CNS Drug Reviews
Vol. 12, No. 2, pp. 123–134

Preclinical and Clinical Pharmacology of DOV 216,303, a "Triple" Reuptake Inhibitor

[1]Phil Skolnick, [1]Philip Krieter, [1]Joseph Tizzano, [1]Anthony Basile, [2]Piotr Popik, [1]Pal Czobor, and [1]Arnold Lippa

[1]DOV Pharmaceutical, Inc, Hackensack, NJ 07601; USA and [2]Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland

Keywords: Antidepressants — Depression — DOV 216,303 — Neurotransmitter uptake inhibitors.

ABSTRACT

DOV 216,303 [(±)-1-(3,4-dichlorophenyl)-3-azabicyclo–[3.1.0]hexane hydrochloride] is the prototype of a class of compounds referred to as "triple" reuptake inhibitors. Such compounds inhibit the reuptake of norepinephrine (NE), serotonin (5-HT), and dopamine (DA), the three neurotransmitters most closely linked to major depressive disorder. DOV 216,303 inhibits [^{3}H]NE, [^{3}H]5-HT, and [^{3}H]DA uptake to the corresponding human recombinant transporters (expressed in HEK 293 cells) with IC_{50} values of ~20, 14, and 78 nM, respectively. DOV 216,303 is active in tests predictive of antidepressant activity including the mouse forced swim test and reversal of tetrabenazine-induced ptosis and locomotor depression. The pharmacodynamic, pharmacokinetic, and toxicological profile of DOV 216,303 in animals prompted us to initiate clinical studies. In both single and multiple dose studies using normal volunteers, DOV 216,303 was safe and well-tolerated. Furthermore, both C_{max} and AUC values were dose-proportional between 5–150 mg. The plasma concentrations of DOV 216,303 at doses >10 mg were in excess of the IC_{50} values for inhibition of biogenic amine reuptake. In a Phase II study designed to explore the safety and tolerability of DOV 216,303 in depressed individuals, patients received either 100 mg DOV 216,303 (50 mg b.i.d.) or 40 mg citalopram (20 mg, b.i.d.) for two weeks. A placebo arm was not employed in this study because several institutional review boards required administration of an active control to severely depressed individuals. Time dependent reductions in HAM-D scores (the primary outcome measure) were observed in both the DOV 216,303 and citalopram groups compared to baseline scores ($p < 0.0001$). The side effect profile was not remarkably different between treatment arms. These findings provide preliminary evidence of a clinically meaningful

Address correspondence and reprint requests to: Dr. Phil Skolnick, DOV Pharmaceutical, Inc., 150 Pierce Street, Somerset, NJ 08873, USA; Tel.: +1 (732) 907-3603; E-mail: pskolnick@dovpharm.com

antidepressant action with a molecule capable of inhibiting the three transmitters most closely linked to major depressive disorder.

INTRODUCTION

The rationale for the design and development of "triple" reuptake inhibitors (molecules inhibiting norepinephrine, dopamine and serotonin reuptake) as antidepressants is firmly grounded in both the clinical and preclinical literature (21,22). Central to the hypothesis that a superior antidepressant effect can be achieved by the addition of a "dopaminergic" component to a norepinephrine and serotonin reuptake inhibitor is the body of evidence that links activation of mesocorticolimbic dopaminergic circuitry to both rewarding events and incentive-driven, goal-oriented behaviors (7,28). Thus, anhedonia, defined as an inability to experience pleasure and diminished interest in all (or most) activities, is central to a diagnosis of major depressive disorder. In both humans and animal models [e.g., the chronic mild stress model (26,27)], anhedonia is associated with deficits in dopaminergic transmission, principally within mesocorticolimbic circuitry (3,21). However, beyond this central role of dopamine in orchestrating goal-directed behaviors and reward-related learning (that are blunted in depressed individuals), there is clinical evidence to indicate that increasing dopaminergic transmission in individuals receiving "standard" antidepressants that inhibit the uptake of serotonin and/or norepinephrine will produce an enhanced therapeutic response. While a full description of these studies is beyond the scope of this review (21,29), it has been demonstrated that co-administration of dopaminergic agents improves depressed mood in patients, including individuals either resistant to, or exhibiting only a partial response to serotonin and/or norepinephrine reuptake inhibitors (4). For example, addition of bupropion (a dopamine reuptake inhibitor), most often to SSRIs (e.g., paroxetine or fluoxetine), produced greater symptomatic improvement than when either drug was used alone (2,8,16,29). Consistent with these reports, Koyama and coworkers (5,6) co-administered dopamine agonists such as bromocriptine and pergolide in open trials to patients resistant to (but concurrently receiving) traditional antidepressants. A clinical improvement was observed in a significant proportion of patients following addition of these dopamine agonists. Using retrospective case review, Sporn et al. (24), reported that the adjunctive use of a D_3 receptor preferring agonist (pramipexole) produced improvement (classified as "moderate to marked") in the CGI-I scale in 40 and 50% of patients with unipolar and bipolar depression, respectively. *In toto*, such studies indicate that increasing dopaminergic tone, either by inhibiting the dopamine transporter or direct stimulation of dopamine receptors, increases the therapeutic response to conventional (single and dual reuptake inhibitors) antidepressants.

The hypothesis that a triple reuptake inhibitor will act more rapidly than the several (usually ≥3) weeks of treatment generally required for conventional antidepressants is based on evidence that a selective sensitization of mesolimbic dopamine receptors is produced by chronic antidepressant treatments (3,21). This observation is among the most consistently reproduced in the preclinical literature following chronic antidepressant treatments. This sensitization can be observed at the behavioral, cellular, and molecular levels, and can be elicited by chronic treatment with structurally diverse antidepressants as well as electroconvulsive shock or REM-sleep deprivation (9,21). Because of the central role

played by mesolimbic dopaminergic neurons in the control of motivation and reward-related behaviors that are frequently blunted in depression (15), the several weeks of antidepressant treatment required to produce this selective perturbation of mesolimbic dopaminergic transmission in animals may model the therapeutic lag in the clinic. Thus, immediate increases in synaptic dopamine levels (via inhibition of dopamine reuptake) may result in a more rapid relief of symptoms associated with anhedonia than produced by drugs blocking norepinephrine and/or serotonin reuptake.

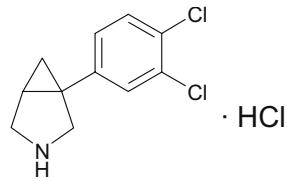

Fig. 1. Structure of DOV 216,303 [(±)-1-(3,4-dichlorophenyl)-3-azabicyclo-[3.1.0]hexane hydrochloride].

Given that there are structurally diverse classes of dual (norepinephrine and serotonin) reuptake inhibitors (Table 1) and that the dopamine, serotonin, and norepinephrine transporters belong to the same twelve transmembrane transporter gene family (18), the synthesis of a triple reuptake inhibitor would appear an almost trivial undertaking. However, the design of molecules active at all three transporters and orally bioavailable, safe, and well-tolerated has been anything but straightforward. In this review, we describe the preclinical and clinical pharmacology of one such molecule, the azabicyclohexane, DOV 216,303 (Fig. 1).

PRECLINICAL STUDIES

The following section presents an overview of the preclinical data most germane to the selection of DOV 216,303 as a clinical candidate. DOV 216,303 inhibits the uptake of [^{3}H]norepinephrine and [^{3}H]serotonin with about equal potency in HEK 293 cells expressing recombinant human NET and SERT, and is ~4-fold less potent as an inhibitor of [^{3}H]dopamine uptake (Table 1). The potency of DOV 216,303 to inhibit norepinephrine and serotonin uptake is well within the range of dual uptake inhibitors such as duloxetine, venlafaxine, and milnacipran (Table 1). Using radioligand binding as a measure of affinity at these transporters, the profile of DOV 216,303 becomes ligand dependent. For example, using [^{125}I]RTI-55 (3β-(4-iodophenyl)tropane-2β-carboxylic acid methyl ester) as the ra-

TABLE 1. Inhibition of [^{3}H]neurotransmitter uptake in recombinant human transporters by DOV 216,303: comparison with other antidepressants

Drug	5-HT	NE	DA
DOV 216,303[1]	14	20	78
Desmethylimipramine[1]	64	4	>10,000
Duloxetine[2]	3.7	20	439
Venlafaxine[2]	145	1420	3070
Milnacipran[2]	151	68	>10,000

Values are expressed in nM; [1] as IC_{50} or [2] K_i.
Data are taken from refs. 22 and 25.

dioligand (measured in HEK cells expressing the human forms of SERT, NET, and DAT, respectively), the potency profile of DOV 216,303 is ~1:2:1, but is ~2:1:1 using radiolabelled paroxetine, nisoxetine, and mazindol, respectively. DOV 216,303 is a racemate, and its enantiomers DOV 21,947 [(+)-1-(3,4-dichlorophenyl)-3-azabicyclo-[3.1.0]hexane hydrochloride] and DOV 102,677 [(–)-1-(3,4-dichlorophenyl)-3-azabicyclo-[3.1.0]hexane hydrochloride]) have been resolved and characterized (17,23). Receptor theory predicts the activity of a racemic mixture to reside in one isomer (i.e., the principle of stereoselectivity). While the predicted stereoselectivity is observed at the norepinephrine and serotonin transporters (based on radioligand binding studies, DOV 21,947 is approximately twice as potent DOV 216,303), there is not a significant difference in potency among the racemate and its enantiomers at the dopamine transporter. This unexpected (particularly in view of the structural homology among the norepinephrine, serotonin, and dopamine transporters) lack of stereoselectivity at the dopamine transporter has been confirmed using both uptake and radioligand binding (17,22,23), and results in three drug candidates with different "potency ratios" at these three transport proteins. While the ideal "potency ratio" of an antidepressant agent is unknown, in Phase I studies, the plasma concentrations of DOV 216,303 (see next section) are sufficiently high (based on these *in vitro* studies) to inhibit the reuptake of all three amines at doses that are both safe and well-tolerated (1).

DOV 216,303 reversed both the motor depression and ptosis induced by the amine depleting agent, tetrabenazine. In this "classical" screen for antidepressant agents, DOV 216,303 proved to be orally active and about equipotent in both measures, while fluoxetine, an SSRI, was approximately 3-fold more potent in inhibiting tetrabenazine-induced ptosis than motor activity (Table 2). DOV 216,303 also exhibited antidepressant-like actions in the murine version of the forced swim test (Fig. 2, top panel), with an MED of 10 mg/kg (22); when administered at 20 mg/kg, the duration of action was ≥3 h (Fig. 2, bottom panel). These *in vivo* and *in vitro* studies indicate that DOV 216,303 elevates extracellular levels of norepinephrine, serotonin, and dopamine. In microdialysis studies performed in rats with DOV 21,947 and DOV 102,677 (17 and unpublished data), the enantiomers of DOV 216,303 produced robust increases in mesocorticolimbic levels of all three biogenic amines at doses ≤20 mg/kg.

In a parallel set of studies, DOV 216,303 did not significantly increase motor activity in rats at doses of up to 50 mg/kg (Fig. 3, left panel), while in mice, modest but statistically significant increases in motor activity were apparent at doses ≥12.5 mg/kg (Fig. 3, right panel). While stimulants can yield false positives in the forced swim test, increases in motor activity are modest at doses of DOV 216,303 that produce meaningful reductions in

TABLE 2. Inhibition of tetrabenzine-induced motor depression and ptosis in mice

Treatment	Motor Depression (MED)	Ptosis (ED_{50})
DOV 216,303	1.6	2.2 (1.2–3.7)
Imipramine	3.1	0.8 (0.6–1.2)
Fluoxetine	25	7.2 (1.8–18.2)

Adult, male Swiss albino mice (10 per group) were administered drugs orally and 30 min later injected (i.p.) with tetrabenazine methane sulfonate (39 mg/kg). Inhibition of tetrabenazine-induced motor depression and ptosis was examined 30 min later. MED, minimum effective dose (mg/kg) required to inhibit motor depression. Values in parentheses represent the 95% confidence interval.

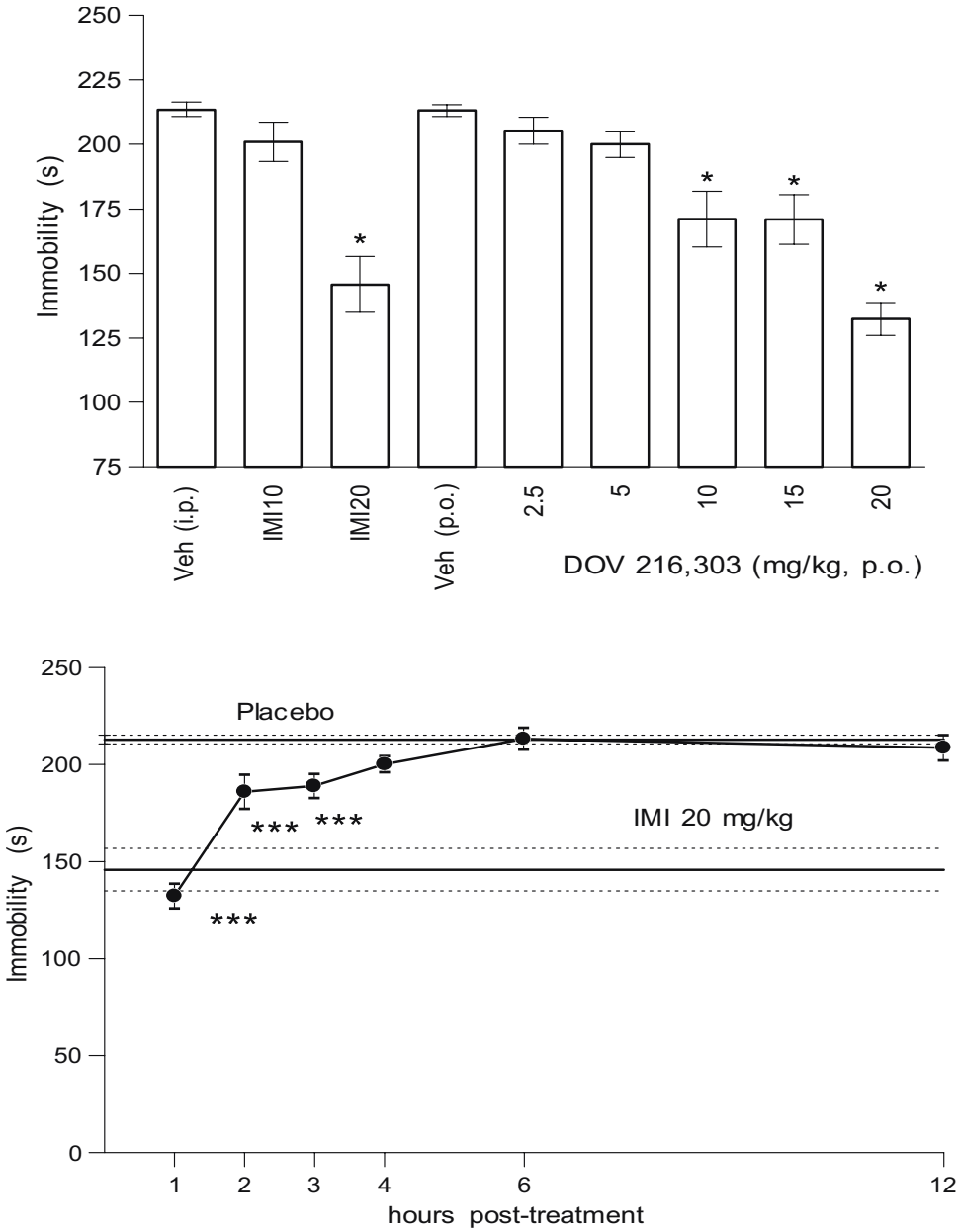

Fig. 2. Effects of DOV 216,303 in the forced swim test. Upper panel: dose response relationship; lower panel: duration of action. Albino Swiss mice were used in this study. For the dose response study, either DOV 216,303 or saline was administered orally 60 min prior to testing. Imipramine, administered intraperitoneally, served as the positive control. * $p < 0.001$, ANOVA followed by Dunnett's multiple comparison test. For the duration of action study, 20 mg/kg of DOV 216,303 was used. The immobility values for the control group are indicated by "placebo." The reduction in immobility produced by imipramine (20 mg/kg, i.p.) 60 min post-injection is included for illustrative purposes; these data were not included in the statistical analyses for duration of action. *** $p < 0.001$, ANOVA followed by Dunnett's multiple comparison test. The time spent immobile was measured for the last four min of a six min test as described. Values represent $X \pm$ S.E.M. of ≥6 mice per group. The top panel is reprinted from ref. 22.

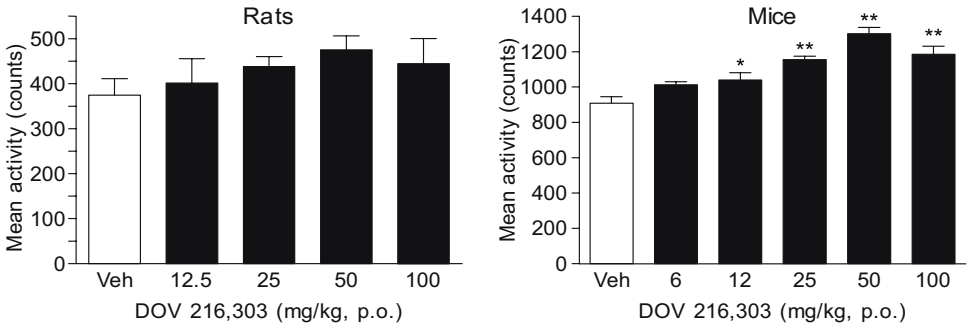

Fig. 3. Effects of DOV 216,303 on locomotor activity in rats (12.5–100 mg/kg) and mice (6.25–100 mg/kg). DOV 216,303 was administered orally and 30 (rats) or 60 (mice) min later, motor activity was monitored for 5 min. Values (counts measured in an actophotometer) represent the $X \pm$ S.E.M. of 10 rats and 6–10 mice/dose group, respectively. * $p < 0.05$; ** $p < 0.01$ compared to vehicle treated mice, ANOVA followed by Bonferonni's multiple comparison test.

immobility in the forced swim test. Moreover, unlike classical stimulants (e.g., amphetamine, cocaine) neither DOV 216,303 nor its enantiomers produce stereotyped behaviors in either rats or mice at any dose tested (unpublished observations).

This pharmacological profile of DOV 216,303 coupled with its oral bioavailability prompted additional pharmacokinetic and toxicological profiling. The oral bioavailability of DOV 216,303 was confirmed in rats and dogs. Furthermore, plasma concentrations were dose-proportional following single oral doses in both species, and no significant accumulation in plasma was observed following two weeks of oral administration. Toxicology dose range-finding studies indicated that DOV 216,303 was well-tolerated in rats and dogs at doses of <100 mg/kg. An acute, intravenous toxicity study of DOV 216,303 in the rat resulted in no notable changes, with the exception of injection site irritation at 10 mg/kg. Acute and multiple-dose oral (gavage or capsule) range-finding studies in rats and dogs indicated that doses at or above 100 mg/kg resulted in dose-related clinical signs attributable to the pharmacology of DOV 216,303. Dose-related increases in liver weights occurred in rats and dogs at doses at or above 30 and 25 mg/kg/day, respectively. Emesis was observed in dogs given oral doses at or above 10 mg/kg. No evidence for genotoxicity was found in two *in vitro* assays. These preclinical findings prompted the first-in-human studies with DOV 216,303.

CLINICAL STUDIES

Safety, Tolerability, and Pharmacokinetic Profile in Normal Volunteers

A complete description of these studies has been published (1). Both the single and multiple dose studies were conducted at the Parexel-CEMAF Clinic (Poitiers, France). The study protocols were approved by an independent institutional review board, and all volunteers provided written, informed consent. Subjects were healthy, male volunteers (18–35 years old) who were within 10% of ideal body weight.

The single dose study was a parallel arm trial examining six doses of DOV 216,303 (ranging from 5 to 150 mg) in ascending dose (7 subjects/arm) and placebo (3 subjects/arm) groups. After an overnight (~10 h) fast, subjects received DOV 216,303 or placebo (as identically appearing capsules) with 240 mL of water between 8–9 a.m. No food was permitted for 4 h following drug administration. Blood was sampled from 0.5 to 24 h after treatment. Subjects were monitored for adverse experiences throughout the day and for at least 24 h after treatment. At single doses DOV 216,303 produced no drug-related effects on vital signs. One adverse event was reported at doses between 5 and 100 mg that was judged as probably not related to study medication. In the 150 mg arm of the study, four adverse gastrointestinal effects (nausea and vomiting, meteorism) were reported. Plasma levels of DOV 216,303 appeared dose-proportional (Fig. 4, top panel); highly significant linear relationships were obtained between C_{max}, AUC, and the dose of medication. The mean elimination half life of DOV 216,303, 3.3 to 4.4 h, did not vary significantly among dose levels.

In multiple dose studies, volunteers received DOV 216,303 at total daily doses of 50, 75, or 100 mg or placebo for 10 days. Subjects were fasted overnight prior to receiving the first dose of drug. Subjects on twice-daily regimens (the 50 mg and 100 mg arms and placebo) received the first dose of the drug between 8 and 9 a.m. and the second dose 12 h later. Subjects receiving 25 mg three times daily were administered the third dose at 2 p.m. Blood samples (10 mL) were drawn at various intervals from 0.5 to 24 h after the first dose on study days 1 and 10. Adverse events were noted only in the highest dosing arm, and, as in the single dose study, were principally related to gastrointestinal disturbance (nausea with dyspepsia, mild diarrhea) (1). Analysis of covariance (ANCOVA) demonstrated that C_{max} values in the single and multiple dose studies were homogeneous with respect to the dose-response relationship, i.e., plasma levels maintained dose proportionality following 10 days of drug administration (Fig. 4, bottom panel). Further, the C_{max} values produced by the 50 mg twice daily regimen (~500 ng/mL) would exceed by more than one order of magnitude the IC_{50} value of DOV 216,303 to inhibit the reuptake of all three biogenic amines (Table 1). The safety, tolerability and bioavailability of DOV 216,303 in normal volunteers prompted a study of its safety, tolerability, and efficacy in depressed individuals.

Safety, Tolerability, and Efficacy of DOV 216,303 in Depressed Individuals

This study was originally designed as a multi-center, double-blind trial comparing the safety, tolerability and efficacy of a two-week regimen of DOV 216,303 (50 mg twice daily) to placebo in depressed individuals. The study duration was constrained by the toxicology data available at the time. All study sites were in Germany, and several of the regional institutional review boards did not approve the use of placebo in severely depressed patients. Therefore, the study compared DOV 216,303 (50 mg twice daily) to an active comparator, citalopram (20 mg twice daily). This dose of citalopram is higher than the widely recommended dose of 20 mg, but based on a meta-analysis of more than 3900 patients reported by Montgomery and Djarv (14), the 40 mg dose appears to confer some advantages to non-responders who are severely depressed.

The principal criteria for study entry was a diagnosis of Major Depressive Disorder, with a score of ≥20 on the Hamilton Depression Scale (HAM-D; 21-item scale) and a

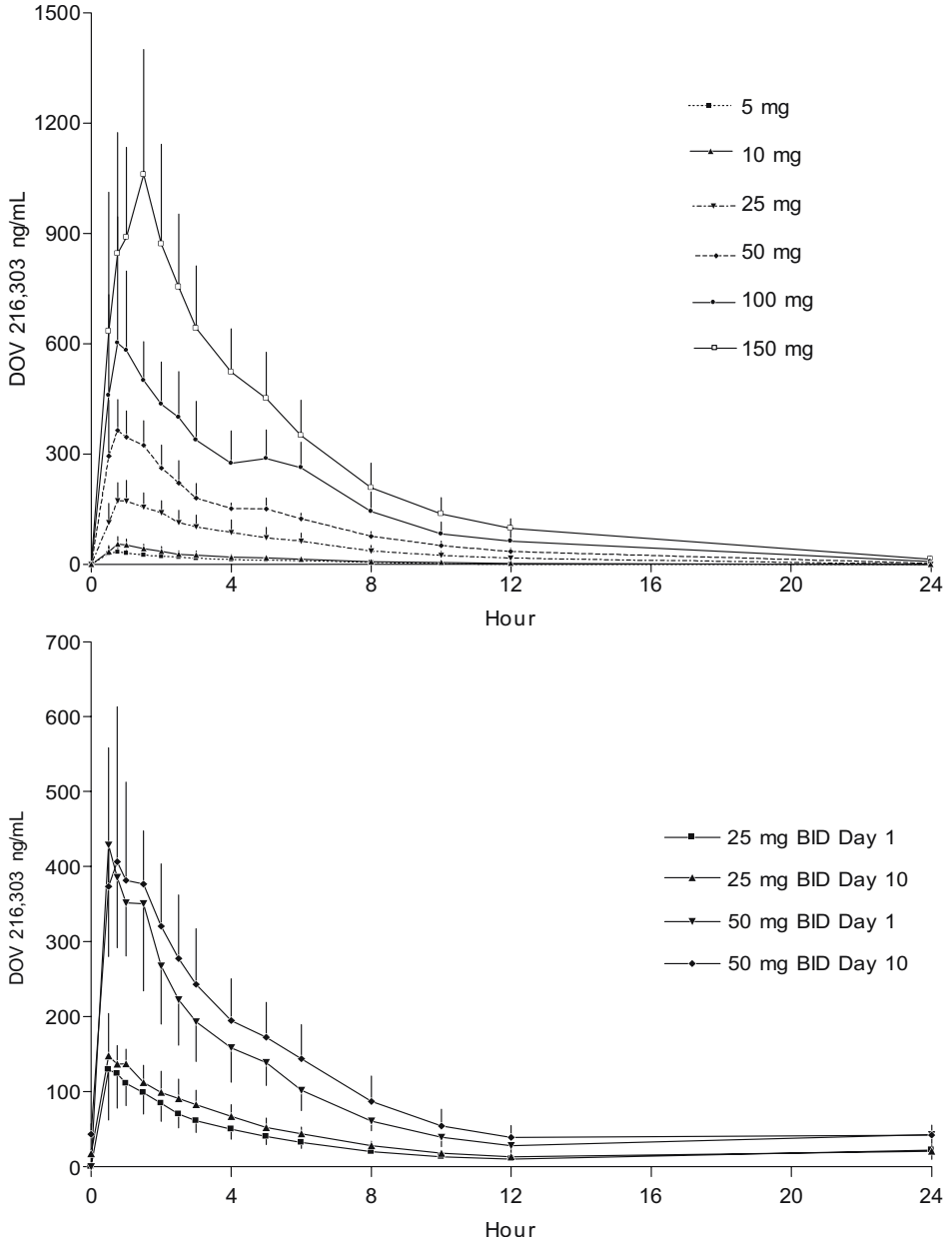

Fig. 4. Plasma concentrations of DOV 216,303 following its administration to healthy male volunteers. Top panel, a single oral dose; Bottom panel: ten days of dosing, with plasma levels measured at days 1 and 10. Values represent the $X \pm$ S.D. of 7 subjects/arm. Modified from ref. 1.

score of <15 on the Hamilton Anxiety scale at baseline. Individuals with either a >20% decrease in HAM-D score between screening and baseline (day 1) or whose HAM-D scores had dropped below 20 were excluded from the study. Initially, the study was

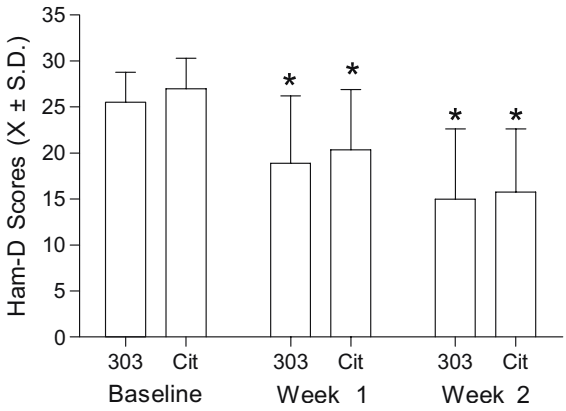

Fig. 5. DOV 216,303 and citalopram produce a time-dependent reduction in Hamilton Depression Scores (HAM-D). Bars represent the $X \pm$ S.D. One week after screening, HAM-D scores were obtained (baseline), and individuals with more than a 10% drop in HAM-D were dropped from the study. Patients received either DOV 216,303 (50 mg/kg twice daily) or citalopram (20 mg twice daily); administered medications were identical in appearance. A total of 67 patients (36 DOV 216,303; 31 citalopram) were enrolled at baseline. At week 1, 64 patients (33 and 31, respectively) were evaluated, and at week 2, 61 patients (30 and 31, respectively). * $p < 0.,0001$, paired *t*-test vs. baseline HAM-D scores.

limited to in-patients, but due to very slow enrollment, the protocol was amended to include out-patients. Males and females between the ages of 18 and 65 within 10% (lower end of weight limit) and 20% (upper end of weight limit) of ideal body weight were enrolled. The population age averaged 42.7 ± 10.8 years, with ~2/3 of the study population being female. The change in HAM-D (administered at screening, Day 1 (baseline), Day 7, and Day 14) was used as the primary outcome measure. Secondary outcome measures (scales administered to patients on the same days as the HAM-D) included the Clinical Global Impression Scales (severity and improvement), Beck Depression Scale, and the Zung Self Rating Depression Scale. Following a complete explanation of the study, each subject was given the opportunity to ask questions and informed of the right to withdraw from the study at any time for any reason. A written, informed consent (approved by the local Research Ethics Committee) on an EC approved consent form was obtained from each subject.

Vital signs, including blood pressure, respiratory rate, temperature, and pulse rate were measured at screening, at baseline and at 2 h after the first dose, and on days 7, 14 and on day 16 (that is, two days after discontinuation of the treatment). Physical examinations were performed at screening and either on day 14 (for outpatients) or day 16 (for inpatients). Clinical chemistry, including blood count and urinalysis, was performed at screening, baseline, and days 7 and 14 of the treatment period. Urine was tested for drugs of abuse at the initial screening, one day before treatment period, and on days 7 and 14.

A total of 67 patients were enrolled. Their HAM-D scores were 25.5 ± 3.3 ($n = 36$) and 27 ± 3.3 ($n = 31$) at baseline in the DOV 216,303 and citalopram groups, respectively. Statistically significant, time-dependent reductions in HAM-D scores were observed in both treatment arms ($p < 0.0001$, paired *t*-test — comparing baseline values to values at weeks 1 and 2) (Fig. 5). Highly significant changes were also observed in all secondary rating scales (data not shown).

The percentage of patients reporting one or more adverse events (AEs) during this two week period were similar, 44.4 and 41.9% in patients treated with DOV 216,303 and citalopram, respectively. Gastrointestinal AEs were the most frequently reported (19.4% in each group), with nausea most frequently reported in the DOV 216,303 and flatulence in the citalopram arm. Most treatment emergent AEs were classified as mild or moderate in both treatment arms; no serious adverse events were reported in this study. Further, no remarkable differences in vital signs were observed in either study group. These findings indicate that DOV 216,303 is safe and generally well-tolerated compared to citalopram in this cohort of depressed patients. DOV 216,303 produced statistically significant changes in HAM-D scores compared to baseline which were detectable as early as one week after initiation of the treatment. While these data suggest an onset of action more rapid than generally reported in most double blind, placebo controlled trials (19), significant effects were also observed following one and two weeks of citalopram administration, albeit at a higher initial dose than is generally prescribed ([14].

DISCUSSION

Inhibitors of serotonin and/or norepinephrine reuptake have been used as antidepressants for more than four decades. The introduction of drugs that "selectively" target the serotonin and/or norepinephrine transport protein(s) is arguably the most significant advance in the pharmacotherapy of depression during the past two decades. These drugs eliminate many of the limiting and even potentially fatal side effects associated with the use of first generation reuptake inhibitors (that is, tricyclic antidepressants and MAOIs). Thus, both serotonin selective reuptake inhibitors (SSRIs; exemplified by paroxetine and citalopram) and serotonin/norepinephrine reuptake inhibitors (SNRIs; exemplified by venlafaxine and duloxetine) are safer, but no more effective than the tricyclics. Several strategies have emerged to improve on the profile of biogenic-amine based antidepressants, including circumvention of the monoaminergic synapse (19,20). However, among biogenic amine based strategies, the use of a triple reuptake inhibitor (or alternatively, using an SSRI or SNRI in combination with either a dopamine reuptake inhibitor or dopamine agonist) is arguably the most compelling means of overcoming the limitations of current antidepressant therapy.

Perhaps the most critical issue for the development of a triple reuptake inhibitor for depression is determining the optimum relative potency at each of the three transporters. Certainly, the relative potencies of antidepressants as inhibitors of serotonin versus norepinephrine uptake can vary over several orders of magnitude (with, for example, citalopram and reboxetine as extremes). Thus, with approximately equal affinity as an inhibitor of norepinephrine versus serotonin uptake, it would be predicted that DOV 216,303 would have antidepressant properties. However, the relative increases in synaptic concentrations of dopamine, norepinephrine, and serotonin will likely affect not only the speed of onset and efficacy of this compound, but also its side effect profile.

One of the hypothesized advantages of a triple reuptake inhibitor not often considered in the literature may be a lower incidence of "serotonin-related" side effects (e.g., changes in libido and related effects on sexual functioning). Thus, it has been reported that a continuous, high occupancy (>75% at trough) of serotonin transporters is required to produce

an antidepressant action across a chemically diverse field of SSRIs (13). Since the side effect profile among SSRIs is quite similar, it is logical to conclude that some or all of these side effects may also require this continuous high occupancy of serotonin transporters. While it is not known if the same high occupancy of serotonin transporters is required for a triple reuptake inhibitor to produce an antidepressant effect, imaging studies indicate that continuous, high occupancy may not be required to produce an antidepressant action through inhibition of, for example, dopamine transporters (10). In addition, there is preclinical evidence demonstrating a marked synergism between SSRIs and dopamine reuptake inhibitors and/or dopamine agonists in behavioral despair paradigms predictive of antidepressive action (12) and elevating extracellular levels of dopamine and norepinephrine (11). Taken together, these clinical and preclinical findings indicate that if this synergism is obtainable in humans, then it is likely that an antidepressant action will be produced at lower SERT occupancy, which could reduce the incidence of side effects typically associated with SSRIs. This hypothesis will require testing in the clinic. Studies in normal volunteers (1) demonstrated that DOV 216,303 is safe and well-tolerated, a finding confirmed in a larger cohort of depressed individuals. Nausea, the most common side effect produced by DOV 216,303, is commonly produced by other antidepressants that inhibit serotonin reuptake. The overall incidence of adverse events produced by DOV 216,303 did not differ remarkably from that of citalopram in depressed patients. However, it should be emphasized that the dose-response curve for both efficacy and tolerability of DOV 216,303 in depressed individuals has not been established. The side effect profile of DOV 216,303 may also be related to its potency as an inhibitor of serotonin uptake relative to its potency to inhibit the uptake of norepinephrine and dopamine. Sibling molecules, including DOV 21,947 (23) and DOV 102,677 (17) with varying potencies at the three transporters are also in clinical development, and may help resolve this issue.

Perhaps the most compelling finding to emerge from the Phase IIA study reviewed here is the robust reduction in HAM-D scores produced by DOV 216,303 and citalopram at both weeks 1 and 2 (Fig. 5). These findings are consistent with the report of Montgomery and Djarv (14) that citalopram (using a flexible 20 to 80 mg regimen) produced a significant separation in HAM-D scores from placebo as early as week 1 of treatment, an effect that was maintained for the 4 week treatment period. The placebo response is a notorious confounding factor in antidepressant trials, and the current study is flawed by the lack of a placebo arm. However, the use of an active comparator in the present study was necessary because several of the local institutional review boards felt that it was unethical to treat severely depressed patients with placebo. Nonetheless, these results are sufficiently encouraging to warrant additional clinical studies of triple reuptake inhibitors in depression.

Acknowledgments. The authors thank B. Beer, G. Beer, S. Langer and J. Stark for their contributions to the clinical studies described in this review.

REFERENCES

1. Beer B, Stark J, Kreiter P, et al. DOV 216,303, a "triple" reuptake inhibitor: Safety, tolerability, and pharmacokinetic profile. *J Clin Pharmacol* 2004;44:1360–1367.
2. Bodkin JA, Lasser RA, Wines JD, et al. Combining serotonin reuptake inhibitors and bupropion in partial responders to antidepressant monotherapy. *J Clin Psychiatry* 1997;58:137–145.

3. D'Aquila PS, Collu M, Gessa GL, et al. The role of dopamine in the mechanism of action of antidepressant drugs. *Eur J Pharmacol* 2000;405:365–373.
4. Fava M. Augmentation and combination strategies in treatment-resistant depression. *J Clin Psychiatry* 2001;62(Suppl 18):4–11.
5. Inoue T, Tscuchiya K, Miura J, et al. Bromocriptine treatment of tricyclic and heterocyclic antidepressant-resistant depression. *Biol Psychiatry* 1996;40:151–153.
6. Izumi T, Inoue T, Kitagawa N, et al. Open pergolide treatment of tricyclic and heterocyclic antidepressant-resistant depression. *J Affect Disord* 2000;61:127–132.
7. Kelley AE. Neural integrative activities of nucleus accumbens subregions in relation to learning and motivation. *Psychobiology* 1999;27:198–213.
8. Kennedy SH, McCann SM, Masellis M, et al. Combining bupropion SR with venlafaxine, paroxetine, or fluoxetine: A preliminary report on pharmacokinetic, therapeutic, and sexual dysfunction effects. *J Clin Psychiatry* 2000;63:181–186.
9. Lammers C-H, Diaz J, Schwartz J-C, et al. Selective increase of dopamine D_3 receptor gene expression as a common effect of chronic antidepressant treatments. *Mol Psychiatry* 2000;5:378–388.
10. Learned-Coughlin S, Bergstrom M, Savitcheva I, et al. In vivo activity of bupropion at the human dopamine transporter as measured by positron emission tomography. *Biol Psychiatry* 2003;54:800–805.
11. Li S, Perry KW, Wong DT. Influence of fluoxetine on the ability of bupropion to modulate extracellular dopamine and norepinephrine concentrations in three mesocorticolimbic areas of rats. *Neuropharmacology* 2002;42:181–190.
12. Maj J, Rogoz Z. Synergistic effects of pramipexole and sertraline in the forced swimming test. *Pol J Pharmacol* 1999;51:471–475.
13. Meyer, JH, Wilson AA, Sagrati S, et al. Serotonin transporter occupancy of five selective serotonin reuptake inhibitors at different doses: An [^{11}C]DASB positron emission tomography study. *Am J Psychiatry* 2004;161:826–835.
14. Montgomery SA, Djarv L. The antidepressant efficacy of citalopram. *Int Clin Psychopharmacol* 1996; 11(Suppl 1):29–33.
15. Naranjo C, Tremblay LK, Busto UE. The role of the brain reward system in depression. *Prog Neuropsychopharmacol Biol Psychiatry* 2001;25:781–823.
16. Nelson JC. Augmentation strategies with serotonergic-noradrenergic combinations. *J Clin Psychiatry* 1998; 59:65–68.
17. Popik P, Krawczyk M, Golembiowska K, et al. Pharmacological profile of the "triple" reuptake inhibitor, DOV 102,677. *Chem Mol Neurobiol* 2006; In press.
18. Povlock SL, Amara SG. The structure and function of norepinephrine, dopamine and serotonin transporters. In: Reith MEA, Ed. Neurotransmitter transporters: Structure, function, and regulation. Totowa: Humana Press, 1997;1–28.
19. Skolnick P. Antidepressants. Skolnick P, Ed. New Pharmacological Strategies. Totowa: Humana Press, 1997.
20. Skolnick P, Legutko B, Li X, et al. Current perspectives on the development of non-biogenic amine-based antidepressants. *Pharmacol Res* 2001;43:411–422.
21. Skolnick P. Dopamine and depression. In: Schmidt W, Ed. Dopamine and glutamate in psychiatric disorders. Totowa: Humana Press, 2005;9:199–214.
22. Skolnick P, Popik P, Janowsky A, et al. "Broad spectrum" antidepressants: Is more better for the treatment of depression? *Life Sci* 2003;73:3175–3179.
23. Skolnick P, Popik P, Janowsky A, et al. Antidepressant-like actions of DOV 21,947: A "triple" reuptake inhibitor. *Eur J Pharmacol* 2003;461:99–104.
24. Sporn J, Ghaemi SN, Sambur MR, et al. Pramipexole augmentation in the treatment of unipolar and bipolar depression: A retrospective chart review. *Ann Clin Psychiatry* 2000;12:137–140.
25. Vaishnavi SN, Nemeroff CB, Plott SJ, et al. Milnacipran: A comparative analysis of human monoamine uptake and transporter binding affinity. *Biol Psychiatry* 2004;55:320–322.
26. Willner P. Validity, reliability and utility of the chronic mild stress model of depression: A 10-year review and evaluation. *Psychopharmacology* 1997;134:319–329.
27. Willner P, Papp M. Animal Models to Detect Antidepressants. In: Antidepressants: Skolnick P, Ed. New pharmacological strategies. Totowa: Humana Press, 1997;213–234.
28. Wise RA. Brain reward circuity: Insights from unsensed incentives. *Neuron* 2002;36:229–240.
29. Zisook S, Rush JA, Haight BR, et al. Use of bupropion in combination with serotonin reuptake inhibitors. *Biol Psychiatry* 2005;59:203–210.