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A randomized, placebo-controlled trial to evaluate the tolerability, safety, pharmacokinetics, and pharmacodynamics of a potent inhibitor of poly(ADP-ribose) polymerase (INO-1001) in patients with ST-elevation myocardial infarction undergoing primary percutaneous coronary intervention: results of the TIMI 37 trial

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Abstract *Background* Reperfusion injury is a significant complication of the management of ST-elevation MI (STEMI). INO-1001 is a potent inhibitor of poly(ADP-ribose) polymerase (PARP), a mediator of oxidant-induced myocyte dysfunction during reperfusion. *Methods & results* We assessed the safety and pharmacokinetics of INO-1001 in a randomized, placebo-controlled, single-blind, dose-escalating trial in 40 patients with STEMI undergoing primary percutaneous coronary intervention within 24 h of onset. INO-1001 was well-tolerated. A trend toward more frequent transaminitis was observed with 800 mg. Plasma from INO1001-treated patients reduced in vitro PARP activity >90% at all doses. Serial C-reactive protein and IL-6 levels showed a trend toward blunting of inflammation with INO-1001. The apparent median terminal half-life ($t_{1/2}$) of INO-1001 was 7.5 (25th, 75th: 5.9, 10.2) h. *Conclusions* The results from this first trial of INO-1001 in STEMI support future investigation of INO-1001 as a novel treatment for reperfusion injury.

Keywords Clinical trials · Myocardial infarction · Reperfusion injury · Poly(ADP-ribose) polymerase

Clinical trial registration: NCT 00271765 at www.clinicaltrials.gov.

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Contemporary management of ST-elevation myocardial infarction (STEMI) is aimed at rapid restoration of blood flow within the infarct-related artery with the goal of minimizing the extent of irreversible myocardial injury [1]. Advances in reperfusion therapy, both pharmacological and mechanical, have improved outcomes in patients with STEMI. However, in large-scale clinical trials, new pharmacological reperfusion strategies have failed to achieve additional reductions in mortality compared with tissue plasminogen activator [1]. Moreover, it is unlikely that additional technical advances in mechanical reperfusion therapy will achieve large incremental gains in survival after primary percutaneous coronary intervention (PCI). For these reasons, novel approaches that act through mechanisms that are complementary rather than similar to current strategies are likely to provide the greatest potential to improve clinical outcomes in patients with STEMI.

Despite the established benefits of reperfusion therapy, restoration of blood flow to the injured myocardium is associated with a number of deleterious effects, including microvascular injury, dysrhythmias, and extension of myocyte necrosis [2, 3]. The causes of this constellation of complications, termed reperfusion injury, are likely multiple. Activation of the nuclear enzyme poly (ADP-ribose)

polymerase (PARP) (also known as a synthetase, PARS) has been shown to be a pivotal step in the pathways mediating oxidant-induced myocyte dysfunction and death during reperfusion in animal models [4, 5]. Moreover, agents that inhibit PARP have demonstrated favorable effects on inflammatory cell recruitment and cellular survival, as well as promising reductions in infarct size and preservation of regional myocardial function in animal models of myocardial reperfusion injury [5–9].

A novel potent PARP inhibitor (INO-1001) with substantial potency (IC50 $<$ 10 nM) has been developed and shown to significantly reduce the extent of tissue infarction in experimental models. In Phase I studies of healthy volunteers, INO-1001 was well-tolerated with the exception of phlebitis when administered in small peripheral veins. However, this agent had not previously been studied in patients with myocardial infarction. The TIMI 37 Trial was a randomized, placebo-controlled, single-blind, multi-center, dose-escalation study designed to asses the tolerability, safety, pharmacokinetics, and pharmacodynamics of INO-1001 in patients with STEMI undergoing primary PCI.

Methods

Study population

Patient enrollment occurred between December 13th, 2004 and April 21, 2006 at 10 centers in the United States and Israel. Eligible participants were men and non-pregnant women, aged 18 years or older, with STEMI with a large territory of myocardium at risk, and planned to undergo primary PCI within 3 h of presentation. A large territory at risk was defined by anterior ST-elevation or inferior ST-elevation with concurrent ST-depression in anterior leads. Patients with cardiogenic shock, an MI due to obvious provoking factors (e.g. severe anemia), history of significant valvular heart disease, malignancy, or hepatic disease were excluded. Additional exclusion criteria included serum creatinine >2 mg/dl, or treatment with a potent inhibitor of hepatic CYP3A4 isoenzyme.

This study complied with the Declaration of Helsinki. The protocol was approved by the ethics committee at each participating center and all patients provided written informed consent prior to the performance of study procedures.

Study protocol

The trial was a randomized, single-blind, dose-escalation study of a single intravenous bolus of INO-1001 mixed in 250 ml of D5W or D5W alone. The trial design included three planned dose tiers (200, 400, and 800 mg) with 10 patients in each active dose group and a 3:1 randomization with placebo. A Safety Committee that included an independent experienced Safety Monitor (Raymond Gibbons, MD, Mayo Clinic, Rochester, MN) reviewed all available safety data at the conclusion of each dose tier and approved escalation to the next planned dose level.

The assigned dose of INO-1001 was to be administered intravenously via the femoral venous sheath or central venous access as a bolus over 15 min, and was to be initiated prior to crossing the lesion with the wire. One subject enrolled in the 200 mg dose group and allocated to placebo did not receive study drug. All participants were treated with aspirin unless contraindicated. All other medications, including heparin, IV glycoprotein IIb/IIIa receptor antagonists, and clopidogrel, were used at the discretion of the managing physician. Patients returned for follow-up evaluations at 2 weeks, and performed a telephone follow-up at 30 days.

Clinical procedures

Blood was to be sampled prior to administration of study drug, and at 20 min, 60 min, 2.5, 6, 12, 24, and at 48 h following the administration of study for measurement of the plasma level of INO-1001. A complete blood count, serum chemistries, and urinalysis were performed pre-dose, on Day 2, Day 3, Day 5 (or hospital discharge, whichever was earlier) and at the Day 14 follow-up visit. Coagulation parameters (PT, aPTT) were measured at screening and on Day 1. Serum and plasma samples for measurement of biomarkers of necrosis (CK-MB and cardiac troponin I), B-type natriuretic peptide (BNP), and inflammation (high sensitivity C-reactive protein, hsCRP, and interleukin-6, IL-6) were obtained at screening, 6, 12, 24 h, and the Day 14 visit (BNP and hsCRP only). Plasma was obtained at screening, 6, and 24 h for pharmacodynamic analysis. Plasma samples were tested for PARP inhibitory activity ex vivo, utilizing an assay in which PARP activity is induced in cultured murine macrophages (RAW cells) by exposure to hydrogen peroxide. PARP inhibition was assessed by the incorporation of radiolabeled NAD^+. Results from the activated RAW cells treated with plasma from subjects treated with INO-1001 were compared to results obtained from control plasma.

End points and statistical analysis

The principal objectives of this study were to assess the safety profile and pharmacokinetics of increasing doses of INO-1001. Secondary objectives were to assess its pharmacodynamic profile using PARP activity and biomarkers of inflammatory response, necrosis, and hemodynamic stress (BNP). All patients who received any dose of study drug were included in the safety analysis. Comparisons of

biomarker values between groups were made using a non-parametric method with the Kruskal–Wallis test. Analyses were performed using STATA 9.2 (STATA Corp., College Station, Texas) and WinNonlin Pro (Pharsight Inc. Mountain View, CA). P-values < 0.05 were considered to indicate statistical significance.

Results

A total of 40 patients were enrolled in the trial. Follow-up through 30 days was complete for all patients enrolled. The baseline characteristics of the study population are described in Table 1.

Safety events

There were no serious adverse events judged as related to study drug by the local investigator (Table 2). Non-serious adverse events assessed by the investigator as possibly related to study drug were nausea (2), vomiting (1), general ache (1), increased QTc (1, 400 mg), cough (1), increased transaminases or lactate dehydrogenase (3). There were no adverse events assessed by the investigator as probably or definitely related to study drug. No clinically significant abnormalities of creatinine or electrolytes were observed. Elevated serum concentrations of aspartate transaminase (AST) were numerically more frequent in patients treated with INO-1001 (Table 3). All transaminase values returned to <2x ULN by 14 days. There were no deaths or new or worsening heart failure events during the 30 days of follow-up.

Table 1 Baseline characteristics

	Control ($n = 10$)	INO-1001 ($n = 30$)
Demographics		
Age, year median (25th, 75th)	52 (49, 61)	55 (48, 63)
Female	1 (10.0)	5 (16.7)
Caucasian	9 (90.0)	28 (93.3)
Medical history		
Current tobacco use	5 (50.0)	15 (50.0)
Diabetes mellitus	0 (0)	4 (13.3)
History of hypertension	6 (60.0)	15 (50.0)
Prior myocardial infarction	2 (20.0)	5 (16.7)
Prior revascularization	2 (20.0)	6 (20.0)
History of heart failure	0 (0)	0 (0)
Presentation		
Anterior MI	2 (20.0)	12.0 (40.0)
Killip II-III	0 (0)	2 (6.7)
Time from symptom onset	3.0 (2.7, 6.4)	2.7 (2.2, 3.6)

Date are shown as n (%) for dichotomous variables and median (25th, 75th percentile) for continuous variables

Table 2 Number of adverse events

	Control ($n = 10$)	Dose INO-1001		
		200 mg ($n = 10$)	400 mg ($n = 10$)	800 mg ($n = 10$)
Serious				
Related[a]	0	0	0	0
Unrelated[b]	4	4	3	0
Non-serious				
Related[a]	0	3	1	8
Unrelated[b]	59	16	49	59

Data are shown as the number of events in each group. [a] Possibly, probably or definitely; [b] None or unlikely. The seven serious events on drug were recorded as chest pain, abnormal ECG, other coronary artery disease leading to revascularization, and cardiac arrest

Table 3 Abnormal liver chemistries

	Fold-elevation >ULN	Control ($n = 9$)[a]	Dose INO-1001		
			200 mg ($n = 10$)	400 mg ($n = 10$)	800 mg ($n = 10$)
AST	2–3x	4 (44)	4 (40)	4 (40)	1 (10)
	>3–5x	0	0	2 (20)	4 (40)
	>5x	1 (11)	2 (20)	0	3 (30)
ALT	2–3x	0	1 (10)	0	4 (40)
	>3–5x	1 (11)	0	0	0
	>5x	0	0	0	0
Total bilirubin	>2x	0	0	0	0

Data are shown as n (%) based upon maximum concentration after randomization. [a] One subject had missing data

Pharmacokinetics

The pharmacokinetic parameters for each dose group are presented in Table 4. Maximum plasma concentrations were obtained after the intravenous bolus with subsequent decline in an apparent bi-exponential manner. The median apparent terminal half-life ($t_{1/2}$) was 7.5 (25th, 75th percentile: 5.9, 10.2) h.

Pharmacodynamics

Plasma from INO1001-treated patients reduced in vitro PARP activity in cultured cells >90% at all doses studied (Fig. 1). Serial C-reactive protein and IL-6 levels showed a trend toward blunting of inflammation post-STEMI with INO-1001 (Fig. 2), with no difference in peak B-type natriuretic peptide (127, 158, 146, 101 with control vs. 200,

Table 4 Median pharmacokinetic parameters

	Dose INO-1001		
	200 mg ($n = 10$)	400 mg ($n = 10$)	800 mg ($n = 10$)
C_{max} (ng/ml)	517 (376, 661)	916 (624, 1,320)	1,972 (1,056, 2,564)
AUC (min * mcg/ml)	594 (541, 725)	1240 (1,230, 1,770)	2,450 (2,270, 3,230)
$t_{1/2}$ (h)	9.7 (5.9, 10.6)	7.4 (6.6, 8.0)	6.3 (5.8, 7.6)
V_{ss} (l)	138 (113, 152)	132 (111, 162)	126 (93, 142)
Cl (ml/min)	325 (264, 361)	305 (224, 318)	325 (244, 349)

Data are presented as median (25th, 75th percentiles)

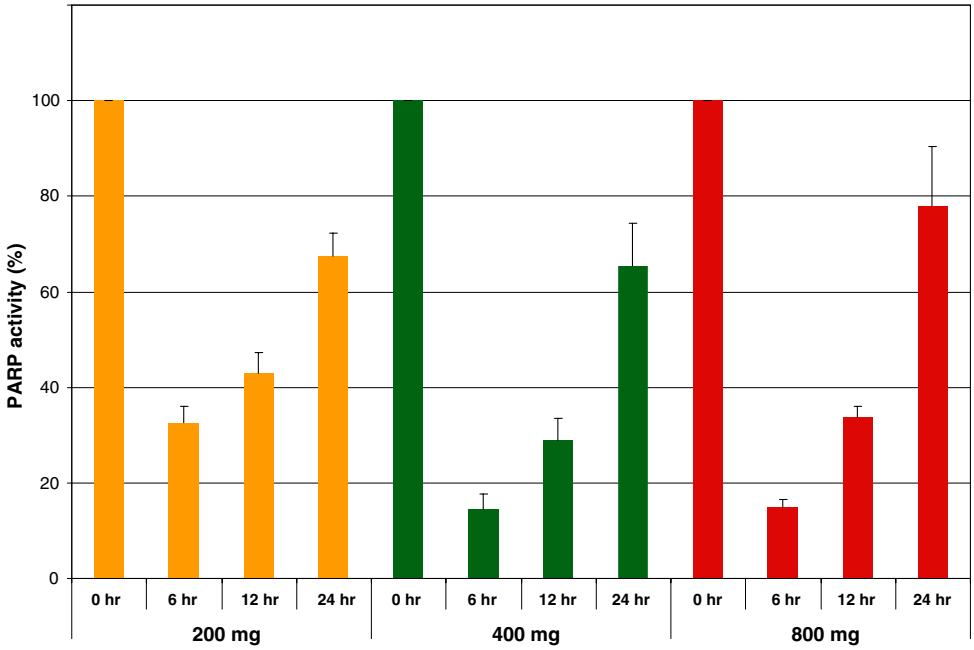

Fig. 1 Ex-vivo PARP activity using plasma (1:10) of INO-1001 treated patients prior to randomization and at 6, 12, and 24 h

400, and 800 mg INO-1001, respectively) or peak CKMB (89, 236, 91, 196 ng/ml with control vs. 200, 400, and 800 mg INO-1001, respectively).

Discussion

In this first pilot trial of INO-1001 in STEMI, INO-1001 was associated with significant in vitro inhibition of PARP activity and a trend toward a reduced inflammatory response. The clinical safety profile from this preliminary experience supports future investigation of INO-1001 as a novel therapy for reperfusion injury.

Although timely restoration of flow in the infarct-related artery is critical to achieving optimal outcomes in patients presenting with STEMI, reperfusion injury is a challenging limitation of current reperfusion therapy. Reperfusion injury includes microvascular and endothelial injury, myocardial stunning, and extension of myocardial necrosis [2, 3]. Endothelial dysfunction results in pathophysiological consequences that are particularly adverse in the setting of acute myocardial injury. These detrimental effects include vasoconstriction, platelet and leukocyte activation, and increased production of toxic oxidant species [2, 3]. In light of the adverse clinical consequences of reperfusion injury, there has been intense interest in identifying therapeutic strategies to mitigate or prevent these processes. Despite encouraging results of pre-clinical studies of numerous putative cardioprotective agents, such as adenosine and cariporide, or approaches, such as therapeutic hypothermia, no convincingly effective strategies have emerged from testing in large-scale trials [2, 3]. Therefore, although viewed with cautious skepticism regarding the potential for success, there remains an unmet need for interventions effective in reducing reperfusion injury.

PARP is an abundant nuclear enzyme that functions in DNA repair by transferring ADP-ribose units to nucleic proteins. Activated PARP is energetically inefficient and rapidly depletes intracellular pools of adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide (NAD^+) [10]. In the setting of massive cellular injury, unchecked activation of PARP can trigger cellular

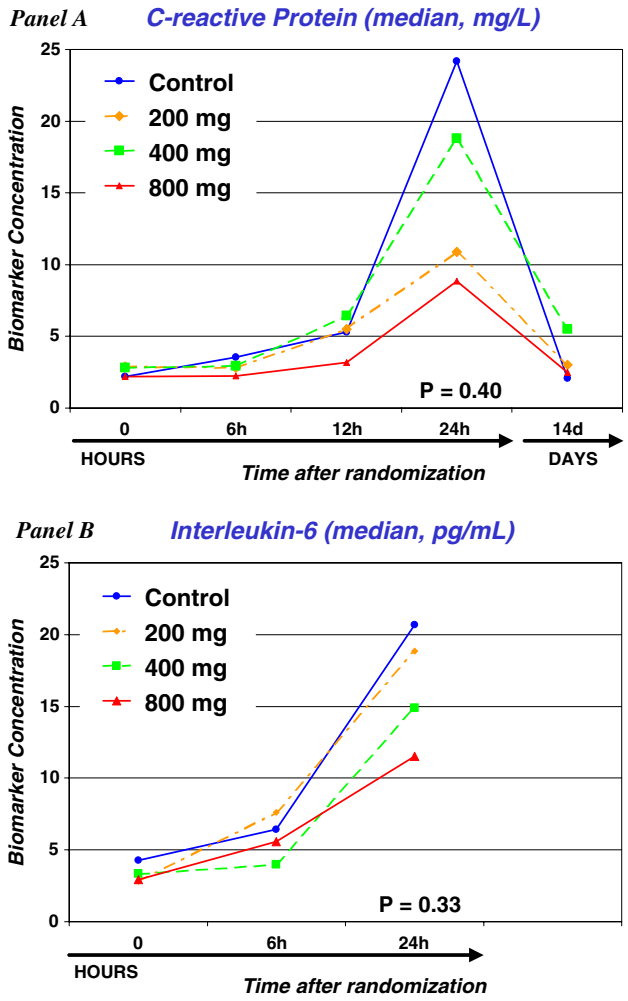

Fig. 2 Inflammatory biomarkers high sensitivity C-reactive protein (hsCRP) and interleukin-6 (IL-6) at each time point by treatment group

energetic collapse and consequent necrosis [11, 12]. Acting in this way, PARP has been implicated in the pathogenesis of myocardial reperfusion injury [7, 13]. PARP has been found to be significantly activated in reperfused myocardium, particularly in the area of necrosis and the ischemic borderzone [7]. Notably, genetically altered animals lacking functional PARP exhibit significant protection from myocardial reperfusion injury [5, 14]. Moreover, pharmacological suppression of PARP in animal models results in diminished expression of inflammatory mediators of endothelial dysfunction, decreased recruitment of neutrophils, preserved tissue content of ATP, diminished infarction size, and improved myocardial contractility [4–9, 13, 15]. Together these findings indicate that inhibition of PARP interferes with a critical mechanism of cellular damage in myocardial reperfusion injury; one that has not yet been targeted by previous experimental therapeutics in humans.

The observations from this preliminary experience with a potent inhibitor of PARP in patients with STEMI detected no major adverse events associated with the use of INO-1001. Although elevation of ALT and AST are expected in the setting of STEMI, the non-significant trend toward more frequent elevation of AST with the highest dose of INO-1001 points toward a need for additional evaluation of liver chemistries in larger studies of this PARP inhibitor. Moreover, our findings provide additional evidence for potent inhibition of PARP with INO-1001.

Our trial was not designed with the objective of defining the clinical efficacy of INO-1001. Exploratory assessment of biomarkers of myocardial necrosis and hemodynamic stress revealed no evidence for benefit with respect to these measures in this small population. However, a pattern of a blunted inflammatory response post-STEMI in patients treated with the highest dose of INO-1001 is interesting when interpreted in the context of the pre-clinical findings of anti-inflammatory effects of INO-1001. In light of the multiple past failures of agents, including those with promising pre-clinical data, to manifest clear efficacy in phase II and phase III clinical trials, trials of INO-1001 designed to test its influence on relevant clinical outcomes would certainly be necessary to establish the potential value of potent inhibition of PARP for reducing reperfusion injury. In addition, in order to be consistent with current patterns of practice, we studied INO-1001 in subjects undergoing primary PCI. This strategy has the experimental advantage of controlling the timing of reperfusion. Additional studies of INO-1001 in the setting of fibrinolytic therapy for STEMI will be valuable in fully characterizing any clinical role for this agent.

Conclusion

Administration of a potent inhibitor of PARP may offer an approach to amelioration of reperfusion injury. Our findings support additional investigation of INO-1001.

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Appendix

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