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Verapamil augmentation of lithium treatment improves outcome in mania unresponsive to lithium alone: preliminary findings and a discussion of therapeutic mechanisms

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Abstract

Objectives—Attenuation of protein kinase C (PKC) is a mechanism common to both established (lithium, valproate) and some novel (tamoxifen) antimanic agents. Verapamil, although primarily known as a calcium channel blocker, also has PKC inhibitory activity. Verapamil has shown antimanic activity in some but not all studies. Therefore, we investigated verapamil, used alone or as an adjunctive treatment, in manic patients who did not respond to an initial adequate trial of lithium.

Methods—Each study phase lasted three weeks. Subjects were treated openly with lithium in Phase 1 (n = 45). Those who failed to respond were randomly assigned to double-blind treatment in Phase 2 with either verapamil (n = 10) or continued-lithium (n = 8). Phase 2 nonresponders (n = 10) were assigned to combined verapamil/lithium in Phase 3.

Results—Response in Phase 2 did not differ significantly between verapamil and continued-lithium. During Phase 3, response to combined treatment was significantly better than overall response to monotherapy in Phase 2 (Fisher's Exact test, p = 0.043). Mania ratings improved during combined treatment in Phase 3 by 88.2% (linear mixed model analysis, $F = 4.34$, p = 0.013), compared with 10.5% improvement during Phase 2.

Conclusions—In this preliminary investigation, verapamil monotherapy did not demonstrate antimanic efficacy. By contrast, the combination of verapamil plus lithium was highly efficacious. Our findings thus suggest that verapamil may have potential utility as an adjunct to lithium. This

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effect may be mediated by additive actions on PKC inhibition, which may be an important mechanism for antimanic agents in general.

Keywords

antimanic; bipolar disorder; lithium; mania; protein kinase C; verapamil

Calcium channel blocking drugs (CCBs) have long been advocated as a treatment for mania, but data on their efficacy have been inconsistent. The literature in this area has been summarized in several recent reviews. Levy and Janicak (1) concluded that while early work on verapamil demonstrated positive results for acute mania, later better-controlled studies (including their own) did not support its efficacy. They noted further that verapamil's efficacy as an augmentation therapy has yet to be adequately studied. Keck et al. (2) pointed out both positive and negative findings for antimanic efficacy of two CCBs (verapamil and nimodipine), and concluded that further studies would be needed to establish CCBs as efficacious in the treatment of mania. In a selected review on the treatment of rapid cycling bipolar disorder, Post et al. (3) suggest a role for CCBs as part of a complex combination treatment strategy for managing this condition. They also pointed to earlier work by Manna (4) demonstrating that the combination of lithium and nimodipine was more effective than either agent alone in long-term administration. In another review on rapid cycling disorder, Dubovsky (5) summarized studies of nimodipine and suggested as a strategy the addition of CCBs to treatment with other mood stabilizers. In the time since these reviews were written, Wisner et al. (6) reported that 9 of 11 women with manic or mixed symptoms showed an antimanic response to verapamil, Yingling et al. (7) described successful treatment of a pregnant bipolar woman with nimodipine, and Giannini et al. (8) reported that combining magnesium oxide with verapamil was more effective for reducing manic symptoms than was verapamil alone.

The theoretical rationale for using CCBs as antimanic agents rests on previous observations that intracellular calcium is elevated in mania (9), a situation that could potentially be corrected by blocking cellular calcium influx. The majority of previous studies on CCBs for treatment of acute mania have used verapamil specifically. However, it is important to note that in addition to its effect on L-type calcium channels, verapamil can inhibit protein kinase C (PKC) activity (10–12). The PKC signaling pathway has an important role in regulating neuronal function, and moreover, preclinical biochemical and behavioral data support the notion that PKC activation may result in manic-like behaviors, whereas PKC inhibition may be antimanic (13–15). Given that the prototypical antimanic agents, lithium and valproate, have been found to bring about isozyme-selective reductions in PKC levels (16–18), attenuation of PKC activity could be an important mechanism of antimanic drug action. Indeed, recent preliminary studies have suggested that tamoxifen, a drug that potently inhibits PKC (in addition to its more well-known effect as a selective estrogen receptor modulator), produces clinical improvement in acutely manic patients (19–21). Moreover, in one of these studies, medroxyprogesterone (a hormonal modulator without PKC inhibition) produced lesser, nonsignificant improvement of manic symptoms (20). Most recently, a whole genome association study of bipolar disorder has been conducted, utilizing North American bipolar pedigrees as a test sample, and German bipolar subjects as the replication sample. This study found a highly significant association ($p \sim 10^{-8}$) of diacylglycerol kinase ϵ (DGKH) with bipolar disorder (22). These findings are particularly noteworthy, since diacylglycerol (DAG) is the major activator of PKC.

Our original purpose in conducting this study was to investigate treatment options for the significant proportion of patients who either do not respond adequately or cannot tolerate the adverse effects associated with therapeutic doses of lithium. While a number of other

treatments are available, including several anticonvulsants and antipsychotics, issues of effectiveness, tolerability, and safety contribute to an ongoing need for the development of new therapeutic alternatives. Thus, the goal of this investigation was to perform a controlled (double-blind, random assignment) comparison of verapamil versus continued-lithium treatment in bipolar manic patients who were unresponsive to three weeks of initial treatment with lithium ($\pm$ perphenazine). Subsequently, patients who failed to respond to the single agents being tested were treated with combined verapamil and lithium. Within this framework, the overall aim of this investigation was to assess the acute treatment efficacy of verapamil, either alone or in combination with lithium, in patients who initially failed to respond to a course of lithium therapy.

Materials and methods

Subjects were inpatients or outpatients at the University of Pittsburgh Medical Center (UPMC), Pittsburgh, PA, USA. Some concurrently participated in the Maintenance Therapies in Bipolar Disorder (MTBD) study (23), and were studied either at the time of a presenting manic index episode, or during a manic recurrence. Other subjects were selected from the general population of patients presenting to the outpatient assessment center, or admitted to the inpatient units at Western Psychiatric Institute and Clinic, UPMC. All subjects gave informed consent for research participation using procedures approved and monitored by the University of Pittsburgh Health Sciences Institutional Review Board, in accordance with the Helsinki Declaration of 1975.

All patients received a comprehensive psychiatric and medical evaluation, including a Structured Clinical Interview for DSM-IV (SCID) by a trained clinician. Patients' eligibility for the study was determined by the consensus of the project coordinator, the SCID evaluator, and at least one of the investigators. To be eligible for the study, patients were required to have a lifetime diagnosis of bipolar I or schizoaffective disorder, a current manic episode meeting DSM-IV criteria, and ratings ≥ 7 on the Raskin Severity of Mania Scale (24) and ≥ 15 on the Bech-Rafaelsen Mania Scale (25). Patients were excluded if they had: (i) a pattern of severe rapid-cycling in which the patient consistently failed to meet the duration criteria for discrete episodes of syndromal mania or depression according to DSM-IV; (ii) sustained drug or alcohol abuse within the past three years; (iii) schizophrenia; (iv) organic affective syndrome; (v) a presenting episode that was secondary to the effect of any pharmacologic agent; (vi) the presence of significant medical illness that would preclude or unduly complicate the intended pharmacologic management of the episode; (vii) in females, refusal to use appropriate contraception; or (viii) pregnancy.

Clinical symptom ratings were completed weekly. An independent evaluator not on the treatment team completed the Bech-Rafaelsen Mania Scale, the Raskin Severity of Mania and Depression Scales, the Hamilton Rating Scale for Depression (17-item) (26), the eight-item Pittsburgh Reversed Vegetative Symptom Scale (27), and an implementation of the seven-point Global Improvement item from the Clinical Global Impressions scale (28) that was operationalized for use in mania.

Phase 1 treatment

Treatment with lithium carbonate was initiated at a dose of 900–1200 mg/day. Serum levels were monitored twice weekly, and the dose was adjusted to attain target levels between 0.8 and 1.0 mmol/L by the end of Week 1, with subsequent levels as high as 1.4 mmol/L permitted if needed. Adjunctive treatment with antipsychotic medication was permitted to control psychotic agitation or related insomnia. The preferred treatment was perphenazine, 4 mg PO or 2 mg IM as needed. For persistent insomnia or nonpsychotic agitation, lorazepam was permitted (0.5 to 1.0 mg individual doses, up to a total of 6.0 mg/day). Nonresponse in

Phase 1 was defined as a rating of ≤ 7 on the Raskin Severity of Mania Scale and ≤ 15 on the Bech-Rafaelsen Mania Scale, after a minimum of three weeks of adequate lithium treatment.

Phase 2 treatment

Qualifying patients were randomly assigned in double-blind fashion to continued-lithium or verapamil. The randomization was stratified according to whether the patient was taking antipsychotic medication in Phase 1, whether they were participating in the MTBD study, and if so, whether they were receiving the study psychotherapy. Two types of tamper-resistant capsules were utilized: one type contained lithium carbonate or placebo; the other contained verapamil or placebo. Lactose was the filler in both capsule types. Patients assigned to continued-lithium were maintained on the same dose utilized in their previous treatment, unless a change was required to maintain maximally tolerated target serum levels between 0.8 and 1.4 mmol/L. The lithium-treated patients also took placebo-verapamil capsules throughout the study. Serum lithium levels were monitored weekly, and blood was drawn from all subjects in the study on the same weekly schedule in order to preserve the double blind. Patients assigned to verapamil were tapered from their previous lithium dose over a nine-day interval (approximately 25% dose reduction every three days) by progressive substitution of identical-appearing placebo-lithium capsules, which were then continued for the duration of the study. At the same time, the patients were started on a second type of capsule containing either verapamil or placebo-verapamil. The initial dose of verapamil was 160 mg/day, and this was raised by 80 mg/day every three days to a maximum of 480 mg/day, unless intolerable side effects were produced at a lower level, in which case the dose was maintained at the highest tolerated amount. The daily verapamil dose was divided b.i.d. (morning and evening) at the starting dose of 160 mg/day, and t.i.d. (morning, noon, and evening) for all higher doses. Any perphenazine previously administered in Phase 1 for management of psychotic symptoms was continued openly at the same dose during the six-week double-blind portion of the study (Phases 2 and 3). Although perphenazine was our preferred antipsychotic treatment, individual circumstances sometimes necessitated substitution of a different antipsychotic medication, which was then kept consistent throughout the protocol. Lorazepam was permitted as in Phase 1, and the individual doses taken were recorded for later use as a secondary outcome measure. At the conclusion of Phase 2, patients were classified as responders if all three of the following criteria were met: a $\geq 50\%$ reduction in Bech-Rafaelsen score (relative to the baseline score at the start of the phase); a total Bech-Rafaelsen score ≤ 10 ; and a rating of 1 or 2 (very much improved or much improved, compared to baseline) on the Clinical Global Impressions scale by Week 3.

Phase 3 treatment

Patients meeting the response criteria above at the conclusion of Phase 2 received three additional weeks of continuation treatment with the same agent that produced the response; patients who failed to meet the response criteria had a second agent added to their therapeutic regimen by progressive substitution of active verapamil or lithium for the corresponding placebo capsules in their treatment regimen. The dose of verapamil was progressively increased as outlined above. Lithium was initiated at a dose of 600 mg/day, and the dose was increased by 300 mg every other day, until target serum levels of 0.8 to 1.2 mmol/L were achieved. Double-blind conditions were maintained, and clinical procedures and mood ratings continued as before. Criteria for clinical response were identical to those used in Phase 2.

Our study hypotheses were: (i) treatment outcome based on the proportion of patients responding as well as the change in mania ratings would be significantly better for verapamil as compared to continued-lithium; (ii) patients who failed to respond to single-

agent treatment with verapamil or lithium would have a favorable outcome when treated with a combination of these two agents; and (iii) use of prn lorazepam would represent a secondary outcome measure that would decrease in parallel with improvement in the primary outcome measures listed above (to be used only in a confirmatory way when findings with the primary outcome measures were significant).

The Mann–Whitney *U*-test was used to compare groups on continuous variables that may not have been normally distributed. Fisher’s Exact test was used with binary outcome measures. Wilcoxon Signed Ranks test was used to compare outcomes from one phase to the next. Also, a full factorial linear mixed model with restricted maximum likelihood estimation and a first order autoregressive variance-covariance structure that included a random effect for the intercept was used to examine the change in Bech-Rafaelsen Mania rating scores over the course of the third phase for each Phase 2 treatment group as well as the full sample. The *p* values were two-tailed except where noted. Statistical analyses were performed using SPSS version 15.0 for Windows (SPSS, Inc., Chicago, IL, USA).

Results

Figure 1 summarizes the treatments administered in each phase of the protocol, the number of subjects and response to treatment in each phase, and the percentage response rates observed. In Phase 1, a total of 45 patients were treated openly with lithium plus additional medications for three weeks as described in the previous section, and of these, 26 (58%) responded. One patient dropped out of treatment and was lost to follow-up; the remaining 18 lithium nonresponsive patients entered Phase 2 of the protocol, and were randomly assigned to treatment with either verapamil or continued-lithium. Mean $\pm$ SD serum lithium levels attained during Phase 1 for these lithium nonresponsive subjects were 0.91 ± 0.25 at the end of Week 2, and 0.91 ± 0.28 at the end of Week 3.

Table 1 summarizes the demographic and clinical characteristics of subjects, categorized by double-blind treatment assignment in Phase 2. Ten patients were assigned to verapamil and eight to continued-lithium in this phase of the study. All but one subject had a diagnosis of bipolar I disorder; the remaining subject was diagnosed with schizoaffective disorder. The subject groups assigned to verapamil or continued-lithium did not differ significantly with respect to age (Mann–Whitney test, $U = 30.5$, $p = 0.42$), gender (Fisher’s Exact test, $p = 0.66$), or number of previous manic episodes (Mann–Whitney test, $U = 25.5$, $p = 0.33$). However, subjects assigned to continued-lithium had a greater number of previous depressive episodes compared to those assigned to verapamil (Mann–Whitney test, $U = 12.0$, $p = 0.038$). Most patients took antipsychotic medication during the study, and there was no difference between the verapamil and continued-lithium treatment groups in the frequency of use for such medication (Fisher’s Exact test, $p = 1.00$).

One verapamil-treated patient terminated in Phase 2 and was classified as a nonresponder. The frequency of response after three weeks of treatment in Phase 2 was 30% with verapamil and 50% with continued-lithium (see Figure 1), and there was no significant difference in outcome between these treatments (Fisher’s Exact test, $p = 0.63$).

Patients who responded to Phase 2 treatment were advanced to Phase 3 on the same double-blind medication for an additional three weeks, and in most cases the response was continued (see Figure 1). The Phase 2 nonresponders were treated with verapamil plus lithium under double-blind conditions for three weeks in Phase 3 by having the second active medication substituted in the placebo capsules they were already taking (as described in the Materials and Methods). The response rate for verapamil plus lithium (80%) was higher than the response rates observed in any other phase of the study. For comparison

purposes, we examined the overall response to Phase 2 treatment (three weeks of single-agent verapamil or continued-lithium) versus the response to Phase 3 combined treatment (three weeks of combined verapamil plus lithium). The ratio of responders to nonresponders was 7:11 for single-agent verapamil or continued-lithium, and 8:2 for combined verapamil plus lithium. The difference between Phase 2 and Phase 3 response was significant by Fisher's Exact test ($p = 0.043$) using one-tailed probability (one-tailed probability was selected because our hypothesis predicted that response to combined verapamil-lithium treatment in Phase 3 would be favorable in patients who had failed to respond to single-agent verapamil or continued-lithium treatment in Phase 2).

Figure 2 illustrates median Bech-Rafaelsen Mania ratings for the 10 nonresponders in Phase 2 who subsequently received combined verapamil-lithium treatment in Phase 3. The median mania score was 19 at the start of Phase 2, after patients had completed (but had not responded to) a three-week course of open treatment with lithium. At the end of Phase 2, after a subsequent three weeks of treatment with either verapamil or continued-lithium, the median score of 17 remained similar. However, during the three weeks of Phase 3 treatment, when subjects received combined verapamil-lithium, the median mania score progressively declined to 2 (see Fig. 2). The percentage improvement in median Bech-Rafaelsen ratings during Phase 3 was 88.2%, compared with 10.5% during Phase 2. Using a linear mixed model, the decrease in mania ratings for all subjects during the three weeks of Phase 3 combined treatment was significant ($F = 4.34$, $p = 0.013$). When subjects were separated into groups based on whether their previous Phase 2 treatment had been single-agent verapamil or continued-lithium, the decrease in mania ratings during Phase 3 remained significant ($F = 3.70$, $p = 0.026$) with no effect of group ($p = 0.177$) or interaction between group and time ($p = 0.516$), suggesting that improvement during Phase 3 occurs regardless of the prior medication utilized in Phase 2. In each analysis, the difference was significant by the second week of combined treatment. In terms of numbers of subjects, three of the four Phase 2 lithium nonresponders (75.0%) and five of the six Phase 2 verapamil nonresponders (83.3%) who entered Phase 3 subsequently responded to combined treatment.

Administration of prn benzodiazepine to control symptoms of nonpsychotic agitation or insomnia was used as a secondary measure of clinical outcome. Our original design called for the use of lorazepam, but because of individual patient factors, clonazepam was substituted for lorazepam in two cases, and alprazolam in one case. Of the 18 subjects who participated in Phase 2 of the study, all but two (89%) required use of prn benzodiazepine. Seventeen of these subjects went on to participate in Phase 3, during which 11 (65%) required such medication. Among these 17 subjects (who thus participated in both Phases 2 and 3), there was no statistically significant difference between Phase 2 and Phase 3 in the number of prn benzodiazepine doses used (Wilcoxon Signed Ranks test, $p = 0.68$); the median number of benzodiazepine doses taken per subject was 12 during Phase 2 and 7 during Phase 3. Five subjects increased their benzodiazepine use during Phase 3 as compared to Phase 2, while 9 subjects decreased or eliminated such use. One subject used the same amount in both phases, and 2 did not use prn medication in either study phase.

For those subjects previously analyzed who were nonresponders in Phase 2 and subsequently received combined verapamil-lithium treatment in Phase 3, use of prn benzodiazepine likewise did not differ significantly between the treatment phases; the median number of benzodiazepine doses taken per subject was 10 during Phase 2 and 13 during Phase 3 (Wilcoxon Signed Ranks test, $p = 0.85$). Three subjects increased their benzodiazepine use during Phase 3 compared to Phase 2, while five subjects decreased or eliminated such use, and two did not use prn medication in either study phase.

Discussion

The findings from this investigation must be considered to be preliminary in view of the limited number of subjects studied. In this context, verapamil as a single-agent mood stabilizer (as administered in Phase 2 of our study) showed minimal efficacy for treating manic patients who previously failed to respond to an initial three-week trial of lithium (30% response rate). There was no significant difference between verapamil treatment and continued administration of lithium during the three weeks of Phase 2, although the outcome with continued-lithium in Phase 2 was actually slightly better (50% response rate) than that with verapamil (30% response rate).

In contrast, patients who were treated with combined verapamil-lithium in Phase 3 showed substantial improvement that was statistically significant, compared to baseline, by the second week of treatment. This improvement was found regardless of whether the previous treatment used in Phase 2 had been verapamil or continued-lithium. It thus appears that combining verapamil with lithium may produce a clinically useful additive or synergistic effect. If these findings are confirmed by larger studies, adjunctive treatment with verapamil may become a novel strategy for effectively treating lithium-nonresponsive manic patients.

It should be noted that additive/synergistic actions such as those found in our study can have negative as well as positive consequences. Combined treatment with verapamil and lithium has been associated with a variety of adverse events, including exacerbation of lithium side effects (29), choreoathetosis (30), bradycardia with possible myocardial infarction (31), and neurotoxicity with ataxia (32, 33). Thus, caution and appropriate monitoring are essential when using this drug combination.

It is important to note that the majority of patients in our study received concurrent treatment with antipsychotic medication. This likely reflects the severity of the manic episodes our subjects experienced, but also affects the interpretation of our findings. Since the antipsychotic medication was held constant in Phases 2 and 3 of the study, this should not have been a confounding variable in our results. Nevertheless, it cannot be determined from our findings whether concomitant use of antipsychotic medication may be essential for effectiveness of the combination treatment strategy reported here.

Historically, the rationale for using CCBs to treat mania rests on observations that baseline and/or stimulated intracellular calcium are elevated in bipolar patients, as measured in platelets (9, 34, 35), lymphocytes (35, 36), and beta lymphoblast cell lines (37, 38). The inhibitory effect of verapamil on calcium uptake via L-type calcium channels is well known, and could potentially offset such increases. Lithium's long-known actions on enzymatic and signal transduction components of the inositol phospholipid signaling system (39–44) can likewise reduce mobilization of intracellular free calcium. For example, chronic exposure of beta lymphoblast cell lines to lithium was found to elevate resting calcium levels while attenuating stimulus-induced calcium mobilization (45). In the hypothetical case where lithium fails to act sufficiently on calcium mobilization, a CCB could perhaps augment the effect on intracellular free calcium needed for therapeutic effectiveness.

However, Pazzaglia et al. (46) suggested that verapamil may not substitute adequately for other CCBs. These authors reported that when verapamil was blindly substituted for nimodipine, two rapid cycling bipolar patients failed to maintain improvement, but responded again when nimodipine was reintroduced or replaced by isradipine. Thus, verapamil may differ from other CCBs in terms of its clinical actions, and it may be useful to consider other therapeutic mechanisms for this drug, especially with respect to combination treatment with lithium. In this regard, we note that the effects of the inositol phospholipid signaling system on intracellular calcium are paralleled by even more direct

changes in the production of the intracellular second messenger DAG, which serves as an allosteric activator of PKC (47).

As noted in a previous section, verapamil and lithium, as well as the antimanic agent valproate, all have actions on PKC. Current data suggest that long-term lithium exposure is accompanied by a down-regulation of specific PKC isozymes (48, 49). Studies in rodents have demonstrated that chronic (but not acute) lithium produces an isozyme-selective reduction in PKC α and ϵ in the frontal cortex and hippocampus in the absence of significant alterations of the β , γ , δ , or ζ isozymes (16, 48, 50). Concomitant studies in immortalized hippocampal cells in culture show a similar reduction in the expression of PKC α and ϵ after chronic lithium exposure (48). Furthermore, chronic lithium has been demonstrated to dramatically reduce the hippocampal levels of a major PKC substrate, myristoylated alanine rich C kinase substrate (MARCKS), a protein that has been implicated in regulating long-term neuroplastic events (51).

It is noteworthy that the structurally dissimilar antimanic agent valproate produces very similar effects to those of lithium on PKC α and ϵ isozymes and MARCKS protein (17, 52–56). Interestingly, lithium and valproate appear to bring about their effects on the PKC signaling pathway by different mechanisms (48, 52). These data further support a role for PKC modulation in the treatment of mania. Given the known inhibitory properties of verapamil on PKC, it seems appropriate to consider the possibility that the observations reported here, as well as other reports of antimanic actions of this drug in the literature, may in fact be related to PKC inhibition rather than calcium channel blockade, and that the actions, clinical specificity, and use in combination treatments of other CCBs lacking effects on PKC may differ from those of verapamil. Unfortunately, very little data exist in terms of the potential isozyme specificity of verapamil's actions. Thus, although a synergistic action for verapamil and lithium could potentially arise from differential effects on PKC isozymes, this must remain speculative at present. Further research on this issue seems warranted.

In addition to the findings on drugs effects discussed above, extensive preclinical and clinical research supports the notion that PKC is an appropriate target for antimanic treatments. This family of closely related enzyme subspecies plays a major role in the regulation of neuronal excitability, neurotransmitter release, and long-term alterations in gene expression and neuronal plasticity (57). Animal studies support a role for PKC in regulating the release of dopamine (56–63), a neurotransmitter widely believed to be implicated in manic episodes. Manic-like behaviors such as increased hedonistic drive, increased tendency to use drugs, hyperactivity, and risk-taking behavior are attenuated by PKC inhibitors (14, 15). Finally, excessive activation of PKC dramatically impaired the cognitive functions of the prefrontal cortex, whereas inhibition of PKC protected cognitive function (13).

A limited number of human studies also support the idea that PKC abnormalities occur in bipolar disorder. Although perhaps an over-simplification, particulate (membrane) PKC can be viewed as the more active form of this enzyme, and thus measurement of the subcellular partitioning of PKC can be utilized to estimate the degree of activation. With this approach, Friedman et al. (64) reported that measures of membrane-bound PKC activity were elevated in platelets from manic patients, and that lithium treatment over a two-week period reduced cytosolic and membrane-associated PKC activities, and attenuated PKC activation. Wang and Friedman (65) measured PKC isozyme levels, activity, and translocation in postmortem brain tissue, and reported increased PKC activity and translocation in bipolar disorder brains compared to controls, with elevated levels of selected PKC isozymes in the cortex. The same group subsequently reported that postmortem brains from bipolar disorder subjects showed increased association with receptor for activated C kinase-1 (RACK1) (66). Since

PKC is anchored to the membrane via RACK1, these results suggest that increased association of RACK1 with PKC isozymes may be responsible for the previously observed increases in membrane PKC and in its activation.

Finally, because activation of PKC by DAG is regulated by diacylglycerol kinases (67), the findings from two recent independent genome-wide association studies of bipolar disorder are of interest. Baum et al. found diacylglycerol kinase ϵ (DGKH) to be the most highly significantly associated ($p \sim 10^{-8}$) bipolar susceptibility gene (22). The second study, from the Wellcome Trust in the UK (68), reported on different single-nucleotide polymorphisms (SNPs) than the Baum et al. study, but several SNPs in DGKH showed association with bipolar disorder at the 10^{-3} level (data available at http://www.wtccc.org.uk/info/summary_stats.shtml). Two of these SNPs were in the same region as those found to be highly significant in the Baum et al. study. Since DAG is the major activator of PKC, these results suggest that PKC signaling abnormalities may well be *etiologically* involved in bipolar disorder, and lend support to the potential utility of pharmacologic strategies targeting PKC in the treatment of mania.

A weakness in the design of our study was that the combined treatment was only administered to subjects after six weeks of protocol participation. Thus, a possibility exists that the findings may have been confounded by spontaneous recovery from the manic episode in some individuals. This issue could be addressed in future studies by including a verapamil-lithium combined treatment arm as one of the initial treatments. However, it should be noted that the response rates as shown in Fig. 1 for Phase 3 single-agent treatments cannot serve as an index of spontaneous recovery, because subjects assigned to single-agent treatment in Phase 3 had by definition already responded to that treatment in the previous phase.

Another potential weakness of our design is that subjects were treated with verapamil for only three weeks in Phase 2. If the optimal response to verapamil occurs only after a delay of more than three weeks, as is observed with several other psychotropic medications, then this could be a confounding factor in the findings regarding combination treatment. Although two of the three patients treated with verapamil alone in Phase 3 showed a positive outcome, these patients were all verapamil responders during Phase 2. We cannot rule out the possibility that treatment of Phase 2 verapamil nonresponders for an additional three weeks with verapamil alone could have produced a positive outcome as a result of longer duration of treatment. On the other hand, our linear mixed model analysis of the Phase 3 data showed that the decrease in mania scores in Phase 3 was significant regardless of whether the previous Phase 2 treatment had been verapamil or lithium. Thus, groups who received verapamil for a total of either three or six weeks both showed significant improvement with Phase 3 combination treatment. Nevertheless, it is possible that due to relatively lower lipid solubility of verapamil, longer exposure time may be needed to build up adequate brain concentrations of the drug.

In conclusion, this study provides new preliminary evidence that augmentation of lithium ($\pm$ antipsychotic) treatment with verapamil can improve therapeutic outcome in manic patients who do not respond to an initial trial of lithium ($\pm$ antipsychotic). As in the treatment of other disorders such as hypertension and epilepsy, our results suggest that rational combination therapy may have considerable utility in the treatment of this difficult disorder. Additional research could also determine whether this combination treatment will allow lower doses of individual agents to be used, potentially reducing the burden of adverse effects. Future investigations should attempt to replicate our findings in a larger sample and study combined verapamil-lithium as an initial and potentially more robust antimanic treatment. Given the evidence for involvement of PKC in the actions of antimanic drugs,

and the ability of verapamil to attenuate PKC activity, the prima facie assumption that this drug's antimanic action is exclusively based on calcium channel blockade should be reconsidered. Further study of PKC and its isozymes in relation to therapeutic outcome with verapamil and more established antimanic agents seems warranted.

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Fig. 1.

Flow diagram illustrating the treatments administered in each phase of the protocol, the number of subjects and response to treatment in each phase, and the percentage response rates observed.

Fig. 2.

Median Bech-Rafaelsen mania scores versus time during the double-blind phases of the study (Phases 2 and 3). Ratings were performed weekly. All subjects had previously failed to respond to open-label lithium treatment during Phase 1. Data shown are for the 10 subjects who were nonresponders in Phase 2 and subsequently were assigned to combined verapamil plus lithium in Phase 3. The solid line represents subjects ($n = 7$) who were treated with verapamil during Phase 2, followed by combined verapamil plus lithium in Phase 3. The dashed line represents subjects ($n = 3$) treated with continued-lithium in Phase 2, then combined verapamil plus lithium in Phase 3.

Table 1

Demographic and clinical characteristics of subjects assigned to double-blind treatment in Phase 2

Subject ID	Phase 2 treatment	Diagnosis	Age (years)	Gender (M/F)	Number of past manias	Number of past depressions	Concurrent antipsychotic used
27210	Verapamil	Bipolar I	32	M	3	2	None
27270	Verapamil	Bipolar I	43	F	6	4	Haloperidol
27801	Verapamil	Bipolar I	20	F	0	1	Risperidone
27802	Verapamil	Schizoaffective	24	F	2	4	Perphenazine
27808	Verapamil	Bipolar I	22	M	2	0	Perphenazine
28301	Verapamil	Bipolar I	33	M	4	3	Thioridazine
28304	Verapamil	Bipolar I	27	M	2	2	Perphenazine
28305	Verapamil	Bipolar I	28	F	99	0	Perphenazine
28306	Verapamil	Bipolar I	46	M	*	*	Perphenazine
73012	Verapamil	Bipolar I	53	F	3	5	None
Median or Count			30	M/F = 5/5	3	2	Proportion on antipsychotic = 8/10
27222	Lithium	Bipolar I	41	F	3	3	Risperidone
27227	Lithium	Bipolar I	33	F	5	5	Perphenazine
27244	Lithium	Bipolar I	28	F	9	6	Perphenazine
27256	Lithium	Bipolar I	40	F	5	4	Perphenazine
27275	Lithium	Bipolar I	31	F	1	4	None
27319	Lithium	Bipolar I	39	M	12	*	Perphenazine
27320	Lithium	Bipolar I	32	M	10	5	Perphenazine
27804	Lithium	Bipolar I	41	M	2	3	None
Median or Count			36	M/F = 3/5	5	4	Proportion on antipsychotic = 6/8

* Data missing.