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Review article

BAY 81-8973, a full-length recombinant factor VIII: Human heat shock protein 70 improves the manufacturing process without affecting clinical safety

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ABSTRACT

BAY 81-8973 is a full-length, unmodified recombinant human factor VIII (FVIII) approved for the treatment of hemophilia A. BAY 81-8973 has the same amino acid sequence as the currently marketed sucrose-formulated recombinant FVIII (rFVIII-FS) product and is produced using additional advanced manufacturing technologies. One of the key manufacturing advances for BAY 81-8973 is introduction of the gene for human heat shock protein 70 (HSP70) into the rFVIII-FS cell line. HSP70 facilitates proper folding of proteins, enhances cell survival by inhibiting apoptosis, and potentially impacts rFVIII glycosylation. HSP70 expression in the BAY 81-8973 cell line along with other manufacturing advances resulted in a higher-producing cell line and improvements in the pharmacokinetics of the final product as determined in clinical studies. HSP70 protein is not detected in the harvest or in the final BAY 81-8973 product. However, because this is a new process, clinical trial safety assessments included monitoring for anti-HSP70 antibodies. Most patients, across all age groups, had low levels of anti-HSP70 antibodies before exposure to the investigational product. During BAY 81-8973 treatment, 5% of patients had sporadic increases in anti-HSP70 antibody levels above a predefined threshold (cutoff value, 239 ng/mL). No clinical symptoms related to anti-HSP70 antibody development occurred. In conclusion, addition of HSP70 to the BAY 81-8973 cell line is an innovative technology for manufacturing rFVIII aimed at improving protein folding and expression. Improved pharmacokinetics and no effect on safety of BAY 81-8973 were observed in clinical trials in patients with hemophilia A.

Contents

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1. Introduction[1]

The development of recombinant technology for expressing factor VIII (FVIII) in mammalian cell lines greatly decreased the likelihood of bloodborne pathogen transmission in patients with hemophilia A, a concern with the use of plasma-derived FVIII products available at the time [1]. Recombinant technology continues to be refined, with current efforts aimed at improving safety and efficacy and producing a purer recombinant FVIII (rFVIII) product. An issue with recombinant techniques for rFVIII manufacture is the generally low level of FVIII expression by the cell line [2,3]. One potential means of enhancing FVIII expression and achieving more consistent posttranslational modification is to reduce aggregation of misfolded proteins and cell apoptosis (programmed cell death) by introducing the gene for human heat shock protein 70 (HSP70) into the cell line. HSP70 is an intracellular chaperone protein that facilitates proper protein folding and enhances cell survival, and coexpression may result in a rFVIII product of high and consistent purity [2,4,5].

BAY 81-8973 (Kovaltry®, Bayer, Berkeley, CA, USA) is a full-length, unmodified, recombinant human FVIII approved for prevention and treatment of bleeding episodes in patients with hemophilia A. BAY 81-8973 has the same amino acid sequence as sucrose-formulated rFVIII (rFVIII-FS; Kogenate® FS/Bayer; Bayer, Berkeley, CA, USA) and is produced using additional advanced manufacturing technologies [6,7]. A key improvement in the manufacturing of BAY 81-8973 compared with rFVIII-FS is the use of an improved cell line into which the human gene for expression of HSP70 has been introduced [6]. Other advances in BAY 81-8973 manufacturing include production without addition of human- or animal-derived raw materials to the cell culture, purification, or formulation processes; use of an optimized and simplified purification process; and the addition of a filtration step that uses a 20-nm pore size filter capable of removing small nonenveloped viruses and potential protein aggregates [6]. These manufacturing changes resulted in a more productive, apoptosis-resistant cell line and a rFVIII product of high, consistent purity with highly branched and sialylated glycans. The resultant rFVIII product exhibited favorable pharmacokinetics in clinical studies [8]. This article discusses the rationale for and implications of the use of HSP70 chaperone protein in the BAY 81-8973 manufacturing process.

2. Role of HSP70 in cellular function

Heat shock proteins are a family of proteins expressed in response to cellular stress, including exposure to heat, cytotoxic drugs, or ultraviolet irradiation [9,10]. Heat shock proteins are categorized by size; at 70 kDa, HSP70 is a high molecular weight HSP [9]. High molecular weight HSPs are distinguished from small HSPs by their dependence on adenosine triphosphate (ATP) for proper functioning, whereas small HSPs appear to be primarily controlled by phosphorylation status [9].

Heat shock protein 70 and other HSPs function as molecular chaperones (proteins that facilitate folding of proteins and provide quality control [11]); they execute essential and protective cellular functions under normal physiologic conditions and in conditions of environmental stress. The functions of HSP70 are accomplished intracellularly by protein-protein interactions and include (1) facilitation of proper folding of newly formed proteins, refolding of denatured or aggregated proteins, and degradation of proteins that cannot be properly refolded [4,5,9,11] (HSP70 and other co-chaperones can aid in protein degradation through interaction with the ubiquitin-proteasome system [9]); (2) facilitation of translocation of proteins across membranes [4]; (3) protection against stress-induced programmed cell death [10] by providing greater resistance to apoptosis-inducing agents and cell culture conditions [4]; (4) regulation of cell cycle [4]; and (5) direct maintenance of genomic stability by enhancing DNA repair [12–14].

The HSP70 gene is highly inducible [15,16], which is consistent with the variable levels of endogenous HSP70 measured in humans [17]. Although its function is intracellular, HSP70 can be released into the extracellular environment, and serum levels of HSP70 in young, healthy individuals have been found in the range of 60–3000 ng/mL [18,19]. HSP70 levels decrease with age [18] but are increased in patients with acute infections, in whom serum levels of approximately 500–6021 ng/mL have been measured [19].

Antibodies to HSP70 have been detected in healthy individuals [17] and in several disease states. Varying levels of anti-HSP70 antibodies have been reported in patients with hypertension [20], atherosclerotic cardiovascular disorders [21], and inflammatory diseases such as Behçet-induced uveitis [22]; in pediatric patients on hemodialysis [23]; and in healthy pregnant women [24]. Anti-HSP70 antibody levels tend to increase with age [18].

3. Role of HSP70 in BAY 81-8973 manufacturing

The feasibility of using HSP70 to increase FVIII expression was demonstrated in a cell culture study comparing an existing baby hamster kidney (BHK)-21 cell line expressing full-length rFVIII (rBHK-21-host) with the same cell line transfected with the human HSP70 gene (rBHK-21-HSP70) [2]. Apoptosis, induced by nutrient deprivation or exposure to cytotoxins, was inhibited in the rBHK-21-HSP70 cells compared with the rBHK-21-host cells [2]. The rBHK-21-HSP70 cells also showed an approximate 2-fold increase in rFVIII productivity and procoagulant activity versus rBHK-21-host cells [2]. A separate study indicated that the anti-apoptotic effects of HSP70 may enhance rFVIII expression by inhibiting adherence of rFVIII to the cell surface (which limits rFVIII productivity) and maintaining higher intracellular levels of FVIII [3]. BAY 81-8973 is the first use of HSP70 coexpression in mammalian cells for production of a licensed recombinant therapeutic protein (US Patent No: US 2005/0048608 AI). The BAY 81-8973 cell line has 2 copies of the HSP70 gene per cell; expression of HSP70 was found to be consistent over a 7-day culture period [2]. No HSP70 was detected in BAY 81-8973 drug substance by a very sensitive western blot assay (limit of detection, 1.5 ng/mL).

4. BAY 81-8973 molecule description

Factor VIII is a highly glycosylated protein that contains several N- and O-linked glycans. Compared with its predecessor, rFVIII-FS, BAY 81-8973 presents a higher proportion of highly branched, sialylated carbohydrates and a consistently high degree of sialic acid capping of N-terminal glycans (BAY 81-8973, a full-length recombinant FVIII: manufacturing processes and product characteristics [Manuscript in preparation]); this posttranslational modification step may affect the half-life of some mammalian proteins [25].

[1] BHK = baby hamster kidney; HSP70 = heat shock protein 70; LEOPOLD = Long-Term Efficacy Open-Label Program in Severe Hemophilia A Disease; rFVIII = recombinant factor VIII; rFVIII-FS = sucrose-formulated rFVIII.

4.1. BAY 81-8973 pharmacokinetics

In the Long-Term Efficacy Open-Label Program in Severe Hemophilia A Disease (LEOPOLD) clinical trials, the pharmacokinetic profile of BAY 81-8973 was compared in a crossover design with rFVIII-FS. The overall pharmacokinetic profile of BAY 81-8973 was more favorable than that for rFVIII-FS [8]. Compared with rFVIII-FS, BAY 81-8973 had a longer half-life (13.8 vs 12.0 h), higher area under the curve (AUC; 1889.2 vs 1583.9 IU·h/dL) and mean residence time (19.3 vs 16.5 h), and slower clearance (0.026 vs 0.032 dL/h/kg; data cited are geometric mean results using the chromogenic assay) [8]. Differences were statistically significant for all of these parameters. Compared with its predecessor, BAY 81-8973 showed significant improvement in pharmacokinetic parameters that are relevant for maintaining a protective FVIII level during prophylaxis treatment. In a phase 1, open-label, crossover clinical study in 18 patients aged 19–64 years, the pharmacokinetic profile of BAY 81-8973 was superior to that of another commercial rFVIII [26]; simulations based on the study data demonstrated that the time to a trough level of 1% was 18–19 hours longer for BAY 81-8973 compared with the other rFVIII in 50% of patients [26].

5. BAY 81-8973 clinical data

5.1. Anti-HSP70 antibodies and clinical safety

The clinical efficacy and safety of BAY 81-8973 were evaluated in the LEOPOLD clinical development program, comprising 3 clinical trials (LEOPOLD I, LEOPOLD II, LEOPOLD Kids) in children, adolescents, and adults with severe hemophilia A [27–29]. HSP70 is not detected in the cell culture medium or in the final BAY 81-8973 product. However, because of the change in manufacturing methods, assessment of the safety of BAY 81-8973 included routine monitoring for the development of anti-HSP70 antibodies in addition to standard assays for anti-drug and anti-host cell antibodies [27]. Anti-HSP70 antibody levels were measured in each study by enzyme-linked immunosorbent assay using a commercial kit; measurements were performed every 3 months for adults (observation time of 1 year) and at the beginning and end of the 6-month study for children. Because anti-HSP70 antibodies are found in the general population, antibody levels above the 95th percentile in a sample of the general population were determined. The cutoff value for antibody negativity or normal levels was set at 239 ng/mL during method validation, with a lower limit of quantification of 25 or 50 ng/mL. The cutoff was determined based on analysis of 50 samples from healthy controls applying standard statistical approaches for evaluation [30], with a 95% CI yielding a 5% false-positive rate. Anti-HSP70 antibody tests were performed by a centralized laboratory (PRA International, Early Development Service, Assen, Netherlands).

In the LEOPOLD I (N = 62; median age, 30.0 years), LEOPOLD II (N = 80; median age, 28.5 years), and LEOPOLD Kids (N = 51; median age, 6 years) trials, all of which enrolled patients previously treated with a FVIII product, most patients had detectable anti-HSP70 antibody levels before first exposure to BAY 81-8973 (pretreatment) but were below the defined assay cutoff for positivity (LEOPOLD I, mean ± SD, 88.4 ± 46.9 ng/mL [range, 25.0–244.0 ng/mL]; LEOPOLD II, mean ± SD, 86.2 ± 99.0 ng/mL [range, 25.0–861.0 ng/mL]). Four of the 193 patients (2.1%) had anti-HSP70 antibody levels above the cutoff level pretreatment; 3 of these patients became negative during the study and 1 patient remained positive. Of the 189 patients who were negative pretreatment, 10 patients (5.3%) had positive anti-HSP70 antibody levels during BAY 81-8973 treatment for at least 1 of the assessment time points during the study; of these, 5 patients were transiently positive at single time points, and 5 patients remained positive until the end of the study with decreasing values (Table 1).

Anti-HSP70 antibody levels at various time points for the patients who had any positive antibody titers during the LEOPOLD trials are shown in Fig. 1. All patients with positive anti-HSP70 antibody levels had a diagnosis of hemophilic arthropathy or chronic synovitis, and 4 patients had a chronic hepatitis C virus (HCV) infection. Several additional pathologic conditions that may be indicative of inflammatory reactions, including upper respiratory tract infections, common cold, increased liver enzymes, caries, high neutrophil count, and joint pain symptoms, were observed at the time of increased anti-HSP70 antibody levels. The observed increased antibody levels were in most cases only slightly above the predefined threshold for positivity (range, 240–584 ng/mL). The highest value was observed before the start of BAY 81-8973 treatment in 1 patient. In the LEOPOLD Kids trial, 1 patient had positive anti-HSP70 antibody levels (1865 ng/mL) pretreatment only, and all subsequent anti-HSP70 values were negative. The patient's clinical history was notable because the pretreatment sample was collected approximately 1 week after the patient was treated for a central venous access device infection, suggesting that the antibodies were present as a part of an inflammatory response against bacterial infection.

6. Discussion

BAY 81-8973 is a full-length, unmodified, recombinant human FVIII approved for prevention and treatment of bleeding episodes in hemophilia A. BAY 81-8973 has the same amino acid sequence as the currently marketed product rFVIII-FS and is produced using additional advanced manufacturing technologies [6]. One of the key advances in the BAY 81-8973 manufacturing process was the introduction of the human HSP70 gene into the cell line to improve cell viability and increase rFVIII yield [6]. The overall changes in BAY 81-8973 manufacturing have resulted in a rFVIII product of high

Table 1
Patients with anti-HSP70 antibody formation in the LEOPOLD Kids, LEOPOLD I, and LEOPOLD II trials.

	LEOPOLD Kids safety pool (n = 51)	LEOPOLD I safety pool (n = 62)	LEOPOLD II safety pool (n = 80)
At least 1 positive[a] anti-HSP70 antibody result pretreatment, n (%)	1 (2.0)	1 (1.6)	2 (2.5)
At least 1 positive result during BAY 81-8973 treatment	0	0	1 (50)
Only negative results during BAY 81-8973 treatment	1 (100)	1 (100)	1 (50)
Only negative results pretreatment, n (%)	50 (98.0)	61 (98.4)	78 (97.5)
At least 1 positive result during BAY 81-8973 treatment	0	2 (3.3)[b]	8 (10.3)
Only negative results during BAY 81-8973 treatment	50 (100)	59 (96.7)	70 (89.7)

HSP70 = heat shock protein 70; LEOPOLD = Long-Term Efficacy Open-Label Program in Severe Hemophilia A Disease.
[a] Positive defined as >95th percentile for normal population (cutoff value, 239 ng/mL).
[b] 1 patient became positive during the 1-year LEOPOLD I extension.

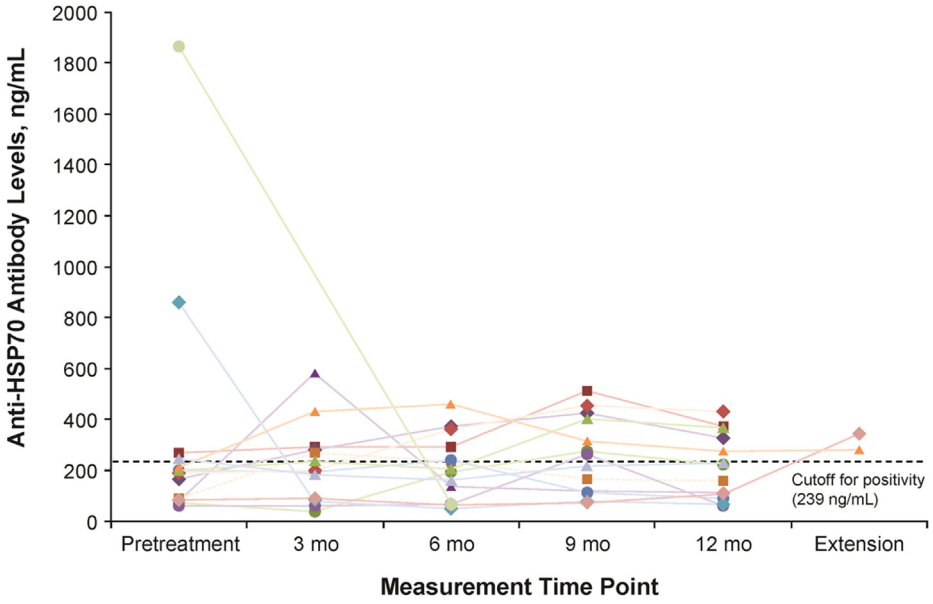

Fig. 1. Anti-HSP70 antibody titers at various time points in patients with at least 1 elevated anti-HSP70 level in the LEOPOLD trials. Symbols represent anti-HSP70 antibody titers in individual patients at each measurement time point; horizontal dashed line indicates the cutoff value for antibody positivity (239 ng/mL). HSP70 = heat shock protein 70; LEOPOLD = Long-Term Efficacy Open-Label Program in Severe Hemophilia A Disease.

and consistent purity and with highly sialylated and branched glycan structure that is relevant for the half-life of some proteins [8,27].

The pharmacokinetics of BAY 81-8973 compared favorably with the predecessor FVIII, rFVIII-FS [8]. In addition, the improved half-life and AUC of BAY 81-8973 resulted in a prolonged time above the threshold of 1% compared with another commercial rFVIII [26]. The enhanced pharmacokinetics of BAY 81-8973 may be one of the reasons for the low bleeding rate observed with a twice-weekly dosing regimen [31].

BAY 81-8973 efficacy and safety were evaluated in 3 clinical trials enrolling pediatric, adolescent, and adult patients with hemophilia A. Although HSP70 is not detectable in the final product, safety assessments in each trial included monitoring for development of anti-HSP70 antibodies in addition to monitoring for FVIII inhibitors and anti–host cell antibodies. Overall, across all BAY 81-8973 studies, any measured increase in anti-HSP70 antibodies was small and only slightly above the assay cutoff, representing both the upper 5% of the normal distribution of anti-HSP70 antibody levels (5% false-positive rate of the validated cutoff) and minor fluctuations of anti-HSP70 antibody levels in single patients over time.

Our work confirms that anti-HSP70 antibodies are commonly found in healthy individuals [17] and most likely result from normal and ongoing exposure to HSP70 released into the blood during infection or other inflammatory processes and that these levels tend to increase with age [18]. Serum HSP70 levels in persons with acute infection have been reported to range from 500 – 6021 ng/mL [19]. Increased expression of HSP70 has also been reported in several disorders, including rheumatoid arthritis, systemic lupus erythematosus, and hypertension [19,32,33]. For the purposes of the LEOPOLD clinical trials, a conservative cutoff level of 239 ng/mL (95th percentile of values in a normal population) was defined to allow a false-positive rate of 5%. This cutoff value may not be clinically relevant but was chosen to ensure that the assay was sufficiently sensitive to detect and characterize potential new immune responses to BAY 81-8973. Overall, treatment with BAY 81-8973 did not result in a meaningful change in anti-HSP70 antibody titers. In the few patients in whom an increase in anti-HSP70 antibody levels was observed, the rise was almost always temporally associated with an acute infection or concomitant inflammatory event. When antibody responses were observed, no other associated hypersensitivity reactions were reported. Thus, it is unlikely that BAY 81-8973 induces any relevant anti-HSP70 antibody response.

In summary, advanced manufacturing technologies including the use of the human HSP70 gene in BAY 81-8973 manufacturing yield a higher-producing cell line with better cell viability and more consistent posttranslational modifications. The expressed FVIII has an improved pharmacokinetic profile and does not affect BAY 81-8973 safety, based on data collected as part of the LEOPOLD clinical trial program.

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