

Is There a Relationship between Baseline and Treatment-Associated Changes in [³H]-IMI Platelet Binding and Clinical Response in Major Depression?

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A peripheral model for the central 5-HT neuron is the characterization of platelet imipramine binding. We studied an outpatient major depressive cohort who fulfilled Research Diagnostic Criteria for agitation. After a 1-week placebo lead-in, subjects were blindly randomized to either imipramine (IMI) or fluoxetine (FLU) during an 8-week, double-blind study period. Thirty-three subjects (15 IMI, 18 FLU) provided both baseline and endpoint samples for the platelet [³H]-IMI assay. Depression efficacy was comparable across the two treatments, whereas FLU was significantly more effective in reducing secondary anxiety (p = .023). Discontinuations due to an adverse event were significantly more frequent with IMI than FLU (p < .01). Baseline affinity (K_D) was mildly predictive of change in the HAMD (r = -.22; p = .07).

Whereas baseline to endpoint density (B_{max}) changes (Δ) were similar for IMI (183 ± 329 fmol/mg) and FLU (196 ± 402 fmol/mg), a statistically significant treatment difference in ΔK_D emerged (IMI -0.005 ± 0.010 pmol/ml versus FLU 0.008 ± 0.013 at p = .004). Moreover, the changes in K_D and HAMD₁₇ trended to a positive correlation among only the FLU-treated subjects (4 = 0.406, p = .095). The clinical effects of 5-HT-based selective antidepressants may be reflected by dynamic changes in the platelet 5-HT uptake apparatus. These data suggest that the baseline confirmational status of the [³H]-IMI:5-HT transporter may reflect a "capacity" for a treatment response. [*Neuropsychopharmacology* 14: 47-53, 1996]

KEY WORDS: ³H-IMI binding; Depression; Response prediction; Platelet; Tricyclic; Fluoxetine

The involvement of biogenic amines in major depression (MD) has been well chronicled (Schildkraut 1965; Maas 1978). Although the relative contribution of any

one neurotransmitter in the modulation of mood states remains obscure, a significant role for the indoleamine serotonin (5-HT) is likely (vanPraag 1986; Baldessarini 1983). However, the systematic study of central amines, such as 5-HT, across large mood-disordered populations is problematic. As an alternative, the utility of peripheral markers has been suggested (Sneddon 1973; Stahl 1977). One such marker is the imipramine (IMI) binding site on platelet (Briley et al. 1980; Langer et al. 1984).

Since the description of the IMI-binding site over 15 years ago, evidence for its functional relevance has accumulated (Langer et al. 1980). As part of a macromolecular complex, IMI and related ligand-binding sites are under allosteric modulation by 5-HT; a biphasic

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Received November 22, 1994; revised April 19, 1995; accepted May 1, 1995.

effect on the disassociation rate for the IMI-site has been described (O'Riordan et al. 1990). Evidence suggests that a strong correlation exists between brain and platelet [^3H]-IMI binding profiles (Paul et al. 1981; Rehavi et al. 1983). Evidence for site specificity includes observations that [^3H]-IMI binding can be inhibited by 5-HT uptake inhibitors in relation to their potency (Langer et al. 1980; Paul et al. 1981).

The characterization of [^3H]-IMI binding sites on human blood platelets has led to a proposed functional relationship to depression (Paul et al. 1981; Raisman et al. 1982; Suranyi-Cadotte 1980; Raisman et al. 1981). Decreased [^3H]-IMI binding, principally receptor density (B_{max}), has been reported in both human brain and platelet preparations from unmedicated depressed patients (Nemeroff et al. 1988).

In this trial, the hypothetical relationship of [^3H]-IMI binding (platelet) and clinical response to antidepressant pharmacotherapy was assessed. We evaluated 33 subjects with a Diagnostic and Statistical Manual of Mental Disorders, 3rd edition-revised (DSM-III-R) (American Psychiatric Association 1987) major depression and Research Diagnostic Criteria (RDC) (Spitzer et al. 1977) compatible agitation who had been randomized to either fluoxetine (20 to 60 mg) or imipramine (50 to 300 mg) for 6 weeks as part of a larger double-blind trial. Platelet [^3H]-IMI binding profiles were analyzed relative to a number of clinical questions including response prediction, between-drug differences, and state-associated phenomena.

METHODS

This was a double-blind, randomized parallel study. The trial was divided into two periods: a 1-week (6- to 10-day), single-blind, placebo lead-in (study period 1) and an 8-week, double-blind, randomized treatment period (study period 2). Visits were scheduled at 1-week (6- to 10-day) intervals. Subjects who met entry screening criteria and did not exhibit a 25% or greater reduction from their Hamilton Depression Rating Scale-17 item (HAMD) (Hamilton 1960) screening value during study period 1 were randomly assigned at baseline to receive either fluoxetine (FLU) 20 mg/day or imipramine (IMI) 150 mg/day (the latter titrated over a 2-week period). Dose escalation beyond the initial fixed targets was permitted after week 4 of double-blind therapy at the investigator's discretion. This study was part of a larger two-site trial ($n = 80$) previously reported (Tollefson et al. 1994); however, the [^3H]-IMI assays were collected at only one site as a study addendum ($n = 33$).

In order to be eligible for screening into the trial, candidates aged 18 to 65 years were required to meet major depression (unipolar) diagnostic criteria according to the DSM-III-R (APA 1987) and the agitated sub-

type per RDC (Spitzer et al. 1977). Subjects were also required to score a minimum of 16 on the HAMD₁₇. Principal exclusionary criteria included serious concomitant medical illness, recent use or coprescription of any centrally active medications, and other DSM-III-R axis-I comorbidity.

PLATELET PREPARATION

Platelets for the binding studies were obtained using a modification of the methods of Briley et al. (1979, 1980) and Asarch et al. (1980). Using evacuated glass tubes containing 2 ml of acid citrate-dextrose solution as an anticoagulant, 35 ml of whole blood was withdrawn from the antecubital vein. Platelet-rich plasma was obtained by centrifugation at 100 g for 30 minutes. The platelet-rich plasma was then centrifuged (16,000 g for 10 minutes at 4°C) and the resulting platelet-rich pellet was suspended in 50 mmol of TRIS buffer (pH, 7.5 at 4°C) containing 15 mmol of sodium chloride and 20 mmol of EDTA acid using Polytron homogenization (Polytron, Brinkmann Instruments, Westbury, NY) medium probe at setting of 4 for 10 to 15 seconds. After centrifugation at 39,000 g for 10 minutes, membranes were prepared by hypotonic lysis in 5 mmol TRIS (pH, 7.5), containing 5 mmol EDTA and then homogenized and centrifuged at 39,000 g for 10 minutes.

The supernatant was discarded and the pellet was washed by resuspension in 70 mmol/L TRIS (pH, 7.5), and centrifuged again at 39,000 g for 10 minutes. The washed pellet was finally resuspended in the assay buffer, which consisted of 50 mmol/L TRIS (pH 7.5 at 4°C) containing 120 mmol/L of sodium chloride and 5 mmol/L of potassium chloride. Aliquots were taken for protein determination according to the method of Lowry et al. (1951) and the remainder frozen at -70°C until used in the binding assay. No differences in [^3H]-IMI binding properties between frozen and unfrozen platelet preparations have previously been seen.

BINDING ASSAY

Platelet [^3H]-imipramine binding was determined using a modification of the procedure of Raisman et al. (1981; 1982). In each sample, total binding was assayed at seven different concentrations (0.25 nmol/L to 8 nmol/L) of tritiated [^3H]-imipramine (specific activity, 45.4 Ci/mmol); the K_D was approximately 1 nmol. Each assay tube (all concentrations were measured in triplicate) contained approximately 100 μg of platelet protein, and the final assay volume was 250 μL . Tubes were incubated with the radiolabel for 60 minutes on ice. The reaction was stopped by addition of 5 ml of ice-cold assay buffer, and bound radioactivity was col-

lected by filtration under vacuum over glass fiber filters presoaked for 3 hours in buffer. The filters were then washed three times with 5 ml of ice-cold assay buffer. Nonspecific binding was defined as binding persisting in the presence of 100 μ mol of desipramine. Specific binding of [³H]-imipramine to platelet membranes was calculated as the difference between total binding minus nonspecific binding. Specific binding represented 80% of total binding, which was less than 5% of the total counts added. Scatchard analysis was used to obtain the B_{max} and K_D for tritiated imipramine (Scatchard 1949). Linear regression of Scatchard plots was used to determine the correlation coefficient r . Assays with r less than 0.90 were excluded. The interassay coefficient of variation for this method is approximately 10% for B_{max} and 5% for K_D .

STATISTICAL PROCEDURES

SAS (SAS Institute Inc. 1990) procedures were used to perform all statistical analyses. Comparisons between treatment groups at baseline, for demographic characteristics, were made using either a 2×2 Pearson χ^2 test or a Wilcoxon rank sum test. Patient disposition, in terms of the reasons for discontinuation from the study, were analyzed using 2×2 Pearson χ^2 tests. Baseline and changes from baseline to endpoint for all clinical measures (HAMD₁₇, HAMA, ASIQ, ARS) were compared between treatment groups using a one-way ANOVA on rank transformed data. For K_D and B_{max} , within-treatment group comparisons were performed using the paired Wilcoxon signed rank test and between-treatment comparisons used a one-way ANOVA on rank transformed data.

Correlations between platelet and clinical measures were made using Spearman's rank-order correlation coefficient. Correlation coefficients within each treatment group, and for both treatments combined, were calculated.

RESULTS

A total of 33 subjects provided baseline and endpoint samples for the platelet [³H]-IMI binding assay. Individual baseline demographic features are summarized in Table 1 and were comparable across treatment assignments.

Of the 33 study participants, 17 FLU (94.4%) and seven IMI (46.7%) subjects completed all 8 treatment weeks ($p < .01$). The most striking explanation for this difference was in those FLU (5.6%) versus IMI (53.3%) subjects who discontinued the trial early due to an adverse event ($p < .01$). Among various event categories, an intolerable central nervous system experience ex-

Table 1. Baseline Descriptions of the Study Population

	Fluoxetine	Imipramine	<i>p</i> -Value
Female	13 (72.2%)	10 (66.7%)	0.730
Male	5 (27.8%)	5 (33.3%)	
Caucasian	18 (100.0%)	13 (86.7%)	—
Black	—	1 (6.7%)	
Other	—	1 (6.7%)	
Age			
Mean (SD)	43.4 (10.1)	38.6 (10.5)	0.143
Range	20–59	21–54	

plained many of the early IMI discontinuances. Patient dispositions are summarized in Table 2.

Baseline to last visit (all subjects with at least one postrandomized visit) change scores across the primary efficacy measures are summarized in Table 3. While statistically significant in its superior anxiolytic profile, FLU was numerically favored on three of the four clinical assessments.

Table 4 summarizes the [³H]-IMI platelet binding results for all randomized subjects who provided a baseline and endpoint sample. No apparent baseline differences were evident between the treatment groups relative to either K_D or B_{max} . A trend for baseline K_D to correlate with HAMD₁₇ ($r = 0.22$; $p = .07$), HAMA ($r = -0.23$; $p = .07$) and agitation rating score ($r = -0.24$; $p = .06$) change across both treatments was evident. Figure 1 displays baseline K_D relative to clinical response outcome. A higher baseline K_D was associated with a greater likelihood of treatment response ($p < .05$). However, no significant between-treatment differences were detected. Within group comparisons using a Wilcoxon-signed rank analysis indicated a possible nonspecific treatment effect on [³H]-IMI receptor density (B_{max}). In both treatment groups, B_{max} median and mean values increased during the trial (see Table 4). However, there was no statistically significant difference between treatment groups ($p = .65$) nor relationship to clinical outcome. In contrast, a statistically significant increase in receptor affinity (K_D) at endpoint characterized the FLU ($p = .024$) but not the IMI study arm. Within both treatment groups pooled endpoint K_D values correlated significantly with treatment-asso-

Table 2. Patient Disposition

Disposition	Treatment Group	
	Fluoxetine	Imipramine
Randomized	18 (100.0)	15 (100.0)
Completed	17 (75.0)	7 (42.5)
Discontinued:		
ADE	1 (5.6)	8 (53.3)
Lack of efficacy	0	0
Other	0	0

Table 3. Baseline, Endpoint, and Change Scores

Variable	Treatment	n	Baseline		Endpoint			Change			p-Value ^d	
			Mean	SD	Media n	Mean	SD	Media n	Mean	SD		Media n
HAMD-17	Flx	18	21.4	2.6	21.5	8.1	6.6	7.0	-13.3	5.3	-15.5	0.145
	Imi	15	20.7	2.7	21.0	11.9	7.6	10.0	-8.7	8.0	-10.0	
HAMA	Flx	18	19.7	3.5	20.0	7.4	6.7	7.0	-12.3	5.2	-13.0	0.023
	Imi	15	19.5	3.3	18.0	12.7	6.7	12.0	-6.8	7.3	-5.0	
HAMD Subscale 5	Flx	18	9.8	1.6	9.5	3.1	3.0	3.0	-6.7	3.2	-7.0	0.236
	Imi	15	10.1	1.6	10.0	5.5	5.3	4.0	-4.5	5.1	-5.0	
ASIQ	Flx	18	20.8	17.8	18.5	13.4 ^b	10.0	17.0	-6.4 ^c	13.6	-2.0	1.000
	Imi	15	33.8	25.8	28.0	24.5	26.4	20.0	-9.3	19.6	-3.0	
ARS	Flx	18	10.1	3.5	9.5	4.0	4.0	3.5	-6.1	4.4	-6.5	0.155
	Imi	15	10.9	2.3	11.0	7.3	4.8	8.0	-3.6	5.2	-4.0	

^a n = 17.^b p-Values are between-group comparisons on rank-transformed data.^c n.b. = No differences between treatments at baseline.

ciated HAMA change ($r = -0.38$; $p = .02$) and trended with ARS change ($r = -0.31$; $p = .06$); however, no differences emerged between the two therapies. In the analysis of baseline to endpoint change (Δ) between-group difference in ΔK_D was highly significant ($p = .004$). ΔK_D was in a positive direction for FLU and conversely, negative for IMI. Of further interest, a correlational trend between ΔK_D and $\Delta HAMD_{17}$ emerged in the FLU arm ($r = 0.41$; $p = .09$) but not the IMI arm ($r = 0.07$; $p = .81$).

DISCUSSION

In this comparative trial in major depression with agitation, both FLU and IMI demonstrated comparable efficacy (see Table 3). This was consistent with previous comparisons reviewed by Benfield and associates (1986). However, the success of pharmacotherapy rests on a favorable risk: benefit ratio. In this cohort, IMI was

significantly less well tolerated than FLU—i.e., more IMI patients discontinued their antidepressant before the end of the eighth treatment week because of one or more adverse events.

Platelet and neuron share a number of common embryologic features. Platelets, like the human neuron, exhibit an amine-precursor-uptake decarboxylation system (Pearse 1986). Accordingly, human platelets represent a promising model to investigate central 5-HT activity. Although the adequacy of the platelet model is still in question (Mellerup et al. 1982; Tang et al. 1985), several groups have demonstrated comparable binding kinetics with the neuron (Rehavi et al. 1980; Langer et al. 1981). More recently the identical composition of the brain and platelet transporters, and their encoding by a single-copy gene, were reported (Lesch et al. 1993). IMI and related ligand-bound sites have been proposed to allosterically modulate 5-HT transport across membranes as part of a macromolecular complex (Phillips and Williams 1983). Accordingly these sites have been investigated for their utility as biologic markers.

Table 4. Descriptive Statistics for B_{max} and K_D

Parameter	Treatment Group	n	Baseline		Endpoint		Change		p-Value ^a
			Mean	Median	Mean	Median	Mean	Median	
B_{max} (fmol/mg)	FLU	18	1693.5	1738.1	1928.4	1920.1	196.5	163.1	.067
	IMI	15	1636.9	1560.3	1870.7	1841.8	182.6	251.3	.035 (.646)
K_D (nmol/L)	FLU	32	0.037	0.031	0.043	0.040	0.008	0.006	.024
	IMI	33	0.037	0.035	0.031	0.030	-0.005	-0.003	.131 (.004)

^a p-Values are within-treatment group comparisons using a Wilcoxon-signed rank test; values in parentheses are between-treatment group comparisons using a one-way ANOVA on rank transformed data.

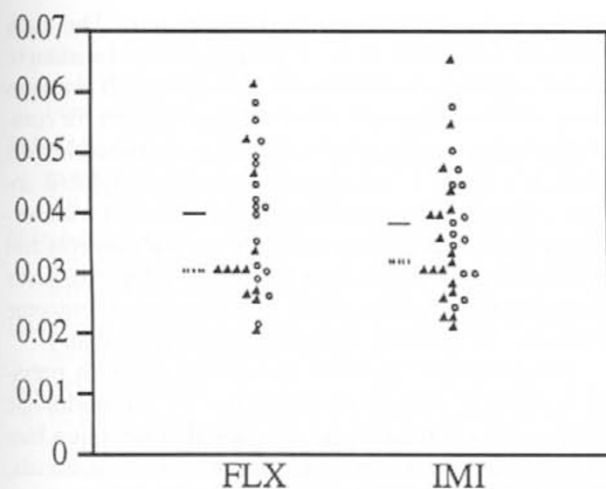


Figure 1. Display of baseline K_D and responder status at endpoint. Significance: no significant difference between treatments ($p = .695$), significant difference between responders and non-responders ($p = .046$), and no significant treatment response interaction ($p = .615$). Legend: Δ non-responders, \circ responders, \cdots median K_D for non-responders, $—$ median K_D for responders.

Affinity

Observation 1. In the present study [³H]-IMI binding profiles were correlated with a series of clinical outcome measures. A key observation was that a higher baseline receptor affinity for [³H]-IMI was related to a greater level of clinical improvement. This relationship held true for subjects assigned to either IMI or FLU. One interpretation could be that pharmacologic response (at least to the two antidepressants examined in this study) is likely to occur only when a certain level of baseline affinity is present. If this minimal threshold is not present, the dynamic capacity of the transport apparatus may be incapable of mediating drug response. In such cases the conventional uptake inhibitors may prove less than optimal in achieving symptomatic improvement.

Observation 2. In this trial was a positive trend between ΔK_D and Δ HAMD among subjects randomized to FLU ($p = .09$) emerged. In contrast, no such relationship occurred among IMI subjects. The latter observation is consistent with previous findings reported for both desipramine and imipramine. A possible understanding of this difference is that although both antidepressants block 5-HT uptake, they may bind at two related but different sites within the uptake complex (Ahtee et al. 1981; Raisman et al. 1982; O'Riordan et al. 1990). The [³H]-IMI binding site and the 5-HT recognition site are linked via a sodium-dependent mechanism (Abott et al. 1982). In contrast to IMI, changes in [³H]-IMI binding induced by SSRIs such as FLU are indirect and mediated via the 5-HT recognition site

(Wennogle and Meyerson 1983). Thus, the divergent results between the two treatments seen in the present study suggest FLUs binding directly at the 5-HT transporter may have better reflected the dynamic process associated with a positive treatment outcome. However, the observation that both FLU and IMI had comparable overall efficacy illustrates that modification of 5-HT transporter affinity is not mandatory for clinical improvement. Obviously the additional effects of IMI and its desmethylated metabolite on noradrenergic systems cannot be dismissed. It is further possible that the conformational state of the 5-HT substrate recognition site could be sensitized through the presence of an endogenous competitive 5-HT antagonist. Barkai and colleagues reported that increasing amounts of a membrane-derived protein that competitively inhibited [³H]-IMI binding (K_D but not B_{max}). Paul et al. (1980) also observed that the addition of plasma to a cortical membrane preparation significantly inhibited binding. Such findings suggest the existence of an endogenous inhibitor in platelet preparations capable of mediating a concentration-dependent effect on transporter site affinity. Furthermore, this could explain the apparent predictive threshold of baseline K_D reported in this present study. Furthermore, if the inhibitor were competitive, a greater treatment-associated change might be expected with a competitive 5-HT uptake inhibitor such as FLU than a noncompetitive one (imipramine).

Observation 3. Baseline and endpoint [³H]-IMI K_D were related to both HAMA and ARS scores. However, there was no apparent relationship between the magnitudes of baseline to endpoint change. Thus, any suggestion that features of anxiety or agitation are reflected at the transporter is tenuous. However, previously suggested relationships between the 5-HT system and adverse drug experiences such as anxiety, hostility, restlessness, etc., make the possible link intriguing and worthy of future investigation.

Density

Baseline B_{max} values in the present study are difficult to interpret in the absence of a control group. The mean study population value of 1665 fmol/ng was somewhat lower than previously reported among matched controls as conducted by the same laboratory (Nemeroff et al. 1988). Briley et al. (1980) reported that depressed subjects demonstrate reduced [³H]-IMI binding density. Although confirmed by others, this observation has not been universal. Significant variability in [³H]-IMI assay methods and an apparent overlap in the distribution of depressed and control subject values renders any conclusions difficult. Other reported variables were controlled for in the present study including subject's age, time of blood draw, seasonality, and confounding

drug treatments. Regardless, some degree of reduced [^3H]-IMI B_{max} among major depressives is defensible (Nemeroff et al. 1988). This is particularly true in view of the recent meta-analysis of platelet [^3H]-IMI binding, which revealed a highly significant reduction in depressed patients.

Of interest B_{max} was altered after pharmacotherapy. The density of [^3H]-IMI binding sites demonstrated an increase from baseline to endpoint in both treatment arms. This was congruent with the conclusion of Healy and colleagues (1991) that "an increase in [^3H]-IMI binding may be common to all antidepressant treatments." The observed direction of change was consistent with that reported after TCA exposure by other investigators (Braddock et al. 1984; Arora and Meltzer 1988; Wagner et al. 1987). However, in the present study, change in B_{max} was not significantly related to any of the clinical outcome measures used. More likely a change in B_{max} during treatment of either depressives or normal controls, as noted by Healy et al. (1991), is independent of a specific reduction in depressive signs and/or symptoms.

CONCLUSIONS

Among RDC compatible major depressives with baseline agitation, IMI and FLU were similarly effective. Of note, FLU was better tolerated as reflected by significantly fewer premature treatment discontinuations due to an adverse event. Because the mode of action among differing antidepressant drugs may shed light on the associated biology of the affective disorders, the platelet findings reported here are of potential interest.

Analyses involving the platelet 5-HT transporter revealed that baseline K_D correlated with endpoint score reductions in depression, anxiety, agitation, and suicidality. This observation was independent of study drug randomization. These results suggested that a certain conformation state of the 5-HT transporter complex may predict or be required for a positive treatment outcome. Future research efforts might be directed toward the identification of one or more competitive endogenous antagonists at the transporter.

A second observation was that only FLU-treated subjects demonstrated an interrelationship between baseline to endpoint changes in [^3H]-IMI binding affinity and HAMD_{17} . Moreover, the direction of treatment-associated K_D change was opposite for FLU versus IMI. Whereas the magnitude of K_D change could be questioned for its biologic significance, the statistically significant relationship to several clinical outcome measures argues for its relevance. Future testing of the hypothesis that the scope of antidepressant response (HAMD_{17}) may be reflected by a modification of the 5-HT transporter's affinity with selective

agents, e.g., fluoxetine, is encouraged. The data reported here provide for a prediction of the sample size necessary to minimize the risk of type II errors in future studies. B_{max} , a more frequent subject for comparison between depressed subjects and controls, was associated with a nonspecific treatment-related increase. The direction of change was consistent with several previous reports among both normal controls and depressed subjects. Because the observed increase was not significantly related to any of the treatment outcome measures, its clinical relevance is questionable.

The observations from this study (as with previous literature) should be tempered by methodologic variance between laboratories, possible selection bias among subjects participating in controlled clinical trials, interindividual variability of [^3H]-IMI binding parameters, platelet heterogeneity, etc. However, this sample was rigorously diagnosed, assessed, and monitored for compliance. Efforts were in place to minimize intrasubject variables. The methods for the [^3H]-IMI assay have been previously validated and were conducted by an experienced group.

In view of the findings, larger sample sizes using similar methodology are encouraged to validate the observed results. If platelet [^3H]-IMI binding affinity, or perhaps more specifically binding at the transporter, reflects corresponding changes within the neuronal 5-HT complex, then such studies present an opportunity to explore and better understand the phenomenon of treatment response and therapeutic resistance.

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