Ganaxolone

Virinder Nohria* and Earl Giller†

*Department of Pediatrics, Emory University School of Medicine, Atlanta, Georgia; †Marinus Pharmaceuticals Inc., Branford, Connecticut; and ‡Yale University School of Medicine, New Haven, Connecticut 06510

Summary: Ganaxolone (3 α -hydroxy-3 β -methyl-5 α -pregnan-20-one) (GNX) is the 3 β -methylated synthetic analog of allopregnanolone; it belongs to a class of compounds referred to as neurosteroids. GNX is an allosteric modulator of GABA_A receptors acting through binding sites which are distinct from the benzodiazepine binding site. It has activity in a broad range of animal models of epilepsy. GNX has been shown to be well tolerated in adults and children. In early phase II studies, GNX

has been shown to have activity in adult patients with partial-onset seizures and epileptic children with history of infantile spasms. It is currently undergoing further development in infants with newly diagnosed infantile spasms, in women with catamenial epilepsy, and in adults with refractory partial-onset seizures. **Key Words:** Ganaxolone, neurosteroids, epilepsy, infantile spasms, catamenial epilepsy, partial-onset seizures.

INTRODUCTION AND HISTORY

Ganaxolone (GNX) is the 3 β -methylated synthetic analog of allopregnanolone (3 α ,5 α -P), a metabolite of progesterone; it belongs to a class of compounds referred to as neurosteroids. These compounds have been shown to be modulators of the GABAergic inhibitory system by acting through GABA_A receptors.¹ These neuroactive steroids lack classical hormonal activity, but have been shown to be allosteric modulators of GABA_A receptors; these actions are mediated through more than one binding site² (distinct from the benzodiazepine binding site). This class of compounds has all of the expected properties of GABA_A receptor modulators in animal models, including anticonvulsant activity and ability to cause sedation.³

The early development of this agent was conducted by Edward Monaghan and his colleagues while working at CoSensys, a biotechnology company based in California. CoSensys was acquired by Purdue Pharmaceuticals in 1998 and development of GNX was suspended. Marinus Pharmaceuticals, Inc., a privately held specialty pharmaceutical company, acquired the development and commercialization rights to GNX from Purdue on 14 September 2004 and is now actively developing this agent as an AED with unique pharmacological properties.

CHEMISTRY AND FORMULATION

Ganaxolone (3 α -hydroxy-3 β -methyl-5 α -pregnan-20-one) is a white, crystalline, nonhygroscopic powder with a melting point of 190–198°C and a molecular weight of 332.54 (FIG. 1).

GNX is insoluble in water, and most of the nonclinical and clinical development thus far has been conducted using either suspensions or tablets. Marinus Pharmaceuticals is currently developing novel liquid and solid formulations, to improve bioavailability and reduce the current pharmacokinetic fed/fasted effect.

PHARMACOLOGY

Ganaxolone inhibited binding of the GABA_A chloride-channel ligand *t*-[³⁵S]butylbicyclopophosphorothionate (TBPS) (IC₅₀ = 80 nmol/L) and enhanced binding of the benzodiazepine site ligand [³H]flunitrazepam (EC₅₀ = 125 nmol/L) and the GABA site ligand [³H]muscimol (EC₅₀ = 86 nmol/L). Electrophysiological recordings showed that nanomolar concentrations of GNX potentiated GABA-evoked chloride currents in *Xenopus* oocytes expressing human GABA_A receptor subunits α_1 , β_2 , γ_{2L} , but direct activation of chloride flux occurred only at micromolar concentrations.

GNX was effective against clonic convulsions induced by subcutaneous pentylenetetrazol administration in mice and rats (ED₅₀ values of 4.3 and 7.8 mg/kg i.p., respectively). GNX also exhibited potent anticonvulsant

Address correspondence and reprint requests to: Virinder Nohria, M.D., Ph.D., 111 Skyline View Road, Franklin, NC 28734. E-mail: vnohria@aol.com.

FIG. 1. Chemical structure of ganaxolone.

activity against seizures induced by subcutaneous bicuculline (ED_{50} of 4.6 mg/kg, i.p.), TBPS (ED_{50} of 11.7 mg/kg, i.p.), and intraperitoneal aminophylline (ED_{50} of 11.5 mg/kg, i.p.) in mice. Although GNX effectively blocked tonic seizures induced by maximal electroshock in mice (ED_{50} of 29.7 mg/kg, i.p.), it did so at doses that produced ataxia on the rotorod (mean toxic dose, TD_{50} of 33.4 mg/kg, i.p.). GNX was effective against fully kindled stage 5 seizures induced by corneal kindling in rats (ED_{50} of 4.5 mg/kg i.p.), producing these effects at doses well below those that resulted in ataxia (TD_{50} of 14.2 mg/kg i.p.).⁴ GNX had anticonvulsant activity against flurothyl-induced seizures in rats; this effect was particularly prominent in immature rats at postnatal age 9 and 15 days.⁵ GNX prevented clonic and behavioral seizures caused by low-frequency (6 Hz) long-duration (3 s) electrical stimulation in mice.⁶ PTZ-induced seizures in rats did not develop tolerance to chronic treatment with GNX.⁷

Nonclinical safety and toxicology

Nonclinical safety and toxicological evaluations have been conducted in standard *in vitro* and *in vivo* models as a part of an overall program to support the chronic use of GNX in humans. This has included assessment of mutagenicity, acute and chronic toxicity in mice, rats and dogs, and reproductive toxicity in rats. GNX has also been administered to neonatal rats for 6 weeks.

The single most striking effect following treatment in all nonclinical studies was dose-related but reversible sedation, an expected effect of the GABAergic actions of this compound. There was little evidence of target organ or systemic toxicity following acute or chronic administration of GNX in mice, rats or dogs. There was no effect on hematologic or chemistry laboratory parameters. High doses of GNX, 40 (mg/kg)/day, administered to female rats over 6 months caused increases in liver and kidney weights without significant histological changes. GNX was devoid of mutagenicity in the mouse lymphoma assay, in the *Salmonella*-*E. Coli*/mammalian-microsome reverse mutation assay, and in the rat *in vivo* micronucleus assay. Treatment with GNX in male rats did not

significantly affect sperm count, sperm motility, mating performance, or litter count. Similarly, treatment with GNX in female rats did not significantly affect fertility, fetal implantation, or viability and development of offspring. A 6-week treatment with oral GNX in neonatal rats did not cause any effect on attainment of postnatal developmental milestones or postweaning behavioral tests.

Absorption, distribution, metabolism, and excretion

Studies of absorption, distribution, metabolism, and excretion with GNX have been conducted in animals and humans, using a variety of formulations. In general, following oral administration in suspension, GNX is rapidly absorbed, with a time to reach peak concentration (T_{max}) of 1.5–2 h. Using radioactive GNX, it has been shown that GNX and its metabolites are widely distributed to all tissues, with tissue to plasma ratios of greater than five. GNX is highly bound to human plasma proteins. With either intravenous or oral administration, GNX is extensively metabolized, to metabolites as yet mostly unidentified. One of the major identified metabolites is 16-OH GNX, which is the result of metabolism by CYP 3A4. Most of the orally administered drug is excreted via the fecal route; approximately 20% is excreted via the renal route.

In healthy human volunteers, GNX, administered in doses ranging from 50 to 1500 mg, either as drug alone or formulated with pharmaceutical grade excipients, is rapidly absorbed from the gastrointestinal tract after oral administration. Pharmacokinetic analysis has shown that GNX administered either as single doses or after once-daily dosing for 14 days results in predictable and proportional plasma concentrations over the dose range of 50–500 mg/day.⁸ The T_{max} following oral administration in suspension is 1.2–2.5 h, with plasma half-life of approximately 20 h.

There were no gender differences in the pharmacokinetic profile of 300 or 900 mg of orally administered GNX.⁸ Effect of food on the pharmacokinetics of GNX was investigated in healthy male volunteers, who were administered neat micronized drug in capsules or in suspension as a single dose of 300 mg either in fasted or fed state (either after a high-fat or high-carbohydrate meal). Both high-fat and high-carbohydrate meals resulted in slight delay of absorption (T_{max} of 5.5 h). The peak plasma levels associated with the high-fat meal were three times higher than those with the high-carbohydrate meal, and nearly 12 times higher than those observed in fasted subjects.

In vitro drug–drug interaction studies have revealed no significant interactions with other AEDs. Although GNX does exhibit high protein binding, no valproic acid protein binding interactions were revealed. Formal *in vivo* drug–drug interaction studies have not yet been con-

ducted. Analyses of available pharmacokinetic data from the pediatric add-on studies revealed no, or limited, drug–drug interactions between GNX and concomitant AEDs. Subjects receiving known CYP 3A4 inducers (phenobarbital, carbamazepine, phenytoin) had similar response rates to those of subjects not receiving these concurrent AEDs.

Clinical efficacy and safety

Both Adult ($n = 631$, male and female, aged 18–69 years old) and pediatric subjects ($n = 79$, male and female, aged 6 months to 15 years) have participated in clinical studies thus far. In migraine, two parallel group, placebo-controlled, dose-ranging studies evaluated the safety, tolerability, and pharmacokinetics of GNX in adult patients with migraine. In these studies, single doses of up to 750 mg were well tolerated, with the most commonly observed adverse events being somnolence and diarrhea. Although no significant reduction in migraine symptoms was observed, the $T_{\max}$ was relatively slow for as-needed treatment of migraine.

A double-blind, randomized, placebo-controlled clinical trial was conducted to examine the safety, tolerability, and antiepileptic activity of GNX in adult patients after withdrawal from other AEDs during presurgical evaluations.⁹ Eligible patients ($n = 52$) were withdrawn from antiepileptic drugs and randomized to receive GNX ($n = 24$) or placebo ($n = 28$) for up to 8 days. GNX was administered at a dose of 1500 mg/d on day 1 and 1875 mg/d on days 2–8, in three divided doses. The primary measure of antiepileptic activity was duration of treatment before withdrawal from the trial. Kaplan–Meier curves depicted a clear separation between treatment groups, with 50% of the GNX-treated patients completing the entire study, compared with 25% of patients treated with placebo. Intent-to-treat survival analyses revealed a trend toward efficacy with GNX ($p = 0.0795$, log-rank test). The tolerability of GNX was similar to that of placebo, with adverse events being reported by 79% of patients in the GNX group and 68% of patients in the placebo group.

Twenty pediatric patients (aged 7 months to 7 years) with refractory infantile spasms or with continuing seizures after a prior history of infantile spasms participated in an open-label study.¹⁰ Concomitant AEDs were maintained throughout the study period. The dose of GNX was progressively increased to 36 (mg/kg)/day (or to the maximal tolerated dose) over a period of 4 weeks, then maintained for 8 weeks before tapering and discontinuation. Of the 20 subjects, 16 completed the study, 15 of whom had refractory infantile spasms at the time of study enrollment. Spasm frequency was reduced by at least 50% in 5 (33%) of these subjects, with an additional 33% experiencing some improvement (25–50% reduction in spasm frequency). Most

commonly observed adverse events were somnolence, diarrhea, nervousness, and vomiting.

In two additional open-label studies, 59 pediatric patients (aged 2–15 years) with refractory epilepsy were dosed for up to 17 weeks. In the first study, the responses of the 13 patients evaluated at maintenance week 4 after a 16-day inpatient dose titration period were considered substantial in 4 patients (1 of whom became seizure free), moderate in 3, and insufficient in 6. Of the 8 patients who were evaluated at maintenance week 8, the responses were considered substantial in 4 patients (1 of whom was a nonresponder at week 4), moderate in 2, and insufficient in 2.

In the second study, of similar design, 45 patients were enrolled. Of the 27 patients whose seizure frequency was available for both baseline and week 8, the responses were considered substantial in 12 patients (1 of whom stayed seizure free from maintenance week 4), moderate in 4, and insufficient in 11.

Of the 48 pediatric subjects eligible for open-label extension treatment for whom GNX was available, 28 continued on GNX therapy; 11 subjects discontinued within 1 year. Of the 17 subjects exposed to GNX for more than 1 year, 4 received treatment for more than 4 years (stopped when GNX was no longer available), 2 for more than 3 years, 1 for more than 2 years, and 11 for more than 1 year. Nine subjects also showed behavioral improvement.

The most commonly observed adverse events in children were agitation and somnolence.

CURRENT STATUS

Marinus Pharmaceuticals is currently reformulating GNX and plans to conduct further controlled studies in infants with newly diagnosed infantile spasms, in women with catamenial epilepsy, and in adults with refractory partial-onset seizures.

REFERENCES

1. Belelli D, Lambert JJ. Neurosteroids: endogenous regulators of the GABA_A receptor. *Nat Rev Neurosci* 2005;6:565–575.
2. Akk G, Bracamontes JR, Covey DF, Evers A, Dao T, Steinbach JH. Neuroactive steroids have multiple actions to potentiate GABA_A receptors. *J Physiol* 2004;558:59–74.
3. Rogawski MA, Reddy DS. Neurosteroids: endogenous modulators of seizure susceptibility. In: Rho JM, Sankar R, Cavazos JE, editors. *Epilepsy: scientific foundations of clinical practice*. New York: Marcel Dekker, 2004:319–355.
4. Carter RB, Wood PL, Wieland S, et al. Characterization of the anticonvulsant properties of ganaxolone (CCD 1042; 3 α -hydroxy-3 β -methyl-5 α -pregnan-20-one), a selective, high-affinity, steroid modulator of the γ -aminobutyric acid_A receptor. *J Pharmacol Exp Ther* 1997;280:1284–1295.
5. Liptakova S, Velisek L, Veliskova J, Moshe SL. Effect of ganaxolone on flurothyl seizures in developing rats. *Epilepsia* 2000;41:788–793.
6. Kaminski RM, Livingood MR, Rogawski MA. Allopregnanolone analogs that positively modulate GABA receptors protect against partial seizures induced by 6-Hz electrical stimulation in mice. *Epilepsia* 2004;45:864–867.

7. Reddy DS, Rogawski MA. Chronic treatment with the neuroactive steroid ganaxolone in the rat induces anticonvulsant tolerance to diazepam but not to itself. *J Pharmacol Exp Ther* 2000;295:1241–1248.
8. Monaghan EP, Navalta LA, Shum L, Ashbrook DW, Lee DA. Initial human experience with ganaxolone, a neuroactive steroid with antiepileptic activity. *Epilepsia* 1997;38:1026–1031.
9. Laxer K, Blum D, Abou-Khalil BW, et al. Assessment of ganaxolone's anticonvulsant activity using a randomized, double-blind, presurgical trial design. Ganaxolone Presurgical Study Group. *Epilepsia* 2000;41:1187–1194.
10. Kerrigan JF, Shields WD, Nelson TY, et al. Ganaxolone for treating intractable infantile spasms: a multicenter, open-label, add-on trial. *Epilepsy Res* 2000;42:133–139.