



# Immunogenicity of Low Molecular Weight Heparins and Their Biosimilars

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# INTRODUCTION

Heparin and low molecular weight heparins (LMWHs) used for the treatment of thrombotic and cardiovascular disorders are heterogeneous mixtures of glycosaminoglycans (GAGs).

**The chemical properties of a LMWH → translate into its biological properties**

Micro-chemical differences in the heparin molecules affect the biological activities of GAGs.

**Chemical properties make each LMWH unique → which translates into biological differences between LMWHs**

# INTRODUCTION

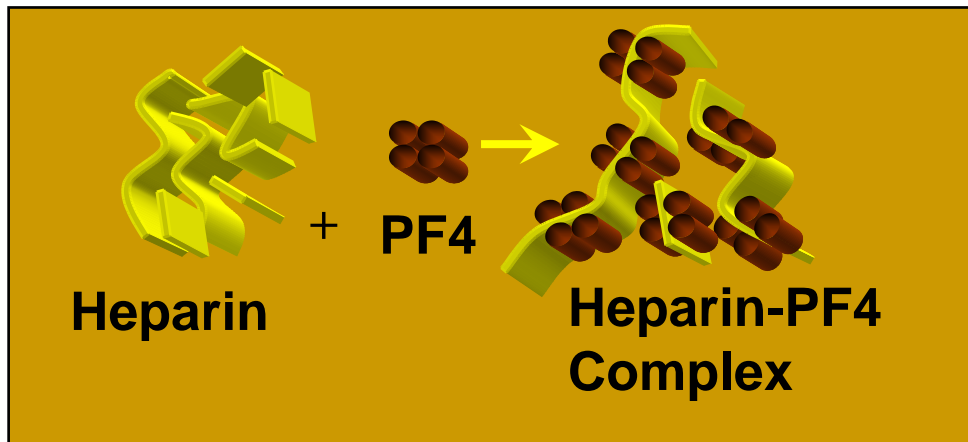
The chemical entities of LMWHs readily bind to numerous plasma proteins. GAGs bound to proteins can promote immunogenic responses.

If compositional variations exist between branded LMWHs, or between an innovator and biosimilar LMWH, then interactions with endogenous proteins such as platelet factor 4 (PF4) would differ.

The result would be a differential immunogenic response between drugs.

# INTRODUCTION

Heparin-induced thrombocytopenia (HIT) is the most common of the known immunogenic responses to heparin.



# INTRODUCTION

Although symptomatic HIT infrequently occurs in patients treated with LMWHs, anti-heparin/PF4 antibodies (HIT antibodies) do develop in up to 25% of treated patients.

There are reports that HIT antibodies that do not generate a symptomatic HIT response may still play a role in the clinical outcome of patients and also contribute to variations in the heparin therapeutic response.

The US Food and Drug Administration has required that comparative data on the immunogenic potential of all biosimilar versions of LMWHs in comparison to the innovator product be provided.

## **STUDY OBJECTIVE**

Since the molecular profile and composition of branded (and perhaps biosimilar) LMWHs varies, it was hypothesized that the immunogenic potential of each LMWH differs.

## METHODS

- The generation of HIT antibodies and their subtypes were evaluated in blood samples obtained from clinical studies of different LMWHs.
- The interaction of branded and biosimilar LMWHs with HIT antibodies was determined in functional platelet activation assays.
- The ability for LMWHs to mobilize PF4 and serotonin from platelets, and interact with these substances, was determined.

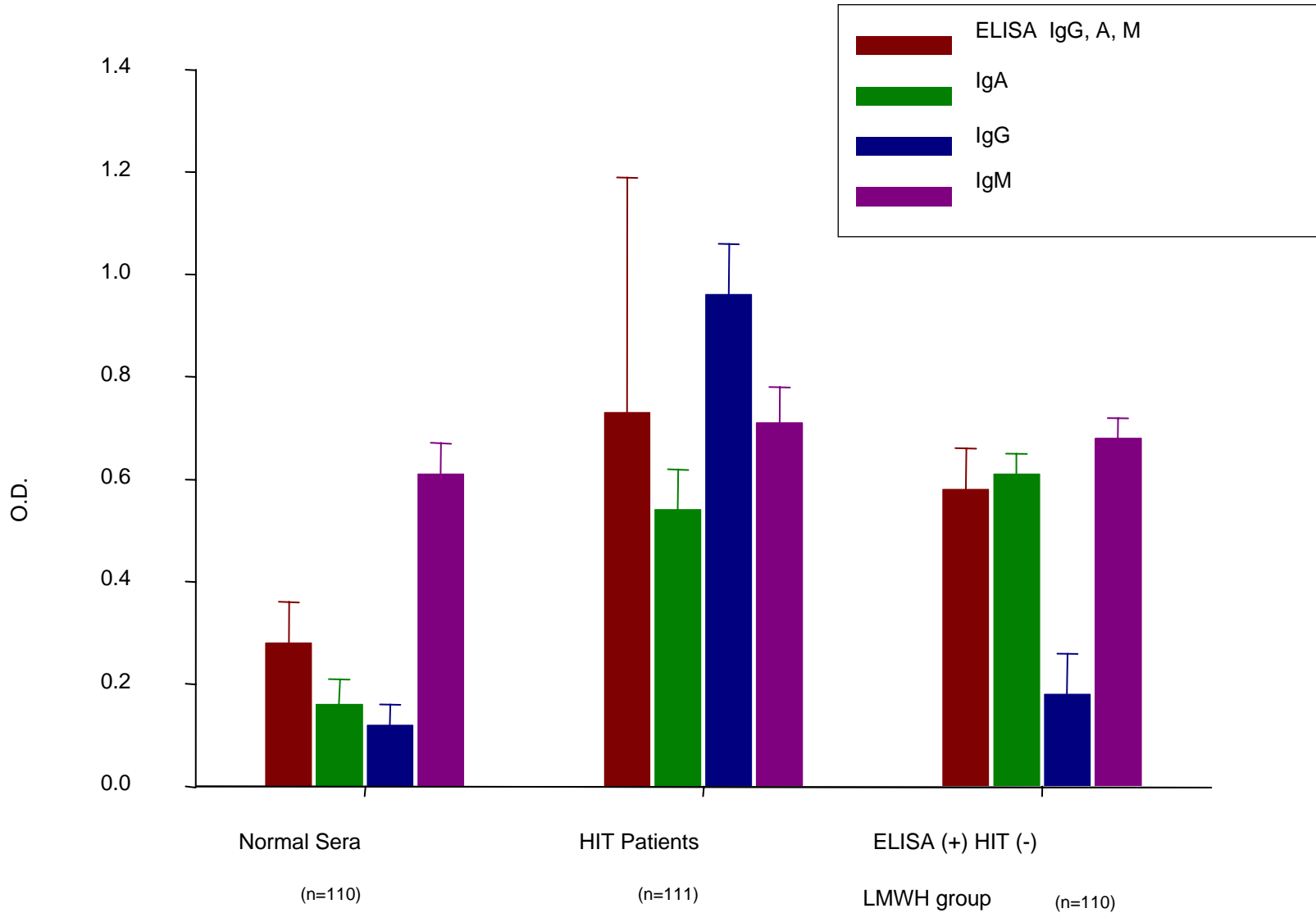
# MATERIAL

1. **Branded LMWHs** included:
  - enoxaparin (Sanofi-aventis)
  - dalteparin (Pfizer)
  - nadroparin (GSK)
  - reviparin (Abbott)
  - parnaparin (Alpha Wasserman)
  - tinzaparin (Leo)
2. **Heparin** was obtained from Gentium
3. **Biosimilar versions of enoxaparin** included:
  - Cutenox
  - Clenox
  - Dripanina
  - Dilutol
  - Fibrinox
  - Lupenox
4. **Biosimilar version of dalteparin** was Daltehep

# MATERIAL

1. Frozen citrated plasma samples from **clinical trial subjects** and frozen serum samples from clinically diagnosed HIT patients (and healthy subjects) were used.
2. The GTI **ELISA** kit (Waukesha, WI) was used to measure the **HIT antibodies** and their subtypes.
3. The interaction of LMWHs with HIT antibodies was studied using **platelet function assays**: HIPA aggregation with platelet rich plasma and  $^{14}\text{C}$ -Serotonin Release Assay (SRA).
4. **PF4 and serotonin released from platelets** [whole blood incubated with drug at 37°C for 30 min with stirring] were measured by ELISAs (PF4, Stago, Parsippany, NJ; serotonin, Alpcos, Salem, NH).
5. Routine **anti-FXa and anti-FIIa** chromogenic assays were used on the ACL (Beckman-Coulter); PF4 was from Hyphen BioMed (Neuville-sur-Oise, France).

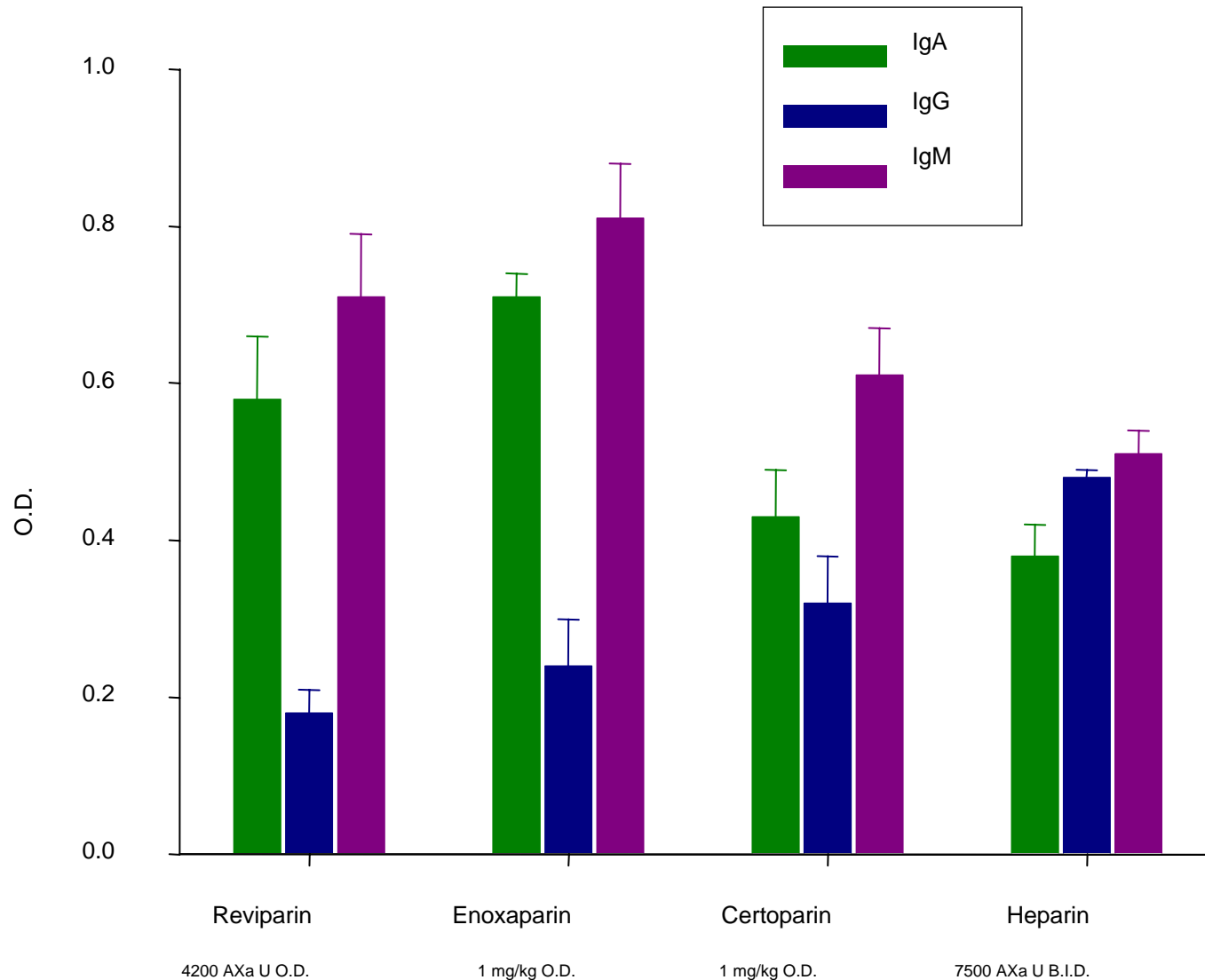
# Comparison of the Immunoglobulin Subtypes in Normals, Clinically Diagnosed HIT Patients, and ELISA Positive Non-HIT Patients

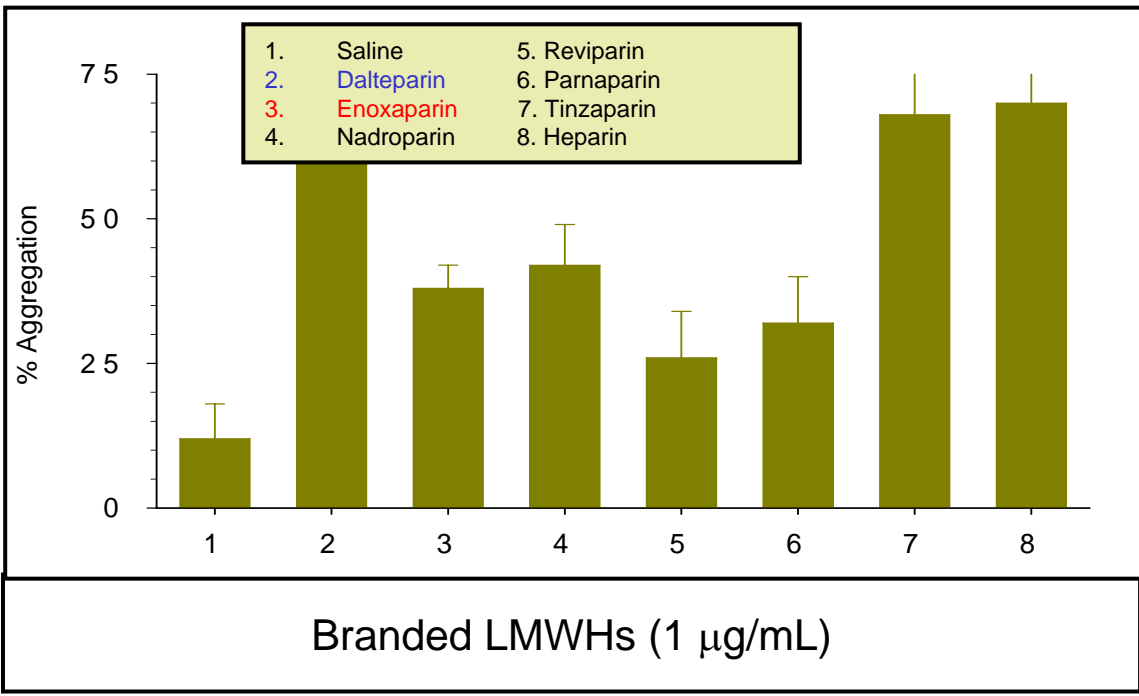


## Prevalence of Anti-Heparin/PF4 Antibodies in Various Patient Groups Treated with Different LMWHs

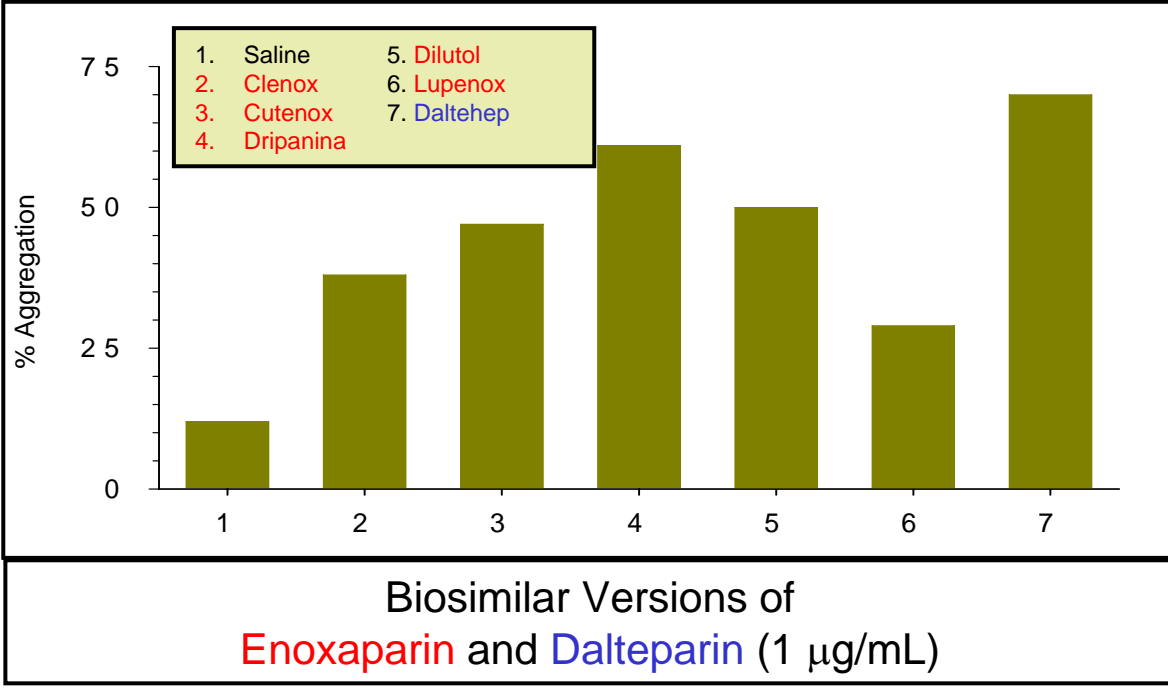
LMWH (Clinical Trial)	Dosage	Patient Group	Prevalence
Reviparin (ECHOS)	4,200 AXa U o.d.	Neurologic patients	12%
Enoxaparin (ONCENOX)	1 mg/kg o.d.	Cancer patients	15%
Certoparin (PARAT)	1 mg/kg o.d.	Acute coronary syndrome	18%
Heparin	7,500 U b.i.d.	DVT	24%

# Antibody Sub-typing of HIT Antibody Positive Samples from Various Branded LMWH Treated Groups

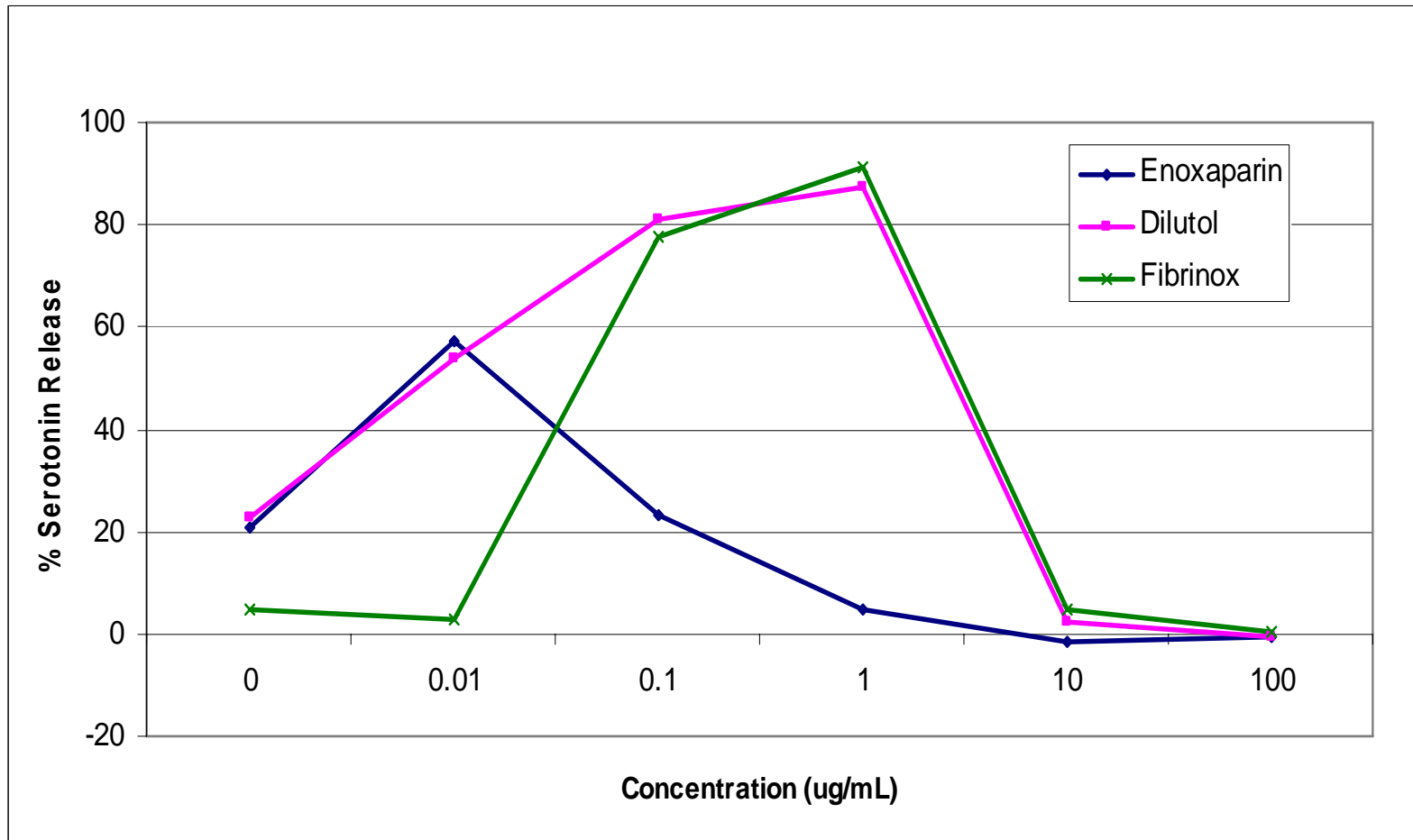




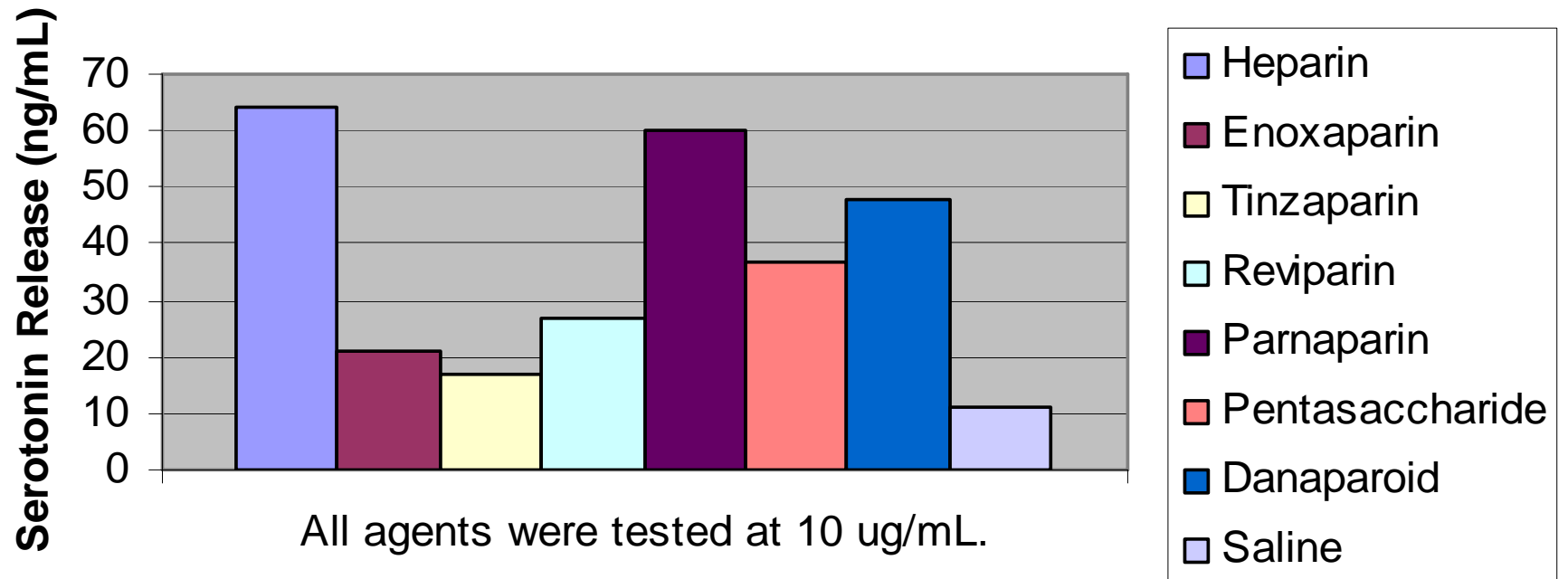
***In Vitro* Cross-Reactivity with HIT Antibodies in the Platelet Aggregation Assay**



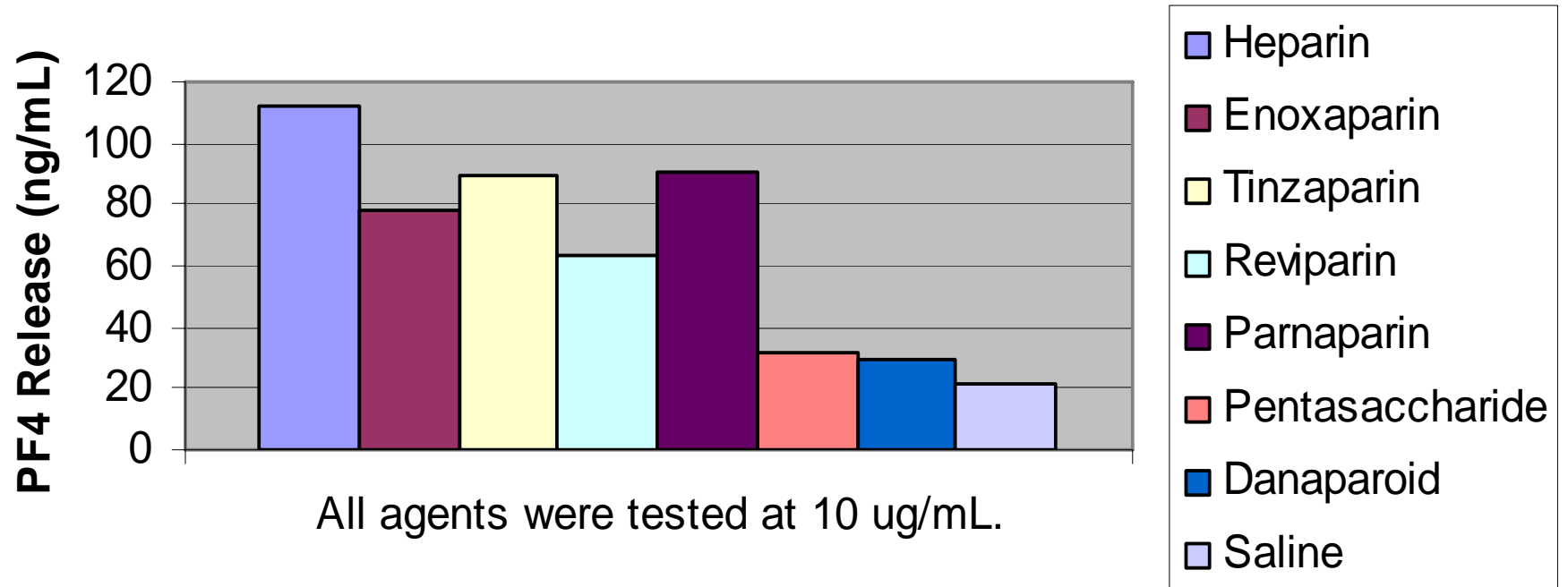
# *In Vitro* Cross-Reactivity of Biosimilar Versions of Enoxaparin with HIT Antibodies in the SRA



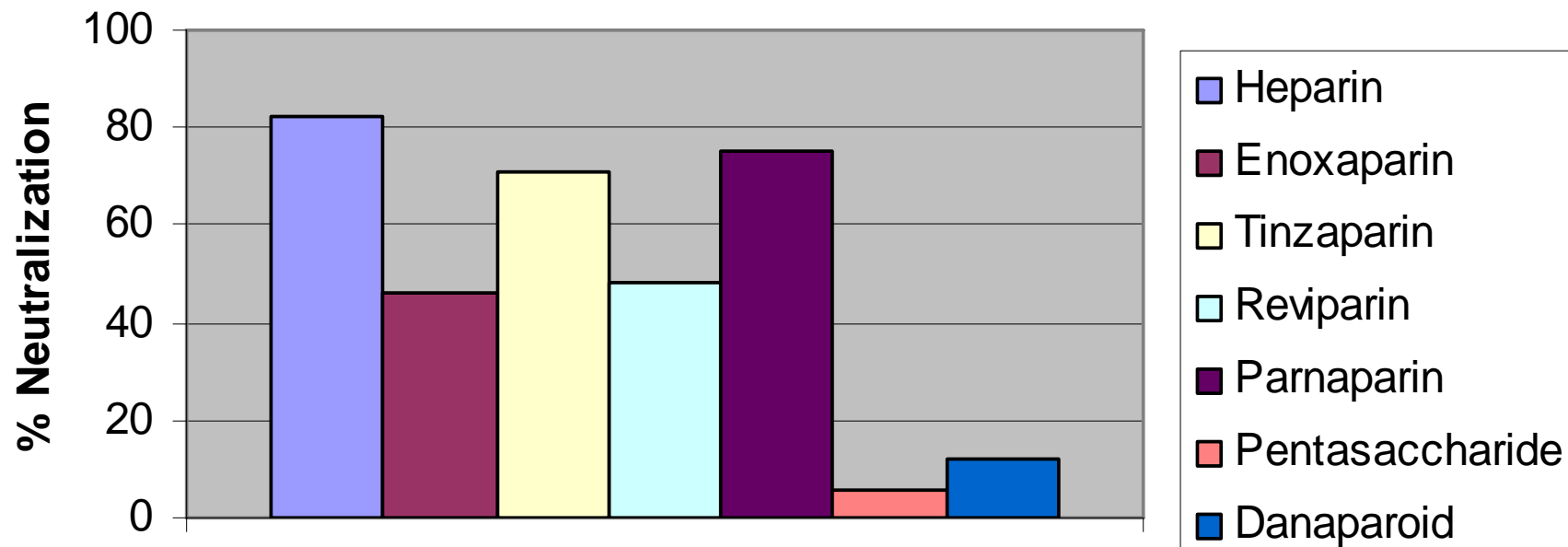
## Differential Serotonin Release in Whole Blood / Platelet Activation



## Differential PF4 Release in Whole Blood / Platelet Activation

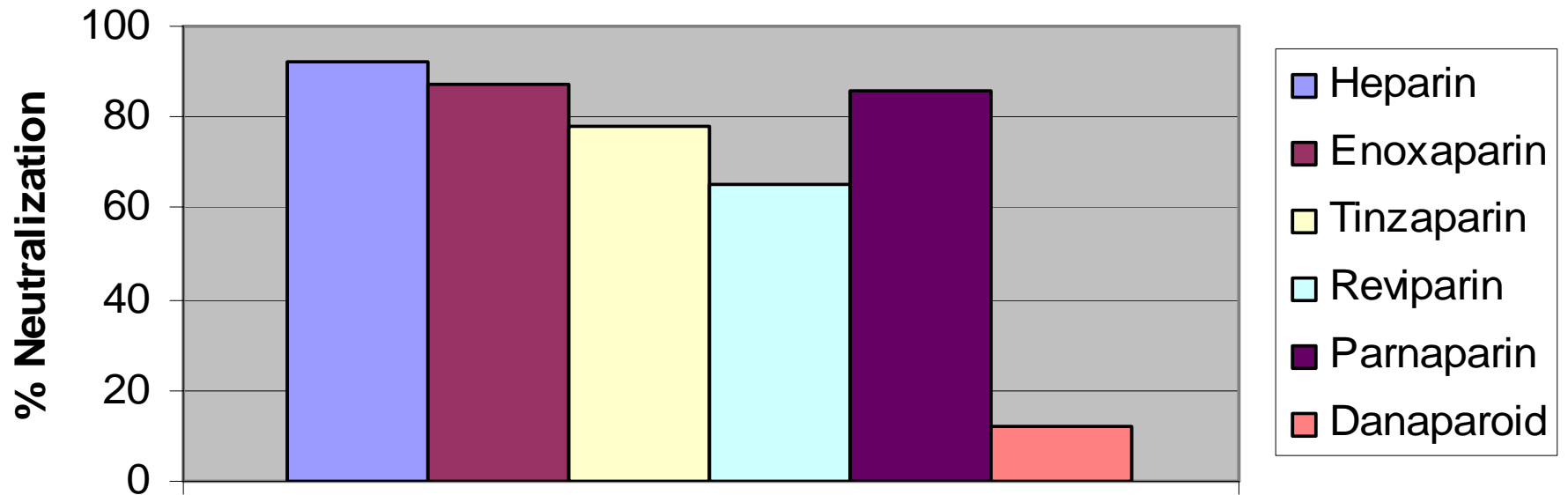


## PF4 Neutralization of the Anti-FXa Activity



All agents were tested at 5 ug/mL.

## PF4 Neutralization of the Anti-FIIa Activity



All agents were tested at 5 ug/mL.

## SUMMARY

1. The prevalence of HIT antibodies varied from 12-18% in patients treated with different branded LMWHs.
2. The antibody sub-typing showed a higher proportion of IgG antibodies in patients with symptomatic HIT.
3. The proportion of IgG to IgA and IgM HIT antibodies generated in LMWH treated patients **differed between the branded products**. Certoparin produced a higher proportion of IgG, whereas enoxaparin and reviparin produced a lower proportion.
4. In the *in vitro* platelet aggregation HIT assay, each of the branded innovator LMWHs produced a response to HIT antibodies, but the **response differed between LMWHs** in terms of onset of platelet aggregation, slope, and time to achieve maximum aggregation.

## SUMMARY

5. In the platelet aggregation HIT assay and SRA, each biosimilar version of enoxaparin showed a response to HIT antibodies, but **differences were observed between biosimilars, as well as in comparison to the branded product.**
6. In the platelet aggregation HIT assay, the biosimilar version of dalteparin showed a response to HIT antibodies that was similar to the response of the branded product.
7. **Differences** were found in the ability of each LMWH to release PF4 and serotonin from platelets.
8. **Differences** were found in the ability of each LMWH to interact with PF4 as shown by the neutralization of both the anti-FXa and anti-thrombin activities of the LMWH.

# CONCLUSIONS

- The branded LMWHs exhibit differential capacities to generate HIT antibodies.
- Differences in the interaction with HIT antibodies are evident for each of the branded LMWHs and also for the various enoxaparin biosimilars.
- Differences are evident in the interaction of each LMWH with platelets and PF4.
- **These studies suggest that compositional differences of each LMWH (branded or biosimilar) are determinants of the immunogenic effects of these drug.**
- Impurities and contaminants that adulterate these drugs can add an additional cause of immunogenicity.

## PROPOSED RECOMMENDATIONS

- Because each LMWH is characterized by its own immunogenic capacity, the immunogenic profile should be used to define the bioequivalence of LMWHs; sub-typing of antibodies generated would provide useful information.
- *In vitro* cross-reactivity studies alone would be insufficient to characterize the immunogenic profile of a LMWH due to the complex and polycomponent nature of these drugs.
- Well-designed, population balanced, clinical trials that include patients particularly at risk for developing HIT (critically ill, septic, multiple pathologies) are desirable.