

A Stabilized Hirudin Solid Premix in the Translational Context of Coronary Thrombosis and Acute Coronary Ischemia

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ABSTRACT

Objective: Persistent thrombin activity contributes to coronary thrombosis and recurrent ischemia in acute coronary syndromes. We measured hirudin activity in a low-water-activity premix and reviewed randomized evidence from unstable angina and non-ST-elevation acute coronary syndromes. **Method:** Optimized BCLC® formulations retained 90.6-92.1% antithrombin activity at water activity values of 0.26-0.34. High-humidity processing produced the highest water activity (0.49) and lowest retention (72.9%); without citrate buffer, retention was 80.1%. An angiographic unstable-angina trial reported dose-related antithrombotic effects of recombinant hirudin. In GUSTO-IIb, 12,142 patients were randomized, and death or myocardial infarction at 24 h occurred in 1.3% with hirudin and 2.1% with heparin. OASIS-2 enrolled 10,141 patients with non-ST-elevation acute myocardial ischemia. **Results:** At 7 days, cardiovascular death or new myocardial infarction occurred in 3.6% with hirudin and 4.2% with heparin; treatment-period analyses favored hirudin, but major bleeding requiring transfusion was higher. These trials establish both pharmacodynamic activity and a dose-safety context. **Novelty:** The premix data permit oral exposure and coagulation biomarkers to be examined from a defined activity level.

INTRODUCTION

Acute coronary syndromes begin with disruption or erosion of an atherosclerotic plaque, followed by platelet recruitment, thrombin generation, and fibrin formation. Thrombin is not only the enzyme that converts fibrinogen to fibrin; once incorporated into a forming clot it continues to support coagulation and platelet activation. This made direct thrombin inhibition attractive in unstable angina and non-ST-elevation ischemia, where recurrent events can occur despite aspirin and heparin. In the early angiographic trial by Topol et al., recombinant hirudin produced a dose-related antithrombotic effect in patients with visible coronary thrombus [1]. The much larger GUSTO-IIb trial then compared 72 h of hirudin with heparin in 12,142 patients with acute coronary syndromes and found the clearest separation in early death or myocardial infarction [2].

The signal was not uniform across dose, endpoint, or follow-up. In the OASIS pilot study, medium-dose hirudin reduced several short-term ischemic outcomes compared with heparin in patients without ST elevation [3]. OASIS-2 later enrolled 10,141 patients and again showed a treatment-period advantage, but the 7-day primary comparison was less definitive and transfusion-requiring bleeding was more frequent with hirudin [4]. Mechanistic sampling within GUSTO-IIb showed that hirudin and heparin produced different patterns of thrombin generation and activity during infusion [5]. Those studies are useful for a formulation paper because they define what an active systemic hirudin

exposure can do, and they also show why exposure cannot be discussed separately from safety.

Direct thrombin inhibitors act differently from heparin. They block thrombin catalytic activity without requiring antithrombin as a cofactor, and bivalent hirudins can inhibit both free and clot-associated thrombin [6]. A later review focused on hirudin pharmacology and clinical use described the same feature together with its dose-dependent anticoagulant behavior [7]. At the acute-coronary-syndrome level, an individual-patient meta-analysis found modest reductions in ischemic events with direct thrombin inhibitors as a class, alongside the expected bleeding trade-off [8]. Earlier clinical-development work had already emphasized that hirudin's potency and selectivity become useful only when the delivered active dose is known with reasonable precision [9].

Clinical use also made additional exposure and safety constraints visible. Antihirudin antibodies can alter the pharmacologic profile of lepirudin during repeated treatment [10], and severe hypersensitivity reactions, although uncommon, have been described after re-exposure [11]. More recent delivery research has therefore treated hirudin stability and exposure control as formulation problems in their own right; thrombin-responsive nanogel systems are one example of attempts to protect hirudin and regulate its release [12]. Parenteral trials outside coronary disease reinforce the point that preserved hirudin remains biologically potent: recombinant hirudin was active in acute deep-vein thrombosis [13], desirudin reduced postoperative thromboembolic events in hip-replacement studies [14], and healthy-volunteer experiments demonstrated dose-related biologic effects after recombinant hirudin administration [15].

The present work addresses the step that comes before those pharmacodynamic questions. BCLC® Hirudin Low-Water-Activity Solid Premix was designed to limit free water, stabilize the local pH environment, and distribute a low-dose protein consistently through a dry powder. We measured retained antithrombin activity and unit-dose consistency under optimized and stress conditions. The clinical literature is used here to define the translational context for later exposure and coagulation studies; it is not used to assign coronary efficacy to the oral premix.

MATERIALS AND METHODS

Optimized powders were processed at low humidity with trehalose/mannitol, preadsorption on colloidal silicon dioxide, geometric dilution with microcrystalline cellulose and citric acid-sodium citrate buffering. Three comparator formulations omitted protective mixing, humidity control or the buffer.

Water activity and retained antithrombin activity were measured after powder equilibration. Activity-distribution and capsule fill-weight uniformity were also assessed so that biochemical stability and manufacturing consistency could be examined together.

Coronary studies were included when recombinant hirudin was tested in unstable angina, non-ST-elevation acute myocardial infarction or broader acute coronary syndromes and angiographic, clinical or thrombin-related outcomes were reported.

RESULTS

Formulation performance

The optimized formulations had water activity values of 0.26-0.34 and retained 90.6-92.1% antithrombin activity. Under high humidity, water activity rose to 0.49 and retention fell to 72.9%. The no-buffer comparator retained 80.1% at water activity 0.31. Moisture control alone therefore did not explain the full stabilization pattern.

In a multicenter randomized angiographic study, patients with unstable angina and visible culprit-vessel thrombus received graded hirudin or heparin regimens. Recombinant hirudin showed dose-related antithrombotic activity in coronary thrombosis [1].

Table 1. Formulation-level physicochemical, activity and capsule-filling performance. F1-F3 are optimized formulations; C1-C3 are mechanistic comparators.

Group	Water %	aw	Activity retention %	Activity RSD %	Angle repose °	of Fill-weight RSD %
F1 optimized	3.2	0.26	92.1	3.8	36.1	2.9
F2 optimized	3.6	0.30	91.4	4.2	37.2	3.1
F3 optimized	4.0	0.34	90.6	4.7	38.4	3.4
C1 no protective preadsorption /s tagged dilution	5.1	0.43	76.4	8.9	42.8	6.8
C2 high-humidity processing	5.8	0.49	72.9	7.6	44.3	7.2
C3 no citrate buffer	3.5	0.31	80.1	4.5	37.6	3.6

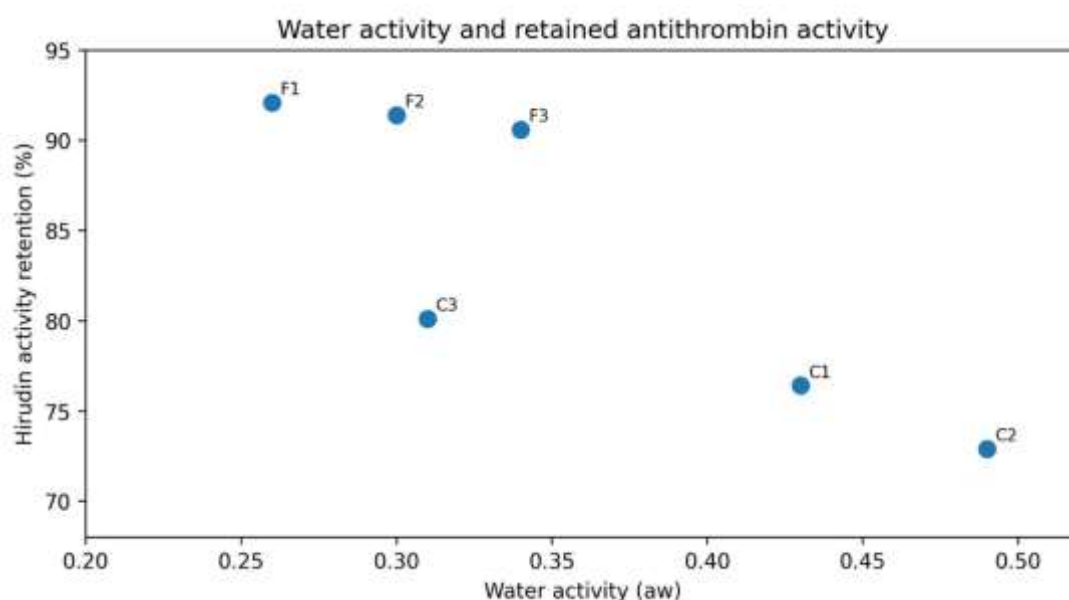


Figure 1. Water activity and retained hirudin antithrombin activity across optimized and comparator formulations.

Published human evidence

GUSTO-IIb randomized 12,142 patients with acute coronary syndromes to 72 h of hirudin or heparin.

Death or myocardial infarction at 24 h occurred in 1.3% and 2.1%, respectively ($p=0.01$ in the subsequent early-event analysis) [2]. OASIS-2 randomized 10,141 patients with non-ST-elevation acute myocardial ischemia to 72 h of either treatment. The 7-day rate of cardiovascular death or new myocardial infarction was 3.6% with hirudin and 4.2% with heparin (RR 0.84, 95% CI 0.69-1.02; $p=0.077$). During treatment, cardiovascular death or MI favored hirudin (RR 0.76, 95% CI 0.59-0.99; $p=0.039$). Major bleeding requiring transfusion occurred in 1.2% and 0.7% ($p=0.01$) [4].

Table 2. Representative randomized coronary studies of recombinant hirudin.

Study	Population	Comparison	Key result
Topol et al., 1994	Unstable angina	Hirudin vs heparin	Randomized angiographic trial
GUSTO-IIb, 1996	12,142 ACS patients	Hirudin vs heparin	24-h death/MI: 1.3% vs 2.1%
OASIS-2, 1999	10,141 NSTEMI acute ischemia patients	Hirudin vs heparin	7-d CV death/new MI: 3.6% vs 4.2%; treatment-period RR 0.76

Discussion

Low-water-activity, buffered dry processing preserved most hirudin activity in the optimized formulations. The comparators show why this matters for dose-response work: degradation before administration would change the active dose even if nominal

powder content remained constant. Both water activity and the local pH environment contributed to the measured pattern.

The coronary trials found early antithrombotic effects together with bleeding risk. GUSTO-IIb reported fewer early deaths or myocardial infarctions with hirudin [2]. In OASIS-2, the treatment-period estimate favored hirudin, the 7-day difference was smaller, and transfusion-requiring bleeding was

more frequent [4]. Any later delivery study therefore needs accurate exposure measurement and prespecified bleeding surveillance.

For the oral premix, the immediate question is how much intact active hirudin reaches the circulation and how variable that exposure is. Plasma hirudin can be assessed together with aPTT, TT, ecarin-derived measures and thrombin-generation markers. These measurements should precede studies of coronary clinical endpoints.

Research Scope and Future Perspectives

A staged program could first define oral absorption and coagulation pharmacodynamics, then characterize exposure and safety across doses. Coronary endpoint studies would require a population and dose selected from those earlier measurements.

CONCLUSION

Fundamental Finding: Protective excipients, citrate buffering and low-water-activity processing maintained high hirudin antithrombin activity with consistent powder performance. **Implication:** Randomized coronary trials show that systemic direct thrombin inhibition can alter early ischemic outcomes while increasing bleeding at some exposures. **Limitation:** Randomized coronary trials show that systemic direct thrombin inhibition can alter early ischemic outcomes while increasing bleeding at some exposures. **Future Research:** The BCLC® premix can now be studied for oral exposure, coagulation response and safety under defined doses.

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