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Comparative subcutaneous repeated toxicity study of enoxaparin products in rats

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ABSTRACT

Enoxaparin is a low-molecular-weight heparin widely used for the prevention and treatment of thromboembolism. With the development of several enoxaparin biosimilars, real medical concerns about their safety and efficacy have been raised. This repeated dose toxicity study consists of preclinical toxicological evaluation of a biosimilar biological version of enoxaparin, the drug product "Enoxa", compared to the enoxaparin reference drug product, "Lovenox". Eighty white Wistar rats were treated with "Enoxa" versus the reference product, using subcutaneous therapeutic and toxic doses, varying from 3.5 to 100 mg/kg/day. Dose levels were adjusted and ultimately fixed at 3.5 and 20 mg/kg/day as therapeutic and toxic doses, respectively. A sodium chloride solution (0.9%) was used as the control, and the comparative study was conducted over periods of 14 and 28 days. Comparable effects were observed with few adverse effects at the administration dose of 20 mg/kg/day, for both enoxaparin biosimilar and reference products. Interestingly, mortality started only at high doses of 40 mg/kg/day and reached 25% at 100 mg/kg/day for both products. These results, as part of the recommended biosimilarity monitoring, demonstrated comparable toxicity profiles of "Enoxa" and "Lovenox" products in rats. Continuing investigation of biosimilarity on humans to confirm safety and efficacy is suggested.

1. Introduction

Enoxaparin is a low-molecular-weight heparin (LMWH) that has been used in clinical practice for several indications, including prophylaxis and treatment of deep vein thrombosis, prophylaxis of ischemic complications of unstable angina and non-Q-wave myocardial infarction, and the treatment of acute ST-segment elevation myocardial infarction (Food and Drug Administration, 2013; Aguilar and Goldhaber, 1999).

Enoxaparin sodium, manufactured by Sanofi-Aventis, is one of the most widely prescribed LMWHs and has been used since 1993. Enoxaparin is considered to be a biological product extracted from heparin porcine mucosa (Mourier et al., 2015). Accelerated development of biosimilar versions of Enoxaparin raised concerns about their safety and efficacy (Drouet, 2012).

In fact, it is well known that the development process for biosimilars is more complex than for true generics, and the demonstration of approvability for biosimilars differs from that of standard generics, as it is based on a comparability exercise rather than demonstration of bioequivalence (Kobbi et al., 2015).

The safety evaluation method, for a new drug product as well as for a biosimilar product, is usually part of the preclinical studies performed before drug testing in humans, and experts must challenge whether one product has the potential to cause serious harm, also referred to as toxicity. Preclinical studies are usually not very large; however, they must provide detailed information on dosing and toxicity levels. In the case of biosimilar products, these studies are usually designed to assess safety profiles between reference products and the biosimilar product under evaluation. After

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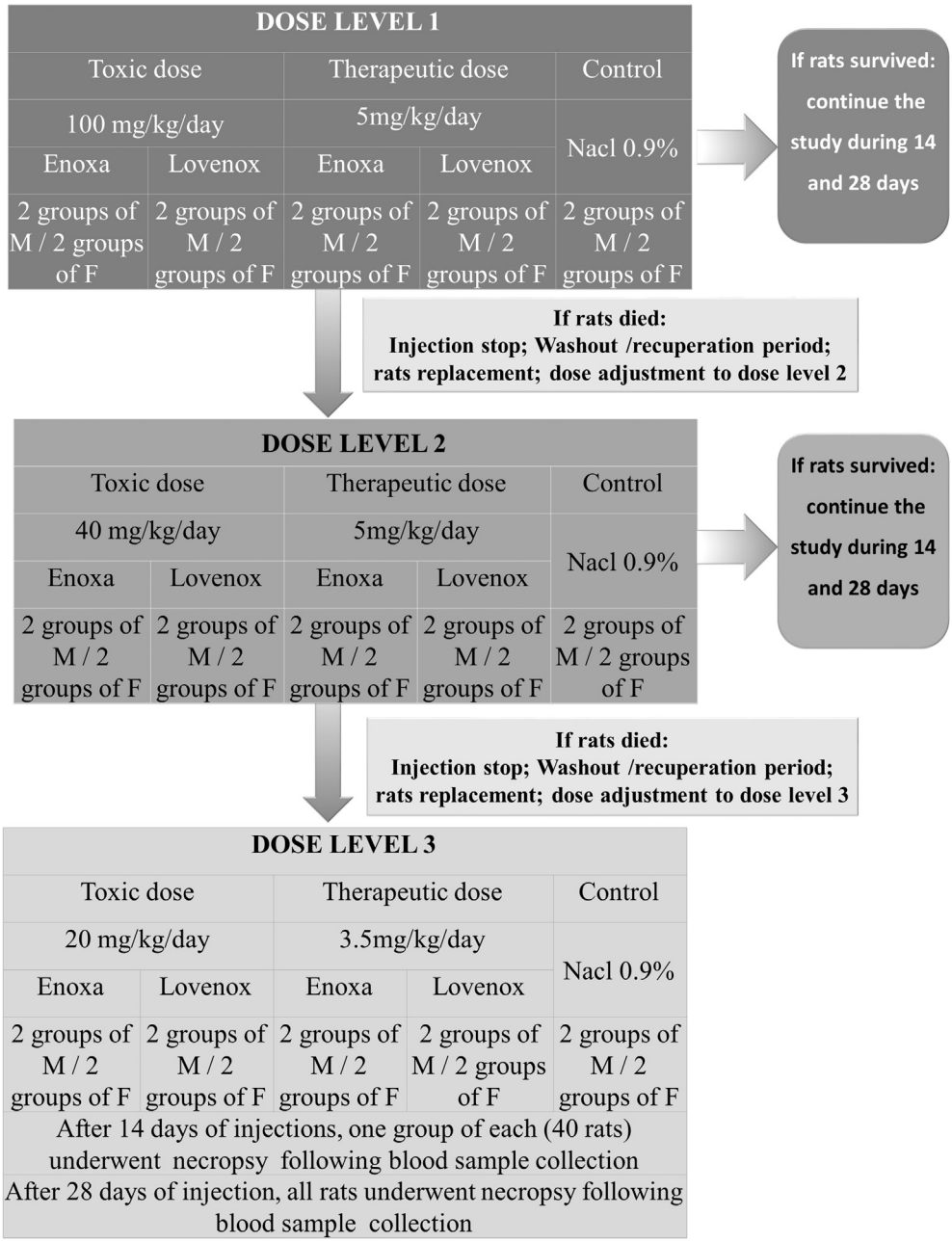

Fig. 1. General protocol study diagram. Briefly, starting with 100 mg/kg/day for the toxic dose (DOSE LEVEL 1) and subsequent to the record of the high level of mortality, the toxic dose was reduced to 40 mg/kg/day (DOSE LEVEL 2) and then to 20 mg/kg/day. The full time period study was conducted using DOSE LEVEL 3, for both toxic and therapeutic doses.

preclinical testing, experts review their findings and decide whether the drug is suitable for human trials (Food and Drug Administration, 2015).

Through the European Medicines Agency "EMA," the European Union (EU) was the first to establish a regulatory framework for biosimilars, in which animal studies are required as part of a step-wise approach to confirm similarity to the reference product (Meeret al, 2015).

Until 2003, there were no regulatory guidelines indicating what non-clinical safety studies could be expected from companies developing biosimilar products, and the only basis for the type of studies that might be required was the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) S6 guideline on preclinical evaluation of novel biotechnology-derived pharmaceuticals of September 1997 (International Conference on Harmonisation, 2008; Parnham et al., 2007).

The EMA guideline was published in 2003 and provided recommendations for non-clinical and clinical comparability of medicinal products containing biotechnology-derived proteins as drug substances. This guideline was updated in 2006 and again 8 years later, in 2014 (European Medicines Agency, 2003; 2006; 2014).

This most recent guideline detailed a battery of in vitro studies and in vivo preclinical or non-clinical studies 'if there are specific uncertainties or concerns regarding safety'. Hence, it is admitted that in vivo toxicology studies must be performed to allow a comparison between the originator and 'varied' products at several dose levels. More product-specific guidelines were issued later by