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Standardization

Biological standardization of human interferon beta: Establishment of a replacement world health organization international biological standard for human glycosylated interferon beta

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Abstract

Human interferon beta (IFN-β) has been developed as a major biotherapeutic agent for the treatment of multiple sclerosis. Since World Health Organization (WHO) international standards (IS) for IFN-β were established several years prior to the development of clinical grade IFN-β products, a number of scientific issues with regard to the biological standardisation of natural and recombinant IFN-β products have emerged. In order to address these issues, an international collaborative study to evaluate WHO IS and candidate international standards (CIS) of IFN-β was instigated by the National Institute for Biological Standards and Control (NIBSC) in 2000 and was carried out in the succeeding year. Sixteen expert laboratories from 8 countries worldwide participated in the study. They performed titrations on 8 different IFN-β preparations, including IS and new CIS, in a variety of mainly antiviral- but also including some antiproliferative- and reporter gene-assays, and contributed raw data from these assays to NIBSC for statistical analysis and calculation of potencies. While both intra- and inter-laboratory variation of potency estimates was evident, overall validity of the study as a whole was clearly shown by comparison of two pairs of internal coded duplicates, which gave the expected relative potency of 1 and the lowest inter-laboratory variability of potency estimates in all assay types. The CIS containing Chinese hamster ovary (CHO) cell- or human fibroblast-derived, glycosylated, IFN-β gave similar low inter-laboratory variation in potency estimates one to another as the coded duplicates, which was significantly less than to the 2nd WHO IS of IFN-β, human fibroblast-derived, Gb23-902-531. One of these CIS, designated 00/572, containing CHO cell-derived IFN-β and formulated with both bovine casein and human serum albumin, could be assigned a potency, consistent for all assay types, of 40,000 international units (IU) per ampoule relative to the IU of the 2nd IS of IFN-β,

Abbreviations: AP, antiproliferative; AV, antiviral; CHO, Chinese hamster ovary; CIS, candidate international standard; ECBS, Expert Committee on Biological Standardization; EMCV, encephalomyocarditis virus; HSA, human serum albumin; IFN, interferon; IL-6, interleukin-6; IS, international standard; ISRE, interferon stimulated response element; JapNS, Japanese National Standard; NIAID, National Institute of Allergy and Infectious Diseases; NIBSC, National Institute for Biological Standards and Control; NIH, National Institutes of Health; RG, reporter gene; SEAP, secreted alkaline phosphatase; SINV, Sindbis virus; VSV, vesicular stomatitis virus; WHO, World Health Organization.

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Gb23-902-531. Other CIS containing glycosylated IFN-β, either CHO cell- or human-fibroblast-derived, could also be assigned potency values that were continuous with the IU of Gb23-902-531 and 00/572. However, greater inter-laboratory variations in estimates were evident from comparisons of Gb23-902-531 or 00/572 with either the 1st IS for *E. coli*-derived, non-glycosylated, IFN-β with serine substitution at position 17 (IFN-β Ser 17 mutein), Gxb02-901-535, or with a CIS (00/574) containing IFN-β Ser 17 mutein. Indeed, variations in potency estimates for preparations containing IFN-β Ser 17 mutein were sufficiently large to indicate that assays could distinguish preparations of IFN-β Ser 17 mutein from preparations of glycosylated IFN-β. Thus, neither the 2nd IS of IFN-β, Gb23-902-531, containing fibroblast-derived IFN-β, nor CIS, 00/572, containing CHO cell-derived IFN-β, was appropriate for standardisation of preparations of IFN-β Ser 17 mutein. Conversely, neither the IS of IFN-β Ser 17 mutein, Gxb02-901-535, or a CIS of IFN-β Ser 17 mutein, 00/574, was appropriate for the standardisation of preparations of glycosylated IFN-β.

CIS 00/572, containing CHO cell-derived, glycosylated IFN-β, was clearly shown to be suitable to serve as a primary standard for glycosylated forms of IFN-β, especially clinical grade IFN-β-1a products. It was further shown to exhibit high thermal and long-term stability. Since the CHO cell-derived IFN-β used for preparation of 00/572 was of a greater purity than the IFN-β used for the 2nd IS of IFN-β, Gb23-902-531, it was recommended by the WHO Informal Consultation on the Standardisation of Cytokines, Growth Factors and Other Endocrinological Substances, which met in October 2003, that 00/572 should replace Gb23-902-531 as the IS for glycosylated IFN-β. This recommendation was accepted by the WHO Expert Committee on Biological Standardization (ECBS) at its annual meeting in November 2003 and 00/572 was established as the 3rd IS for human glycosylated IFN-β with an assigned potency of 40,000 IU. As this study identified no advantage to replacing the existing 1st IS for IFN-β Ser 17 mutein, Gxb02-901-535, WHO ECBS accepted that this should continue to serve as the IS for this material.

1. Introduction

Human IFN-β is a 166-amino acid protein with an α-helical bundle structure and ~30% sequence homology to the consensus sequence of human IFN-α (Taniguchi et al., 1980; Karpusas et al., 1997; Meager, 1998). Unlike IFN-α, IFN-β contains a single site, asparagine 80, through which N-linked glycosylation can occur and which has been shown to vary according to the cells in which IFN-β is synthesized (Kagawa et al., 1988; Utsumi et al., 1989). It is thought that glycosylation plays important roles in facilitating IFN-β secretion from cells, in increasing its solubility and stability, and in its metabolism in vivo (Edy et al., 1977; Knight and Fahey, 1982; Watanabe and Kawade, 1983; McCullagh, 1983; Satoh et al., 1984; Utsumi et al., 1989). However, non-glycosylated IFN-β, e.g., produced by genetically engineered *E. coli*, although more hydrophobic than glycosylated IFN-β produced by either human fibroblasts or genetically modified mammalian cells, retains biological activity although with lower specific activity (Utsumi et al., 1989; Runkel et al., 1998).

The WHO ECBS had previously established two IS for IFN-β. The 2nd IS for glycosylated IFN-β (fibroblast-derived), Gb23-902-531, serves as primary calibrant for preparations of glycosylated IFN-β. The 1st IS for IFN-β Ser 17 mutein, a stable recombinant IFN-β molecule expressed in *E. coli* following site-specific mutagenesis of the IFN-β gene (Mark et al., 1984), serves as primary calibrant for the standardization of preparations of IFN-β Ser 17 mutein (Standardization of interferons, 1987; Pestka and Meager, 1997). As these IS were established prior to the development of recombinant IFN-β products, particularly CHO cell-derived, glycosylated IFN-β (IFN-β-1a), licensed for the treatment of relapsing and remitting multiple sclerosis, their suitability to serve as IS for the marketed IFN-β products required evaluation. The WHO 2nd IS for glycosylated IFN-β, Gb23-902-531, contains human fibroblast-derived IFN-β that was partially purified by control pore glass affinity adsorption (De Ley et al., 1980). The IFN-β content was approximately 1% of total protein, and the preparation has subsequently been shown to contain cytokines other than IFN-β, most notably interleukin-6 (IL-6), which are also likely to be adsorbed to control pore glass (Content et al., 1982; Van Damme and Billiau, 1982; Le and Vilcek, 1989; A. Meager, unpublished results). The cytokine contaminants present in this IS might mod-