

Article

Stabilized Hirudin Activity and Unit-Dose Uniformity in a Dry Premix: Translational Evidence from Venous Thrombosis and Thromboembolism Trials

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Abstract: Recombinant hirudins have been tested clinically in the treatment and prevention of venous thromboembolism. We evaluated the activity retention and unit-dose performance of a low-water-activity hirudin premix, then reviewed randomized evidence from deep-vein thrombosis (DVT) treatment and postoperative prophylaxis. Optimized BCLC® premixes retained 90.6-92.1% antithrombin activity. Activity-distribution RSD was 3.8-4.7%, and capsule fill-weight RSD was 2.9-3.4%. Removal of staged protective mixing or humidity control increased variability and reduced retention. In parenteral studies, subcutaneous recombinant hirudin was compared with intravenous heparin in acute lower-limb DVT. After hip replacement, desirudin reduced confirmed DVT relative to unfractionated heparin (7% vs 23%) and major thromboembolic events relative to enoxaparin (4.9% vs 7.6%). These trials describe systemic hirudin pharmacology; the premix results describe the manufacturing controls required before oral exposure can be studied.

Keywords: Hirudin; venous thromboembolism; deep-vein thrombosis; desirudin; thrombin inhibition; unit-dose uniformity; dry premix.

Introduction

Venous thrombosis is driven by persistent thrombin generation and fibrin formation, but the clinical importance of thrombin extends beyond clot assembly. Once formed, thrombin amplifies coagulation by activating platelets and several coagulation factors, which made direct thrombin inhibition an attractive alternative to agents that act indirectly through antithrombin. Reviews of the direct thrombin inhibitor class and of hirudin pharmacology describe recombinant hirudins as among the earliest agents to translate this mechanism into human treatment [1], [2], [3].

That translation was established largely with parenteral dosing. In acute lower-limb DVT, subcutaneous recombinant hirudin was compared with intravenous heparin in a prospective randomized dose-ranging trial [4]. In orthopedic prophylaxis, desirudin was evaluated against unfractionated heparin and enoxaparin after hip replacement, and an earlier randomized hip-replacement study also examined recombinant hirudin as a direct antithrombin strategy [5], [6], [7]. These studies are clinically useful because they show what can be achieved when active hirudin reaches the systemic circulation; they do not, however, answer whether a dry oral unit can preserve activity or deliver reproducible exposure.

Human pharmacology also shows why formulation and dose control matter for a molecule with a narrow functional target. Volunteer studies documented dose-related changes in coagulation and bleeding-time measures after recombinant hirudin exposure [8], [9]. Other clinical programs extended hirudin investigation to unstable angina and acute coronary syndromes [10], [11]. At the same time, repeated exposure introduced issues that are less visible in short efficacy trials, including antihirudin antibodies and rare severe hypersensitivity reactions [12], [13]. Thus, consistency of the delivered amount is not a manufacturing detail detached from pharmacology; it is part of the conditions under which exposure and safety can be interpreted.

Oral delivery creates an additional problem. Hirudin is a polypeptide, so processing stress, moisture, gastrointestinal degradation and uncertain epithelial transfer can all separate labeled dose from active dose. An earlier oral-route investigation demonstrated the practical interest in this route [14], while later delivery work has continued to explore ways of controlling hirudin exposure [15]. Before such questions are taken into an oral pharmacokinetic study, the starting solid has to be stable enough that loss of antithrombin activity and between-unit variability are not confounded with absorption.

The present work therefore focuses on the part of that chain that can be measured directly in the dosage-form platform. We quantified retained antithrombin activity, multipoint powder uniformity and capsule fill consistency in a low-water-activity BCLC® premix, using comparators that removed individual protective steps. The formulation data were then set beside randomized venous-thrombosis and thromboprophylaxis trials to define the intended systemic pharmacology without attributing parenteral clinical outcomes to the oral premix.

Materials and Methods

Three optimized premixes and three mechanistic comparators were tested. Optimized processing combined low humidity, preadsorption of hirudin on colloidal silicon dioxide, trehalose/mannitol protection, geometric dilution with microcrystalline cellulose, citrate buffering and brief low-speed lubrication. C1 omitted protective preadsorption and staged dilution, C2 used high humidity, and C3 omitted the citrate buffer.

Retention was calculated against theoretical activity before processing. Activity-distribution RSD was measured at ten powder locations. Continuous size-0 filling was assessed over 1000 capsules, from which fill-weight RSD was calculated.

Randomized trials were included when recombinant hirudin or desirudin was used in established DVT or orthopedic thromboprophylaxis. Their clinical outcomes were summarized separately from the oral-premix measurements.

Results

Formulation performance

Activity-distribution RSD was 3.8%, 4.2% and 4.7% in the optimized formulations; fill-weight RSD was 2.9%, 3.1% and 3.4%. Without staged protective mixing, the corresponding values rose to 8.9% and 6.8%. High-humidity processing produced activity and fill-weight RSD values of 7.6% and 7.2%.

The no-buffer powder remained relatively uniform but retained only 80.1% activity. Uniform distribution did not by itself ensure biochemical stability.

In established DVT, subcutaneous recombinant hirudin HBW 023 was compared with intravenous heparin in a multicentre prospective randomized dose-ranging study. Hirudin produced better anticoagulant control and fewer new ventilation/perfusion mismatches after treatment [4].

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 of repose °	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

Group	Water %	aw	Activity retention %	Activity RSD %	Angle of repose °	Fill-weight RSD %
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

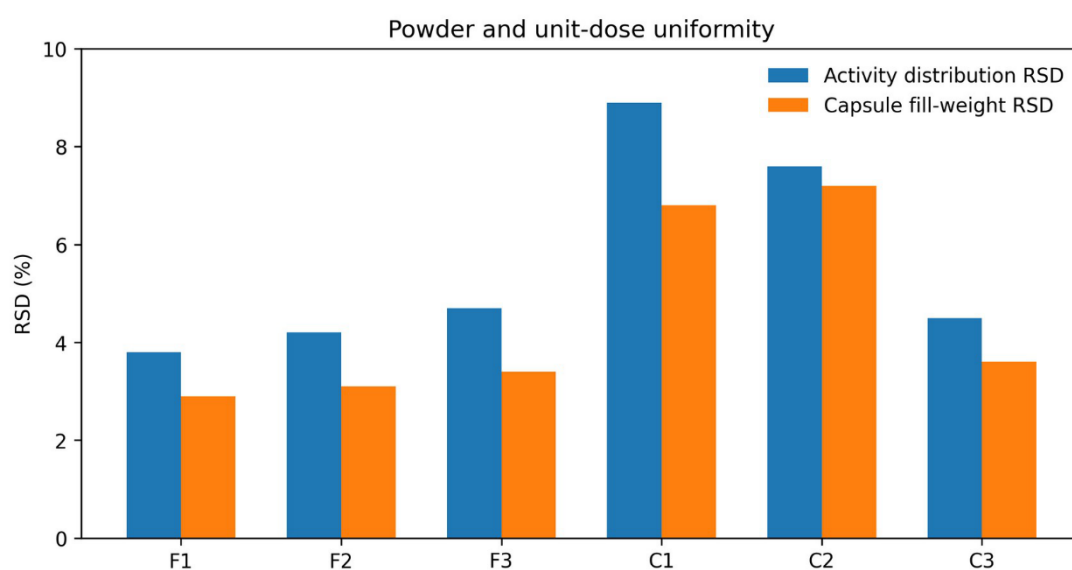


Figure 1. Activity-distribution and capsule fill-weight variability across the formulation groups.

Published human evidence

After hip replacement, confirmed DVT occurred in 13 of 174 desirudin recipients (7%) and 41 of 177 unfractionated-heparin recipients (23%; $p < 0.0001$) [5]. In the larger enoxaparin comparison, major thromboembolic events occurred in 39 of 802 patients given desirudin (4.9%) and 60 of 785 given enoxaparin (7.6%; $p = 0.02$) [6].

Table 2. Representative randomized human studies of hirudin/desirudin in venous thrombosis

Study	Clinical setting	Comparison	Key result
Schiele et al., 1997	Acute lower-limb DVT	SC hirudin vs IV heparin	Randomized dose-ranging treatment study
Eriksson et al., 1997 (JBJS)	Elective hip replacement	Desirudin vs UFH	Confirmed DVT: 7% vs 23%
Eriksson et al., 1997 (NEJM)	Primary hip replacement	Desirudin vs enoxaparin	Major thromboembolic events: 4.9% vs 7.6%

Discussion

The premix addresses two separate manufacturing requirements: preservation of thrombin-inhibitory activity and control of variation between doses. The no-buffer comparator remained fairly uniform but lost activity. Under high humidity, both activity and powder performance deteriorated. Both dimensions therefore need to be measured.

The venous-thromboembolism trials show the effects of systemically available hirudin. Desirudin reduced postoperative DVT relative to unfractionated heparin [5] and reduced major thromboembolic events relative to enoxaparin [6]. The established-DVT trial supplied evidence in a treatment setting [4].

Those studies used subcutaneous or intravenous dosing, whereas the BCLC® platform is a dry oral premix. They define the intended pharmacology rather than the performance of this formulation.

Subsequent experiments can examine gastrointestinal protection, intact absorption, exposure variability and thrombosis-related biomarkers after defined oral doses.

Research Scope and Future Perspectives

The next experiments should link premix quality with oral pharmacokinetics and coagulation pharmacodynamics under defined dosing conditions. Clinical endpoint studies would follow only after exposure and safety relationships have been characterized.

Conclusion

BCLC® low-water-activity premixes retained high hirudin activity and showed low variability during blending and capsule filling. Parenteral trials demonstrate clinically active direct thrombin inhibition in defined DVT and postoperative settings. The premix is suitable for staged evaluation of oral exposure and thrombin-related pharmacodynamics.

REFERENCES

- [1] K. L. Kaplan and C. W. Francis, "Direct thrombin inhibitors," *Semin. Hematol.*, vol. 39, no. 3, pp. 187–196, 2002, PMID: 12124681.
- [2] A. Greinacher and T. E. Warkentin, "The direct thrombin inhibitor hirudin," *Thromb. Haemost.*, vol. 99, no. 5, pp. 819–829, 2008, PMID: 18449411.
- [3] P. H. Johnson, "Hirudin: Clinical potential of a thrombin inhibitor," *Annu. Rev. Med.*, vol. 45, pp. 165–177, 1994, PMID: 8198374.
- [4] Schiele F, Lindgaerde F, Eriksson H, Bassand JP, Wallmark A, Hansson PO, Grollier G, Sjo M, Moia M, Camez A, Smyth V, Walker M; International Multicentre Hirudin Study Group, "Subcutaneous recombinant hirudin (HBW 023) versus intravenous sodium heparin in treatment of established acute deep vein thrombosis of the legs: a multicentre prospective dose-ranging randomized trial," *Thromb. Haemost.* 1997;77(5):834-838. PMID:9184388.
- [5] Eriksson BI, et al., "Prevention of thromboembolism with use of recombinant hirudin: results of a double-blind, multicenter trial comparing desirudin with unfractionated heparin in patients having a total hip replacement," *J. Bone Joint Surg. Am.* 1997;79(3):326-333. doi:10.2106/00004623-199703000-00002.
- [6] Eriksson BI, Wille-Jørgensen P, Kälebo P, Mouret P, Rosencher N, Bösch P, Baur M, Ekman S, Bach D, Lindbratt S, Close P, "A comparison of recombinant hirudin with a low-molecular-weight heparin to prevent thromboembolic complications after total hip replacement," *N. Engl. J. Med.* 1997;337(19):1329-1335. doi:10.1056/NEJM199711063371901.
- [7] B. I. Eriksson, S. Ekman, P. Kalebo, B. Zachrisson, D. Bach, and P. Close, "Prevention of deep-vein thrombosis after total hip replacement: direct thrombin inhibition with recombinant hirudin, CGP 39393," *Lancet*, vol. 347, no. 9002, pp. 635-639, 1996, doi:10.1016/S0140-6736(96)91200-3.
- [8] Schenk JF, Glusa E, Radziwon P, Butti A, Markwardt F, Breddin HK, "A recombinant hirudin (IK-HIR02) in healthy volunteers. I. Effects on coagulation parameters and bleeding time," *Haemostasis*. 1996;26(3):140-149. doi:10.1159/000217199.
- [9] M. Verstraete et al., "Biologic effects of recombinant hirudin in human volunteers," *J. Am. Coll. Cardiol.*, vol. 22, no. 4, pp. 1080–1088, 1993, doi:10.1016/0735-1097(93)90419-2.
- [10] E. J. Topol et al., "Recombinant hirudin for unstable angina pectoris: A multicenter, randomized angiographic trial," *Circulation*, vol. 89, no. 4, pp. 1557–1566, 1994, doi:10.1161/01.CIR.89.4.1557.

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- [11] Direct Thrombin Inhibitor Trialists' Collaborative Group, "Direct thrombin inhibitors in acute coronary syndromes," *Lancet*, vol. 359, no. 9303, pp. 294–302, 2002, PMID: 11830196.
 - [12] P. Eichler et al., "Antihirudin antibodies in patients with heparin-induced thrombocytopenia treated with lepirudin," *Blood*, vol. 96, no. 7, pp. 2373–2378, 2000, PMID: 11001886.
 - [13] A. Greinacher et al., "Anaphylactic and anaphylactoid reactions associated with lepirudin," *Circulation*, vol. 108, no. 17, pp. 2062–2065, 2003, PMID: 14568897.
 - [14] Cen X, Ni J, Tan T, et al., "Investigation on recombinant hirudin via oral route," *Peptides*. 2006;27(4):836-840. doi:10.1016/j.peptides.2005.08.015.
 - [15] X. Xu et al., "Self-regulated hirudin delivery for anticoagulant therapy," *Sci. Adv.*, vol. 6, no. 41, 2020, PMID: