



## Antiepileptic potential of ganaxolone

## Antiepileptički potencijal ganaksolona

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### Key words:

ganaxolone; epilepsies, partial; neurotransmitter agents; child; adult.

### Ključne reči:

ganaksolon; epilepsije, parcijalne; neurotransmiteri; deca; odrasle osobe.

### Introduction

With its estimated prevalence of 0.52% in Europe, 0.68% in the United States of America and up to 1.5% in developing countries, epilepsy makes a heavy burden on individuals, healthcare systems and societies in general all over the world<sup>1,2</sup>. Despite long history of epilepsy treatment with medication, efficacy and effectiveness of available antiepileptic drugs as monotherapy were unequivocally proven in clinical trials only for partial-onset seizures in children and adults (including elderly), while generalized-onset tonic-clonic seizures in children and adults, juvenile myoclonic epilepsy and benign epilepsy with centrotemporal spikes are still waiting for optimal therapy<sup>3-5</sup>. It is estimated that 19–30% of epilepsy patients suffer from drug resistant epilepsy, which could not be controlled with available drugs, and they have to consider surgical treatment options<sup>6-9</sup>. Besides, antiepileptics are drugs with narrow therapeutic window, and control over epilepsy could easily be lost if the patients are switched from brand-name to generic, or from one to another generic antiepileptic. Although bioequivalence of generic drugs with their brand-name counterparts has to be confirmed prior to marketing authorization, generic antiepileptic drugs actually do not have the same bioavailability as brand-name drugs, and plasma concentration fluctuations could have much different pattern, leading to loss of seizures control<sup>2-4</sup>. Development of new antiepileptic drugs with mechanisms of action different from that of available anticonvulsants and with wide therapeutic window is one of the main ways to satisfy the unmet needs of patients with epilepsy. Ganaxolone, a positive allosteric modulator of gamma-aminobutyric acid-A (GABA-A) receptor, is one of the drugs with new mechanism of action which are currently in the process of clinical testing<sup>2-4, 6, 7, 10</sup>.

### New anticonvulsants

The drug resistant epilepsy has been recently defined by the International League Against Epilepsy as “a failure of adequate trials of two tolerated, appropriately chosen and used antiepileptic drug schedules (whether as monotherapies or in combination) to achieve sustained seizure freedom”<sup>11</sup>. The mechanisms of drug resistance in epilepsy are still incompletely understood, and none of the anticonvulsants with current marketing authorization has demonstrated superior efficacy in the treatment of drug resistant epilepsy<sup>12</sup>. Using new anticonvulsants as add-on therapy lead to freedom from seizures in only 6% of patients with drug resistant epilepsy<sup>13</sup>. This huge unmet need could be satisfied in the future only by synthesis and development of anticonvulsants with new mechanisms of action.

There are several anticonvulsants besides ganaxolone which are currently in the stage of clinical development: brivaracetam, seletacetam, talampanel, fluorofelbamate, carisbamate, and losigamone<sup>14</sup>. Being analogues of levetiracetam, brivaracetam and seletacetam bind with high-affinity for synaptic vesicle protein 2A (SV2A) and brivaracetam also inhibits voltage-gated sodium channels; talampanel is non-competitive allosteric blocker of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors and weak inhibitor of kainite receptors for glutamate<sup>15</sup>. Fluorofelbamate has similar mechanism of action as felbamate, *ie* decreases responses of N-methyl-D-aspartate (NMDA) and kainate receptor to activation and blocks voltage-dependent sodium channels<sup>15</sup>. While the mechanism of carisbamate action remains unknown, losigamone is both sodium channel blocker, suppressor of NMDA-induced depolarization and enhancer of chloride uptake into neurons<sup>15</sup>. The mechanism of action of ganaxolone is different: it prevents seizures through positive allosteric modulation of synaptic

and extrasynaptic GABA-A receptors, mimicking action of endogenous neurosteroids. Such subtle mechanism of action provided for beneficial efficacy/safety ratio of ganaxolone.

### The compound

Ganaxolone is a synthetic molecule with the steroid backbone which resembles endogenous neurosteroids that are synthesized and acting in the brain. There are three groups of endogenous neurosteroids: pregnane neurosteroids (allopregnanolone and allotetrahydrodeoxycorticosterone), androstane neurosteroids (androstenediol and etiocholanolone) and sulfated neurosteroids (pregnenolone sulfate). Neurosteroids from the pregnane group show anti-seizure activity in various animal models due to their positive allosteric modulation of GABA-A receptors and increase of chloride influx<sup>16</sup>. The development of ganaxolone was initiated by Kelvin Gee and Nancy Lan at CoSensys company<sup>17</sup>, and then continued by Edward Monaghan and his associates<sup>18</sup>. Later development took place at Marinus Pharmaceuticals, Inc., after this company obtained development and commercialization rights from Purdue Pharmaceuticals, who acquired CoSensys in 1998<sup>18</sup>. Ganaxolone is currently in phase 3 clinical trials, and the results are expected in 2016.

### Chemistry

Reduction of progesterone at the 5- and 3-positions of the steroid A-ring leads to formation of endogenous neurosteroid allopregnanolone (through an intermediate metabolite 5 $\alpha$ -dihydroprogesterone) which has modulating effect on GABA-A receptors, and lacks progestogenic effect<sup>19</sup>. However, allopregnanolone could be oxidized back to 5 $\alpha$ -dihydroprogesterone which has progestogenic properties. Ganaxolone (3 $\alpha$ -hydroxy-3 $\beta$ -methyl-5 $\alpha$ -pregnan-20-one) is synthesized from allopregnanolone by methylation at position 3 of its A-ring (Figure 1). The methyl group added prevents conversion of ganaxolone back to a steroid with hormonal (progestogenic) properties, improving its safety profile.

Ganaxolone (molecular weight 332.52) is present at room temperature as white powder which is insoluble in water<sup>20</sup>. Due to its insolubility in water, significant efforts were made to prepare suitable oral formulations of ganaxolone with acceptable bioavailability. Successful formulation efforts resulted in a patent issued to Marinus Pharmaceuticals Inc. covering an oral suspension and capsule formulation. The new formulations achieved bioavailability of 300–400% compared to conventional ganaxolone formulations<sup>21</sup>.

### Pharmacodynamics

#### Mechanism of action

Allopregnanolone is a positive allosteric modulator of action of GABA on its A-type receptors, but various part of the brain differ in rate of allopregnanolone synthesis. Tissue concentrations of allopregnanolone are higher in mice olfactory bulb than in frontoparietal cortex or cerebellum. The study on patch-clamped neocortical pyramidal neurons of mice showed that blocking synthesis of allopregnanolone decreases chloride ion currents elicited by GABA-A receptor agonist muscimol; this effect was reversed with addition of allopregnanolone<sup>22–24</sup>. The mechanism of action of ganaxolone is similar to the mechanism of action of endogenous neurosteroid allopregnanolone: it binds for unique recognition site on the GABA-A receptor which is different from the binding sites of GABA, benzodiazepines and barbiturates. After binding, ganaxolone probably potentiates inhibitory action of GABA on neurons which carry the GABA-A receptor. *In vitro* studies on xenopus oocytes expressing the human GABA-A receptors showed that ganaxolone increased chlorine influx only after  $\gamma$ -aminobutyric acid exhibited its basal activity, while direct effect in the absence of GABA was of minor extent. Although action of ganaxolone was not dependent on subunit composition of the GABA-A receptor in this study (it was exhibited across all three GABA-A receptor subtypes tested:  $\alpha 1\beta 2\gamma 2$ ,  $\alpha 2\beta 2\gamma 2$ , and  $\alpha 3\beta 2\gamma 2$ )<sup>25</sup>, numerous other studies have shown that delta subunit enhances sensitivity to neurosteroids including ganaxolone. Neurosteroids bind for two sites on alpha

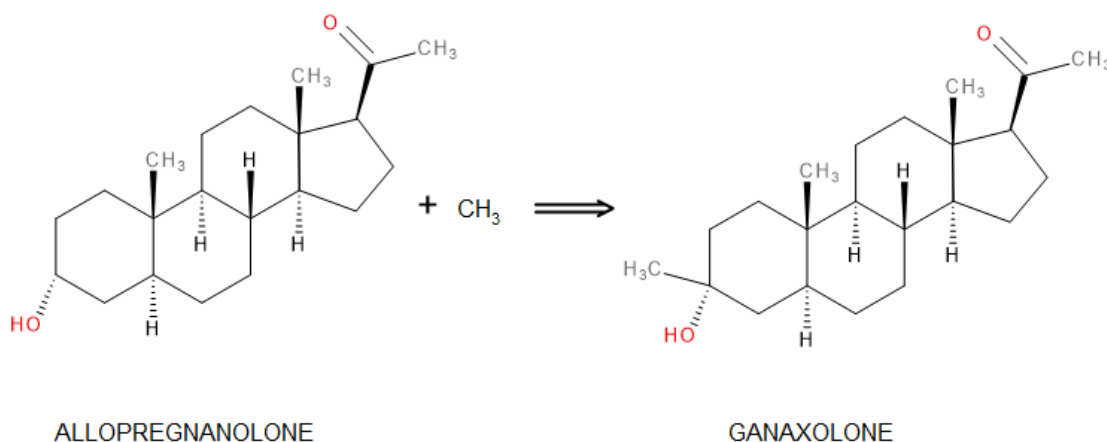


Fig. 1 – Chemical structures of ganaxolone and its precursor allopregnanolone.

subunit of GABA-A receptor: one is located in transmembrane domain and is essential for potentiation of responses to GABA, and another is placed on contact surface between alpha and beta subunits, causing activation of the receptor. However, the GABA-A receptor could be activated only after both sites have been occupied by a neurosteroid<sup>26</sup>.

#### Anti-epileptic effects

In a variety of animal models of epilepsy ganaxolone shows potent anti-seizure activity which is comparable to that of valproate: it prevents pentylenetetrazol (PTZ)-induced seizures in mice and rats, and bicuculline, tert-butylbicyclophosphorothionate (TBPS) or aminophylline-induced seizures in mice. In a rat cornea-kindled seizures model ganaxolone efficiently prevents seizures, and it significantly elevates seizure threshold in mice receiving pentylenetetrazol (PTZ)<sup>25, 27</sup>. Besides anticonvulsant activity against PTZ-induced clonic and tonic seizures in mice, ganaxolone shows anti-epileptogenic action against sensitization of the kindled mice to the convulsive and lethal effects of PTZ; its efficacy in this animal model was better than that of diazepam and valproate<sup>28</sup>. Both anticonvulsant and anti-epileptogenic effects of ganaxolone were recorded in cocaine-kindled seizures in male mice, too; this dual action gives important advantage to ganaxolone over conventional anticonvulsive drugs which mostly lack anti-epileptogenic action<sup>29</sup>. Ganaxolone was more potent than diazepam in exhibiting protection against cocaine-induced seizures in mice; when co-administered with diazepam, it acts synergistically to protect against both cocaine and pentylenetetrazol-induced seizures in mice. Although high doses of ganaxolone produce motoric impairment similar to that induced by diazepam, the same was not observed at lower doses of ganaxolone which produce anticonvulsant action<sup>27</sup>. Beneficial ratio was also observed between doses of ganaxolone that prevent prolongation of cortical epileptic after discharges in rats caused by low-frequency stimulation of the sensorimotor cortical area through epidural electrodes, and doses that compromise motor activity, suggesting acceptable safety profile of the drug<sup>30</sup>. In a model of primarily generalized seizures in developing rats, where seizures were induced by inhalation of flurothyl, ganaxolone showed dose-dependent anticonvulsant effect<sup>31</sup>.

Protective effect of ganaxolone against seizures was also shown in an animal model of infantile spasms. The rats were at first prenatally primed with betamethasone, and then on the day 15 after birth convulsions were initiated with NMDA. When given 30 min before the NMDA, ganaxolone delayed the onset of spasms and decreased the number of spasms or suppressed their occurrence<sup>32</sup>.

In a mice model of complex partial seizures induced by low-frequency (6 Hz), long-duration (3 s) electrical stimulation ganaxolone showed strong protective effect, comparable to that of clonazepam. Potency of ganaxolone in this model was similar to its potency in models of PTZ-induced seizures (ED<sub>50</sub> value = 6.3 mg/kg)<sup>33</sup>. The same protective effect with almost identical potency (ED<sub>50</sub> value = 6.6 mg/kg) ganaxolone exerted in fully amygdala-kindled female mice

(by means of the electrodes implanted into the right amygdala complex). The seizures were nearly completely prevented with the highest doses of ganaxolone, and its effect was comparable to protective effect of clonazepam. The potential advantage of ganaxolone over benzodiazepines lies in the absence of tolerance for protection against seizures, which is regularly observed in experiments with the latter drugs<sup>34, 35</sup>.

Ganaxolone showed a specific protective anticonvulsive effect in a rat model of catamenial epilepsy (a kind of epilepsy with the exacerbation of seizures immediately before, during or after menstruation), which is believed to be caused by perimenstrual decrease in brain levels of progesterone metabolite allopregnanolone<sup>36</sup>. Almost 70% of women in reproductive age with epilepsy experience increase in seizure frequency around menstruation. Female rats were maintained at high levels of progesterone, then subsequently deprived of allopregnanolone by administration of finasteride. Ganaxolone gave protection against PTZ-induced seizures in much lower doses (*ie*, with higher potency) than in non-deprived pregnant or non-pregnant animals. Similar phenomenon was not observed with diazepam or valproate, which indicated that ganaxolone could be specific and potent drug for treatment of catamenial epilepsy in humans. Greater efficacy of neurosteroids including ganaxolone compared to benzodiazepines in the treatment of catamenial epilepsy in animal models could be explained by temporary increase in expression of delta subunit of GABA-A receptor caused by progesterone. GABA-A receptors which contain a delta subunit are located mostly perisynaptically/extrasynaptically, and GABA is less efficacious at such receptors. While benzodiazepines require gamma-2 subunit to act on GABA-A receptor, neurosteroids positively modulate GABA-A receptors with all kinds of subunits, especially those with delta-subunits which are more sensitive to them. These temporary changes in the composition of GABA-A receptors during and around menstruation give to neurosteroids a unique opportunity to enhance inhibitory effect of GABA and prevent exacerbation of seizures during and around menstruation<sup>37</sup>.

On the other hand, ganaxolone was not only ineffective in animal models of absence seizures, but it showed seizure-potentiating activity. Pretreatment of rats with ganaxolone prolonged absence seizures caused by low-doses of PTZ or gamma-hydroxybutyric acid (GHB), and ganaxolone alone (> 20 mg/kg) caused occasional bilateral synchronous spike wave complexes in EEG<sup>38</sup>. When during *in vivo* experiments ganaxolone was focally micro-injected into WAG/Rij rats, genetically modified animals that suffer from absence-like epilepsy, with characteristic recordings of spike-wave complexes, it significantly increased frequency of spike-wave complexes when injected into thalamic but not somatosensory cortical nuclei<sup>39</sup> (Table 1).

#### Other central effects of ganaxolone

An anxiolytic-like effect of ganaxolone was observed on pentylenetetrazol-treated mice: ganaxolone administered 15 minutes before pentylenetetrazol prevented PTZ-induced

Table 1

Anti-epileptic effects of ganaxolone observed in pre-clinical studies	
Experimental model	Observed effects
PTZ - induced seizures in mice	Elevates seizure threshold <sup>27</sup>
Rat cornea - kindled seizures model	Prevents seizures <sup>25</sup>
PTZ - induced seizures in kindled mice	Prevent sensitization of the kindled mice to PTZ <sup>28</sup>
Cocaine - kindled seizures in male mice	Anticonvulsant and anti-epileptogenic actions <sup>29</sup>
Primarily generalized seizures in developing rats induced by flurothyl	Dose-dependent anticonvulsant effect <sup>31</sup>
Rat model of infantile spasms	Prevents convulsions induced by NMDA <sup>32</sup>
Mice model of complex partial seizures	Prevents the seizures <sup>33</sup>
Fully amygdala - kindled female mice	Prevents complex partial seizures <sup>34,35</sup>
Rat model of catamenial epilepsy	Prevent the seizures <sup>36</sup>
Absence seizures caused by low-doses of PTZ or gamma-hydroxybutyric acid	Ineffective <sup>38</sup>
WAG/Rij rats model of absence seizures	Increases frequency of spike-wave complexes <sup>39</sup>
Audiogenic seizures in Fmr1 knockout mice	Anti-convulsant effect <sup>40</sup>

PTZ – pentylenetetrazol; WAG/Rij – Wistar Albino Glaxo/Rij; NMDA – N-methyl-D-aspartate.

decrease in behaviors like sitting or lying without moving, lying with at least one of the back limbs clearly visible, the occurrence of small twitches of the body, the tail straightened backwards or pressed along a wall of the cage and sitting or lying with the nose turned to the corner of the cage. When compared with well-established anti-convulsants (phenobarbital, ethosuximide, clonazepam, diazepam and valproate), ganaxolone was more efficient in prevention of PTZ-induced behaviors than the majority of comparators except phenobarbital. The effect of ganaxolone is dose-dependent, and it occurs within the dose range that is not associated with motor toxicity like circling or uncoordinated walking <sup>41</sup>. Ganaxolone produced similar effects in mice treated by other pro-convulsive and anxiogenic drugs: it reversed locomotor depression caused by bicuculline, picrotoxin and yohimbine (it was the least potent against yohimbine) <sup>42</sup>. Interestingly, ganaxolone failed to decrease cocaine-induced hyperactivity in mice and motor stimulation caused by methamphetamine, dizocilpine, and phencyclidine, which suggests complex and regionally specific role of neurosteroids in control of locomotion <sup>43</sup>. Indeed, in a study on rat hippocampal CA1 pyramidal neurons and dentate granule cells using whole-cell patch-clamp recordings it was shown that the cells from the two hippocampal regions are differentially sensitive to neurosteroids in regard to enhancement of GABA-A receptor conductance due to both variations of the subunit composition and phosphorylation of the GABA-A receptor, and the differences in local steroid metabolism <sup>44</sup>.

A possible antidepressant action of ganaxolone was hypothesized after *in vivo* experiments on dorsal raphe nucleus serotonergic neurons in female rats, where ganaxolone and endogenous neurosteroid allopregnanolone strongly increased spontaneous firing activity. When co-administered with a serotonin-uptake inhibitor citalopram, ganaxolone prevented the reduction of firing activity usually caused by citalopram after 3 or more days of treatment. This observation sets rationale for further testing of augmenting properties of ganaxolone in regard to the antidepressant effect produced by selective serotonin reuptake inhibitors <sup>45</sup>. Interestingly,

fluoxetine and fluvoxamine have been shown to increase allopregnanolone levels at doses below those effective at serotonin transporters <sup>46</sup>.

Ganaxolone, as well as endogenous neurosteroids, has certain effect on regulation of ethanol consumption in experimental rats. When administered systemically to rats trained to self-administer ethanol, ganaxolone at first shortens the latency until the animals start licking ethanol, and then decreases overall ethanol consumption <sup>47-49</sup>. The same effect was achieved after stereotaxic infusion of ganaxolone to nucleus accumbens shell <sup>50</sup>, and it results from positive modulation of both synaptic and extra-synaptic GABA-A receptors. On the other hand, ganaxolone induces reinstatement of ethanol seeking behavior in mice that previously were trained to self-administer alcohol, and then extinguished <sup>40</sup>. These effects of ganaxolone should be taken into account if this drug is going to be used in patients with epilepsy and concomitant alcohol dependence.

A number of new areas where ganaxolone could offer therapeutic benefit were recently studied through animal models of fragile X syndrome, posttraumatic stress disorder, spinal analgesia, Niemann-Pick Type C disease and multiple sclerosis. In rodents (and humans) with the fragile X mental retardation gene (Fmr1) mutation the ensuing intellectual disability is accompanied with down-regulation of GABA transmission (decreased synthesis and increased catabolism of GABA, decreased number of GABA receptors). Ganaxolone effectively rescued audiogenic seizures in Fmr1 knockout mice through its positive modulation of GABA-A receptors <sup>51</sup>. In mice with mutation in the NPC1 gene and signs and symptoms which resemble Niemann-Pick Type C disease in humans activity of the neurosteroidogenic enzymes is decreased; administration of allopregnanolone or ganaxolone in such mice delay the onset and progression of neurological symptoms <sup>52</sup>. As the level of allprenanolone in cerebrospinal fluid is reduced in premenopausal women with post-traumatic stress disorder <sup>53</sup>, beneficial effect of ganaxolone on this disorder was supposed by researchers who tried it on socially isolated (SI) mice, which have allop-

regnanolone deficiency and post-traumatic stress-like behaviors. Brain areas of the SI mice which control emotions (frontal cortex, hippocampus and basolateral amygdala) have decreased allopregnanolone levels, and GABA-A receptors on neurons in these areas have distinct subunit composition, with the decreased presence of gamma 2, alpha 1 and alpha 2 subunits. Such GABA-A receptors are less sensitive to benzodiazepines, but retain sensitivity to neurosteroids, including ganaxolone. Unlike benzodiazepines, ganaxolone improved anxiety, aggression, and other posttraumatic stress disorder (PTSD) like behaviors in SI mice, without causing sedation or locomotor impairment<sup>54, 55</sup>.

In models of pain, peripheral nerve injury causes changes in K(+)/Cl(-) cotransporter isoform 2 (KCC2) expression on spinal neurons from the dorsal horn; these changes lead to accumulation of chloride ion within the neurons, decreasing the flux of that ion through GABA-A channels, preventing hyperpolarization of neuronal membrane and creating allodynia (hypersensitivity). Intrathecal administration of ganaxolone within the framework of tail flick assay produces significant analgesic effect<sup>56</sup>. Finally, it has been shown in human material from multiple sclerosis patients as well as in mice with induced experimental autoimmune encephalomyelitis (EAE) that neuroinflammation increases expression of GABA transporter type 2, which then decreases concentration of extracellular GABA. On the other hand, GABA and ganaxolone decrease expression of receptors for inflammatory mediators on surface of activated macrophages, improving behavior of the animals and reducing demyelination and injury of nerve fibers<sup>57</sup> (Table 2).

metabolite 16 $\alpha$ -hydroxyganaxolone. Only 20% of dose is eliminated through kidneys, and the rest is eliminated in feces<sup>10</sup>. The elimination half-life is approximately 10–30 h based on formulation and dose tested<sup>1, 58</sup>.

It has linear kinetics of elimination, and after repeated dosing 500 mg three times a day (*tid*) steady-state was achieved after 48 h (significant accumulation of ganaxolone was not observed in clinical trials)<sup>58</sup>. Maximal ganaxolone concentrations in the steady state range from 32 ng/mL (the dosing regimen of 50 mg *bid*) to 376 ng/mL (the dosing regimen of 500 mg *bid*) with early formulations of oral suspension<sup>59</sup>. The newer formulation, on oral capsule has maximum concentration (C<sub>max</sub>) of 239 ng/mL while maintaining area under the curve (AUC)<sup>58</sup>, which may have a positive impact on tolerability. Total clearance of ganaxolone was not affected by creatinine, urea or aminotransferases serum levels according to population pharmacokinetic analysis from a clinical trial<sup>60</sup>.

Ganaxolone neither induces nor inhibits activity of CYP3A4/5. However, strong inducers (*eg* carbamazepine) and inhibitors (*eg* ketoconazole) of CYP3A4/5 may increase and decrease clearance of ganaxolone, respectively<sup>61</sup>.

### Clinical efficacy

#### Phase I studies

Safety, tolerability, and pharmacokinetics of ganaxolone were reported from 7 phase I studies conducted on 87 healthy adult male and 9 healthy adult female volunteers<sup>59</sup>. The first

**Table 2**

**Other central effects of ganaxolone observed in pre-clinical studies**

Experimental model	Observed effects
Behaviors of pentylentetrazol-treated mice	Anxiolytic-like effect <sup>41</sup>
Locomotor depression of mice by bicuculline, picrotoxin and yohimbine	Reversal of the depression <sup>42</sup>
Cocaine-induced hyperactivity in mice	Ineffective <sup>43</sup>
Electrical activity of dorsal raphe nucleus serotonergic neurons in female rats	Increases spontaneous firing activity <sup>45</sup>
Rats trained to self-administer ethanol	Decreases overall ethanol consumption <sup>46–49</sup>
Mice model of Niemann-Pick Type C disease	Delays onset and progression of neurological symptoms <sup>51</sup>
Socially isolated (SI) mice model of post-traumatic stress disorder	Improves anxiety, aggression, and other PTSD-like behaviors <sup>53, 54</sup>
Tail flick assay in rats as model of nerve injury	Analgesic effect <sup>55</sup>
Autoimmune encephalomyelitis in mice	Decreases expression of receptors for inflammatory mediators and reduce demyelination <sup>56</sup>

#### PTSD – posttraumatic stress disorder.

##### Pharmacokinetics

After oral administration ganaxolone is rapidly and completely absorbed from the gastrointestinal tract: maximal plasma concentration after single oral dose is achieved after 1 to 4 hours<sup>21</sup>. Food increases bioavailability of ganaxolone which is formulated with submicron particulates in suspension or capsules, and area under the curve plasma concentration/time when ganaxolone is taken with food is 1.5 to 3 times greater than when it is taken on empty stomach<sup>21</sup>. Ganaxolone is 99% bound to plasma proteins and metabolized in the liver, by cytochromes CYP3A4/5, to an inactive

study was open-label, single dose study on 15 male volunteers, testing the following oral doses: 50, 150, 300, 450, and 600 mg. In the second study on 16 volunteers higher single doses (900, 1,200 and 1,500 mg) of ganaxolone were tested using a double-blind, placebo-controlled design. The first-single-then-multiple doses design was used in two studies, one being double-blind, placebo-controlled (12 volunteers, 50, 200, and 500 mg/day), and another open-label study (6-volunteers, 300 mg *bid*). The excretion pathways and pharmacokinetics were studied on 6 male volunteers receiving single oral 300 mg dose of [<sup>14</sup>C]-ganaxolone. The differences in ganaxolone pharmacokinetics

among sexes were tested in double-blind, single oral dose (300 or 900 mg), cross-over trial involving 8 males and 9 females. Finally, the seventh study assessed influence of food on absorption of ganaxolone, using different formulations (24 volunteers).

These studies show that ganaxolone in beta-cyclodextrin formulations has linear pharmacokinetics at the doses tested with rapid absorption and bi-exponential elimination, characterized by shorter initial, and a long terminal half-life (18–28 h and 37–70 h, respectively); reports with a newer formulation state a 7–10 h initial half-life<sup>58</sup>. The drug does not accumulate in the body after multiple doses. The safety of ganaxolone in these early studies was excellent. The only serious complaint of the volunteers was somnolence, but it was pronounced only after the highest doses (900, 1,200 and 1,500 mg), which could have been expected taking into account the mechanism of action of ganaxolone. No serious adverse events were recorded in the phase I studies.

### Phase II studies

The first clinical trial on adult patients was conducted on 52 adults with a history of complex partial seizures with or without secondary generalization, who have been withdrawn from antiepileptic drugs during diagnostic evaluation for surgical treatment of seizures<sup>61</sup>. The study was double blind, randomized, placebo controlled, and lasted for 8 days. The patients started to take orally either ganaxolone (500 mg *tid* on the day 1 and 625 mg *tid* on the days 2 to 8) or placebo only after plasma concentrations of previously used anti-convulsive drugs dropped to the levels less than 25% of lower limit of the therapeutic range. The primary measure of antiepileptic activity was duration of treatment before withdrawal from the trial. Fifty percent of the patients treated with ganaxolone completed the study, in comparison with 25% of the patients on placebo. However, the study was underpowered to prove a significant difference ( $p = 0.0795$ ) in the duration of treatment before withdrawal from the study due to one of the following: four seizures of any type except simple partial, three generalized tonic-clonic seizures in the patients who had such seizures before and one in the patients without such experience, and status epilepticus. Ganaxolone was well tolerated, with similar pattern and frequency of adverse events in the two groups. It was also observed that through plasma concentrations of ganaxolone did not correlate with anti-convulsive effect though most responders had trough levels above 20 ng/mL.

Ganaxolone was also tested in a small open-label study in children (7 months to 7 years old) with either refractory infantile spasms or continuing seizures after treatment for infantile spasms<sup>62</sup>. Ganaxolone was added to existing anti-convulsive therapy for 12 weeks, in oral doses that were gradually increased up to 36 mg/kg/d. The frequency of spasms was reduced for 50% or more in 33% of the patients, while another 33% of the patients had 25–50% less spasms. The patients did not experience any serious adverse event that could be attributed to ganaxolone.

The study with children 4 to 24 months of age suffering from infantile spasms and already treated unsuccessfully with 3 anti-epileptic drugs was designed as double-blind, placebo-controlled and randomized study. In total 56 patients participa-

ted in the study for two weeks, and then the study was extended for further 99 weeks in an open-label manner. The outcomes set before the study were not significantly different between the groups, but there were beneficial trends toward decrease in seizure clusters, better responder rates, global assessment of the patients and decrease of hypsarrhythmia<sup>58</sup>.

Another small, open-label study with ganaxolone was conducted on 15 children 5–15 years of age with partial or generalized seizures (myoclonic seizures and epileptic spasms, too) that were not controlled with 2 anti-epileptic drugs<sup>63</sup>. Ganaxolone was given as add-on oral therapy, and in the first 16 days the doses were titrated from 1 mg/kg *bid* up to the maximal tolerated dose or to 12 mg/kg, *tid*; after the titration period, the patients were receiving the last titrated dose for the next 8 weeks. Although only 8 patients completed this study, an intention-to-treat analysis showed that after 8 weeks 27% of the patients had more than 50% reduction in the seizures frequency (responders), and 13% of the patients between 25 and 50%. Three of the responders continued to take ganaxolone, from 4 months to 3.5 years, maintaining the same level of response. The most frequent adverse events were somnolence (9 patients), convulsions (3 patients) and agitation (2 patients).

There is only one published clinical study (*ie*, case report) on usage of ganaxolone in catamenial epilepsy. Oral ganaxolone (300 mg/day, *bid*) was given to two women from the 21st day of the menstrual cycle to the third day of the menstruation, and it decreased the number of seizures<sup>64</sup>.

A larger phase II clinical study, double-blind, placebo controlled and randomized, was conducted with 147 patients (100 women and 47 men, 18 to 69 years old) suffering from partial onset seizures with or without secondary generalization and refractory to previously used anti-convulsants. The dose of oral ganaxolone was 500 mg/8 hours. During the 10-weeks study ganaxolone decreased the mean seizure frequency *per week* for 18%, while placebo increased it for 2%. In the ganaxolone group there were 26% of the patients with more than 50% reduction in seizure frequency, and in the placebo group only 13%. The study was extended in a way that the patients from both placebo and ganaxolone group continued to take ganaxolone, and even 38 patients remained in the extension phase for more than 52 weeks. Even 24% of all the patients had a decrease in seizure frequency for more than 50% in the extension study. The only adverse events which were more frequent in the ganaxolone group were somnolence (13% vs 2%), dizziness (16% vs 8%) and fatigue (16% vs 8%)<sup>60,65</sup>.

### Phase III studies

There is only one phase III clinical trial with ganaxolone, which is currently ongoing, and its completion is planned for the year 2016. It is a multicenter, randomized, double-blind and placebo-controlled study investigating efficacy and safety of ganaxolone as add-on antiepileptic therapy for adult patients with partial-onset seizures that were not controlled with previous therapy. In Cohort 1, the study lasts for 9 weeks, and then the patients will enter open-label phase for 52 weeks. In the first 9 weeks, placebo or two doses of ganaxolone will be tested (1,200 mg/day and 1,800

mg/day), and in the open-label phase the dose of 1,800 mg/day open label ganaxolone will be used. A second cohort will receive 1,800 mg/day ganaxolone or placebo for 12 weeks and then enter the one-year open label phase. Primary outcome of the study is the percentage change in seizure frequency *per* 28 days relative to baseline in the second cohort, and the outcomes in the open-label phase are the change in seizure frequency, responder rate, percentage of seizure-free patients, change in percentage of seizure-free days and clinician's and patient's global impression of change<sup>66</sup>.

#### *Safety and tolerability*

When tried on usual battery of preclinical safety tests, ganaxolone caused only dose-dependent and reversible sedation of experimental animals, while the other findings were unremarkable. Ganaxolone did not show mutagenicity or reproductive toxicity when given to both male and female rats<sup>18</sup>.

Although human experiences with ganaxolone are still limited (about 1000 patients received this drug until now), from what was published so far it appears that its safety profile is rather beneficial. No serious adverse reactions that could be ascribed to ganaxolone with certainty were described in published reports, and the adverse reactions that were described were actually the extension of pharmacological action of ganaxolone, and depend on its dose. Since this drug enhances GABA-mediated inhibitory transmission in the central nervous system, it is not surprising that the most frequent adverse effect is somnolence, which affects less than 20% of patients taking ganaxolone. In small children, paradoxical irritability and agitation were noted in less than 10% of cases, what is analogous to similar paradoxical effects observed in the same population with classic sedatives as benzodiazepines. Only in a few cases these adverse effects were severe enough to require withdrawal from the therapy with ganaxolone. While these experiences seem promising, a larger picture about safety profile of ganaxolone will have to wait for completion of new clinical trials.

#### **Regulatory affairs**

Ganaxolone is currently tested in the third phase, multicentric clinical trial as add-on therapy for the treatment of partial onset seizures in adults with epilepsy. The results from phase II trials in adults with partial seizures were encouraging, and it is expected that after the third phase studies ganaxolone file will be submitted to the FDA for marketing approval. The Marinus Pharmaceuticals is also trying to obtain clinical evidence for efficacy and safety of ganaxolone in other indications: it is a conducting phase 2 study with ganaxolone as a treatment for behavior disturbances in Fragile X Syndrome and preparing initiation of another phase II study on children of female sex with PCDH19 gene mutation epilepsy, who have deficient GABA-ergic neurotransmission<sup>67,68</sup>.

Summary of the drug characteristics is given in Addendum.

#### **Conclusion**

Ganaxolone is an allopregnanolone analogue devoid of hormonal activity which allosterically potentiates inhibitory action of GABA on neurons carrying the GABA-A receptor on their membranes. It prevented seizures in animal models of partial seizures and generalized tonic-clonic seizures, while in the models of the absence of seizures it was either ineffective or prolonged spike wave discharge. Phase I clinical trials pointed to linear pharmacokinetics of ganaxolone, its high protein-binding and metabolism in the liver, and predominant excretion through feces. Ganaxolone was the most efficient as add-on therapy against partial seizures with or without secondary generalization in adult patients, and it is this indication with which the sponsor proceeded to the phase III clinical trials. Although tried in several studies on children suffering from infantile spasms, beneficial effects never reached a statistical significance. Due to its beneficial safety profile (somnolence being the most prominent adverse effect until now) and considerable efficiency in partial onset seizures, it is likely that ganaxolone will be approved as useful adjunct to existing anti-epileptic therapy which could not achieve satisfactory seizure control in adult patients with partial onset seizures.

#### **Acknowledgements**

This study was partially financed through the grant No 175007 given by the Serbian Ministry of Education, Science and Technological Development and through the grant No 404 given by the Ministry of Science, Montenegro.

#### **Addendum**

##### **Drug summary box**

<i>Drug name (generic) and route of administration:</i> ganaxolone, oral.
<i>Clinical trial phase (for indication under discussion):</i> phase III.
<i>Pharmacology description/mechanism of action:</i> Ganaxolone binds for unique recognition site on the GABA-A receptor which is different from the binding sites of GABA, benzodiazepines and barbiturates. After binding, ganaxolone potentiates inhibitory action of GABA on neurons which carry the GABA-A receptor.
<i>Indication:</i> drug-resistant, partial onset seizures in adult patients, with or without secondary generalization.
<i>Chemical structure:</i> 3alpha-hydroxy-3beta-methyl-5alpha-pregnan-20-one.
<i>Key trial(s):</i> <b>Phase I:</b> 7 phase I studies conducted on 87 healthy adult male and 9 healthy adult female volunteers <sup>59</sup> ; <b>Phase II:</b> Assessment of ganaxolone's anticonvulsant activity against complex partial seizures in adults with epilepsy using a randomized, double-blind, pre-surgical trial design, and another double-blind, placebo controlled and randomized study conducted with 147 patients suffering from partial onset seizures with or without secondary generalization and refractory to previously used anti-convulsants <sup>61</sup> . <b>Phase III:</b> An ongoing multicenter, randomized, double-blind and placebo-controlled study investigating efficacy and safety of ganaxolone as add-on antiepileptic therapy for adult patients with partial-onset seizures that were not controlled with previous therapy <sup>66</sup> .

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Received on June 26, 2015.

Revised on August 27, 2015.

Accepted on August 28, 2015.

Online First July, 2016.