

Initial Human Experience with Ganaxolone, a Neuroactive Steroid with Antiepileptic Activity

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Summary: *Purpose:* Studies were conducted to establish the safety, tolerability, and pharmacokinetics of the antiepileptic drug (AED) ganaxolone. Ganaxolone belongs to a novel class of neuroactive steroids called epalons, which specifically modulate the γ -aminobutyric acid type A (GABA_A) receptor in the central nervous system (CNS). Chemically related to progesterone but devoid of any hormonal activity, the epalons have potent antiepileptic, anxiolytic, sedative, and hypnotic activities in animals.

Methods: Ninety-six healthy male and female volunteers received ganaxolone in a variety of formulations, doses, and dosing regimens. The pharmacokinetics of ganaxolone were systematically characterized, and adverse events associated with drug use were documented.

Results: Ganaxolone was well tolerated after single doses ($\leq 1,500$ mg) and after multiple doses (≤ 300 mg b.i.d. for 10 days). Steady-state plasma levels (trough) occurred after ~7

days of dosing, with mean steady-state plasma concentrations (C_{max}) in multiple dose studies of between 32 ng/ml (50-mg doses) and 376 ng/ml (500-mg doses). No serious or life-threatening adverse events attributed to the drug were observed. The majority of adverse events reported were mild (82%) to moderate (14%) and were limited to headache, dizziness, somnolence, gastrointestinal disturbances, and malaise.

Conclusions: Ganaxolone alone or formulated with pharmaceutical-grade excipients is rapidly absorbed from the gastrointestinal tract after oral administration in doses ranging from 50 to 1,500 mg. Pharmacokinetic analysis revealed a linear and proportional increase in the area under the curve (AUC) and C_{max} values with increasing dose within the expected therapeutic dose range. Safety and tolerability in the clinical program were unremarkable. **Key Words:** Ganaxolone—Neurosteroid—Antiepileptic—Epalon—GABA agonist.

Ganaxolone is a member of a novel class of neuroactive steroids called epalons, which allosterically modulate the γ -aminobutyric acid (GABA_A) receptor complex (GRC) in the brain through a unique recognition site. The compound was developed after endogenously occurring metabolites of progesterone, such as 3α -hydroxy- 5α -pregnan-20-one ($3\alpha,5\alpha$ -P), were observed to have prominent anticonvulsant effects in animal models of epilepsy (1–3).

Ganaxolone (3α -hydroxy- 3β -methyl- 5α -pregnan-20-one) is the 3β -methylated, synthetic analog of $3\alpha,5\alpha$ -P. $3\alpha,5\alpha$ -P is formed endogenously with its 5β -isomer when progesterone undergoes 5α -reduction of the 4-5 double bond to form $5\alpha(b)$ -pregnan-3,20-dione followed by the reduction of the 3-ketone to a 3α -hydroxyl. This

reduction of progesterone to $3\alpha,5\alpha$ -P results in the loss of progestational activity (i.e., loss of hormonal activity) (3,4).

Endogenously occurring epalons were initially characterized as potent allosteric modulators of the GABA_A receptor by using in vitro binding (5,6). In radioligand-binding studies, ganaxolone allosterically displaced [³⁵S]*t*-butylbicyclophosphorothionate (TBPS) binding to the chloride channel and enhanced [³H]flunitrazepam binding to the benzodiazepine receptor in the presence of GABA. Electrophysiological studies demonstrate the ability of ganaxolone to potentiate, at nanomolar concentrations, the GABA-evoked currents in *Xenopus* oocytes expressing the human GABA_A-receptor subunits α_1 , β_1 , and γ_{2L} . Direct activation of chloride currents by ganaxolone is minimal and is evident only at micromolar drug concentrations. Collectively these data provide strong evidence that ganaxolone is a positive allosteric modulator of the GRC and that it acts at a unique receptor site.

In vivo pharmacology studies demonstrated the ability

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of ganaxolone to provide protection against animal seizure models (7). In pentylenetetrazol (PTZ) seizure models, ganaxolone is effective both in mice and rats with median effective dose (ED₅₀) values <5 and 8 mg/kg, respectively. In other chemical convulsant models sensitive to GABAergic modulators, ganaxolone also is potent; these include bicuculline- and aminophylline-induced seizures in mice. In contrast, ganaxolone is inactive against tonic seizures induced by the glycine antagonist strychnine. This antiepileptic profile in chemically induced seizure models is similar to that of ethosuximide (ESM) and valproate (VPA), suggesting efficacy in humans against primary generalized seizures of the absence type (1,8,9). Further characterization of the antiepileptic profile of ganaxolone was obtained with studies of TBPS-induced tonic-clonic convulsions. These seizures are insensitive to GABAergic modulation by benzodiazepines (BZDs) and barbiturates (10) but are potentially blocked by epalons (4) and by ganaxolone in particular. Ganaxolone, like VPA, is highly effective against cornea-kindled seizures in rats, suggesting efficacy in treating partial seizures in humans. It is also active against maximal electroshock (MES) seizures in mice but at ataxic doses, indicating potential activity against generalized tonic-clonic seizures. Collectively, the data from the PTZ-, bicuculline-, aminophylline-, TBPS-, corneal kindling-, and MES-induced seizure models suggest that the antiepileptic potential of ganaxolone may extend to generalized absence, generalized tonic-clonic, and partial seizures (8,9).

The studies reported herein were conducted to establish the safety, tolerability, and pharmacokinetics of ganaxolone.

SUBJECTS AND METHODS

Subjects

Clinical trials evaluating ganaxolone were conducted in 87 healthy adult male volunteers by using both single-dose (50–1,500 mg) and multiple-dose (≤300 mg b.i.d. for 10 days or 500 mg once daily for 14 days) regimens and in nine healthy adult female volunteers by using a single-dose (300 and 900 mg) regimen. These studies were designed to evaluate the maximal tolerated dose, safety, tolerability, and pharmacokinetics after single and multiple doses.

Formulation

Details of the formulation and dosing regimen used in each study are listed in Table 1. Ganaxolone (C₂₂H₃₆O₂; molecular weight, 332.53) is highly insoluble in aqueous mediums, including acidic, basic, or neutral pHs. To enhance bioavailability, ganaxolone was administered in solution complexed with 2-hydroxypropyl-β-cyclodextrin (HPβCD; 20% for doses ≤450 mg; 40% for 600 mg) or complexed with β-cyclodextrin (β-CD) and suspended in Ora Plus/Ora Sweet to improve palatability. Both formulations were administered after a high-fat meal to assure good absorption.

Study designs

The initial clinical experience in humans involved both open-label and double-blind studies (Table 1). Initially an open-label study with an increasing dose schedule helped to establish the safety, tolerability, and pharmacokinetics of ganaxolone in healthy adult male volunteers (study 1). Groups of three male volunteers were treated at doses of 50, 150, 300, 450, and 600 mg each.

TABLE 1. Studies conducted in healthy volunteers

Study no.	Design	Subjects ^a	Dose/formulation of ganaxolone
1	Open-label, increasing single-dose evaluation	15 (3 per dose level)	50, 150, 300, 450, or 600 mg suspended in 2-hydroxypropyl-β-cyclodextrin (HPβCD)
2	Double-blind, placebo-controlled, increasing single-dose evaluation	16 Active, 8 placebo (4 active and 2 placebo per dose)	900, 1,200, or 1,500 mg (15 and 30 mg/ml) ganaxolone-βCD complex suspended in Ora Plus/Ora Sweet
3	Double-blind, placebo-controlled, single-dose followed by multiple dose (q.d.) for 14 days	12 Active, 5 placebo (4 active and 2 placebo per dose) ^b	Placebo, 50, 200, or 500 mg ganaxolone-βCD complex
4	Open-label, single morning dose followed by single evening dose followed by multiple dose (b.i.d.) for 10 days	6	300 mg ganaxolone-βCD complex
5	Open-label evaluation of absorption, metabolism, and excretion after a single dose	6	300 mg [¹⁴ C]-ganaxolone formulated with HPβCD administered in water
6	Double-blind, two-dose crossover comparison involving men and women	8 Male, 9 female	300 or 900 mg ganaxolone-βCD complex suspended in Ora Plus/Ora Sweet
7	Series of open-label comparisons of formulations, excipients, and effect of food on absorption	24	300 mg formulated with βCD, HPβCD, or sodium lauryl sulfate; 300 mg neat drug administered after 10-h fast, or high-carbohydrate or high-fat meal; 600 mg ganaxolone-βCD complex suspended in yogurt or Ora Plus/Ora Sweet

^a Men unless otherwise indicated.

^b Incomplete recruitment for 50-mg group: five of six entered.

A later study of ganaxolone in male volunteers was designed to investigate the safety, tolerability, and pharmacokinetic profile after oral administration of increasing doses (900, 1,200, and 1,500 mg) of ganaxolone- β CD complex (30 mg/ml in Ora Plus/Ora Sweet) in a randomized, double-blind, placebo-controlled design (study 2). Six volunteers (four active and two placebo) were randomized into each dose group.

To assess the tolerability and pharmacokinetics of ganaxolone after single and multiple oral doses, a double-blind, placebo-controlled study was conducted with healthy, male volunteers (study 3). This randomized trial evaluated single- and multiple-dose administrations at three levels of ganaxolone (50, 200, and 500 mg/day). Two groups of six subjects (four active and two placebo) and one group of five subjects (four active and one placebo) were randomized.

An additional open-label study evaluated the safety, tolerability, and pharmacokinetics of ganaxolone when administered in a single (morning vs. evening) or twice daily (300 mg b.i.d.) treatment regimen (study 4). Six healthy adult male volunteers were studied. Pharmacokinetic profiles were obtained after single oral doses administered at 8 a.m. and 8 p.m. before initiation of the twice daily dosing. The single-dose pharmacokinetic comparisons between morning and evening dosing were separated by 60 h to allow the plasma drug concentration to return to baseline.

An excretion balance and pharmacokinetic study (ADME) with [^{14} C]-ganaxolone was performed after administration of a single oral dose to healthy, male volunteers (study 5). The study was designed to determine the extent and rate of absorption, metabolism, and excretion of [^{14} C]-ganaxolone and to determine the metabolite profile after single-dose, oral administration of 300 mg ganaxolone as a suspension (HP β CD). Six subjects were dosed with 300 mg of ganaxolone (~50 mCi of radioactivity) in 20% HP β CD after ingestion of a high-fat breakfast.

An open-label, randomized, two-way crossover study investigated the safety, tolerability, and pharmacokinetics of ganaxolone in male and female volunteers following a single oral dose (300 or 900 mg suspended in Ora Plus/Ora Sweet; study 6).

Additional pilot studies were conducted to assess the absorption of ganaxolone in a variety of formulations and in fed and fasted states (study 7). The details of these studies are not discussed here, but the safety data from the 24 subjects involved in these studies is included in the adverse-event summary.

Analytic methods

Ganaxolone levels in plasma were determined by a validated gas-liquid chromatography (GC) plus electron-capture detection assay (Wisconsin Analytical Research

Services, Madison, WI, U.S.A.). Human plasma samples with internal standards were extracted in hexane and derivatized by using pentafluorobenzyl hydroxylamine. Ganaxolone was chromatographed by a Hewlett-Packard 5890, Series II GC system equipped with an electron-capture detector (Avondale, PA, U.S.A.). With a 1-ml sample volume, the method was validated over a range of 1.00–500 ng/ml. The limit of quantification was 1.00 ng/ml, with an accuracy of $\pm 6\%$ over the assay range and a precision of 3.9% at the low control (4 ng/ml) and 5.8% at the high control (200 ng/ml).

Pharmacokinetic analysis

Descriptive pharmacokinetic analysis was performed by model-independent methods (7) based on the plasma concentration-time data. $C_{\max}$ and $C_{\min}$ are the observed maximum and trough (multiple dose) concentrations, respectively. $T_{\max}$ is the time required to reach maximum concentration. The area under the plasma concentration-time curve ($AUC_{0-\infty}$) was calculated by the linear trapezoidal rule from time zero to the last sampling time, with extrapolation to time infinity by using the least-squares terminal slope. Half-life ($T_{1/2}$) was calculated by $0.693/\text{terminal slope}$. Results are presented as means and standard deviations.

RESULTS

The increasing-dose tolerance data (study 1) indicate that ganaxolone was safe and well tolerated when administered as single oral doses of 50, 150, 300, 450, and 600 mg to healthy men. Pharmacokinetic analysis of

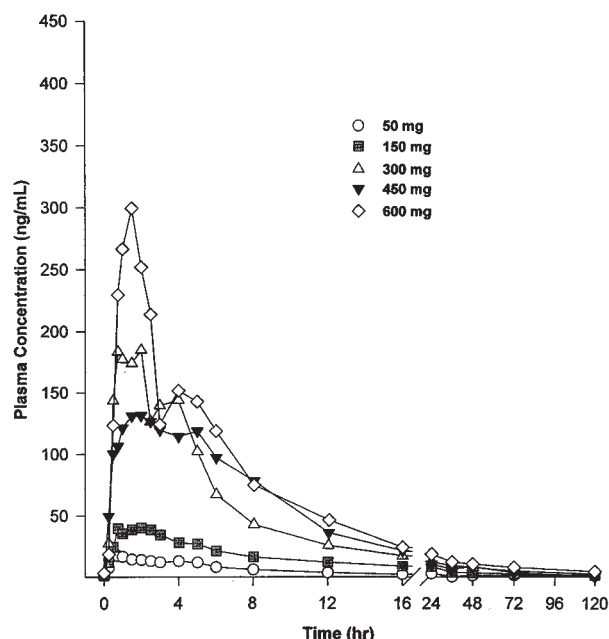


FIG. 1. Mean plasma ganaxolone concentrations at sampling times after a single dose of 50, 150, 300, 450, or 600 mg of ganaxolone.

plasma samples indicates linear and proportional pharmacokinetics characterized by rapid absorption and bi-exponential decay with a long terminal half-life (Fig. 1). The plasma concentrations increased rapidly, reaching peak levels within 1–3 h. Drug levels declined in a bi-exponential fashion with a terminal half-life of 37–70 h.

The plasma concentration–time profiles of higher doses of ganaxolone from a separate study (study 2) are depicted in Fig. 2. The plasma concentration–time profiles are similar in shape to those seen with lower doses, with peak levels obtained rapidly (T_{max} , 1.5–3 h) and then decreasing over the first 12 h. There is a general increase in ganaxolone plasma levels as dose increases, although the levels observed at the higher doses are somewhat lower than would have been predicted from the earlier study. There is an approximately proportional increase in AUC with increasing dose; AUC increases ~80% with a 67% dose increase (900–1,500 mg; Table 2). For the same dose escalation, peak levels increase only 30%.

The plasma concentration–time profiles after multiple doses of ganaxolone (study 3) were similar in shape to those observed after single doses. Peak levels were achieved rapidly and were followed by a sharp decrease in plasma levels over the first 12 h. The plasma levels increased nearly proportionally with increasing dose. There was limited accumulation, as evidenced by a lack of increasing trough levels during the multiple dose studies (data not shown). Plasma samples obtained during the study exhibited a linear increase in $AUC_{0-\infty}$, with increasing dose after single and multiple drug-administration periods (Table 3). Over the course of the 14-day administration period, C_{max} values (without correction for C_{min}) increased proportionally with dose from 32 ng/ml (50-mg group) to 106 and 376 ng/ml for the 200- and 500-mg groups, respectively. The C_{min} values also increased proportionally with dose from a value of 2.34 ng/ml (50-mg group) to 23.79 and 56.71 ng/ml for the 200- and 500-mg groups, respectively. The calculated terminal half-life of 40 to 65 h is generally consistent after both single and multiple dosing over the dosing range of 50–500 mg/day.

Results from the single- and multiple-dose studies are presented together to compare pharmacokinetic param-

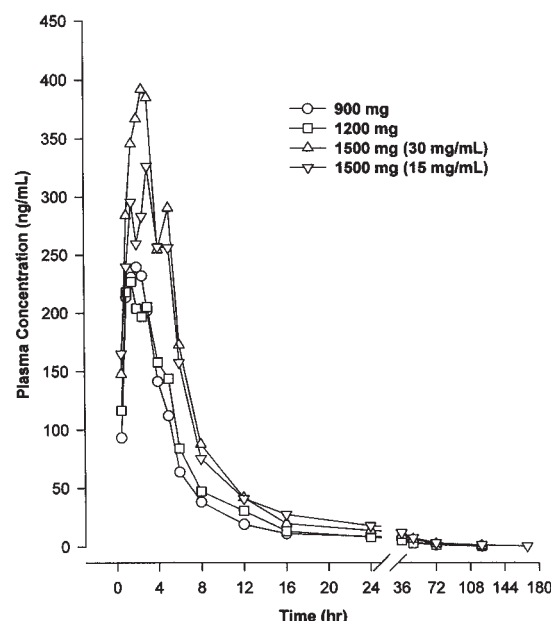


FIG. 2. Mean total radioactivity in plasma at sampling times after a single dose of 300 mg [^{14} C]-ganaxolone.

eters across studies and to assess both linearity and dose proportionality. Figure 3 presents both $AUC_{0-\infty}$ and C_{max} data as a function of dose and demonstrates that these parameters increase with increasing dose. When normalized by dose, the results demonstrate proportionality for both $AUC_{0-\infty}$ and C_{max} over the dose range (once or twice daily) from 50 to 600 mg. These findings confirm that there is no unexpected accumulation of drug.

Measurement of total [^{14} C] radioactivity in plasma (study 5) revealed that the absorption of orally administered [^{14} C]-ganaxolone was rapid (Fig. 4). Total [^{14} C] radioactivity demonstrated an exponential decline with an elimination half-life of ~230 h. This is 3–6 times the terminal half-life of nonlabeled ganaxolone, suggesting that much of this radioactivity was associated with metabolites.

The study with both male and female volunteers did not reveal a gender difference in the pharmacokinetic profile of ganaxolone at 300 or 900 mg (Fig. 5).

Adverse-event summary

Ganaxolone was safe and well tolerated after as much as 14 days of once-daily dosing or 10 days of repeated

TABLE 2. Summary of pharmacokinetic parameters in a single increasing-dose study (mean $\pm$ SD)

	Dose (mg)			
	900	1,200	1,500 (30 mg/ml)	1,500 (15 mg/ml)
$AUC_{0-\infty}$ (ng/h/ml)	1,564 $\pm$ 566	1,692 $\pm$ 177	2,871 $\pm$ 142	2,826 $\pm$ 316
C_{max} (ng/ml)	293.3 $\pm$ 63.8	247.2 $\pm$ 41.0	413.2 $\pm$ 137.9	353.8 $\pm$ 65.8
T_{max}^a (h)	1.2 (1.0–3.0)	1.8 (1.0–3.0)	2.5 (1.5–3.0)	2.0 (1.5–3.0)
$T_{1/2}$ (h)	19.2 $\pm$ 5.2	20.7 $\pm$ 3.3	18.1 $\pm$ 3.2	28.3 $\pm$ 4.0

^a Median (range).

TABLE 3. Pharmacokinetic profile of ganaxolone after single and multiple dosing (q.d. for 14 days; mean $\pm$ SD)

	50 mg	200 mg	500 mg
$AUC_{0-\infty}$ (ng/h/ml)			
Single dose	166.96 $\pm$ 24.07	977.93 $\pm$ 69.48	2,639.34 $\pm$ 294.87
Multiple dose	235.53 $\pm$ 4.20	1,093.13 $\pm$ 148.09	3,103.04 $\pm$ 497.85
C_{max} (ng/ml)			
Single dose	23.02 $\pm$ 2.79	75.58 $\pm$ 6.14	285.55 $\pm$ 36.90
Multiple dose	32.34 $\pm$ 3.33	106.23 $\pm$ 9.53	376.03 $\pm$ 39.22
$C_{min,ss}$ (ng/ml)			
Single dose	NA	NA	NA
Multiple dose	2.34 $\pm$ 0.72	23.79 $\pm$ 4.15	56.71 $\pm$ 8.15
T_{max} (h)			
Single dose	2.5 (2.0–3.0) ^a	2.75 (2.0–3.0)	2.25 (1.5–5.0)
Multiple dose	2.0 (2.0–2.5)	2.25 (1.5–3.0)	2.5 (2.0–5.0)
$T_{1/2}$ (h)			
Single dose	ND	39.1 $\pm$ 9.8	38.1 $\pm$ 6.8
Multiple dose	ND	65.1 $\pm$ 18.1	37.7 $\pm$ 9.6

For the single doses, $AUC_{0-\infty}$, whereas for the multiple-dose setting, $AUC_{0-\tau}$ refers to $AUC_{0-\tau}$; NA, not applicable; ND, not done.

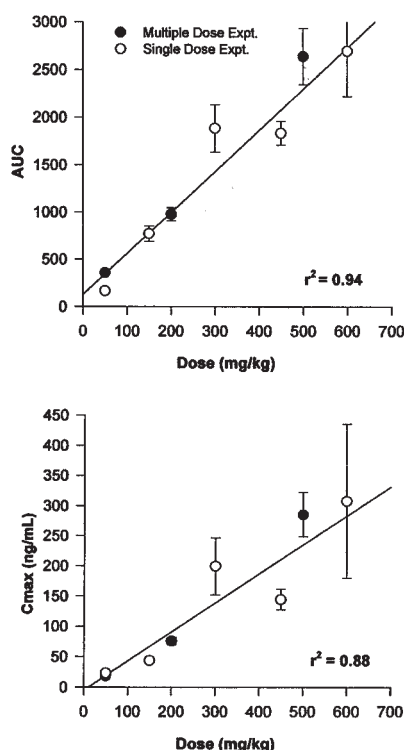
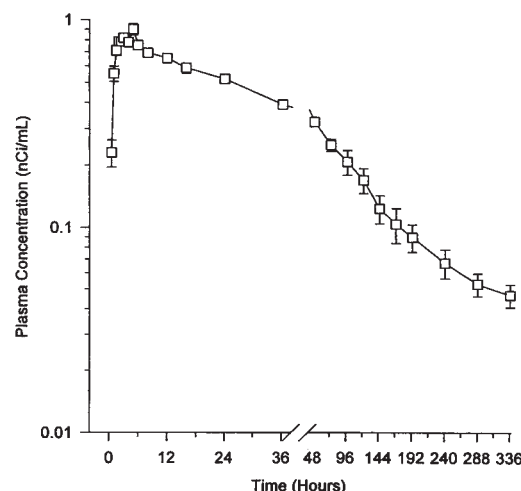
^a Median (range).

(b.i.d.) dosing in healthy adult male volunteers. Clinical evaluations of subjects, including electrocardiograms (ECGs), vital signs, neurologic and physical examinations, serum chemistries, and hematologic studies were unremarkable. Reports of adverse events included nausea, diarrhea, flatulence, abdominal pain, lightheadedness, tiredness, somnolence, and headache. All adverse events resolved spontaneously.

The severity of the complaints attributed to ganaxolone in all studies ranged from mild in 82% to severe in 4% of cases. Of the 14 severe complaints, there were 12 reports of somnolence and one report each of dizziness and unsteady gait. Of the severe complaints, >70% were associated with the 900- and 1,200-mg doses. Profiles of reported adverse events in the healthy volunteers have been consistent with the predicted pharmacology of the drug [i.e., central nervous system (CNS) effects including sedation, dizziness, and ataxia]. No serious or life-threatening episodes were observed in any of the healthy volunteer studies. All adverse events resolved without intervention.

DISCUSSION

Human trials have been initiated, and 96 healthy volunteers have been treated with ganaxolone. The dose has

**FIG. 3.** Mean plasma ganaxolone concentrations at sampling times after a single dose of 900, 1,200, or 1,500 mg of ganaxolone.**FIG. 4.** Comparison of mean area under the plasma concentration–time curve (AUC) and maximum concentration (C_{max}) after oral treatment of ganaxolone in single- and multiple-dose studies.

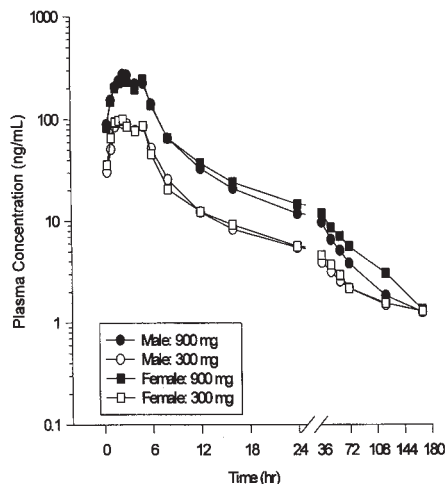


FIG. 5. Mean plasma ganaxolone concentrations at sampling times after single doses of 300 and 900 mg of ganaxolone in men and women.

been systematically escalated, based on evaluation of safety and tolerability data obtained. At present, the maximum dose administered has been 1,500 mg/subject/day; this was well tolerated, with no serious or life-threatening adverse events. The studies performed consistently demonstrate a benign safety profile, with the predominantly mild adverse events an extension of the pharmacologic activity of the drug.

These studies also demonstrate that ganaxolone administered as drug alone or formulated with pharmaceutical grade excipients, in doses ranging from 50 to 1,500 mg, is rapidly absorbed from the gastrointestinal tract after oral administration. Pharmacokinetic analysis demonstrated that ganaxolone 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/daily. Evaluation of safety parameters was unremarkable, with ganaxolone having no apparent effect on vital signs or ECGs. The adverse events reported include dizziness, unsteady gait, impaired coordination and concentration, tiredness, somnolence, and malaise. A comparison of predose and postdose vital signs and laboratory evaluations showed no changes of clinical significance.

In summary, ganaxolone has been tolerated by all

healthy subjects tested and appears to be safe after single doses of $\leq 1,500$ mg and after multiple doses (≤ 500 mg once daily for 14 days or 300 mg b.i.d. for 10 days). Based on these positive safety data and the putative antiepileptic activity observed in preclinical work, initiation of patient trials is warranted.

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REFERENCES

1. Selye H. The antagonism between anesthetic steroid hormones and pentamethylenetetrazol (Metrazol). *J Lab Clin Med* 1942;27:1051–3.
2. Figdor SK, Kodet MJ, Bloom BM, Agnello EJ, P'an SY, Laubach GD. Central activity and structure in a series of water-soluble steroids. *J Pharmacol Exp Ther* 1957;119:299–309.
3. Craig CR. Antiepileptic activity of steroids: separability of antiepileptic from hormonal effects. *J Pharmacol Exp Ther* 1966;153:337–43.
4. Belelli D, Bolger MB, Gee KW. Antiepileptic profile of the progesterone metabolite 5 α -pregnan-3 α -ol-20-one. *Eur J Pharmacol* 1989;166:325–9.
5. Gee KW, Bolger MB, Brinton RE, Coirini H, McEwen BS. Steroid modulation of the chloride ionophore in rat brain: structure-activity requirements, regional dependence and mechanism of action. *J Pharmacol Exp Ther* 1988;246:803–12.
6. Gee KW, McCauley LD, Lan NC. A putative receptor for neurosteroids on the GABA_A receptor complex: the pharmacological and therapeutic potential of epalons. *Crit Rev Neurobiol* 1995;9:207–27.
7. 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 GABA_A receptor. *J Pharmacol Exp Ther* 1997;280:1284–95.
8. Swinyard EA, Woodhead JH. Experimental detection, quantification, and evaluation of anticonvulsants. In: Woodbury DM, Penry JK, Schmidt RP, eds. *Antiepileptic drugs*. New York: Raven Press, 1982:111–26.
9. White HS. New mechanisms of antiepileptic drugs. In: Porter R, Chadwick D, eds. *Epilepsies II*. Boston: Butterworth-Heinemann, 1997:1–30.
10. Holland KD, McKeon AC, Canney DJ, Covey DF, Ferrendelli JA. Relative antiepileptic effects of GABA mimetic and GABA modulatory agents. *Epilepsia* 1992;33:981–6.