REVIEW ARTICLE

Longer-acting factor VIII to overcome limitations in haemophilia management: the PEGylated liposomes formulation issue

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Summary. Injected factor VIII (FVIII), the current treatment for haemophilia A, leads to major improvements in the quality of life and life expectancy of individuals with this disorder. However, because injected FVIII has a short half-life in vivo, this strategy has major limitations for highly demanding regimens (e.g. prophylaxis, immune tolerance induction, surgery). Newer formulations of longer-acting FVIII are presently under investigation. The use of low molecular weight polyethylene glycol (PEG)-containing liposomes as carriers for recombinant FVIII (rFVIII) results in the prolongation of haemostatic efficacy. Data from preclinical experiments in mice, early clinical evaluations, and pharmacokinetics and pharmacodynamics results indicate that an rFVIII pegylated liposomal formulation may provide potential clinical benefit to patients with severe haemophilia A by prolonging the protection from bleeding. In light of this potential clinical benefit, a multicentre, randomized, active-controlled, non-inferiority phase II trial with two parallel treatment arms and equal randomization after stratification for the presence or absence of target joints in patients and for ages ≥18 years vs. <18 years is currently being conducted. The study will test the hypothesis that rFVIII-Lip once-weekly prophylaxis is not inferior to rFVIII-water for injection thrice-weekly prophylaxis. A total of 250 patients will be enrolled with severe haemophilia A (<1% FVIII) on on-demand or secondary prophylaxis treatment and with documented bleeds or injections during the 6 months before study entry. Sixty-four centres in 14 different countries are involved in the study; recruitment is underway. In Italy, six centres have already included 15 patients (no screening failure). Eight of these patients have completed the run-in phase and have begun the home treatment. No unexpected serious adverse events have been reported thus far. Data emerging from this phase II study will help collect relevant data to overcome current limitations in haemophilia management by employing treatment with longer-acting rFVIII.

Keywords: clinical trial, efficacy, haemophilia A, PEGylated liposomes, rFVIII, safety

Introduction

Haemophilia A is an X-linked congenital bleeding disorder with a frequency of 1 in 10 000 births, caused by a functional deficiency of coagulation factor VIII (FVIII). Individuals with severe haemophilia A (<1% functional FVIII) often experience bleeding and recurrent spontaneous bleeds into soft tissues and joints. This leads to joint damage and disability, which in turn impacts the patients’ quality of life, their physical state and their financial circumstances [1,2].

Injected FVIII (plasma-derived or recombinant FVIII), the current treatment of choice for patients with haemophilia A, decreases the bleeding tendency in such individuals [1–3], with major improvements in their quality of life and life expectancy. However,
the treatment regimen itself has not improved sub-
stantially since FVIII injections became the mainstay
of haemophilia care. Because injected FVIII has a
short half-life in vivo, the necessity of frequent
infusion is a barrier to highly demanding regimens
such as prophylaxis, immune tolerance induction and
protection during major surgery [3]. There may also
be consequences with respect to cost of treatments,
inhibitor development and the availability of FVIII
concentrates [4].

Strategies for formulations of longer-acting FVIII
and their potential advantages and limitations are
summarized in the Table 1. Investigational ap-
proaches in addition to pegylation and polisalisation
include stabilization of the FVIII molecule through
the introduction of disulfide bonds (DSBs) between
the A2 and A3 domains, and mutagenesis of binding
sites such as low-density lipoprotein receptor-related
protein (LRP) that mediate FVIII catabolism [5].

Formulations that would require less frequent
injections may lead to extended protection from
bleeding, reduced frequency of bleeding episodes,
avoidance of the need for central catheter implanta-
tion and reduced total factor consumption.

Liposomes, PEG polymers and the search for
new recombinant factor VIII formulations
Polyethylene glycol polymers have been successfully
used for the preparation of long-acting drugs such as
interferon-β1a and interleukin-11 [6,7]. PEG conjuation
has been associated with molecular modifications
in interleukin-11 that may enhance protein
activity [7]. Liposomes are small lipid vesicles
(diameter ~100 nm) that serve as vehicles for drugs
[8]. When drugs are encapsulated within the aqueous
phase or conjugated to the lipid bilayer of liposomes,
degradation of the drug is prevented and its release
into the bloodstream is delayed [8]. Liposomes have
been demonstrated to improve drug targeting of
amphotericin B, doxorubicin, daunorubicin and
interleukin-2 [9–12] but are rapidly cleared from
the circulation by the reticuloendothelial system [13].
The incorporation of PEG polymers into liposomes
stabilizes them and reduces their rate of clearance
from the circulation [14]. In view of this, PEGylated
liposomes have been prepared and successfully
employed with doxorubicin and interleukin-2
[15,16]. A PEG-containing liposome that acts as a
carrier (functional excipient) for recombinant FVIII
formulated with sucrose (rFVIII-FS) [17] has been
developed. The specific non-covalent interaction of
rFVIII with PEG on the outer surface of liposomes
(distearyl phosphatidylethanolamine conjugated
with PEG 2000) has been shown to prolong the
haemostatic efficacy of rFVIII [17].

The investigational new drug, BAY 79-4980, con-
sists of rFVIII-FS reconstituted with a liposome solvent
(BAY 79-4979). Preclinical studies in normal and
haemophilic mice have shown that, compared with

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<th>Table 1. Current strategies for achieving longer-acting FVIII.</th>
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<td><strong>Strategy</strong></td>
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<td>PEG conjugation</td>
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<td>Polysialic acid modification</td>
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<td>LRP-binding site mutagenesis</td>
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IR8, DSB-FVIII and LRP muteins are considered, in theory, to be long acting. Presently,
no report has demonstrated that they have significantly prolonged efficacy in vivo vs.
standard rFVIII.

Adapted with permission from Saenko et al. [5].
aPC, activated protein C; DSB, disulphide bond; FVIII, factor VIII; IR8, intron 8; LRP,
low-density lipoprotein receptor-related protein; PEG, polyethylene glycol; PK, phar-
cmacokinetics; rFVIII, recombinant factor VIII; vWF, von Willebrand factor.

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Haemophilia (2010), 16 (Suppl. 1), 2–6
rFVIII-FS reconstituted in water for injection (WFI), the protein half-life is greatly prolonged with BAY 79-4980 [17]. This effect is not fully explained by the plasma pharmacokinetics of FVIII [18–20]. Toxicology studies, including acute studies in rats, rabbits, and beagle dogs, as well as repeated-dose studies in rats and rabbits, indicate that both BAY 79-4979 and BAY 79-4980 are well tolerated [18–20].

**Phase I clinical studies**

A patient-blinded, controlled crossover-design study [21] conducted in 23 adults with severe haemophilia A (FVIII <1%) evaluated the safety and efficacy of prophylaxis with BAY 79-4980 reconstituted with BAY 79-4979 at 22 mg kg−1 compared with rFVIII-FS. BAY 79-4980 and rFVIII-FS were each tested at two different doses (35 and 25 IU kg−1). Eleven of the 23 subjects received 35 IU kg−1 and 12 received 25 IU kg−1 of BAY 79-4980. All patients had been previously treated with on-demand therapy. The length of the bleeding-free period was evaluated. In the 35 IU kg−1 cohort, the mean number of bleeding-free days was 13.3 ± 6.2 days for BAY 79-4980 compared with 7.2 ± 1.7 days for rFVIII-FS. The mean difference of 6.1 days [median 5.0; 95% confidence interval (CI) 3.2–9.1] was statistically significant (P < 0.001). In the 25 IU kg−1 cohort, the mean number of bleeding-free days was 10.9 ± 2.9 days for BAY 79-4980 compared with 5.9 ± 1.7 days for rFVIII-FS. The mean difference of 5.0 days [median 3.8; 95% confidence interval (CI) 2.3–7.6] was statistically significant (P = 0.002).

A double-blinded, randomized, controlled, crossover-design phase I study [18] investigated the safety, pharmacokinetics and pharmacodynamics of BAY 79-4980 at a fixed rFVIII-FS dose of 35 IU kg−1 reconstituted with 22 and 13 mg kg−1 of liposomes in 26 patients with severe haemophilia A. The results were consistent with a mechanism of BAY 79-4980 that could not be explained by the extended presence of circulating plasma levels of FVIII, similar to the preclinical findings in haemophilic mice [18–20]. Other mechanisms, such as interaction with prohaemostatic cells, storage in blood or endothelial cells, and/or liposome-mediated accumulation in injured tissues are being investigated to elucidate further the mechanism of action of BAY 79-4980. A liposome-related dose-dependent elevation of low-density lipoprotein (LDL) and total cholesterol was also observed in this study and may be related to the above mechanisms. The increased mean levels of LDL and total cholesterol, which never exceeded the upper limit of normal, were unlikely to be due to de novo synthesis, but may represent reverse cholesterol transport of unesterified cholesterol from internal stores, as published for similar liposomal drugs. BAY 79-4980 does not contain cholesterol. Finally, a marked liposome dose-dependent complement C3a elevation was observed in nearly all patients studied immediately after the injection, even though only one patient had signs of a moderate hypersensitivity reaction (facial flushing and higher breathing rate, but no changes in vital signs or any other sign of hypersensitivity). The complement system is involved in the clearance of liposomes [22] and, in some patients, an excessive complement activation has been described in association with liposomal drugs. This pseudo-allergic reaction, referred to as complement activation-related pseudo-allergy (CARPA), usually occurs after the first exposure and disappears with repeated exposures [23]. Because slow infusion can prevent this reaction to liposomal drugs, BAY 79-4980 was infused over 20–30 min in this study. A different study on 18 patients who were infused at rates up to 2.42 mL min−1 for 5 min (comparable with usual infusion during home treatment) found that the drug was safe and well tolerated [24].

Taken together, the available phase I study data indicate that BAY 79-4980 may provide clinical benefit to patients with haemophilia by extending the length of time over which they are protected from bleeding and, thus, may potentially allow for less frequent administration. Therefore, a phase II trial was designed to test this hypothesis.

**Phase II trial**

A randomized, multicentre, active controlled, double-blind, parallel design study of 1-year duration was designed to evaluate the efficacy and safety of a once-weekly (QW) prophylaxis treatment with BAY 79-4980 (35 IU kg−1) compared with thrice-weekly prophylaxis with rFVIII-FS (25 IU kg−1) [25]. The study was designed to test the hypothesis that rFVIII-Lip QW prophylaxis is not inferior to rFVIII-WFI after stratification for the presence or absence of target joints and age (≥18 years vs. <18 years). The study will enrol 250 male patients aged 12–70 years with severe haemophilia A (<1% FVIII:C) who were receiving on-demand or secondary prophylaxis treatment and who had documented bleeds or injections in the 6 months before study entry (Fig. 1).

The primary objective was to evaluate the effect of prophylaxis on total bleeds using an endpoint of percentage of patients with <9 total bleeds per year. The secondary objective was to evaluate the effect of prophylaxis on joint bleeds. Patients will document
all bleeding events and all injections via electronic patient diaries. Any bleeding episode that occurs during prophylaxis and that needs treatment will be managed with a single injection of BAY 79-4980 for the test group and with palmitoyl-oleo-ylphosphatidylcholine (POPC)-rFVIII for the control group. An additional objective was to assess the 72-h pharmacokinetic data in a subset of patients in each treatment group at 6 months. The main safety variables are the short- and long-term safety and tolerability of BAY 79-4980 and the effect on cholesterol and lipoprotein blood levels.

State of the art and perspectives

The phase II study is presently being carried out in Europe, North America and Israel. As many as 62 centres in 14 different countries have been included in the preliminary phase, and recruitment is underway. In Italy, six centres have begun and have included 15 patients (no screening failure thus far). Eight patients have completed the run-in phase and have started the home treatment. A Data Safety Monitoring Board involved in a monthly review of the data and evaluation of safety, has not identified any serious safety concerns to date.

In addition to their obvious pathophysiological significance, data emerging from this phase II study are likely to contribute information to long-term safety and efficacy of this new drug. In particular, the effect of PEG and liposomes, if any, on tissue infiltration and/or interference with lipid metabolism is likely to be elucidated. Regarding clinical significance, data emerging from this phase II study may provide relevant information on a longer-acting formulation of rFVIII that may help overcome current limitations in haemophilia management.

Disclosures

F.P. is a Medical Director for Bayer Schering Pharma, Italy. A.C. has received speaker fees from Baxter, Bayer Schering Pharma and CSL Behring. The other authors have no conflicts.

References

25 Randomized, active-controlled, double-blind, parallel design study to evaluate the efficacy and safety of a once-a-week prophylaxis treatment with BAY 79-4980 compared to three times-per-week prophylaxis with rFVIII-FS in previously treated patients with severe hemophilia A. Available at: http://clinicaltrials.gov/ct2/show/NCT00623727. Accessed September 30, 2009.