



**BRAZILIAN JOURNAL**  
OF MEDICAL AND BIOLOGICAL RESEARCH

[www.bjournal.com.br](http://www.bjournal.com.br)

ISSN 0100-879X  
Volume 42 (11) 993-1118 November 2009

BIOMEDICAL SCIENCES  
AND  
CLINICAL INVESTIGATION

Braz J Med Biol Res, November 2009, Volume 42(11) 1002-1014

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The Brazilian Journal of Medical and Biological Research is partially financed by



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# Glutamate-N-methyl-D-aspartate receptor modulation and minocycline for the treatment of patients with schizophrenia: an update

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## Abstract

Growing consistent evidence indicates that hypofunction of N-methyl-D-aspartate (NMDA) transmission plays a pivotal role in the neuropathophysiology of schizophrenia. Hence, drugs which modulate NMDA neurotransmission are promising approaches to the treatment of schizophrenia. The aim of this article is to review clinical trials with novel compounds acting on the NMDA receptor (NMDA-R). This review also includes a discussion and translation of neuroscience into schizophrenia therapeutics. Although the precise mechanism of action of minocycline in the brain remains unclear, there is evidence that it blocks the neurotoxicity of NMDA antagonists and may exert a differential effect on NMDA signaling pathways. We, therefore, hypothesize that the effects of minocycline on the brain may be partially modulated by the NMDA-R or related mechanisms. Thus, we have included a review of minocycline neuroscience. The search was performed in the PubMed, Web of Science, SciELO, and Lilacs databases. The results of glycine and D-cycloserine trials were conflicting regarding effectiveness on the negative and cognitive symptoms of schizophrenia. D-serine and D-alanine showed a potential effect on negative symptoms and on cognitive deficits. Sarcosine data indicated a considerable improvement as adjunctive therapy. Finally, minocycline add-on treatment appears to be effective on a broad range of psychopathology in patients with schizophrenia. The differential modulation of NMDA-R neurotransmission, in particular synaptic versus extrasynaptic NMDA-R activation and specific subtypes of NMDA-R, may be the key mediators of neurogenesis and neuroprotection. Thus, psychotropics modulating NMDA-R neurotransmission may represent future monotherapy or add-on treatment strategies in the treatment of schizophrenia.

Key words: Schizophrenia; NMDA agonist; Glycine; Glutamate; Clinical trial; Minocycline

## Introduction

Schizophrenia is a disabling illness that affects approximately 1% of the world population (1) and is characterized by positive, negative and cognitive symptoms. This psychiatric disorder encompasses symptoms such as delusions, hallucinations, disorganized thinking, apathy, and deterioration of social role functioning (1). In addition, patients with schizophrenia experience severe suffering and close to 10% of them commit suicide (1). The typical onset of this disease is in young adults, and its course is often chronic (1).

Disruption of the dopaminergic system is implicated in the pathophysiology of schizophrenia. Conventional dopamine receptor-blocking antipsychotics are effective for positive symptoms, but they have limited effects on negative and cognitive symptoms (2). These symptoms possibly involve other neurotransmitter systems besides dopamine.

Emerging data indicate that hypofunction of glutamatergic N-methyl-D-aspartate receptor (NMDA-R) transmission plays a central role in the pathophysiology of schizophrenia (3,4).

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Received March 14, 2009. Accepted September 15, 2009. Available online October 26, 2009.

### Hypothesis of NMDA-R hypofunction in schizophrenia

Emerging data indicate a direct involvement of the glutamatergic system in the pathophysiology of schizophrenia core symptoms. Complementary to the dopamine hypothesis, the hypofunction of the ionotropic glutamate NMDA-R has been proposed as a model of schizophrenia in humans (4). Thus far, there have been many clinical trials with novel compounds acting on the NMDA-R.

The glutamatergic hypothesis of schizophrenia postulates that the dysfunction of neurotransmission mediated by the NMDA-R might represent a primary deficit in this disorder (4). The ability of phencyclidine, ketamine, and other NMDA-R antagonists to induce schizophrenia-like symptoms in healthy volunteers and to exacerbate psychosis in schizophrenic patients is the most compelling evidence of a relationship between NMDA-R function and schizophrenia (4,5).

The NMDA-R antagonist-induced psychosis models are closer to certain clinical aspects of schizophrenia than the amphetamine/dopamine model, mainly by the generation of negative symptoms and cognitive deficits (4,5). Thus, the potentiation of NMDA-R transmission might be a promising treatment approach to schizophrenia.

### Drugs that modulate NMDA-R neurotransmission

Since glutamate and direct-acting NMDA-R agonists may be neurotoxic and are known to cause excitotoxicity, the strategy used in the last decade has relied on agonists at the strychnine-insensitive glycine receptor site of the NMDA-R complex (6). Therefore, experiments have been carried out on full agonists like glycine, D-serine and D-alanine, on the partial agonist D-cycloserine, and on sarcosine.

### NMDA-R full agonists

Glycine is a small naturally occurring amino acid that functions as an endogenous co-agonist at the strychnine-insensitive glycine binding site, being a prerequisite for the activation of the NMDA ion channel (7). Glycine is not synthesized by glutamatergic neurons and a small amount is released from glycine neurons, so that the neighboring glial cells are the main source of the glycine available for the NMDA-R (8). The permeation of glycine into the brain barrier after peripheral administration is the lowest for any naturally occurring amino acid and high oral doses of glycine must be administered to achieve significant elevations in its central nervous system (CNS) levels (7).

D-serine is an endogenous co-transmitter enriched in corticolimbic regions and distributed in parallel with the NMDA-R (9,10), acting as an endogenous full agonist at the NMDA-R-associated glycine site (9,10). D-serine can be derived from L-serine by serine racemase (SR) enzymatic conversion (8,9). Although initial studies have suggested that this enzyme was localized in glial cells, a recent investigation using novel SR knockout mice as negative controls pointed out that SR was co-localized with neuron-specific

nuclear proteins, with SR expression being observed in GABAergic and glutamatergic neurons, but not in glia (9). In addition, serum and cerebrospinal fluid levels of D- and L-serine and D-serine ratio (D-serine/total serine) may be altered in schizophrenia, as demonstrated by reports of reduction of the D-serine ratio in the cerebrospinal fluid of drug-naïve schizophrenic patients (3) and by decreased serum levels of D-serine in patients with schizophrenia (5). These data suggest that D-serine synthesis or metabolism may be altered in schizophrenia, highlighting a possible reduction of SR activity (3,5). Moreover, D-serine has high affinity for the glycine site on NMDA-R and has the advantage of being more permeable than glycine through the blood-brain barrier, thus requiring a reduced amount per dose (8,10).

D-alanine, as well as D-serine, is an endogenous D-amino acid present in the human CNS (11) and is a selective and potent agonist at the NMDA-R glycine site (11).

### NMDA-R partial agonist

D-cycloserine, an analog of D-serine, is an antituberculous drug rarely used nowadays due to its adverse psychiatric effects including anxiety, psychosis and seizures at doses of 500-2000 mg (12). D-cycloserine freely crosses the blood-brain barrier and has good bioavailability to the brain (13). It is a partial agonist at the glycine recognition site at lower doses, with agonist properties over a narrow dose range; hence, it can exhibit antagonist activity at higher concentrations. Additionally, D-cycloserine has approximately 40-60% activity compared to glycine and the glycine modulatory site of the NMDA-R (13).

### Glycine transporter-1 inhibitor

Sarcosine (or N-methylglycine), a nonessential amino acid, is an endogenous antagonist of glycine transporter-1 (glycine-T1). Glycine-T1 is located in the glial cells and its reuptake pump is the main route of inactivation of synaptic glycine. Therefore, the inhibition of glycine-T1 reuptake can increase glycine levels in the glutamatergic synapses and consequently augment NMDA-R transmission (8). Sarcosine is a natural occurring glycine-T1, which potentiates the action of glycine on the NMDA glycine site and can enhance NMDA neurotransmission (14).

Glycinergic agents and targets of NMDA-R modulation are summarized in Figure 1.

### NMDA-R augmentation

Recent studies have supported the idea that increasing NMDA-R function may be an effective approach to the treatment of schizophrenia (15). Two recent reviews have been published about the use of glycinergic agents for this purpose (15,16). Tuominen et al. (15) published a systematic review and meta-analysis that comprised eighteen short-term trials using glycine, D-serine or D-cycloserine to increase the antipsychotic action. These investigators also

included ampakine CX516, an experimental drug that acts as an  $\alpha$ -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor agonist. Glycine and D-serine were successful in reducing the negative symptoms of schizophrenia and the magnitude of the effect was moderate. D-cycloserine had a less efficient action on negative symptoms. On the other hand, positive symptoms failed to respond to glycinergic medication and there were no significant effects of glycine or D-serine on cognitive functioning.

Shim et al. (16), in a review of the results of glycinergic agents in the treatment of schizophrenia, proposed a polypharmacy of glycine site potentiators in addition to antipsychotic medication to stop neurodegeneration in schizophrenia. In addition, they suggested that drugs acting on other sites of the NMDA-R could also be effective in the treatment of the disorder.

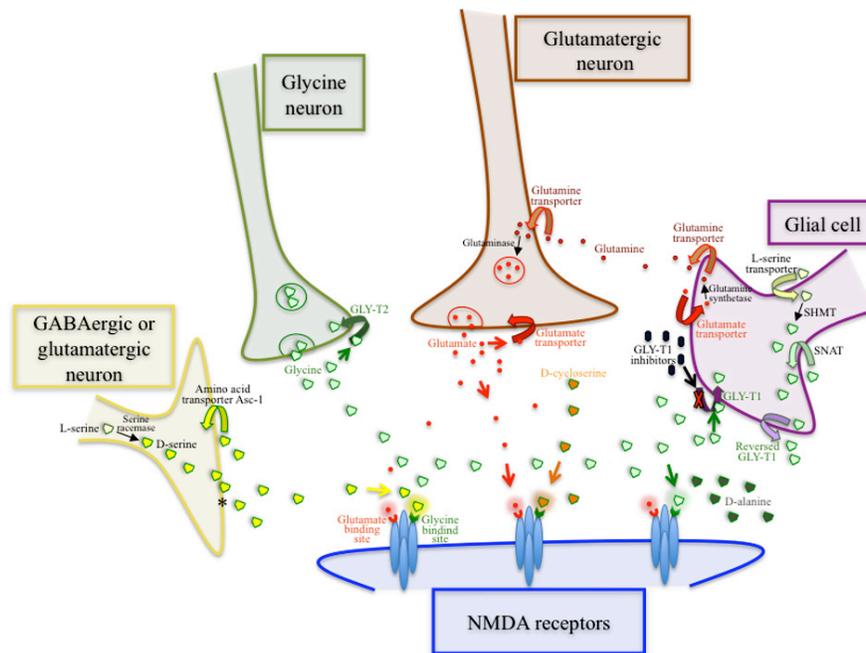
### Hypothesis of NMDA-R modulation by minocycline

Minocycline is a second-generation tetracycline with a broad spectrum of antimicrobial activities and anti-inflammatory properties. There is growing evidence showing neuroprotective effects of minocycline in several animal and human models of neurological diseases, including Parkin-

son's disease, amyotrophic lateral sclerosis, Huntington's disease, and ischemia (17).

Translational neuroscientific data from animal and human studies indicate that minocycline may be a potential treatment for schizophrenia. In the published literature, the first evidence indicating the antipsychotic efficacy of minocycline was observed in a patient with Huntington's disease, a case report from Canada (18). This clinical observation indicated the potential antipsychotic efficacy of this drug in schizophrenia. Zhang et al. (19) observed that pretreatment of mice with minocycline (40 mg/kg) significantly attenuated the level of dopamine in the striatum after repeated administration of methamphetamine. The same group (20) found that pretreatment with minocycline (40 mg/kg) reduced the increase of extracellular dopamine levels in the frontal cortex and striatum of mice after administration of the NMDA-R antagonist dizolcipine. Moreover, subsequent subchronic administration of minocycline considerably reduced the cognitive deficits induced by the NMDA-R antagonist phenylcyclidine in mice (21) and reversed the cognitive effects of the NMDA-R antagonist MK801 in rats (22).

The neuroprotective action of minocycline can occur indirectly by inhibition of microglial activation and prolif-



**Figure 1.** Allosteric modulation of NMDA-R transmission by glycinergic agents. NMDA = N-methyl-D-aspartate; GLY-T1 = type-1 glycine transporter; GLY-T2 = type-2 glycine transporter; SHMT = serine hydroxymethyl transferase; SNAT = specific neutral amino acid transporter. \*The pathway for D-serine release from neurons is unknown. Glycinergic agents (glycine, D-serine, D-alanine or D-cycloserine) are obligatory co-agonists at the strychnine-insensitive glycine binding site of NMDA receptors. Small amounts of glycine are released from glycinergic neurons and are taken back up by a GLY-T2 located in these neurons. However, most of the glycine available in the synaptic cleft is released by glial cells through a reversed GLY-T1. Glycine can be taken up into glial cells by GLY-T1 or through a glial specific neutral amino acid transporter. Glycine can also be converted from L-serine in glial cells through SHMT action. It is noteworthy that glycine reuptake by GLY-T1 in glial cells limits the availability of glycine in the synaptic cleft. L-serine can also be converted to D-serine by serine racemase in GABAergic or glutamatergic neurons. D-serine availability in the synaptic cleft is limited through its reuptake by amino acid transporter Asc-1 located in these neurons.

eration and nitric oxide synthase inhibition (17). There are also influences on caspase expression and cytochrome c release, with an anti-apoptotic property, among others (17). Although the precise mechanism of action of minocycline remains unclear, the latest studies conducted with this agent suggest effects related to the glutamatergic system, in addition to its action as an anti-apoptotic drug. In fact, minocycline reversed several NMDA-R antagonist effects in animal studies and showed good results in the treatment of patients with schizophrenia (23,24). Thus, the hypothesis was raised that minocycline indirectly modulates NMDA-R transmission.

Furthermore, minocycline may exert a differential action on NMDA-R signaling pathways (described in the Discussion section). Consequently, the data about the three studies of minocycline in patients with schizophrenia were included in the present review.

The objective of this review is to identify and analyze clinical trials with NMDA-R transmission modulation and minocycline for the treatment of patients with schizophrenia.

## Methods

The search was carried out in the PubMed, Web of Science, SciELO, and Lilacs databases using the key words schizophrenia, NMDA agonist, glycine, serine, glutamate, clinical trial, and minocycline, up to September 2008. It contemplated only clinical trials with NMDA-R transmission modulation in the treatment of schizophrenia, with no year or language limits. We also inspected the reference lists of the reviewed articles to identify additional trials. We excluded animal or *in vitro* studies, review or meta-analysis articles, and studies on healthy volunteers. The present review covered 36 clinical trials.

## Results

There were 27 randomized placebo-controlled double-blind studies (2,6,7,10-12,14,23,25-42), 8 non-randomized trials (43-51), and 1 case report. Except for two trials (42,49) with drug-free patients, all other studies involved adjuvant therapy for antipsychotic treatment. Clinical assessments were performed biweekly throughout most of the investigations.

The most used inclusion criteria were schizophrenia diagnosis according to the Diagnostic and Statistical Manual (DSM), primary deficit syndrome (using the Schedule for the Assessment of Negative Symptoms (SANS)), and use of a stable antipsychotic dose for more than three months. Exclusion criteria comprised comorbidity with substance abuse, major depression, serious clinical diseases, neurological diseases, and additional use of other psychotropic medications.

A combination of various scales was used for the evalu-

ation of clinical efficacy: Positive and Negative Symptoms Scale (PANSS), Brief Psychiatric Research Scale (BPRS), SANS, Global Assessment Scale (GAS), and Clinical Global Impression (CGI). The PANSS and/or SANS were used in almost all of the studies. Eleven trials (10,12,23,28,31,34,37,38,47,48,50) assessed specific cognitive tests.

To facilitate the presentation of the results, studies were divided into four groups: 1) glycine, 2) D-cycloserine, 3) D-alanine, D-serine and sarcosine, and 4) minocycline.

### Glycine

Twelve articles were selected regarding the addition of glycine to the antipsychotic treatment as usual (Table 1), including 9 placebo-controlled double-blind trials (divided into 4 parallel and 5 crossover studies) (2,7,25-31), and 3 open-label trials (43-45). The duration of the studies ranged from 6 to 28 weeks. The first studies (25,43,44) used low doses, including 10, 15 and 30 g/day. However, the glycine dose needs to be higher to sufficiently cross the blood-brain barrier, and most of the trials subsequently administered higher doses (60 or 0.8 g·kg<sup>-1</sup>·day<sup>-1</sup>).

For heuristic purposes, the results shown in Table 1 are analyzed in three distinct groups according to the type of antipsychotic used.

### Glycine added to conventional antipsychotics

Eight trials evaluated the addition of glycine to typical antipsychotics (25-27,29,31,43-45) and none of them observed any beneficial effect of glycine on positive symptoms. Rosse et al. (43) published a pilot study with 6 patients in which no significant results were obtained regarding the negative symptoms of schizophrenia. However, this study (43) was done with a low dose of glycine (10.8 g/day), which could explain the lack of effectiveness. The seven other investigations showed a beneficial effect on negative symptoms.

In 2007, Buchanan et al. (31) published the Cognitive and Negative Symptoms in Schizophrenia Trial (CONSIST), a randomized double-blind study with a duration of 16 weeks and with the participation of 4 sites in the United States and one site in Israel. A total of 157 patients were recruited and randomized to glycine, D-cycloserine and placebo groups. The study suggested that glycine is not an effective therapeutic option for treating negative symptoms. Nevertheless, *post hoc* analyses revealed that, among subjects receiving conventional antipsychotics, the 8 subjects randomly assigned to glycine had a greater decrease in the SANS total score compared to the 7 subjects randomly assigned to placebo. This trial also showed no significant difference in treatment response between patients with and without deficit syndrome. Nonetheless, tendencies to greater negative symptom reductions for both the glycine and D-cycloserine groups compared with placebo were observed in inpatients, but not in outpatients.

Moreover, the CONSIST (31) was the only study that

**Table 1.** Glycine adjuvant therapy for schizophrenia.

Reference	Design	Groups	Subjects		Mean age ± SD (years)	Duration of illness (years)	Antipsychotic (N)	Daily Gly dose	Duration (weeks)*	Rating scales	Results of symptoms		
			All (N)	Completers N (M/F)							Negative	Positive	Cognitive
<b>Randomized placebo-controlled trials</b>													
Javitt et al., 1994 (25)	double-blind, placebo-controlled, parallel	Gly placebo	7 7 (7/0)		36.0 ± 9.7 38.1 ± 7.2	15.5 ± 8.1 20.0 ± 6.6	typical	30 g	16	PANSS	↑	↔	NA
Heresco-Levy et al., 1996 (26)	double-blind, placebo-controlled, crossover	Gly + placebo 1st group 2nd group	12 6 (ND) 6 5 <sup>a</sup>		41.4 ± 12.9 ND	19.8 ± 10.7 ND	typical (7) clozapine (4)	0.8 g/kg	6	BPRS, PANSS	↑	↔	↑ <sup>c</sup>
Heresco-Levy et al., 1999 (27)	double-blind, placebo-controlled, crossover	Gly + placebo 1st group 2nd group	22 11 11		38.8 ± 11.0 <sup>d</sup> ND	ND	typical (15) clozapine (7)	0.8 g/kg	6	BPRS, PANSS	↑	↑	↑ <sup>c</sup>
Potkin et al., 1999 (2)	double-blind, placebo-controlled, parallel	Gly placebo	12 12	9 (8/1) 10 (10/0)	35.3 ± 5.3 <sup>d</sup> 34.4 ± 4.8 <sup>d</sup>	ND	clozapine	30 g	12	BPRS, SANS	↔	↓	NA
Evins et al., 2000 (28)	double-blind, placebo-controlled, parallel	Gly + placebo Gly placebo	30 15 15	27 (21/6) 14 (ND) 13	39 ± 7 ND	16 ± 7 ND	clozapine	60 g	8	BPRS, PANSS, SANS, Stroop, WAIS, CVLT	↔	↔	↔
Javitt et al., 2001 (29)	double-blind, placebo-controlled, crossover	Gly + placebo 1st group 2nd group	12 6 6	12 (8/4) 6 (ND) 6	39.6 ± 5.5 ND	20.5 ± 6.2 ND	typical (2) olanzapine (6) clozapine (4)	0.8 g/kg	14	PANSS	↑	↔	↑ <sup>c</sup>
Heresco-Levy et al., 2004 (7)	double-blind, placebo-controlled, crossover	Gly + placebo 1st group 2nd group	17 ND 7	14 (ND) 7	44.7 ± 10.8 <sup>d</sup> ND	22.9 ± 7.0 <sup>d</sup> ND	olanzapine (10) risperidone (4)	0.8 g/kg	14	BPRS, PANSS	↑	↑	↑ <sup>c</sup>
Diaz et al., 2005 (30)	double-blind, placebo-controlled, crossover	Gly + placebo 1st group 2nd group	12 6 6	11 (9/2) 5 <sup>b</sup> (ND) 6	39.5 ± 12.4 ND	ND	clozapine	60 g	28	BPRS, PANSS	↔	↔	NA
Buchanan et al., 2007 (31)	double-blind, placebo-controlled, parallel	Gly placebo	54 55	42 (ND) 45	42.6 ± 10.8 <sup>d</sup> 43.4 ± 11.4 <sup>d</sup>	20.2 ± 10.0 <sup>d</sup> 20.2 ± 11.0 <sup>d</sup>	typical (ND) atypical (ND) (no clozapine)	60 g	16	BPRS, SANS, neuropsychological tests	↔	↔	↔
<b>Non-randomized trials</b>													
Rosse et al., 1989 (43)	open-label trial	Gly	6	6 (6/0)	38.0 ± 15.0	12.6 ± 8.6	typical	10.8 g	8	BPRS, SANS	↔	↔	NA
Costa et al., 1990 (44)	open-label trial	Gly	6	4 (4/0)	34.8 <sup>d</sup> (ND)	16.3 <sup>d</sup> (ND)	typical	15 g	6	BPRS	↑	NA	NA
Leiderman et al., 1996 (45)	open-label trial	Gly	5	5 (ND)	45.0 ± 7.6	24.2 ± 5.9	haloperidol (1) risperidone (2) clozapine (2)	0.8 g/kg	8	PANSS, SANS	↑	↔	NA

\*For the crossover trials, the length of the entire study was included. <sup>a</sup>Drop out from placebo group; <sup>b</sup>drop out from glycine group; <sup>c</sup>assessed by PANSS cognitive subscale; <sup>d</sup>including non-completers' data. ↑, improved; ↓, worsened; ↔, no effect; BPRS, Brief Psychiatric Rating Scale; CVLT, California Verbal Learning Test; Gly, glycine; N, number of subjects; NA, not applied; ND, not described; PANSS, Positive and Negative Syndrome Scale; SANS, Scale for the Assessment of Negative Symptoms; WAIS, Wechsler Adult Intelligence Scale.

assessed specific cognitive tests, detecting no significant results compared to placebo. Three other trials (26,27,29) evaluated the cognitive effects of glycine based on clinical ratings (PANSS cognitive subscale) rather than neuropsychological examination, showing a positive result in cognitive functioning. Nevertheless, it is noteworthy that these three investigations (26,27,29) included a relatively small patient sample size and short-term glycine treatment.

#### **Glycine added to second-generation antipsychotics**

Four trials appraised the addition of glycine to second-generation antipsychotics (7,29,31,45). A 6-week double-blind crossover trial (7) registered a significant reduction of positive symptoms while the other three (29,31,45) did not exhibit a significant change of positive symptoms.

Three studies (7,29,45) provided evidence for a reduction of negative symptoms. However, these clinical trials were conducted on small samples over short periods of time, while the CONSIST (31) found no significant effect compared to placebo.

Once more, the CONSIST (31) was the only study that applied specific cognitive tests, obtaining no significant results compared to placebo. Two other short-term trials (7,29) measured the cognitive effects of glycine based on clinical ratings (PANSS cognitive subscale) rather than on neuropsychological examination, pointing to a positive result in cognitive functioning.

#### **Glycine added to clozapine**

Seven trials (2,26-30,45) evaluated the addition of glycine to clozapine. Five of them (26,28-30,45) did not find a large change of positive symptoms with the adjuvant use of glycine in combination with clozapine. One study (2) found worsening of positive symptoms. Although one trial (27) reported a reduction of positive symptoms, the authors did not specify whether the results were related to the typical treatment or to the clozapine group.

Although four trials (26,27,29,45) included patients on clozapine with a further decrease of negative symptoms, most of them (26,27,45) did not distinguish between patients taking clozapine and patients taking other antipsychotics. Three other investigations (2,28,30) showed no beneficial effects on negative symptoms.

Cognitive functioning was assessed in only four trials (26-29). In one investigation (28), specific cognitive tests were applied and no effect on cognition was observed. The other three studies used the PANSS cognitive subscale, indicating beneficial results.

#### **D-cycloserine**

Fourteen articles were selected involving the addition of D-cycloserine to antipsychotic treatment as usual (Table 2), including 9 placebo-controlled double-blind trials (6,12,31-37) and 5 non-randomized trials (46-50). These studies had a variable length of 4 to 24 weeks. Since D-cycloserine is a

partial agonist, four studies (47-50) compared the effectiveness of 5, 15, 50 and 250 mg/day D-cycloserine in an attempt to obtain the optimal dosing. The initial results suggested that the dosage of 50 mg/day yielded the best results, with worsening of symptoms at higher doses (100-250 mg/day) and lack of effect at lower doses (5-30 mg/day).

#### **D-cycloserine added to conventional antipsychotics**

Ten trials evaluated the addition of D-cycloserine to typical antipsychotics (6,12,31-34,36,37,46,47). Seven of them (6,12,31-34,47) did not find any significant change in positive symptoms and two (with higher dosages, namely 100 and 250 mg/day) reported worsening of positive symptoms (36,46).

Beneficial effects on negative symptoms were obtained in four trials (6,33,34,47), while four other studies (12,31,32,37) did not find a significant change. Two studies (36,46) with doses of 100 and 250 mg/day showed worsening of negative symptoms.

In addition, one trial (47) demonstrated improvement of cognitive functioning at the dose of 50 mg/day, while three other trials (12,34,37) did not find significant changes in cognitive symptoms. The CONSIST (31) was the largest trial evaluating the addition of D-cycloserine to typical antipsychotics and showed no effect on positive, negative or cognitive symptoms.

#### **D-cycloserine added to second-generation antipsychotics**

Four trials evaluated the effect of D-cycloserine added to second-generation antipsychotics (6,31,33,50) and none of them detected any significant change in positive symptoms. Three studies (6,33,50) demonstrated a decrease of negative symptoms at the dose of 50 mg/day. On the other hand, these trials were conducted on a small numbers of participants and the CONSIST (31) showed no significant effect of the increase of D-cycloserine to 50 mg/day on negative symptoms. Only two trials (31,50) assessed cognitive functioning and showed no significant effects.

#### **D-cycloserine added to clozapine**

Only three trials evaluated the addition of D-cycloserine to clozapine (33,35,48), showing no significant effects on cognitive or positive symptoms. One trial (33) observed amelioration of negative symptoms, but did not distinguish the type of antipsychotics used and consisted of only 9 participants. The other two investigations showed worsening of negative symptoms when D-cycloserine was added to clozapine.

#### **D-alanine, D-serine and sarcosine**

Eight articles involving the addition of these drugs to the antipsychotic treatment were selected (Table 3).

#### **D-alanine**

There is only one trial involving the addition of D-alanine to habitual antipsychotic treatment (Table 3), with the addi-

**Table 2.** D-cycloserine adjuvant therapy for schizophrenia.

Reference	Design	Groups	Subjects		Mean age ± SD (years)	Duration of illness (years)	Antipsychotic (N)	DCS dose (mg/day)	Duration (weeks)*	Rating scales	Results of symptoms		
			All (N)	Completers N (M/F)							Negative	Positive	Cognitive
<b>Randomized placebo-controlled trials</b>													
Rosse et al., 1996 (32)	double-blind, placebo-controlled, parallel	DCS + placebo DCS (10 mg) DCS (30 mg) placebo	13 3 6 4	13 (ND) 3 6 4	38.1 ± 6.8 ND	15.6 ± 5.5 ND	mollindone	10 and 30	4	BPRS, SANS	↔	↔	NA
Heresco-Levy et al., 1998 (33)	double-blind, placebo-controlled, crossover	DSC + placebo 1st group 2nd group	9 5 4	8 (ND) ND ND	39.2 ± 14.7 <sup>a</sup> ND	20.2 ± 12.9 <sup>a</sup> ND	typical (5) <sup>a</sup> risperidone (1) <sup>a</sup> clozapine (3) <sup>a</sup>	50	16	PANSS	↑	↔	NA
Goff et al., 1999 (34)	double-blind, placebo-controlled, parallel	DCS placebo	23 23	16 (ND) 23 (19/4)	46.8 ± 12.3 <sup>a</sup> 41.2 ± 8.1 <sup>a</sup>	22.3 ± 13.3 <sup>a</sup> 18.5 ± 8.6 <sup>a</sup>	typical	50	8	PANSS, SANS, SIRP, Stroop, MST, other neuropsychological tests	↑	↔	↔
Goff et al., 1999 (35)	double-blind, placebo-controlled, crossover	DSC + placebo 1st group 2nd group	17	11 (9/2) ND	36.6 ± 9.6 ND	14.8 ± 8.4 ND	clozapine	50	13	PANSS, SANS	↓	↔	NA
van Berckel et al., 1999 (36)	double-blind, placebo-controlled, parallel	DCS placebo	13 13	12 (10/2) 13 (12/1)	45 ± 6 <sup>a</sup> 44 ± 9 <sup>a</sup>	26 ± 8 <sup>a</sup> 22 ± 10 <sup>a</sup>	typical	100	8	PANSS	↓	↓	NA
Heresco-Levy et al., 2002 (6)	double-blind, placebo-controlled, crossover	DSC + placebo 1st group 2nd group	24 12 12	16 (6/10) 8 (ND) 8	40.0 ± 12.1 ND	28.2 ± 11.2 ND	typical (8) risperidone (3) olanzapine (5)	50	6	PANSS	↑	↔	NA
Goff et al., 2005 (12)	double-blind, placebo-controlled, parallel	DCS placebo	27 28	14 (ND) 12	45.9 ± 7.4 <sup>a</sup> 47.0 ± 8.6 <sup>a</sup>	22.9 ± 5.8 <sup>a</sup> 22.4 ± 8.1 <sup>a</sup>	typical	50	24	PANSS, SANS, ANART, CVLT, Stroop, WAIS, WCST	↔	↔	↔
Duncan et al., 2004 (37)	double-blind, placebo-controlled, parallel	DCS placebo	10 12	10 (10/0) 12 (12/0)	48.7 ± 12.1 54.4 ± 11.8	ND	typical	50	4	ATSEB, BPRS, SANS, CPT, MMSE, SSTMSP	↔	↔	↔
Buchanan et al., 2007 (31)	double-blind, placebo-controlled, parallel	DCS placebo	53 52	46 (ND) 45	44.4 ± 10.4 <sup>a</sup> 43.4 ± 11.4 <sup>a</sup>	21.8 ± 11.1 <sup>a</sup> 20.2 ± 11.0 <sup>a</sup>	typical (ND) atypical (no clozapine)	50	16	BPRS, SANS, neuropsychological tests	↔	↔	↔
<b>Non-randomized trials</b>													
Cascella et al., 1994 (46)	open-label	DCS	7	2 (ND)	33.3 ± 5.4 <sup>a</sup>	14.6 ± 5.2 <sup>a</sup>	typical	250	6	BPRS, SANS	↓	↓	NA
Goff et al., 1995 (47)	single-blind, rater-blind, dose-finding	DCS	10	9 (8/1)	43 ± 12	21 ± 6	typical	5-250	10	BPRS, SANS, SIRP	↑ (50 mg)	↔	↑ (50 mg)
Goff et al., 1996 (48)	single-blind, rater-blind, dose-finding	DCS	10	8 (8/0)	40.4 ± 6.1	ND	clozapine	5-250	10	BPRS, SANS, SIRP	↓	ND	↔
van Berckel et al., 1996 (49)	single-blind, dose-finding	DCS	13	7 (ND)	30.0 ± 9.8 <sup>a</sup>	10.3 ± 6.2 <sup>a</sup>	none	15-250	3.5	PANSS	↑ (100 mg)	↔	NA
Evins et al., 2002 (50)	single-blind, dose-finding	DCS	10	10 (9/1)	42 ± 11	19 ± 11	risperidone	5-250	10	BPRS, SANS, cognitive tests	↑ (50 mg)	NA	↔

\*For the crossover trials, the length of the entire study was included. <sup>a</sup>Including non-completers' data. ↑, improved; ↓, worsened; ↔, no effect; ANART, Adult North American Reading Test; ATSEB, Abrams and Taylor Scale for Emotional Blunting; BPRS, Brief Psychiatric Rating Scale; CPT, Continuous Performance Test; CVLT, California Verbal Learning Test; DCS, D-cycloserine; MMSE, Mini-Mental State Exam; MST, Miller-Setfridge Test; N, number of subjects; NA, not applied; ND, not described; PANSS, Positive and Negative Syndrome Scale; SANS, Scale for the Assessment of Negative Symptoms; SIRP, Sternberg's Item Recognition Paradigm; SSTMSP, Sternberg Short Term Memory Scanning Paradigm; WAIS, Wechsler Adult Intelligence Scale; WCST, Wisconsin Card Sort Test.

**Table 3.** D-alanine, D-serine and sarcosine adjuvant therapy for schizophrenia.

Reference	Design	Drug	Daily drug dose	Groups	Subjects		Mean age ± SD (years)	Duration of illness (years)	Antipsychotic (N)	Duration (weeks)*	Rating scales	Results of symptoms		
					All (N)	Completers N (M/F)						Negative	Positive	Cognitive
Tsai et al., 2006 (11)	double-blind, placebo-controlled, parallel	D-alanine	100 mg/kg	D-alanine placebo	14 (4/10)	14 (4/10)	30.9 ± 6.5	9.5 ± 6.1	typical (21)	6	PANSS, SANS	↑	↑	↑ <sup>b</sup>
Tsai et al., 1998 (10)	double-blind, placebo-controlled, parallel	DSR	30 mg/kg	DSR placebo	18 (17 ND)	17 (ND)	31.8 ± 7.4 <sup>a</sup>	8.8 ± 6.0 <sup>a</sup>	typical (24)	6	PANSS, SANS, WCST	↑	↑	↑
Tsai et al., 1999 (36)	double-blind, placebo-controlled, parallel	DSR	30 mg/kg	DSR placebo	10 (10/0)	10 (6/4)	42.6 ± 3.6	20.6 ± 6.1	clozapine	6	PANSS, SANS, WCST	↔↔	↔↔	↔↔
Heresco-Levy et al., 2005 (39)	double-blind, placebo-controlled, crossover	DSR	30 mg/kg	DSR + placebo 1st group 2nd group	39 (ND)	37 (ND)	44.9 ± 12.5 <sup>a</sup> ND	ND	risperidone (21) <sup>a</sup> olanzapine (18) <sup>a</sup>	15	BPRS, PANSS, SANS	↑	↑	↑ <sup>b</sup>
Tsai et al., 2004 (14)	double-blind, placebo-controlled, parallel	sarcosine	2 mg	sarcosine placebo	17 (16 ND)	16 (ND)	29.8 ± 7.2 <sup>a</sup> 33.4 ± 8.3 <sup>a</sup>	10.5 ± 5.6 <sup>a</sup> 8.9 ± 5.6 <sup>a</sup>	typical (6) <sup>a</sup> risperidone (20) <sup>a</sup>	6	BPRS, PANSS, SANS	↑	↑	↑ <sup>b</sup>
Lane et al., 2005 (40)	double-blind, placebo-controlled, parallel	sarcosine DSR	2 g	sarcosine DSR placebo	21 (21)	18 (ND) 19	36.1 ± 10.2 <sup>a</sup> 31.8 ± 10.4 <sup>a</sup> 34.1 ± 8.7 <sup>a</sup>	ND	risperidone	6	PANSS, SANS	↑(sarcosine) ↔↔(DSR)	↑(sarcosine) ↔↔(DSR)	↑(sarcosine) <sup>b</sup> ↔↔(DSR) <sup>b</sup>
Lane et al., 2006 (41)	double-blind, placebo-controlled, parallel	sarcosine	2 g	sarcosine placebo	10 (10/0)	10 (7/3)	36.7 ± 10.1 35.5 ± 6.6	ND	clozapine	6	PANSS	↔↔	↔↔	↔↔ <sup>b</sup>
Lane et al., 2008 (42)	double-blind, placebo-controlled, parallel	sarcosine	1 and 2 g	1 g group 2 g group	9 (9)	6 (ND)	31.3 ± 10.4 <sup>a</sup> 34.3 ± 11.2 <sup>a</sup>	6.4 ± 8.4 <sup>a</sup> 8.5 ± 8.4 <sup>a</sup>	none	6	PANSS, SANS	↔↔	↔↔	NA

\*For the crossover trials, the length of the entire study was included. <sup>a</sup>Including non-completers' data; <sup>b</sup>assessed by PANSS cognitive subscale. ↑, improved; ↔, no effect; BPRS, Brief Psychiatric Rating Scale; DSR, D-serine; N, number of subjects; NA, not applied; ND, not described; PANSS, Positive and Negative Syndrome Scale; SANS, Scale for the Assessment of Negative Symptoms; WCST, Wisconsin Card Sort Test.

tion of D-alanine (100 mg·kg<sup>-1</sup>·day<sup>-1</sup>) to typical antipsychotics and to risperidone (11). This study showed effectiveness of D-alanine adjuvant treatment to both typical antipsychotics and risperidone on the positive, negative and cognitive domains of schizophrenia. Cognitive functioning was assessed by the cognitive subscale of PANSS, with no specific cognitive tests. Furthermore, this trial had a small sample size, with larger trials being needed to confirm these initial results.

### **D-serine**

There were four trials with the addition of D-serine to the usual antipsychotic treatment (10,38-40). One trial (10) evaluated the addition of D-serine to typical antipsychotics, with amelioration of positive, negative and cognitive symptoms. Three trials (10,39,40) evaluated the addition of D-serine to second-generation antipsychotics, and two of them (10,39) demonstrated good outcomes regarding positive, negative and cognitive symptoms. However, one study (40) did not find beneficial results in any of the three domains. One trial with addition of D-serine to clozapine (38) showed no significant effect on the three core symptoms of schizophrenia.

### **Sarcosine**

The first trial (14) evaluated the addition of 2 g sarcosine to typical antipsychotics and to risperidone, revealing an improvement of the positive, negative and cognitive symptoms of schizophrenia. The second trial (40) evaluated the addition of sarcosine to risperidone in patients with acute exacerbation of schizophrenia, observing an overall reduction of symptoms.

These results encouraged research on sarcosine as add-on therapy to clozapine (41), which led to no significant change in schizophrenia symptoms. Moreover, Lane et al. (42) evaluated the effect of sarcosine monotherapy on 12 acutely symptomatic drug-free patients with schizophrenia, who were randomly assigned to take 1 or 2 g sarcosine. Overall, there was no significant effect of sarcosine dose. However, the 5 responders were drug-naïve patients.

### **Minocycline**

To the best of our knowledge, the antipsychotic efficacy of minocycline was first observed in a patient with Huntington's disease (18). There is one case report (24) with addition of minocycline to the antipsychotic treatment as usual, using a dose of 150 mg/day in two cases of acute catatonic schizophrenia. Only the PANSS was used weekly for the evaluation of clinical efficacy, showing a marked reduction of positive, negative and cognitive symptoms. There were no considerable side effects, which were clinically evaluated, without using scales. The authors reported a considerable decrease of psychiatric symptoms, followed by a worsening of

symptoms after discontinuation of minocycline. However, improvement was observed again after the reintroduction of minocycline. Both patients remained on minocycline co-treatment and were practically symptom-free after one year of follow-up, even though the dose of haloperidol was reduced.

Levkovitz et al. (23) examined the efficacy of minocycline as add-on treatment in a double-blind, randomized placebo-controlled study conducted on patients with early schizophrenia, who were treated with minocycline (N = 36) or placebo (N = 18). The patients were followed-up for 6 months with clinical evaluations by SANS and CGI. The cognitive assessment consisted of a computerized battery. This trial demonstrated improvement of negative symptoms and executive functioning. It is noteworthy that positive symptoms were not rated.

Miyaoka et al. (51) published an open-label study of minocycline as adjunctive therapy for schizophrenia. The researchers evaluated the addition of minocycline (150 mg/day) to second-generation antipsychotics in 22 patients with schizophrenia for a period of 4 weeks. The clinical ratings were performed using the PANSS, and the positive and negative symptom subscales were reduced to 40.4 and 44.0%, respectively, after 4 weeks of minocycline co-treatment. The PANSS general psychopathology subscale was reduced to 52.1%. All the improvements on the PANSS subscales were maintained when scored at the 4-week follow-up time.

## **Discussion**

### **Possible limitations of glycinergic agents**

#### **NMDA agonists**

Initial results with glycine and D-cycloserine in addition to typical and second-generation antipsychotics have demonstrated a decrease in negative and cognitive symptoms. However, the CONSIST (31) results suggested that neither glycine nor D-cycloserine was effective for the treatment of negative symptoms or cognitive impairment. Nevertheless, this trial had a low percentage of subjects treated with typical antipsychotics.

Additionally, the CONSIST (31) data showed no correlation between plasma glycine concentration and negative symptom response, despite previous findings that plasma glycine levels predicted the reduction of negative symptoms. Thus, the correlation of plasma glycine levels and negative symptom response requires further studies.

Overall, the CONSIST (31) results contested the previous hypotheses of amelioration of negative symptoms and cognitive impairment by glycine and D-cycloserine, particularly when added to second-generation antipsychotics.

There is only one trial (11) with the addition of D-

alanine to treatment with typical antipsychotics and risperidone. This research showed effectiveness on the positive, negative and cognitive domains of schizophrenia.

The trials of D-serine added to both typical and second-generation antipsychotics (10,39) indicated a broad symptomatic response in the treatment of chronic schizophrenia, but not for acute exacerbation (40).

### ***Glycine transporter-1 inhibitors***

The presence of endogenous mechanisms that regulate the impact of amino acids administered exogenously (8), in addition to the need for high oral doses and relative lack of penetration into the CNS (52), is a possible limitation of glycinergic agents. Moreover, glycine transporters regulate glycine levels in the synaptic cleft. This may limit the impact of dietary glycine in the same way that the serotonin precursor L-tryptophan is less efficient in increasing serotonergic transmission in comparison to the selective serotonin reuptake inhibitors. As a result, glycine-T1 antagonists may be more effective in increasing synaptic glycine and effectively potentiating NMDA-R transmission (52). Several glycine-T1 inhibitors are in clinical and preclinical testing, and inhibitors with greater potency are expected to be even more effective (8). In view of the early stage of development of these compounds, clinical findings are still limited to sarcosine.

Adjunctive therapy with sarcosine has provided evidence of broad amelioration of schizophrenia symptoms, but with no significant results in monotherapy (42). As a result, sarcosine has a good potential as adjunctive therapy in addition to typical antipsychotics and risperidone.

### ***Clozapine and NMDA-R***

The trials that attempted to enhance the effect of clozapine have not been effective or have worsened the psychotic frame, suggesting that glycinergic drugs interfere with the mechanism of action of clozapine. These results also indicate that clozapine has a particular efficacy related in part to the potentiation of neurotransmission mediated by NMDA receptors. In fact, clozapine has been effective against the neurotoxicity of NMDA antagonists in animal models (53). Additionally, a partial agonist of muscarinic M1 receptors and an agonist of dopamine D1 receptors have been shown to enhance NMDA-R function (53). Hence, clozapine is a potent partial agonist of M1 receptors and a partial agonist of D1 receptors, which may increase NMDA-R transmission, explaining the unique efficacy of this agent (53).

### ***Minocycline***

The trials with minocycline add-on treatment to both typical and second-generation antipsychotics have shown a broad symptomatic response in schizophrenic patients.

These results warrant further investigation of minocycline add-on treatment for patients with schizophrenia.

### ***Mechanisms of action of minocycline***

The exact mechanism of action of minocycline remains elusive. In addition to inhibiting microglial activation, decreasing nitric oxide synthase and having anti-apoptotic properties, minocycline has been recently suggested to be a positive modulator of the GluR1 subunit receptors of AMPA. Although it has been previously suggested that AMPA antagonists may offer neuroprotection, Black (54) stated that some positive AMPA modulators increase brain-derived neurotrophic factor production, which in turn has been reported to have neuroprotective effects. Furthermore, AMPA receptors are distinguished on the basis of subunit composition (Glu R1-4) and isoform conformation. The subunit composition of the AMPA receptor shows striking discrepancy across the brain (54) and has different properties depending on AMPA subunit or splice variant complement. Consequently, the clinical effect of an AMPA modulator may vary according to the action of different subunits.

### ***NMDA-R signaling dichotomy***

Papadia and Hardingham (55) discussed pro-survival and pro-death signaling from the NMDA-R, arguing that both excessive and poor NMDA-R activities are deleterious and cause apoptosis in neurons. For this reason, NMDA-R activity shows a bell-shaped curve of neuronal response.

Thus, the phosphoinositide-3-kinase/Akt kinase cascade is an essential pathway for the pro-survival effects of NMDA-R stimulation (55). NMDA-R activity also robustly induces cAMP-response-element-binding protein-dependent gene expression, which promotes neuronal survival (55).

On the other hand, NMDA-R activity triggers several downstream pathways of cell death by  $Ca^{2+}$  influx. One of these mechanisms is p38 mitogen-activated protein kinase (MAPK) activation, which can be inversely stimulated depending on NMDA-R subtype, subunit composition and localization (56).

Therefore, the magnitude of NMDA-R activation, differential synaptic versus extrasynaptic NMDA-R effects and specific subtypes of NMDA receptors can provide the key mediators of neuroprotection instead of neurodegeneration (55).

### ***Minocycline and NMDA-R***

It is important to point out that minocycline, in addition to having the ability to reverse the neurotoxic effects of NMDA antagonists, also prevented NMDA-induced injury (57).

Actually, minocycline does not have a direct action on NMDA-R and does not regulate intracellular  $Ca^{2+}$  influx (58). However, minocycline seems to exert a differential control of NMDA-R signaling by preserving pro-survival pathways and reducing upstream mechanisms of apoptosis. Accordingly, minocycline inhibits p38 MAPK and maintains

the activation of phosphoinositide-3-kinase/Akt, which are prominently associated with NMDA-R neurochemistry and physiology (59).

These mechanisms may underlie the action of minocycline on caspase and cytochrome c, since they are later events in cell death. Minocycline is an anti-microglial agent and may reduce the hypothetical inflammatory component of schizophrenia. Besides, minocycline inhibits the activation of all MAPKs. It is important to emphasize that these enzymes are essential for microglia activation.

Thus, the aforementioned effects of minocycline may play an important role in the broad effect of this drug in schizophrenia as well as in a wide range of neurological disorders.

### Future trends and psychopharmacological targets

The modulation of NMDA-R transmission is a promis-

ing therapeutic approach to the treatment of schizophrenia. However, the majority of trials had small sample sizes (less than 20 patients) and short durations. Future studies with larger numbers of subjects and longer follow-ups are needed to corroborate the initial findings.

Moreover, evaluations of potent glycine-T1 inhibitors along with other potential mechanisms for the modulation of NMDA-R neurotransmission are promising approaches to the treatment of schizophrenia. In addition, metabotropic glutamate (mGlu) receptors may be another pharmacological target for the enhancement of NMDA-R transmission. Accordingly, positive modulators of mGlu5 receptors enhance NMDA-R transmission and agonists of mGlu2/3 receptors regulate the release of glutamate (60). Another possibility lies in the combination of drugs with different mechanisms of NMDA-R potentiation.

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