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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.