of diazepam.

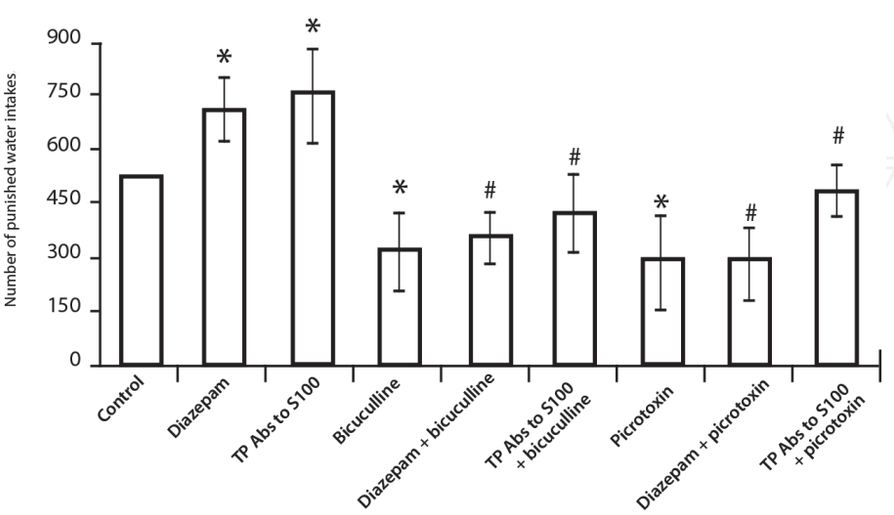

Figure 4.
*Influence of GABA-A-ergic agents on anxiolytic activity of TP Abs to S100 and diazepam in the Vogel conflict test. Note: animals were intragastrically administered distilled water (2.5 ml/kg, control), TP Abs to S100 (2.5 ml/kg) or diazepam (2 mg/kg) at a single dose alone or simultaneously with GABA-A receptor antagonist bicuculline (1 mg/kg) or GABA-benzodiazepine receptor complex chloride channel blocker picrotoxin (1 mg/kg) 30 minutes prior to testing. Data are expressed as M ± SD. * $p < 0.05$ versus control, # $p < 0.05$ versus TP Abs to S100 or diazepam. TP Abs to S100, technologically processed highly diluted antibodies to S100 protein.*

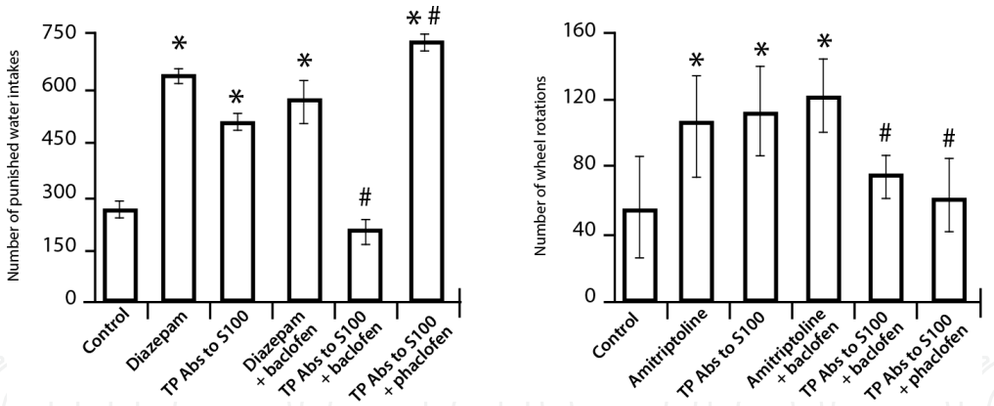

Figure 5.
*Influence of GABA-B-ergic agents on anxiolytic and antidepressant activity of TP Abs to S100, diazepam, and amitriptyline in the Vogel conflict test and the Nomura test. Note: animals were intragastrically administered distilled water (2.5 ml/kg, control), TP Abs to S100 (2.5 ml/kg), diazepam (2 mg/kg), or amitriptyline (10 mg/kg) at a single dose. GABA-B receptors agonist baclofen (1 mg/kg) or antagonist phaclofen (10 mg/kg) were intraperitoneally administered 40 min prior to testing and 10 min prior to the administration of the drugs. Data are expressed as M ± SD. * $p < 0.05$ versus control, # $p < 0.05$ versus TP Abs to S100. TP Abs to S100, technologically processed highly diluted antibodies to S100 protein.*

In the forced swim test, baclofen and phaclofen reduced the antidepressant effect of TP Abs to S100 by 1.5 and 2-fold, respectively, whereas these ligands did not affect the effectiveness of amitriptyline.

Thus, it was shown that the GABA-B-ergic system is involved in the realization of both the anxiolytic and antidepressant effects of TP Abs to S100.

In an *in vitro* study, the ability of TP Abs to S100 to influence binding of the standard radioligands to the corresponding GABA receptors and to change the effect of the standard GABA-$B_{1A/B2}$ receptor agonist (using functional analysis—measuring [^{35}S]GTPγS incorporation into G-proteins) was investigated [41]. The study was performed on the cell membranes of Chinese hamster cells (CHO) and human embryonic kidney cells (HEK293) that expressed human recombinant GABA-$B_{1A/B2}$ receptors.

In the presence of TP Abs to S100, a 25.8% decrease in standard ligand binding to GABA-$B_{1A/B2}$ receptor was observed, as well as 30.2% inhibition of the GABA-$B_{1A/B2}$ receptor's agonist-induced response was observed.

2.2.2 Serotoninergic system involvement in TP Abs to S100 mechanisms of action

Similarly, this hypothesis was studied in experiments *in vivo* and *in vitro*.

For the *in vivo* experiments, ketanserin, a blocker of 5-HT_2/5-HT_{1C} receptors involved in the development of both anxiety and depression, and the 5HT precursor, 5-hydroxytryptophan (5HTP), were used [79].

The anxiolytic effect of TP Abs to S100 was studied using the Vogel conflict test [30]. The antidepressant effect of the drugs was determined using the Nomura test [30]. Outbred white male rats weighing 200–250 g were pretreated with ketanserin or 5HTP, and before testing, they received a single dose of TP Abs to S100 or diazepam.

Ketanserin and 5HTP reduced both anxiolytic (2 and 1.3-fold, respectively) and antidepressant effects of TP Abs to S100 (2- and 1.6-fold, respectively) (**Figure 6**).

Thus, it was demonstrated that the 5HT system is involved in the realization of both the anxiolytic and antidepressant effects of TP Abs to S100.

In an *in vitro* study, the ability of TP Abs to S100 to influence binding of standard radiolabeled ligands to the corresponding 5HT receptors and the ability to change the magnitude of the effect on binding of standard ligands to their receptors

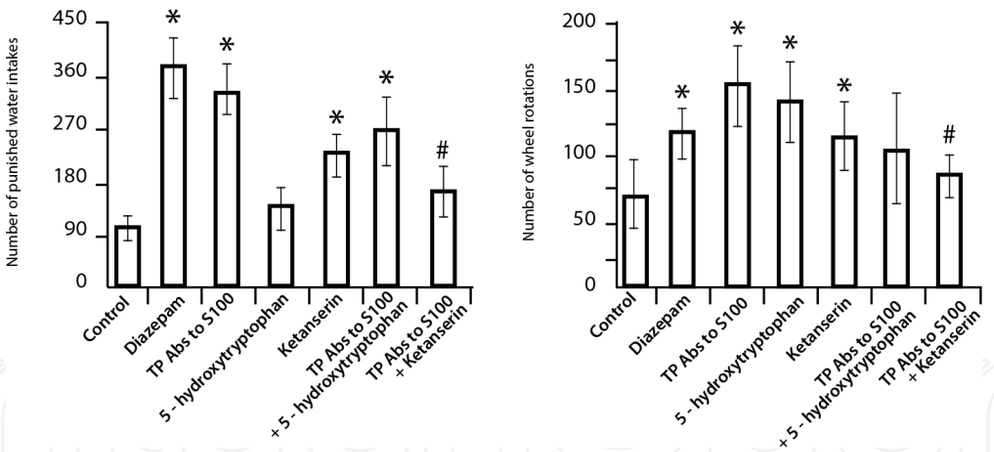

Figure 6.
*Influence of serotoninergic agents on anxiolytic and antidepressant activity of TP Abs to S100, diazepam, and amitriptyline in the Vogel conflict test and the Nomura test. Note: animals were intragastrically administered distilled water (2.5 ml/kg, control), TP Abs to S100 (2.5 ml/kg), diazepam (2 mg/kg), or amitriptyline (15 mg/kg) at a single dose. 5-HT2 receptors antagonist ketanserin (1 mg/kg) or the serotonin precursor 5-hydroxytryptophan (5-HTP, 50 mg/kg) were intraperitoneally administered 40 min prior to testing and 10 min prior to administration of the drugs. Data are expressed as M ± SD. * $p < 0.05$ versus control, # $p < 0.05$ versus TP Abs to S100. TP Abs to S100, technologically processed highly diluted antibodies to S100 protein.*

were tested. The latter was investigated using a functional analysis of the binding of [^{35}S]GTPγS, calcium mobilization assay, and dielectric spectroscopy or by measuring the intracellular concentration of cAMP using HTRF (Homogenous Time Resolved Fluorescence) technology. The experiments were performed on CHO cells stably expressing human $5HT_{1A}$, $5HT_{1B}$, $5HT_{1D}$, $5HT_{1E}$, $5HT_{1F}$, $5HT_{2A}$, $5HT_{2B}$, $5HT_{2Cedited}$, $5HT_3$, $5HT_4$, $5HT_6$, or $5HT_7$ receptors [41].

TP Abs to S100 increased binding of the corresponding standard ligands to $5HT_{1A}$ (19.0%), $5HT_{1F}$ (42.0%), $5HT_{2B}$ (31.9%), $5HT_{2Cedited}$ (49.3%), and $5HT_3$ (20.7%) receptors. Moreover, the drug enhanced the effect of $5HT_{1A}$ receptor agonist by 27.8% and reduced the effect of $5HT_{1B}$ receptor agonist by 27.5%.

2.2.3 Dopaminergic system involvement in TP Abs to S100 mechanisms of action

The *in vitro* experiment was carried out similar to the study of the effect of TP Abs to S100 on dopamine receptors [41].

The study was performed on CHO, HEK293, and pituitary rat tumor cells (GH4) stably expressing human D_1, D_{2L}, D_{2S}, D_3, $D_{4.4}$ or D_5 receptors.

TP Abs to S100 increased binding of the standard ligand to the human D_3 receptor by 26.3% and reduced the effect of an agonist of this type of receptor by 32.8%.

2.2.4 Glutamatergic system involvement in TP Abs to S100 mechanisms of action

In this study that was performed *in vitro* using rat cerebral cortex cells, TP Abs to S100 significantly reduced binding of the standard radiolabeled ligand to the glycine site of NMDA receptors [80].

2.2.5 Sigma$_1$ receptors involvement in TP Abs to S100 mechanisms of action

The study was carried out *in vitro* using MCF-7 or Jurkat cells [41].

TP Abs to S100 significantly (by 24.7–56.7%) reduced binding of the standard radiolabeled ligand to human sigma$_1$ receptors (**Figure** 7).

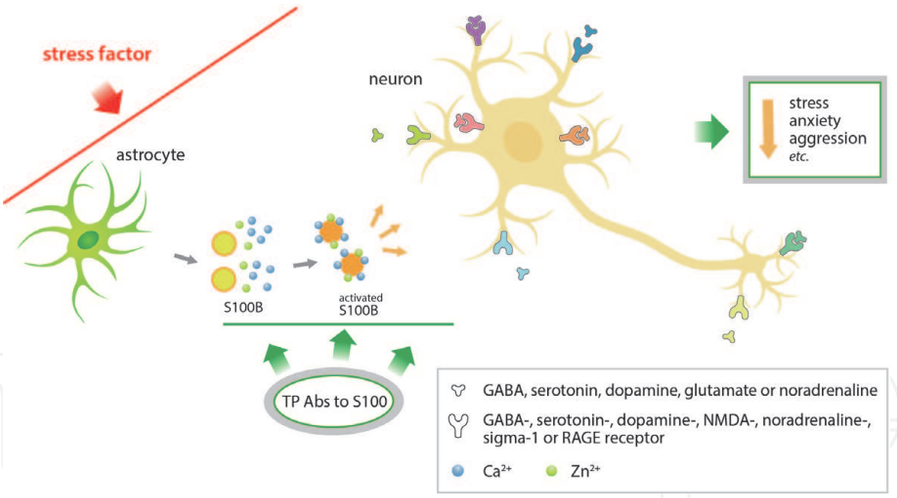

Figure 7.
Schematic representation of TP Abs to S100 mechanisms of action. Note: TP Abs to S100 (technologically processed highly diluted antibodies to S100 protein) molecular target—brain-specific S100 protein. This protein is secreted mainly by astrocytes in the CNS and considered to be an important regulator of many intracellular and extracellular processes (e.g., protein phosphorylation, activity of various enzymes, the dynamics of cytoskeleton components, binding of transcription factors, calcium homeostasis, cell proliferation and differentiation, generation and transmission of nerve impulses, and synaptic transmission [81]). Moreover, S100 proteins interact with almost every neurotransmitter system (serotonin-, dopamine-, GABA-, glutamatergic, etc.) and sigma$_1$ receptors [74–78]. TP Abs to S100 *possess their pharmacological effects via modulating activity of brain-specific S100 protein and influencing functions of the major neurotransmitter systems as well as sigma$_1$ receptors.* In vivo *studies [29, 40] revealed 5-HT$_{2a}$, GABA$_A$, and GABA$_B$ receptor involvement in the drug psychotropic effects. Also, the drug was shown to normalize noradrenaline level [82].* In vitro *studies [41, 42] have shown that* TP Abs to S100 *increase standard radioligand binding to 5-HT$_{1F}$, 5-HT$_{2B}$, 5-HT$_{2Cedited}$, 5-HT$_3$, NMDA, and D$_3$ receptors. In addition, the drug inhibits binding of specific radioligands to GABAB$_{1A/B2}$ and sigma$_1$ receptors and exerts antagonism at GABAB$_{1A/B2}$, 5-HT$_{1B}$, and D$_3$ receptors and agonism at 5-HT$_{1A}$ receptor. The above listed* TP Abs to S100 *activities at the molecular level are involved in maintaining both emotional and physiological homeostasis, and thereby, the drug exerts its stress-protective, anxiolytic, antiamnestic, antidepressant, neuroprotective, and other activities.*

2.3 Safety investigation

2.3.1 Assessment of a possible sedative effect

The study was performed on outbred white male rats weighing 230–250 g. Prior to testing (in OF test), animals were administered TP Abs to S100 or diazepam. The sedative effect was evaluated by a decrease in the horizontal activity of rats [7].

TP Abs to S100 did not decrease the motor activity of animals, while diazepam decreased this parameter by 1.5 times.

2.3.2 Assessment of a possible muscle relaxant effect

This activity was investigated in the rotarod test on outbred white male rats weighing 230–250 g [33]. Before testing, animals were administered TP Abs to S100 or diazepam. Then, the time before falling off the rotating rod and the number of rats that fell off were recorded.

TP Abs to S100 did not affect the coordination of movements and did not have a muscle relaxant effect. In contrast, only 30% of rats from diazepam group were able to keep balance.

2.3.3 Toxicological studies of TP Abs to S100

The drug safety investigation was performed in accordance with principles of Good Laboratory Practice. It included studies of the single and repeat dose

toxicities, genotoxicity, reproductive and developmental toxicity, immunotoxicity, and local tolerance.

TP Abs to S100 exerted no toxic effects even at a dose significantly exceeding the human recommended daily dose. The drug was shown to be well tolerated and thereby considered to be a low-hazard substance.

3. Clinical efficacy and safety of TP Abs to S100 protein in the treatment of NDs

3.1 Treatment of AD, AjD, SD, and neurasthenia

To date, 453 patients with AD, AjD, and neurasthenia took part in double-blind randomized controlled CTs (n = 2), and open-label comparative randomized CTs (n = 4) conducted in the Russian Federation and Kazakhstan according to International Conference on Harmonisation Good Clinical Practice and Declaration of Helsinki [55, 57–60, 83]. Two studies were registered and approved by the regulatory agency (Ministry of Health of the Russian Federation) [55, 57].

3.1.1 Placebo-controlled studies

3.1.1.1 CT in patients with AD and neurological diseases

A double-blind placebo-controlled CT of TP Abs to S100 in the treatment of AD in patients with neurological diseases [Parkinson's disease (PD) (G.20) and chronic cerebrovascular diseases (CCD)—cerebral atherosclerosis (I67.2), hypertensive encephalopathy (I67.4), unspecified sequelae of cerebral infarction (I69.3)] was conducted in 2010 ([55], unpublished data). Sixty-two patients of both sexes aged 18–75 years were enrolled and randomized in two groups to receive TP Abs to S100 (n = 32) 10 tablets/day or placebo 10 tablets/day. Data from all 62 patients were included in the analysis, so that intention-to-treat and per-protocol sets were equal. The use of any antidepressants, antipsychotics, or antianxiety medications was prohibited in CT. The therapy of concurrent somatic and neurological diseases was permitted.

The study duration was 4 weeks with a 4-week follow-up period. Inclusion criteria were: manifested AD, the Hospital Anxiety and Depression Scale-Anxiety (HADS-A) score ≥ 11, signed informed consent form (ICF). The percentage of patients with a ≥50% decrease in the severity of anxiety according to the Hamilton Anxiety Rating Scale (HAM-A) after 4 weeks of treatment and 4-week follow-up was set as a primary efficacy endpoint. Other efficacy endpoints were: mean decrease in HAM-A, HADS-A, and State-Trait Anxiety Inventory (STAI) scores after 4 weeks of treatment and 4-week follow-up. Safety was assessed based on the results of laboratory tests (blood and urine analysis) and adverse events reports. Mann-Whitney U test, Wilcoxon signed-rank test, Student t-test, and Fisher's exact test were used for analysis.

The mean age of patients enrolled was 59.5 ± 2.0 years in the TP Abs to S100 group and 60.0 ± 1.9 years in the placebo group. The mean duration of neurological disease was 6.13 ± 1.2 years in the TP Abs to S100 group and 6.55 ± 0.89 years in the placebo group. No differences in demographic and clinical characteristics of patients were found (**Table 4**).

The percentage of patients with a ≥50% decrease in HAM-A total score was 41.3% in the TP Abs to S100 group and 6.7% in the placebo group ($p < 0.05$ compared to placebo) after 4 weeks of therapy (**Table 4**). After 4 weeks of therapy, the total HAM-A score significantly decreased in the TP Abs to S100 group [a 1.8-fold decrease (−45.63 ± 2.61%) from baseline in the TP Abs to S100 group

	TP Abs to S100			Placebo		
	Total (n = 32)	**Patients with PD (n = 16)**	**Patients with CCD (n = 16)**	**Total (n = 30)**	**Patients with PD (n = 15)**	**Patients with CCD (n = 15)**
Demographic and clinical characteristics						
Age, years	59.5 ± 2.0	61.4 ± 3.0	57.9 ± 2.5	60.0 ± 1.9	61.1 ± 2.9	58.9 ± 2.6
Duration of neurological disease, years	6.13 ± 1.22	6.13 ± 1.15	5.94 ± 2.03	6.55 ± 0.89	8.0 ± 1.48	5.29 ± 2.56
Baseline data						
HADS-A, score	14.75 ± 0.46	15 ± 0.68	14.24 ± 0.63	15.7 ± 0.41	16.93 ± 0.34	14.47 ± 0.59
STAI, trait anxiety, score	62.28 ± 0.97	62.69 ± 1.76	60.82 ± 0.88	59.5 ± 1.25	61.53 ± 1.82	57.47 ± 1.61
STAI, state anxiety, score	60.09 ± 1.05	59.31 ± 1.71	59.82 ± 1.24	60.8 ± 1.07	63 ± 1.06	58.6 ± 1.7
HAM-A, score	27.28 ± 0.66	26.38 ± 0.82	28.19 ± 1.01	26.37 ± 0.6	27.13 ± 0.65	25.6 ± 0.9
After 4 weeks of treatment						
HADS-A, score	7.74 ± 0.53	8.8 ± 0.97	6.75 ± 0.39	12.93 ± 0.8	16.0 ± 0.53	9.87 ± 1.0
STAI, trait anxiety, score	50.58 ± 1.25	53.2 ± 2.27	48.13 ± 0.82	55.93 ± 1.55	61.07 ± 1.55	50.8 ± 1.95
STAI, state anxiety, score	43.48 ± 1.06	46.4 ± 1.57	40.75 ± 1.07	54.0 ± 1.58	58.6 ± 1.59	49.4 ± 2.19
HAM-A, score	14.74 ± 0.74	16.4 ± 1.21	13.19 ± 0.7	22.83 ± 1.05	26.07 ± 1.05	19.6 ± 1.41
After 4 weeks of follow-up						
HADS-A, score	7.61 ± 0.49	8.8 ± 0.74	6.5 ± 0.52	13.1 ± 0.69	15.6 ± 0.36	10.6 ± 0.97
STAI, trait anxiety, score	49.45 ± 1.04	51.0 ± 1.91	48.0 ± 0.84	56.47 ± 1.33	60.6 ± 1.43	52.33 ± 1.69
STAI, state anxiety, score	43.65 ± 0.85	46.67 ± 1.13	40.81 ± 0.78	56.67 ± 1.27	60.47 ± 1.19	60.47 ± 1.19 52.87 ± 1.8
HAM-A, score	14.13 ± 0.68	15 ± 1.01	13.31 ± 0.9	24.03 ± 0.89	26.78 ± 0.64	21.2 ± 1.31

Note: Data are expressed as M ± SD. HAM-A, Hamilton Anxiety Rating scale; HADS-A, Hospital Anxiety and Depression Scale-Anxiety; STAI, State-Trait Anxiety Inventory; CCD, chronic cerebrovascular disease; PD, Parkinson's disease; TP Abs to S100, technologically processed highly diluted antibodies to S100 protein.

Table 4.
Demographic and clinical characteristics, baseline, and post-treatment data on patients in double-blind placebo controlled CT of TP Abs to S100.

versus a 1.1-fold (or −13.09 ± 3.3%) decrease in the placebo group; Student's t-test $p < 0.05$]. The result of therapy persisted during the follow-up period in the TP Abs to S100 group. The anxiety level additionally decreased by 3% (−48.19 ± 2.1% from baseline in total) by the end of the follow-up period ($p < 0.05$ compared to placebo). The percentage of patients with a ≥50% decrease in HAM-A total score additionally increased by 3.3% after 4 weeks of follow-up ($p < 0.05$ compared to placebo) (**Figure 8**).

A significant decrease in the severity of anxiety was shown in patients receiving TP Abs to S100 according to HADS-A after 4 weeks of therapy and 4 weeks of follow-up ($p < 0.05$ compared to the placebo group). There was a 1.4-fold decrease

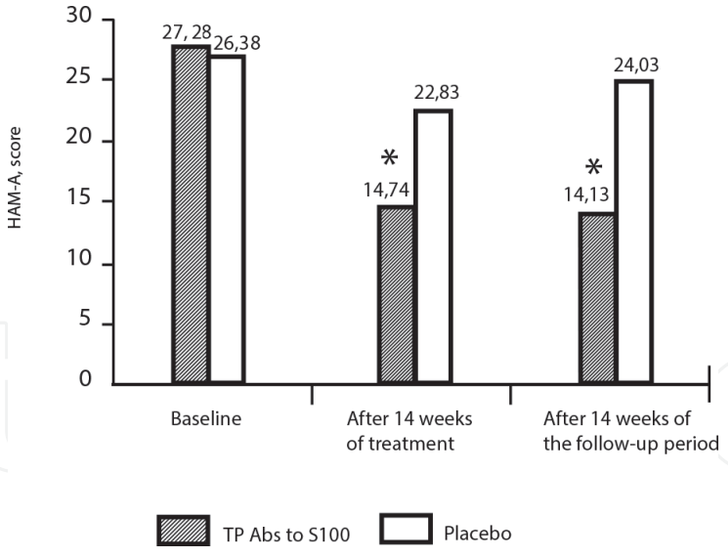

Figure 8.
*Dynamics of the severity of anxiety in TP Abs to S100 and placebo groups. *p < 0.05 versus placebo (Student's t-test). HAM-A, Hamilton Anxiety Rating scale; TP Abs to S100, technologically processed highly diluted antibodies to S100 protein.*

(60.09 ± 1.05 vs. 43.65 ± 0.85) in state anxiety according to STAI in the TP Abs to S100 group after 4 weeks of therapy and result of therapy persisted during the follow-up period. The efficacy rate in reduction of the anxiety was higher in CCD patients than in PD patients according to STAI.

Data from 62 patients were included in the safety analysis. There were two AEs (pyrosis and burping) in one patient received TP Abs to S100 and one AE (pyrosis) in one patient in the placebo group. There was no significant difference in the frequency of AEs between groups. Neither TP Abs to S100 nor placebo influenced results of blood or urine tests in patients. All AEs were of medium severity and had no definite relationship with the study drug. No serious AEs were registered.

TP Abs to S100 were shown to be an effective drug for the treatment of AD in adult patients with concurrent neurological diseases.

3.1.1.2 Clinical trials in patients with SD

An international multicenter double-blind randomized placebo-controlled study in 390 patients of both sexes aged 18–45 years with SD (mostly), AjD, or neurasthenia and ≥11 HADS-A points was conducted in 2017–2019 in the Russian Federation and Kazakhstan [57]. There were four treatment groups receiving TP Abs to S100 or placebo in two dosage regimens: 4 or 8 tablets/day. Preliminary (yet unpublished) data on primary efficacy endpoint showed the decrease in the mean HAM-A score by 11.25 points in TP Abs to S100 group (4 tablets/day) and by 11.91 points in TP Abs to S100 8 tablets/day groups observed after 12 weeks of treatment (vs. 9.71 points in merged placebo group; ANCOVA: p_{TP} Abs to S100 4 tablets per day/placebo = 0.0055, p_{TP} Abs to S100 8 tablets per day /placebo < 0.0001). A detailed analysis of the results is currently being prepared for a publication. Complete information on the study design is available at clinicaltrials.gov NCT 03036293 [57].

3.1.2 Comparison of the TP Abs to S100 efficacy and safety with benzodiazepines

To evaluate the advantages and limitations of novel medication, especially in the treatment of mental disorders, it is necessary to compare its efficacy and safety not only with placebo but also with the "golden standard" treatment [84].

Benzodiazepines are usually chosen as such a standard in CTs in patients with NDs and, in particular, ADs. Therefore, four CTs with the use of bromdihydrochlorphenylbenzodiazepine, diazepam, clonazepam, and tofisopam as the control therapy were conducted [58–60].

3.1.2.1 Open-label comparative randomized CT of TP Abs to S100 and bromdihydrochlorphenylbenzodiazepine

Outpatients aged 18–65 years (n = 59) with a diagnosis of GAD (F41.1), AjD (F43.2), or neurasthenia (F48.0) who signed ICF participated in this open-label randomized CT [58]. One group of patients (n = 32) received TP Abs to S100 4 tablets/day, and the other (n = 27) was administered bromdihydrochlorphenylbenzodiazepine 1.5 mg/day for 28 days. Exclusion criteria were other mental diseases, severe somatic diseases, pregnancy, or lactation period. Any medications that could influence the emotional state of participants were prohibited for use for 1 week prior to the initiation of CT and during the study.

Efficacy was evaluated based on the results of the HAM-A test and Clinical Global Impression-Improvement scale (CGI-I) after 7, 14, and 28 days of treatment. The frequency of AEs and any deviations from the reference ranges in blood and urine tests was used for safety assessment. The Kruskal-Wallis test, ANOVA, and Mann-Whitney U-test were used for statistical analysis.

The mean age of patients was 34.8 ± 3.6 years in TP Abs to S100 and 36.3 ± 4.6 years in bromdihydrochlorphenylbenzodiazepine group. The mean duration of disease was 0.8 ± 0.6 years and 0.9 ± 0.5 years in TP Abs to S100 and bromdihydrochlorphenylbenzodiazepine groups, respectively (**Table 5**). No significant differences between groups in any demographic and clinical characteristics were found.

After 7 days of treatment, the severity of anxiety was reduced by 41% (from 18.2 ± 3.91 to 10.73 ± 5.02) in the TP Abs to S100 group and by 56.2% (from 21.24 ± 3.25 to 9.29 ± 4.24) in the comparison group according to HAM-A scale. No significant differences between groups were found after the first week of treatment (p = 0.41), and TP Abs to S100 were shown to be as effective as bromdihydrochlorphenylbenzodiazepine in the short-term period. After 14 and 28 days the anxiolytic effect in the group, receiving benzodiazepine drug was superior to that in the TP Abs to S100 group ($p < 0.05$ between groups). In accordance with CGI-I results, the level of improvement was found to be similar in both groups (p = 0.004) on the 7th and 14th days, but later, bromdihydrochlorphenylbenzodiazepine led to a significant decrease in the severity of illness after 28 days of treatment ($p > 0.05$ between groups).

The frequency of AEs was higher in the benzodiazepine group. There were several cases of severe daytime sleepiness, disturbance of accommodation, and muscle weakness in patients that received bromdihydrochlorphenylbenzodiazepine. Some patients in the study group reported mild sleepiness. No severe AEs were registered in the TP Abs to S100 group. Neither TP Abs to S100 nor benzodiazepine administration affected results of blood or urine tests in patients.

Thus, TP Abs to S100 were as effective as the control medication only in the short-term period according to HAM-A but caused no severe AEs in patients with GAD, AjD, and neurasthenia comparing to benzodiazepine.

3.1.2.2 Open-label comparative randomized CT of TP Abs to S100 and diazepam

Diazepam is the most frequent standard drug used in CTs of anxiolytic agents [85]. This open-label randomized CT was conducted under the regulation of the

	TP Abs to S100	Bromdihydrochlorphenylbenzodiazepine	*p*
Demographic and clinical characteristics			
Age, years	34.8 ± 3.6	36.3 ± 4.6	>0.05
Disease duration, years	0.8 ± 0.6	0.9 ± 0.5	>0.05
Baseline data			
HAM-A, score	18.2 ± 3.91	21.24 ± 3.25	0.41
After 7 days of treatment			
HAM-A, score	10.73 ± 5.02	9.29 ± 4.24	0.46
CGI-I, score	3.41 ± 1.1	3.05 ± 0.97	0.28
After 14 days of treatment			
HAM-A, score	11.14 ± 5.49	6.62 ± 2.80	0.003
CGI-I, score	3.14 ± 1.13	2.33 ± 1.39	0.004
After 28 days of treatment			
HAM-A, score	9.59 ± 6.08	5.62 ± 2.18	0.000023
CGI-I, score	2.86 ± 1.58	2.33 ± 1.06	0.21

Note: Data are expressed as M ± SD. HAM-A, Hamilton Anxiety Rating scale; CGI-I, Clinical Global Impression-Improvement scale; TP Abs to S100, technologically processed highly diluted antibodies to S100 protein.

Table 5.
Demographic and clinical characteristics, baseline, and post-treatment data of patients in comparative CT of TP Abs to S100 and bromdihydrochlorphenylbenzodiazepine.

Ministry of Health of the Russian Federation [unpublished data]. Outpatients aged 18–65 years with GAD (F41.1), AjD (F43.2), neurasthenia (F48.0) (total n = 272), and mixed anxiety and depressive disorder (mADD) (F41.2) signed ICF and then were randomized to receive TP Abs to S100 (n = 142) 6 tablets/day or diazepam (n = 130) 15 mg/day for 28 days. All medications influencing the emotional state were prohibited for use 1 week prior to CT initiation and during the study. Diagnosis of any other psychiatric disorder, pregnancy, lactation period, substance abuse, and severe somatic diseases were set as the exclusion criteria. Efficacy was measured using the HAM-A scale and STAI. Safety was assessed based on the AE reports and results of blood and urine tests.

The mean age of patients was 40.4 ± 1.13 in TP Abs to S100 group and 39.6 ± 1.06 in the diazepam group. The mean duration of NDs was 31.9 ± 4.1 and 29.2 ± 3.23 months in the TP Abs to S100 and diazepam groups, respectively. In the TP Abs to S100 group, 27.5% of patients had GAD, 31.3%—neurasthenia, 24.4%—AjD, and 17%—mADD. Among patients administered diazepam 31.6% had GAD, 37%—neurasthenia, 17.5%—AjD, and 14.8%—mADD. Mean HAM-A score was 27.1 ± 0.5 in the TP Abs to S100 group and 28.1 ± 0.46 in the diazepam group (p = 0.3). No differences in baseline characteristics were observed.

The total HAM-A score decreased to 22.0 ± 0.5 in TP Abs to S100 group at the end of the first week of therapy ($p < 0.001$ compared to baseline). There was a 57.2% decrease in total HAM-A score in the TP Abs to S100 group after 28 days of treatment (vs. 63% in the diazepam group, p = 0.02) (**Figure 9**).

The percentage of patients with a ≥50% decrease in HAM-A total score was 72.6% in the TP Abs to S100 group (vs. 65.8% in the diazepam group) after 28 days of treatment. There were 12.8% of patients in TP Abs to S100 group with anxiety remission (less than 7 HAM-A scores) (vs. 22.1% in diazepam group). There were no significant differences between the TP Abs to S100 and diazepam groups on 7th

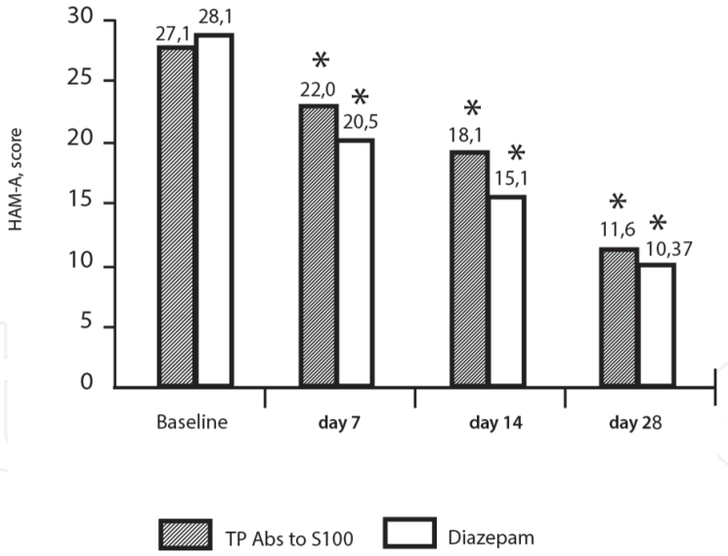

Figure 9.
*Dynamics of the severity of anxiety in the TP Abs to S100 and diazepam groups. * p < 0.05 versus baseline. HAM-A, Hamilton Anxiety Rating Scale; TP Abs to S100, technologically processed highly diluted antibodies to S100 protein.*

and 28th days of treatment according to the HAM-A section "anxiety mood" (p = 0.2 and p = 0.1 between groups). Treatment with diazepam was more effective only at the 14th day of treatment [48.0 ± 0.62 (diazepam) vs. 50.0 ± 0.52 (TP Abs to S100), p = 0.02 comparing to the TP Abs to S100 group] according to STAI (state anxiety). The influence of TP Abs to S100 and diazepam on state anxiety was equal on the 7th and 28th days of therapy (p = 0.2 between groups). Diazepam and TP As to S100 were of equal efficacy in reducing the trait anxiety after 14 days [49.7 ± 0.60 (TP Abs to S100) vs. 51.0 ± 0.55 (diazepam); p = 0.1 between groups].

Only eight (5.6%) patients in the TP Abs to S100 group reported AEs (sleepiness, dizziness, dry mouth, pyrosis, bloating, excessive sweating, decreased libido, and tachycardia) of mild and moderate severity. In contrast, in the diazepam group, 51 (39.2%) patients had AEs (most frequent—daytime sleepiness, muscle relaxation, orthostatic hypotension) (p < 0.01).

To summarize the data, we consider TP Abs to S100 less effective than diazepam, though TP Abs to S100 were well tolerated by patients with GAD, AjD, neurasthenia, or mADD and exerted significantly less AEs in contrast to diazepam.

3.1.2.3 Open-label comparative randomized CT of TP Abs to S100 and clonazepam

In this open-label CT, 60 patients with AjD (n = 35) or SD (n = 25) and cardiovascular diseases (CVDs) (coronary heart disease (CHD), arterial hypertension (AH) grades II–III, postmyocarditis cardiosclerosis, dyshormonal myocardial dystrophy with cardiac arrhythmias, ventricular and supraventricular extrasystoles, atrial fibrillation, and heart defects) were randomly prescribed to receive TP Abs to S100 6 tablets/day (n = 30) or clonazepam 0.5–1 mg/day (n = 20) as an anxiolytic treatment in addition to standard therapy of CVD for 28 days after signing an ICF [59]. The control group (n = 10) was not administered any antianxiety medication. No drugs influencing the mental status of participants were allowed 1 week prior to CT and after the onset of CT. The reduction of HAM-A score was set as an efficacy endpoint. Safety was assessed based on the number of reported AEs, changes in electrocardiogram (in TP Abs to S100 group), and results of blood and urine tests.

At the baseline mean, HAM-A score was 20.75 ± 8.3 in the TP Abs to S100 group, 22.3 ± 8.1 in the clonazepam group, and 14.7 ± 5.6 in control. After 28 days

of treatment, the mean HAM-A score was reduced by 30.1% in patients that received TP Abs to S100 (to 14.5 ± 5.6; $p < 0.01$ vs. baseline), by 30.04% in the clonazepam group (to 15.6 ± 6.2; $p < 0.01$ vs. baseline) and 24.5% in the control group (to 11.1 ± 4.1; $p > 0.05$ vs. baseline). Patients in the TP Abs to S100 group reported no AEs and no changes were found on electrocardiogram or blood and urine tests. On the contrary, the extrasystoles in two participants with dyshormonal myocardial dystrophy that received TP Abs to S100 became less frequent (from 3122 to 2040) after 14 days of treatment. Patients in the clonazepam group (n = 5) noted a slowdown in mental and motor reactions, a feeling of tiredness, and daytime sleepiness.

Thus, TP Abs to S100 appeared to be slightly less effective than clonazepam but at the same time exerted less AEs that are important for patients with not only the AjD alone but also for those with CVD.

3.1.2.4 Open-label comparative randomized CT of TP Abs to S100 and tofisopam

Patients (n = 51) with GAD or mADD and CVD (CHD or AH grades II–III) signed ICF and then were randomized into two groups. The first group (n = 31) received TP Abs to S100 4 tablets/day, the second (n = 20)—tofisopam 100 mg/day for 4 weeks in addition to standard CVD therapy [60]. After 4 weeks of treatment, patients were followed up for the next 4 weeks. Patients that previously received antianxiety or antidepressant medications, diagnosed with other mental diseases, having a history of substance abuse or lactose intolerance were not included in CT. The changes in HAM-A score after 2 and 4 weeks of treatment and after 4-week follow-up were set as efficacy endpoints. Safety was evaluated by analysis of AEs.

The mean age of patients in the TP Abs to S100 group was 49.3 ± 7.0 years, and the mean duration of CVD was 8.2 ± 4.5 years. In the tofisopam group, the mean age was 54 ± 5.2, and the duration of CVD was 7.6 ± 2.9 years. No differences in baseline characteristics were registered. During the treatment, anxiety was reduced by 63% after 1 week, by 73.1% after 2 weeks, and by 78.5% after 4 weeks in the TP Abs to S100 group according to HAM-A. There was a decrease in HAM-A scores by 62.5% after 1 week, by 75% after 2 weeks, and by 78.5% after 4 weeks in the tofisopam group. A positive effect of TP Abs to S100 on anxiety state was maintained for 4 weeks during follow-up, while there was a tendency for an increase in HAM-A score in the tofisopam group. The addition of TP Abs to S100 to standard CVD therapy helped to decrease the mean systolic blood pressure (SBP) by 25% (from 161.5 ± 18.5 mmHg to 122 ± 5.0 mmHg) after 4 weeks of treatment, whereas only 15.9% decrease in mean SBP was shown in the tofisopam group. No serious AEs were registered in both groups. In the TP Abs to S100 and tofisopam groups, 3.2 and 10% of patients, respectively, discontinued the treatment for personal reasons.

Thus, TP Abs to S100 were shown to be as effective as tofisopam. The compliance in the TP Abs to S100 group was slightly higher than that in the tofisopam group. The addition of TP Abs to S100 to standard CVD treatment led to a more prominent decrease in the mean SBP than the addition of tofisopam. TP Abs to S100 achieved more prolonged action on anxiety state than tofisopam.

3.2 Treatment of anxiety, accompanying somatic diseases

The use of anxiolytic treatment in the patients with chronic somatic diseases is challenging [86]. Many side effects of benzodiazepines such as drowsiness, sleepiness, cognitive impairment, dizziness, and addiction can be crucial for these

patients [87]. Polypharmacy is also an unwanted phenomenon. The negative interaction of antianxiety medications with standard therapy of somatic diseases is frequently observed [88]. For instance, the use of SSRI in combination with nonsteroidal anti-inflammatory drugs increases the risk of gastrointestinal tract bleeding [89]. Some authors described the association between high risk of myocardial infarction and the use of benzodiazepines and antidepressants [90]. So, the search for a possible role of TP Abs to S100 in treatment of patients with the somatic disease is relevant.

3.2.1 Cardiovascular diseases

Around 40% of patients with CVD experience anxiety that can have a negative impact on the risk of adverse cardiovascular events [91, 92]. Thus, it is important to reduce anxiety symptoms in CVD patients.

Two CTs that compared TP Abs to S100 with clonazepam and tofisopam in patients with CVD were described above [59, 60]. The results showed equal efficacy of TP Abs to S100 and tofisopam in CVD patients.

In another open-label randomized comparative CT by Nikol'skaya et al., TP Abs to S100 were used in combination with the standard treatment of patients with AH grades II–III and anxiety (n = 60, 23 women, 37 men; mean age—61.4 ± 6.9, mean AH duration—10.6 ± 4.1 years) [93]. All patients received diuretics, β blockers, and angiotensin-converting-enzyme inhibitors (ACE inhibitors). Randomly chosen participants (n = 30) were additionally prescribed TP Abs to S100 6 tablets/day for 4 weeks. At the baseline, there were 40% of patients with severe anxiety and 60% with the anxiety of moderate severity in the TP Abs to S100 group according to the Taylor Manifest Anxiety Scale (TMAS) modified by Nemchinov. Sixty percent of patients with severe anxiety and 56.6% with the anxiety of moderate severity made up the control group were receiving no antianxiety therapy.

The 1.3-fold reduction of severity of anxiety (from 23.76 ± 2.81 to 18.83 ± 2.75 TMAS points) after 2 weeks of therapy was shown in the TP Abs to S100 group ($p < 0.0001$ vs. baseline and the control group). There was a 24.28% decrease in SBP from 181.7 ± 10.8 to 140.0 ± 8.3 ($p < 0.0001$ vs. baseline and control) after 4 weeks of therapy in the TP Abs to S100 group. There was a 17.7% decrease in diastolic blood pressure from 102.3 ± 4.3 to 85.0 ± 5.7 in the study drug group after 4 weeks of therapy ($p < 0.0001$ vs. baseline and the control group). No negative interactions with standard therapy were registered for TP Abs to S100.

Matyushin et al. demonstrated the efficacy and safety of TP Abs to S100 in an open-label randomized comparative CT in patients (n = 60) with anxiety measured with HAM-A and CHD, AH grades II–III, angina pectoris I–III functional classes by Canadian Cardiovascular Society Classification, heart rhythm disturbances (extrasystole, paroxysmal supraventricular tachyarrhythmias) receiving standard CVD therapy (β blockers, amiodarone, sotalol, lappaconitine hydrobromide, diethylaminopropionylethoxycarbonylaminophenothiazine, etc.) [94]. The study drug group (n = 30) received TP Abs to S100 6 tablets/day, and the control group (n = 30) was administered only CVD treatment for 8 weeks. The mean age of patients in TP Abs to S100 and control groups was 64.4 ± 8.6 and 63.1 ± 8.5, respectively.

The addition of TP Abs to S100 to the standard therapy in patients with angina pectoris I–III functional classes helped to decrease the severity of anxiety (50.4% decrease vs. 32.3% decrease in the TP Abs to S100 group vs. the control group; $p < 0.05$) and caused cardiac rhythm normalization [80% patients with more than 75% decrease in the frequency of daily episodes of rhythm disturbances in the study group ($p < 0.05$ vs. control)]. There were 60% of patients with a decrease in angina pectoris functional class in the study drug group (vs. 33.3% in

control; $p < 0.05$ between groups). No AEs and negative drug interactions were registered.

An open-label placebo-controlled study in 85 patients with acute coronary syndrome and anxiety (diagnosed with HADS-A) showed significant improvement in the quality of life assessed with the Short Form-36 in the TP Abs to S100 group after 6 month of therapy [95]. The 1.7-fold decrease in HADS-A score (from 12.1 [9;17] to 7.1 [6;8]) was observed in patients receiving TP Abs to S100 in combination with standard therapy after 6 months ($p = 0.00008$ vs. baseline). No reduction of anxiety according to HADS-A was found in the placebo group after 6 months ($p = 0.07$ vs. baseline). No negative interaction with standard therapy (acetylsalicylic acid, clopidogrel, enoxaparin, statins, ACE inhibitors, β blockers, nitrates, calcium agonists) was registered.

Thus, TP Abs to S100 is an effective anxiolytic medication that helps to reduce the severity of anxiety as well as to avoid drug interaction, polypharmacy and increase the quality of life. According to some CTs mentioned above, TP Abs to S100 increase the efficacy of standard treatment in patients with AH, angina pectoris, and some heart rhythm disturbances due to their antianxiety action. Due to reduction in severity of anxiety, the improvement of compliance in CVD patients is possible, though this consideration requires further investigation.

3.2.2 Gastrointestinal diseases

Anxiety is common in 20% of patients with gastrointestinal (GI) problems [96] and in 27% of patients with gastritis in particular [97]. Some publications revealed an association between mood disorders and the risk of carcinogenesis in patients with GI diseases [98, 99]. The necessity for antianxiety therapy in these patients is justified.

An open-label comparative study by Tsukanov et al. in patients with anxiety (diagnosed with HAM-A) complicating ulcerative gastritis associated with *Helicobacter pylori* and duodenal ulcers was conducted [100]. One hundred and two participants received standard helicobacter eradication therapy (clarithromycin, amoxicillin, omeprazole, algeldrate—magnesium hydroxide combination drug), and 49 of them were prescribed TP Abs to S100 6 tablets/day for 20 days. Mean age of participants was 41.8 ± 2.4 in the TP Abs to S100 group ($n = 49$) and 42.3 ± 2.8 in the control group ($n = 53$). The dynamics of HAM-A scale scores was evaluated.

Anxiety was significantly reduced in the TP Abs to S100 group after 20 days of treatment. The mean HAM-A score decreased by 55.2% from 23.43 ± 1.8 to 10.5 ± 0.98 (vs. 28% in the control group; $p < 0.001$ vs. baseline; $p < 0.001$ vs. control group). No serious AEs were registered in both groups.

According to another open-label noncomparative CT by Karpin et al. in patients with chronic gastritis and duodenal ulcers, the addition of TP Abs to S100 to standard treatment leads to a prominent reduction in GI symptoms (pain, intestinal dyspepsia, appetite changes) ($p = 0.003$ vs. baseline for pain and dyspepsia, $p = 0.045$ for appetite changes) [101].

So, the treatment of patients with GI diseases with TP Abs to S100 helps to reduce anxiety and indirectly decrease the severity of somatic symptoms via its anxiolytic action.

4. Conclusions

In this review, the data obtained from experimental and clinical studies of TP Abs to S100 efficacy, safety, and mechanisms of action are summarized.

In nonclinical trials, TP Abs to S100 were shown to exert stress-protective, anxiolytic, antidepressant, antiamnestic, and neuroprotective activities. All these effects were manifested at the same level as the activity of comparator drugs. At the same time, toxicological studies have shown a high safety level of TP Abs to S100: there was no any toxic activity of drug reviled even when it was administered to laboratory animals at the maximal dose for 6 consecutive months (every day).

The mechanisms of action studies confirmed the hypothesis that TP Abs to S100 biological effects are realized via recruiting of GABA-, serotonin-, dopamine-, noradrenaline-, and glutamatergic systems, as well as via $sigma_1$ receptors.

Clinical efficacy and safety of TP Abs to S100 were demonstrated in multicenter double-blind randomized placebo-controlled trials and in open-label randomized comparative trials. In all conducted placebo-comparative studies or studies with nonmedicated control group, the main symptom of most NDs—the anxiety—was significantly reduced in TP Abs to S100 action. It should be stressed that in these CTs, the equal efficacy of TP Abs to S100, tofisopam, and bromdihydrochlorphenylbenzodiazepine (in the short-term use) with a notably higher tolerance level was demonstrated. Meanwhile, TP Abs to S100 increased the efficacy of standard treatment of somatic diseases (due to its anxiolytic activity), and there was a lower number of AEs and lack of drug interactions observed in the TP Abs to S100 group.

Thus, the discussed drug—TP Abs to S100—has been extensively studied and demonstrated favorable efficacy and safety profile. The presented evidence justifies TP Abs to S100 to be a promising treatment option for patients with NDs.

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Conflict of interest

All authors are the employees of OOO NPF Materia Medica Holding pharmaceutical company. The substance TP ABS to S100 is produced and marketed by OOO NPF Materia Medica Holding.

Abbreviations

ACE	angiotensin-converting-enzyme
AD	anxiety disorder
AE	adverse event
AH	arterial hypertension
AjD	adjustment disorder
CCD	chronic cerebrovascular disease
CHD	coronary heart disease
CGI-I	global impression-improvement scale
GI	gastrointestinal
CNS	central nervous system
CT	clinical trial
CVD	cardiovascular diseases
DSM	Diagnostic and Statistical Manual of Mental Disorders
HADS-A	Hospital Anxiety and Depression Scale-Anxiety

HAM-A	Hamilton Anxiety Rating Scale
HPAA	hypothalamic-pituitary-adrenal axis
HTP	hydroxytryptophan
GABA	γ-aminobutyric acid
GAD	generalized anxiety disorder
ICD	International Disease Classification
ICF	informed consent form
mADD	mixed anxiety and depressive disorder
ND	neurotic disorder
NMDA	N-methyl-D-aspartate
OCD	obsessive-compulsive disorder
OF	open field test
PD	Parkinson's disease
SBP	systolic blood pressure
SD	somatoform disorder
SSRI	selective serotonin reuptake inhibitors
STAI	State-Trait Anxiety Inventory
TCA	tricyclic antidepressants
TMAS	Taylor Manifest Anxiety Scale

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