Formulation and Stability of Cytokine Therapeutics

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ABSTRACT: Cytokines are messenger proteins that regulate the proliferation and differentiation of cells and control immune responses. Interferons, interleukins, and growth factors have applications in cancer, autoimmune, and viral disease treatment. The cytokines are susceptible to chemical and physical instability. This article reviews the structure and stability issues of clinically used cytokines, as well as formulation strategies for improved stability. Some general aspects for identifying most probable stability concerns, selecting excipients, and developing stable cytokine formulations are presented. The vast group of cytokines offers possibilities for new biopharmaceuticals. The formulation approaches of the current cytokine products could facilitate development of new biopharmaceuticals. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci

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INTRODUCTION

Cytokines are small secreted proteins that enable receptor-mediated communication between cells and act as key modulators of immune responses by controlling growth, differentiation, and activation of various cell types. Hundreds of cytokines have been identified[1] and some of these proteins are currently used in therapeutic products, mostly for treating cancer, autoimmune, and viral diseases (Table 1). The biological mechanisms and clinical use of cytokines have been well reviewed,[1–5] but aspects of formulation and stability have been less widely studied. Here, we review cytokine therapeutics marketed in the United States and in Europe, aspects related to their structural instability, and the role of formulation excipients toward improving stability. There are decades between the market introduction of the first and the most recent cytokine products. Formulation strategies have evolved during that time and some of the first-generation products have been reformulated. The main difference to the older approaches is the current preference of albumin-free formulations. In addition, cytokines with conjugated polyethylene glycol (PEG) chains have been introduced to market. These pegylated proteins have greatly increased half-lives compared with the unmodified cytokines, allowing more convenient administration regimes.

Cytokines bind to the cell surface cytokine receptors with high affinity, making them high-potency molecules. Thus, cytokines are generally administered at low doses and they have a narrow therapeutic index. For this reason, structural stability is a critical factor in ensuring biological function and efficacy. Low concentrations of active ingredient cause difficulties characteristic to cytokine products. Analysis of the cytokine protein structure and stability becomes challenging, especially if the product additionally contains albumin as a stabilizing excipient. Another important result is loss of protein because of adsorption on container or other surfaces. Most cytokines have a helical bundle fold, generally associated with marked hydrophobicity.[6] Hydrophobicity can lead to problems related to solubility, tendency to aggregate and adsorption on surfaces, and consequent challenges in manufacturing and long-term storage.

Physical and chemical instabilities may also lead to the formation of immunogenic degradation products. Oxidation is a major chemical degradation pathway for cytokines.[7–13] Cytokine aggregates, especially those formed by oxidized proteins, have been linked to immunogenicity.[9,10] Immunogenicity of therapeutic proteins is a topic of concern that has gained wide interest lately.[14–17] Many of the cytokine products are intended for long-term use, and many patients eventually develop an antibody response to the protein drug.[15] Antibodies formed against therapeutic proteins may cause serious adverse effects if they cross-react with endogenous proteins.[18] Protein aggregates are a key risk factor for immunogenicity.[16] Therefore, the hydrophobicity and aggregation tendency of cytokines is a matter of concern, and a major aim in formulation development is to inhibit unfolding and aggregation.

In this review, we have covered the available information on structural characteristics (Table 2) and degradation pathways of interferon-alpha (IFN-α), interferon-beta (IFN-β), interferon-gamma (IFN-γ), interleukin-2 (IL-2), IL-11, granulocyte colony-stimulating factor (G-CSF), and granulocyte-macrophage colony-stimulating factor (GM-CSF). As a result, we attempt to identify common properties within these molecules and formulation strategies, and to provide a rationale for developing stable cytokine products.

Abbreviations used: CHO, Chinese hamster ovary; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; EDTA, ethylenediaminetetraacetic acid; EMA, European Medicines Agency; Fimea, Finnish Medicines Agency; HSA, human serum albumin; MAH, marketing authorization holder; PEG, polyethylene glycol; SDS, sodium dodecyl sulfate.

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Table 1. Cytokine Products Approved for Clinical Use in the United States and Europe[145–147]

Cytokine	Trade Name (Company)	Active Ingredient	Production Host	Indication	Year of Authorization (Responsible Agency)
IFN-α	IntronA (US: Schering, EU: Merck Sharp and Dohme[a])	IFN-α-2b	*E. coli*	Multiple myeloma[b] Chronic myelogenous leukaemia[b] Chronic hepatitis B Carcinoid tumor[b] Hairy cell leukemia Follicular lymphoma Malignant melanoma Chronic hepatitis C Condylomata acuminata[c] AIDS-related Kaposi's sarcoma[c]	1986 (FDA) 2000 (EMA)
	Roferon-A (Roche)	IFN-α-2a	*E. coli*	Hairy cell leukemia AIDS-related Kaposi's sarcoma[b] Chronic myelogenous leukaemia Cutaneous T cell lymphoma[b] Chronic hepatitis B[b] Chronic hepatitis C Follicular lymphoma[b] Renal cancer[b] Malignant melanoma[b]	1986 (FDA) 2001 (Fimea, Finnish Medicines Agency)
	PegIntron (EU: Merck[a],US: Schering)	Peginterferon-α-2b	*E. coli*	Chronic hepatitis C	2000 (EMA) 2001 (FDA)
	ViraferonPeg (Merck[a])	Peginterferon-α-2b	*E. coli*	Chronic hepatitis C	2000 (EMA)
	Sylatron (Schering)	Peginterferon-α-2b	*E. coli*	Melanoma	2011 (FDA)
	Pegasys (Roche)	Peginterferon-α-2a	*E. coli*	Chronic hepatitis B Chronic hepatitis C	2002 (EMA) 2002 (FDA)
	Infergen (US: Intermune Pharms)	Interferon-alfacon-1	*E. coli*	Chronic hepatitis C	1997 (FDA)
	Alferon N Injection (Interferon Sciences)	IFN-α-n3	Human leucocytes	Condylomata acuminata	1989 (FDA)
IFN-β	Betaseron (US)/Betaferon (EU) (Bayer)	IFN-β-1b	*E. coli*	Multiple sclerosis	1993 (FDA) 1995 (EMA)
	Extavia (Novartis)	IFN-β-1a	CHO cells	Multiple sclerosis	2008 (EMA) 2009 (FDA)
	Avonex (Biogen Idec)	IFN-β-1a	CHO cells	Multiple sclerosis	1996 (FDA) 1997 (EMA)
	Rebif (US: Serono, EU: Merck Serono Europe)	IFN-β-1a	CHO cells	Multiple sclerosis	1998 (EMA) 2002 (FDA)
IFN-γ	Actimmune (US) (Intermune/Vidara Ther.) /Imukin (EU) (Boehringer Ingelheim)	IFN-γ-1b	*E. coli*	Chronic granulomatous disease Osteopetrosis	1994 (Fimea) 1999 (FDA)
IL-2	Proleukin (EU: Novartis, US: Chiron)	Aldesleukin (rhIL-2)	*E. coli*	Renal cell carcinoma Melanoma[c]	1992 (FDA) 1992 (Fimea)
	Ontak (Eisai)	Denileukin diftitox	*E. coli*	T-cell lymphoma	1999 (FDA)
IL-11	Neumega (Wyeth Pharms Inc.)	Oprelvekin (rhIL-11)	*E. coli*	Chemotherapy-induced thrombocytopenia	1997 (FDA)

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