# Comparison of the oral delivery of three marketed Low Molecular Weight Heparins encapsulated in nanoparticles

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## INTRODUCTION

Advances in anticoagulant treatment led to low molecular weight heparins (LMWH) as improved antithrombotic drugs compared to unfractionated heparins (UFH) for the prevention and treatment of deep vein thrombosis, mainly due to a longer half-life and less hemorrhagic complications. Their half-life and clearance remain constant, regardless the administered dose, involving an improved predictibility of their pharmacodynamic effect without laboratory monitoring. In addition, LMWHs which interact less with platelet factor 4 are less immunogenic, leading to a lower risk of heparin-induced thrombocytopenia.

However, the main disadvantage of LMWHs therapy consists, as UFH, in their parenteral route of administration. In an effort to make the treatment more tolerable and less constraining for patients, several attempts to develop effective oral heparin formulations have been made (1-4).

Indeed, it would be an important breakthrough in the care of patients to conveniently administer LMWH orally. It has been previously demonstrated (with doses that were similar to those administered by intravenous infusion or subcutaneous injection in humans) that the administration of UFH encapsulated in polymeric particles have led to the GI absorption of the drug in rabbits (3,4).

With a view to developing an oral dosage form of LMWH, LMWH-loaded nanoparticles (NP) were prepared with three marketed LMWH and polymers accepted worldwide in the pharmaceutical field and were compared in terms of oral bioavailability after oral administration of each formulation in rabbits (600 IU/Kg) versus the various LMWH solutions administered subcutaneously (200 IU/Kg).

## EXPERIMENTAL

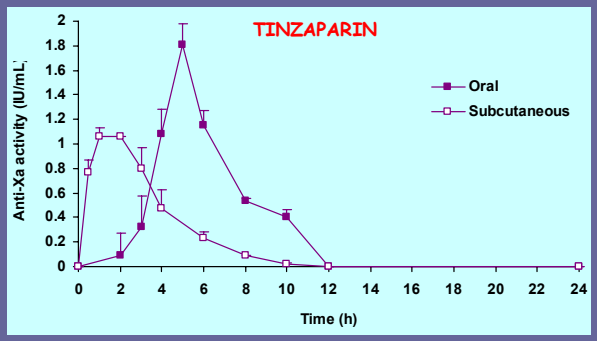
Nanoparticles were prepared by the w/o/w emulsion and solvent evaporation method. Briefly, 1 ml of each marketed LMWH (5000 anti-Xa U, Tinzaparin, Leo laboratories, Nadroparin, Sanofi-Synthelabo, Enoxaparin, Aventis) was emulsified with an ultrasound probe (15 sec) in methylene chloride (10 ml) containing a blend (250 mg, ratio 1/1) of a biodegradable polymer (poly-ε-caprolactone, PCL) and a polycationic polymethacrylate (Eudragit® RS) . This first emulsion was then emulsified into 40 ml of a PVA aqueous solution (0.1%). After sonication for 1 min following evaporation of methylene chloride under reduced pressure, the polymers precipitated involving the solidification of NP which were then isolated by centrifugation.

The encapsulation efficiency was determined by nephelemetry.

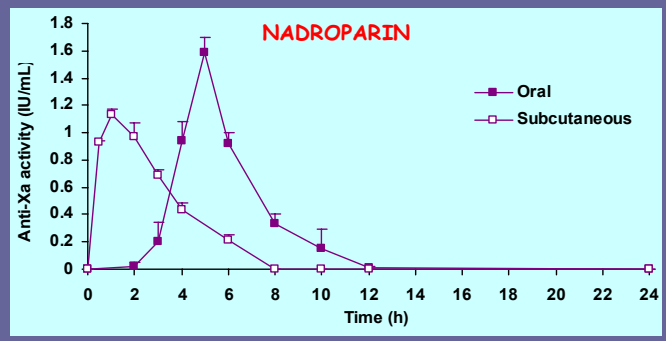
The in vivo study was performed on New Zealand rabbits fasted overnight before a single oral dose of LMWH-loaded particles (600 anti-Xa U/Kg) versus each LMWH solutions administered subcutaneously (200 anti-Xa U/Kg). Blood samples were withdrawn from the marginal ear vein at determined times over 24 hours, in sodium citrate vials (9/1, v/v). The presence of heparin recovered in blood plasma was evaluated by the anti-factor Xa activity with a chromogenic assay (Stachrom®, Stago, France).

## RESULTS

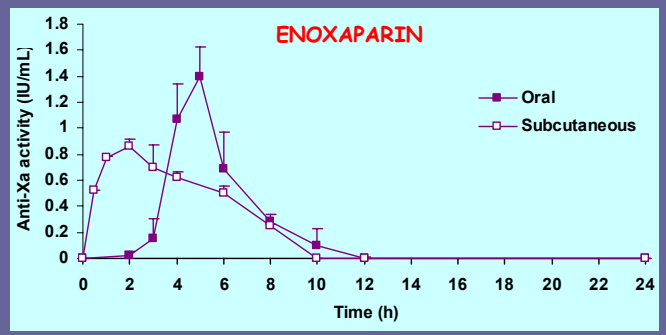
Plasma anti-factor Xa levels after oral administration in rabbits of Tinzaparin-loaded NP (600 anti-Xa U/Kg) prepared with PCL and Eudragit® RS versus the Tinzaparin solution administered subcutaneously (200 anti-Xa U/Kg) (n=4)




Plasma anti-factor Xa levels after oral administration in rabbits of Nadroparin-loaded NP (600 anti-Xa U/Kg) prepared with PCL and Eudragit® RS versus the Nadroparin solution administered subcutaneously (200 anti-Xa U/Kg) (n=4)




Plasma anti-factor Xa levels after oral administration in rabbits of Enoxaparin-loaded NP (600 anti-Xa U/Kg) prepared with PCL and Eudragit® RS versus the Enoxaparin solution administered subcutaneously (200 anti-Xa U/Kg) (n=4)




Pharmacokinetic parameters after oral absorption in rabbits of LMWHs-loaded NP (600 anti-Xa U/Kg) prepared with PCL and Eudragit® RS versus the LMWH solutions administered subcutaneously (200 anti-Xa U/Kg) (n=4)

| Formulations | Mean $C_{max}$ Anti-Xa U/mL | Mean $T_{max}$ (h) | AUC/Kg $_{0\text{-}24\ h}$ U.h/mL/Kg | Relative bioavailability (%) |
|---|---|---|---|---|
| Tinzaparin NP | 1.8 ± 0.2 | 5 | 2.0 ± 0.2 | 60.3 ± 7.3 |
| Tinzaparin Solution | 1.1 ± 0.06 | 1-2 | 1.2 ± 0.1 | - |
| Nadroparin NP | 1.6 ± 0.1 | 5 | 1.7 ± 0.2 | 47.5 ± 5.5 |
| Nadroparin Solution | 1.1 ± 0.04 | 1 | 1.3 ± 0.04 | - |
| Enoxaparin NP | 1.4 ± 0.2 | 5 | 1.7 ± 0.2 | 33.4 ± 3.6 |
| Enoxaparin solution | 0.8 ± 0.06 | 2 | 1.8 ± 0.04 | - |

In vitro characteristics of Tinzaparin, Nadroparin and Enoxaparin NP prepared with a blend of PCL and Eudragit RS (50/50) by the double emulsion method (n=4)

| Formulations PCL/RS | Size (nm) | Zeta potential (mV) | Encapsulation (%) |
|---|---|---|---|
| Tinzaparin NP | 370 ± 15 | -28 ± 7 | 58 ± 5.7 |
| Nadroparin NP | 385 ± 22 | -31 ± 5 | 57 ± 4.4 |
| Enoxaparin NP | 364 ± 17 | -41 ± 4 | 51 ± 6.9 |

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- After subcutaneous administration of each LMWH solution :

Influence of the LMWH salt on the AUC : Enoxaparin (sodium salt) AUC > Tinzaparin and Nadroparin (calcium salts) AUCs, involving an influence on the relative bioavailabilities.
Similar Cmax after subcutaneous administration of Tinzaparin and Nadroparin, and lower with Enoxaparin.

- After oral administration of each LMWH nanoparticles in rabbits :

Cmax Tinzaparin>Nadroparin>Enoxaparin.
However, Tmax were similar for each LMWH (5h).
No influence of the heparin salt on the AUC : AUCs similar

PCL/RS nanoparticles allowed the oral absorption of LMWH with a relative bioavailability ranging from 30 to 60% in rabbits. These results confirm the potential of multiparticulate systems based on the association of a biodegradable polyester and a polycationic polymer.