Effectiveness of Imazodan for Treatment of Chronic Congestive Heart Failure

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A 12-week, multicenter, double-blind, randomized, placebo-controlled trial of imazodan, a type III phosphodiesterase inhibitor, was conducted in 147 patients with congestive heart failure to determine clinical efficacy and safety. Patients were randomized to placebo or 2, 5 or 10 mg of imazodan administered twice daily. Patients were maintained on their standard therapy including diuretics, digoxin and an angiotensin-converting enzyme inhibitor. The mean ejection fraction was 23 ± 10%. Exercise time increased from baseline in all 4 groups. There was no significant difference observed between the placebo group and any of the treated groups with regard to exercise time, ejection fraction, frequency of ventricular premature complexes or ventricular tachycardia. When analyzed by intent to treat, the placebo mortality was 7% (3 of 44) and the imazodan mortality was 8% (8 of 103) (p = not significant). This study failed to demonstrate that imazodan provided any benefit in exercise performance when compared with placebo.

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The optimal treatment of congestive heart failure (CHF) remains a major clinical issue despite treatment with digitalis and diuretics. The use of vasodilators,[1] especially the angiotensin-converting enzyme inhibitors,[2,3] have improved both symptoms and prognosis. Nevertheless, the mortality of patients with advanced CHF remains high and many patients continue to be severely symptomatic despite medical therapy. Type III phosphodiesterase inhibitors may provide an alternative or additional treatment for CHF. These agents have demonstrated hemodynamic efficacy in the acute setting.[4–10] However, long-term trials studying the effect on exercise performance have generally been disappointing.[11–16]

Imazodan hydrochloride (CI 914), a selective inhibitor of type III phosphodiesterase, blocks the degradation of cyclic adenosine monophosphate leading to increased intracellular concentrations, thereby increasing myocardial contractility. Imazodan also acts as a peripheral vasodilator in a variety of animal models.[17] Initial human studies[8] indicated that after intravenous or oral administration of imazodan, cardiac output increases and pulmonary capillary pressure and systemic vascular resistance decrease. Pharmacokinetic studies suggested a half-life of 15 to 25 hours, allowing twice-daily administration.

The primary objective of this study was to determine whether, during a 12-week period, 1 or more dosing regimens of orally administered imazodan were more effective than placebo at increasing exercise time on a treadmill in patients with moderate to severe chronic CHF.

METHODS

Patient population: The study was designed to enroll 160 patients. Recruitment was discontinued after 147 patients were randomized after an analysis of the initial 80 patients indicated that a positive effect of the drug was unlikely to be achieved. Each patient was required to provide written informed consent, be aged >18 and <75 years and have chronic CHF in which dyspnea or fatigue limited their exercise. Patients were allowed to continue receiving antiarrhythmic agents except for disopyramide. Short-acting nitrates and constant doses of digitalis and angiotensin-converting enzyme inhibitors were permitted. Diuretic doses could be decreased, but

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TABLE I Baseline Characteristics of Patient Population

	No. of Pts. (n = 147)	Placebo (n = 44)	Imazodan 2 mg BID (n = 34)	Imazodan 5 mg BID (n = 37)	Imazodan 10 mg BID (n = 32)	p Value
Men	128 (87)	39 (89)	32 (94)	30 (81)	27 (84)	0.39
Age	59 ± 9.7	57.5 ± 9.8	59.6 ± 12.2	60.9 ± 7.2	58.3 ± 9.2	0.43
NYHA						0.28
Class III	72 (49)	22 (50)	12 (35)	18 (49)	20 (63)	
Class IV	7 (5)	2 (5)	1 (3)	3 (8)	1 (3)	
Etiology						0.19
CAD	78 (53)	20 (45)	23 (68)	20 (54)	15 (47)	
SH	16 (11)	7 (16)	0 (0)	5 (14)	4 (13)	
IDC	35 (24)	12 (27)	7 (21)	10 (27)	6 (19)	
Other	18 (12)	5 (11)	4 (12)	2 (3)	7 (22)	
Exercise time(s)	506 ± 145	529 ± 152	526 ± 128	462 ± 160	502 ± 127	0.18
Ejection fraction (%)	23 ± 10	24 ± 11	22 ± 7	20 ± 9	25 ± 11	0.18
Holter						
VPCs/hour	153 ± 253	189 ± 270	170 ± 313	106 ± 185	140 ± 228	0.52
24-hour HR	86 ± 12	88 ± 11	85 ± 11	84 ± 13	85 ± 12	0.36
V runs/day	2.3 ± 9.2	4.7 ± 14.1	1 ± 2.5	2.2 ± 9.0	0.5 ± 0.9	0.19
Concurrent medications						
Digitalis	124 (84)	38 (86)	27 (79)	32 (86)	27 (84)	0.83
Lasix dose mg/day	91 ± 67	98 ± 73	86 ± 68	91 ± 61	87 ± 65	0.86
ACE inhibitors	81 (55)	25 (57)	20 (59)	20 (54)	16 (50)	0.90

ACE = angiotensin-converting enzyme inhibitors; BID = twice daily; CAD = coronary artery disease; HR = heart rate; IDC = idiopathic dilated cardiomyopathy; NYHA = New York Heart Association; Other = valvular disease (5), congenital heart disease (3), heart disease not specified (10); SH = systemic hypertension; VPCs = ventricular premature complexes; V runs = ventricular runs > 4 beats at a rate > 100 beats/min.

not increased from that taken during the placebo baseline phase. Women with child-bearing potential were excluded.

Study design: This study was a double-blind, parallel-group comparison of imazodan therapy (2, 5 and 10 mg, administered twice daily) with placebo in patients with chronic CHF. The study began with a 2- to 4-week, single-blind, placebo phase during which routine laboratory and cardiac studies were performed.

During the baseline phase, treadmill exercise tests were performed each week using the Naughton protocol modified by Weber et al.[18] Before randomization, 2 consecutive symptom-limited maximal exercise tolerance tests had to be performed between 4 and 12 minutes and had to be reproducible to within 10%. Patients were then randomly assigned to 1 of the 4 treatment groups and were evaluated every week for 12 weeks. Exercise tests were performed at baseline and at weeks 4, 8 and 12. Ejection fraction was measured by gated radionuclide angiography at baseline and 12 weeks. Twenty-four-hour Holter monitorings were performed at baseline and weeks 1, 4 and 10, and were analyzed at a central site (Cardiodata Inc.). Each tape was analyzed for average 24-hour heart rate, ventricular premature complexes per hour and ventricular runs (>4 beats at a rate >100 beats/min/day).

Patients who withdrew from the study because of lack of efficacy before completing the 12 weeks were eligible to enter an open-label treatment program in which imazodan could be initiated or increased for symptomatic relief of CHF.

Differences in baseline variables between treatment groups were analyzed by chi-square analysis for discontinuous variable and analysis of variance. Changes in exercise times, ejection fraction, 24-hour heart rate, frequency of ventricular premature complexes and ventricular runs were analyzed by 1 factor analysis of variance using treatment group as the independent factor. A p value <0.05 was considered significant. Definitions from the Cardiac Arrhythmia Pilot Study[19] were used for determining proarrhythmia.

Results were analyzed by intent to treat and also by end point analysis of the last laboratory determinant before the time the double-blind phase was broken because of increased symptoms or death. For the double-blind phase and through 84 days, the relation of the treatment group to survival was examined by estimating survival curves for each treatment using the product-limit method of Kaplan and Meier. The equality of the 4 survival distributions was tested by the log rank (Mantel-Cox) statistic.

Data collection was performed by Parke-Davis Pharmaceutical Research Division. Data were analyzed at the Division of Cardiology, Henry Ford Heart and Vascular Institute with the assistance of the Department of Biostatistics, Henry Ford Hospital.

A safety committee (see Appendix), established before the study initiation, was charged with reviewing

the safety data. The members of the committee were unaware of patient characteristics and had access to the dose of drug each patient was receiving, description of deaths and other adverse events, 24-hour Holter monitor results and clinical laboratory findings.

RESULTS

The baseline characteristics of the 147 patients who entered the study are listed in Table I. The treatment groups were similar with respect to all baseline demographic variables (Table I).

Exercise response: Increases in exercise tolerance were seen in all groups compared with baseline (Figure 1). There was no significant difference between the 4 treatment groups in the change in exercise time from baseline to weeks 4, 8 and 12 (Table II). There was no significant change in ejection fraction between the treatment groups during the study period (Table III).

Of the 146 patients who were classified by New York Heart Association class during both baseline and double-blind phases, 122 (84%) remained in the same class, 12 patients (8%) improved, and 12 were worse. There was no significant difference in class change between the 4 treatment groups ($p = 0.7$).

Arrhythmias: In the imazodan 5- and 10-mg groups, a significant increase in 24-hour average heart rates was observed at 1, 4 and 10 weeks (Table IV).

No significant change occurred in the average frequency of ventricular premature beats per hour (Table V) or runs of ventricular ectopic beats compared with baseline in any treatment group at 1, 4 or 10 weeks. According to the Cardiac Arrhythmia Pilot Study[19] definitions there were 15 patients who had proarrhythmia on the week 1 Holter. These were distributed evenly through the 4 treatment groups.

Safety: Patient withdrawals during the double-blind phase are listed in Table VI. There was a tendency for greater withdrawal from the placebo group for lack of efficacy than from the imazodan treatment groups. No adverse effects related to imazodan were reported. Investigators reported 7 patients with severe adverse effects requiring withdrawal from the study: These included stomach irritation, resuscitated sudden death,

TABLE II Change in Exercise Time (seconds) from Baseline

Visit (week)	Placebo	Imazodan (2 mg BID)	Imazodan (5 mg BID)	Imazodan (10 mg BID)	p Value
4	24 ± 108	83 ± 79	75 ± 121	32 ± 123	0.08
8	45 ± 114	65 ± 103	67 ± 127	0 ± 127	0.16
12	48 ± 158	83 ± 129	99 ± 128	52 ± 161	0.58

TABLE III Change in Ejection Fraction (%)

	Placebo	Imazodan (2 mg BID)	Imazodan (5 mg BID)	Imazodan (10 mg BID)
No. of pts.	31	29	28	23
Baseline (%)	26 ± 11	21 ± 8	22 ± 9	25 ± 12
End point (%)	24 ± 11	22 ± 9	22 ± 12	25 ± 14
Difference (%)	−2.4 ± 9.9	0 ± 6.5	0.25 ± 6.9	−0.09 ± 6.7
p Value	0.18	1	0.85	0.95

TABLE IV Change in Average 24-Hour Heart Rate

Week	Placebo	Imazodan (2 mg BID)	Imazodan (5 mg BID)	Imazodan (10 mg BID)	p Value
1	1.5 ± 4.7	0.5 ± 6.0	4.8 ± 7.4	4.8 ± 6.0	<0.005
4	−0.5 ± 6.6	2.0 ± 9.0	4.3 ± 8.8	6.4 ± 7.0	0.005
10	−2.2 ± 6.6	−0.2 ± 6.7	4.3 ± 10.6	6.0 ± 10.3	<0.005

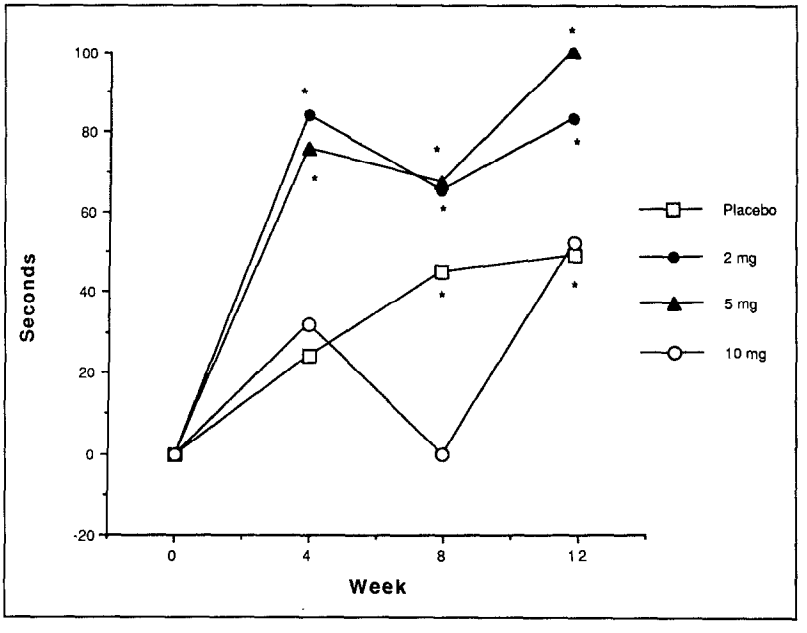

FIGURE 1. Change in exercise time during the follow-up period in the groups taking placebo and 2.5, 5 or 10 mg of imazodan twice daily. *p <0.05 change from baseline.

TABLE V Frequency of Ventricular Premature Complexes Per Hour

	Placebo	Imazodan (2 mg BID)	Imazodan (5 mg BID)	Imazodan (10 mg BID)	p Value
Baseline	189 ± 270	170 ± 313	106 ± 185	140 ± 228	0.52
Week 1	190 ± 237	188 ± 327	143 ± 179	109 ± 158	0.49
Week 4	221 ± 287	189 ± 373	163 ± 172	98 ± 114	0.33
Week 10	197 ± 266	167 ± 339	163 ± 253	122 ± 172	0.82

TABLE VI Reasons for Patient Withdrawal During Double-Blind Treatment and Numbers of Patients Completing the 12-Week Period

	Placebo	Imazodan (2 mg BID)	Imazodan (5 mg BID)	Imazodan (10 mg BID)
Death	0	0	4	3
Adverse experience	2	0	3	2
Lack of efficacy	3	1	0	0
Concurrent illness	2	2	0	1
Other withdrawal	6	2	6	4
Completed week 12	32	30	26	22

lightheadedness and headache, nausea, anorexia and weakness, postural dizziness and hypotension, worsening CHF and ventricular arrhythmias.

There were 13 patients with ventricular arrhythmias reported as adverse events by the investigators. Of these, 6 were receiving placebo, 1 was receiving imazodan 2 mg, 4 imazodan 5 mg and 2 imazodan 10 mg twice daily.

By intent-to-treat analysis the mortality in the placebo group was 7% (3 of 44) and in the imazodan groups 8% (8 of 103) (p = not significant). During the double-blind phase of the study there were 7 deaths, 5 of them sudden. Four deaths occurred in the imazodan 5 mg and 3 in the imazodan 10 mg groups. There were 4 additional deaths during the 12-week period in patients who were withdrawn from the double-blind phase. Three were originally in the placebo group and at the time of death 1 of these was taking imazodan 5 mg and another 15 mg twice daily. One was initially in the imazodan 10-mg group, but was withdrawn while hospitalized for CHF and died 11 days later. Patients who died had lower ejection fraction (14 vs 23%, p <0.02), lower baseline exercise times (379 vs 512 seconds, p <0.02), and were less likely to be taking an angiotensin-converting enzyme inhibitor (14 vs 57%, p <0.03).

DISCUSSION

This study examined the efficacy and safety of a new type III phosphodiesterase inhibitor over a range of doses. During the 3 months of follow-up, exercise performance and ejection fraction did not significantly improve when compared with the effect of the placebo. The drug appeared to be well tolerated. Frequency of ventricular ectopy did not increase, although there was the increase in heart rate seen in the patients taking the larger doses of imazodan. The cause of this chronotropic response has not been previously described and warrants further investigation.

Previous studies with imazodan (CI-914),[8] its analog CI-930[9] and other phosphodiesterase inhibitors indicate that intravenous and oral therapy results in an increase in cardiac output and a reduction in peripheral vascular resistance. Although most studies suggest that these drugs have a positive inotropic action, it is difficult, in humans, to separate the effect on contractility from the direct vasodilatation. Patients treated with CI-930[9] were observed to have an increase in frequency of ventricular ectopy which was not found in this study.

Other phosphodiesterase inhibitors have been evaluated for the treatment of chronic CHF. Amrinone[4] and milrinone,[5] both bipyridine derivatives, have significant inotropic and vasodilator effects in the acute setting. The effects of long-term amrinone therapy on exercise performance have been disappointing. Massie et al[12] compared amrinone with placebo in 99 patients with chronic CHF and found no difference in exercise ability over a 12-week period. A multicenter placebo-controlled comparison of milrinone with digoxin[13] found an improvement in exercise performance in patients given either milrinone or digoxin when compared with placebo, but no additional benefit to the combination of milrinone and digoxin.

Enoximone[6] and piroximone,[7] like imazodan, are imidazol derivatives with acute hemodynamic effects similar to amrinone and milrinone. Whereas there have been numerous acute and chronic studies of enoximone[20] in patients with heart failure, few have been controlled. Uretsky et al,[16] in a study of 102 patients with chronic CHF, found no improvement in exercise capacity or symptoms compared with placebo.

The design of this study would have permitted an analysis of mortality over 12 weeks although the power was not calculated for studying survival. However, patients who discontinued the double-blind study early were allowed to begin the open-label phase immediately. This resulted in the administration of imazodan, before 84 days, to some patients randomized to placebo.

By intent-to-treat analysis for the 84 days, there was no significant difference in mortality between the placebo group (7%) and those treated with imazodan (8%). The fact that all the deaths that occurred during the double-blind phase were in patients taking large doses of imazodan is disturbing. The effect of phosphodiesterase inhibitors on survival has not been studied in a rigorous manner. Most of the initial hemodynamic studies were conducted in patients with severe heart failure in whom the expected mortality was high. In the few placebo-controlled studies,[12,13,16] as in this study, there has been a disturbing and consistent trend toward increased mortality in the treatment group. Colucci[21] reviewed 571 patients enrolled in 3 placebo-controlled studies of milrinone and concluded that there was no evidence for an adverse effect of that drug on mortality. In the largest of these studies, however, there was a trend ($p = 0.08$) toward reduced survival in the milrinone-treated group. The investigators attributed this to a baseline imbalance in ejection fraction which suggested that milrinone was given to a sicker population. After post hoc adjustment of this covariant, the trend toward increased mortality was no longer present. Recently, a mortality study of milrinone in patients with class III and IV heart failure was prematurely terminated because of increased mortality in the milrinone-treated group (Schwartz R, personal communication, Sterling Winthrop, Inc.). Uretsky et al[16] found a higher mortality in enoximone-treated patients than in a placebo-treated group. This increase in mortality was significant when the study was analyzed by double-blind ($p < 0.05$) or intention-to-treat ($p < 0.05$) analyses. In our study, the increased mortality rate was observed in the large dose ranges, suggesting a dose effect.

REFERENCES

1. Cohn JN, Archibald DG, Ziesche S, Franciosa JA, Harston WE, Tristani FE, Dunkman WB, Jacobs W, Francis GS, Flohr KH. Effect of vasodilator therapy on mortality in chronic congestive heart failure. Results of a Veterans Administration Cooperative Study. *N Engl J Med* 1986;314:1547–1552.

2. Captopril-Digoxin Multicenter Research Group. Comparative effects of captopril and digoxin in patients with mild to moderate heart failure. *JAMA* 1988;259:539–544.

3. CONSENSUS Study Trial Group. Effects of enalapril on mortality in severe heart failure. Results of the CONSENSUS Trial (Cooperative North American Enalapril Survival Study). *N Engl J Med* 1987;316:1429–1435.

4. Benotti JR, Grossman W, Braunwald E, Davolos DD, Alousi AA. Hemodynamic assessment of amrinone. *N Engl J Med* 1978;299:1373–1377.

5. Baim DS, McDowell AV, Cherniles J, Monrad ES, Parker JA, Edelson J, Braunwald E, Grossman W. Evaluation of a new bipyridine inotropic agent — milrinone — in patients with severe congestive heart failure. *N Engl J Med* 1983;309:748–756.

6. Crawford MH, Richards KL, Sodums MT, Kennedy GT. Positive inotropic and vasodilator effects of MDL 17,403 in patients with reduced left ventricular performance. *Am J Cardiol* 1984;53:1051–1053.

7. Petein M, Levine TB, Cohn JN. Hemodynamic effects of a new inotropic agent, piroximone (MDL 19205), in patients with chronic heart failure. *J Am Coll Cardiol* 1984;4:364–371.

8. Jafri SM, Burlew BS, Goldberg AD, Rogers A, Goldstein S. Hemodynamic effects of a new type III phosphodiesterase inhibitor (CI-914) for congestive heart failure. *Am J Cardiol* 1986;57:254–259.

9. Jafri SM, Burlew BS, Goldberg AD, Olsen S, Froelich JW, Goldstein S. Hemodynamic, pharmacokinetic and clinical response to CI-930 in congestive heart failure due to ischemic or dilated cardiomyopathy. *Am J Cardiol* 1987;59:1126–1130.

10. Maskin CS, Sinoway L, Chadwick B, Sonnenblick EH, LeJemtel TH. Sustained hemodynamic and clinical effects of a new cardiotonic agent, WIN 47203, in patients with severe congestive heart failure. *Circulation* 1983;67:1065–1070.

11. Packer M, Medina N, Yushak M. Hemodynamic and clinical limitations of long-term inotropic therapy with amrinone in patients with severe chronic heart failure. *Circulation* 1984;70:1038–1047.

12. Massie B, Bourassa M, DiBianco R, Hess M, Konstam M, Likoff M, Packer M, for the Amrinone Multicenter Trial Group. Long-term oral administration of amrinone for congestive heart failure: lack of efficacy in a multicenter controlled trial. *Circulation* 1985;71:963–971.

13. DiBianco R, Shabetai R, Kostuk W, Moran J, Schlant RC, Wright R, for the Milrinone Multicenter Trial Group. A comparison of oral milrinone, digoxin and their combination in the treatment of patients with chronic heart failure. *N Engl J Med* 1989;320:677–683.

14. Shah PK, Amin DK, Hulse S, Shellock F, Swan HJC. Inotropic therapy for refractory congestive heart failure with oral fenoximone (MDL-17,043): poor long-term results despite early hemodynamic and clinical improvement. *Circulation* 1985;71:326–331.

15. Petein M, Levine TB, Cohn JN. Persistent hemodynamic effects without long-term clinical benefits in response to oral piroximone (MDL 19,205) in patients with congestive heart failure. *Circulation* 1986;73(suppl III):III-230–III-236.

16. Uretsky BF, Jessup M, Konstam MA, Dec GW, Leier CV, Benotti J, Murali S, Herrmann HC, Sandberg JA for the Enoximone Multicenter Trial Group. Multicenter trial of oral enoximone in patients with moderate to moderately severe congestive heart failure. *Circulation* 1990;82:774–780.

17. Weishaar RE, Quade M, Schenden JA, Boyd DK, Evans DB. Studies aimed at elucidating the action of CI-914, a new cardiotonic. *Eur J Pharmacol* 1985;119:205–215.

18. Weber KT, Kinasewitz GT, West JS, Janicki JS, Reichek N, Fishman AP. Long-term vasodilator therapy with trimazosinin chronic cardiac failure. *N Engl J Med* 1980;303:242–250.

19. The Cardiac Arrhythmia Pilot Study. *Am J Cardiol* 1986;57:91–95.

20. Hood WB. Controlled and uncontrolled studies of phosphodiesterase III inhibitors in contemporary cardiovascular medicine. *Am J Cardiol* 1989; 63:46A–53A.

21. Colucci WS. Antagonist's viewpoint. *J Am Coll Cardiol* 1988;12:566–569.

APPENDIX

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