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Research paper

Quantitative detection of therapeutic proteins and their metabolites in serum using antibody-coupled ProteinChip® Arrays and SELDI-TOF-MS

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Abstract

One of the important steps in developing protein therapeutics is the determination of their preliminary PK *in vivo*. These data are essential to design optimal dosing in animal models prior to progressing to clinical trials in man. The quantitative detection of protein therapeutics in serum is traditionally performed by ELISA, which has the prerequisite of the availability of the appropriate monoclonal antibodies. We have developed an alternative method using polyclonal antibodies immobilized on ProteinChip Arrays and SELDI-TOF mass spectrometry. This method has an advantage over ELISA since it provides simultaneously information on the clearance rate of the protein and it's *in vivo* processing. We compared these two methods using a RANTES variant, [^{44}AANA^{47}]-RANTES as the test protein in this study. Using SELDI-TOF mass spectrometry, we were able to establish that the protein is readily oxidized in serum, and moreover is processed *in vivo* to produce a truncated 3–68 protein, and undergoes a further cleavage to produce the 4–68 protein. These modifications are not identified by ELISA, whilst the serum exposure profiles determined by the two methods show essentially similar protein concentration values.

Keywords: Protein processing *in vivo*; SELDI; RANTES; Pharmacokinetics

1. Introduction

Protein therapeutics or biologicals play a major role alongside small molecule therapeutics in treating many diseases, and in fact many strategies using biologicals are now replacing or supplementing therapies such as steroids that have been used for decades. Protein therapeutics are generally applied to interactions that are not easily targeted by small molecules, the most common example being the interaction between cytokines and their receptors (Johnson-Leger et al., 2006). In the immune system for example, the interaction of cytokines, interleukins and growth factors with their receptors

Abbreviations: SELDI-TOF-MS, Surface Enhanced Laser Desorption Ionization time-of-flight mass spectrometry; MALDI, matrix-assisted laser desorption/ionization; PK, pharmacokinetic; GAG, glycosaminoglycan; RANTES, Regulated on Activation, Normal T Expressed and Secreted; CCL5, chemokine (CC motif) ligand 5.

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involve large surfaces, which are not amenable to blockade by small molecules, but can be inhibited by binding proteins or neutralizing antibodies. Natural agonists have similarly given birth to their own class of protein therapeutics: the activation of cytokine-, interleukin-, and growth factor-receptors often requires activation that may be best achieved with the endogenous ligand, and many such therapies are on the market, including interleukin-1α for Hepatitis-C infection, interferon-β for multiple sclerosis, and the biggest block buster in the protein therapeutic field, Epoetin alpha for chronic renal failure.

An essential step in the development of protein therapeutics is the determination of their bioavailability by various routes of administration such as either intravenous (i.v.) or subcutaneous (s.c.) suitable for injection in man, or the intraperitoneal (i.p.) route for testing of efficacy in animal models. Classically this is determined by the ELISA technique, but this methodology is time consuming to develop since it first requires the production of suitable antibodies, ideally monoclonals. Secondly, problems of species cross-reactivity are often a hindrance since ELISAs are required to identify the candidate therapeutic protein in a number of species during the pre-clinical development.

To develop an alternative method we used a variant of RANTES/CCL5 with targeted amino acid mutations in its principal GAG binding site (Proudfoot et al., 2001). RANTES/CCL5 is a member of the chemokine family of proteins, which play an important role in leukocyte trafficking and homing (Schall et al., 1990). RANTES mediates its effects via several 7 transmembrane G protein-coupled chemokine receptors, CCR1, CCR3, and CCR5 (Pakianathan et al., 1997). We have demonstrated that in addition to the well characterized interaction that RANTES has with its receptors, a second, less well understood interaction with immobilized glycosaminoglycans appears to be essential in order for RANTES to mediate its chemotactic effects *in vivo* (Proudfoot et al., 2003). Furthermore, this variant has been shown to inhibit the recruitment capabilities of RANTES *in vivo*, an observation which has been extended to show amelioration of disease in experimental allergic encephalomyelitis (EAE), a mouse model of multiple sclerosis (MS) (Johnson et al., 2004). In order to further understand this inhibitory capacity we used both an ELISA as well as tracing of iodinated proteins and showed that differences in *in vivo* distribution over time following administration are directly related to the GAG binding properties of the protein, i.e. RANTES remains essentially at the local site of injection for a period of up to 4 h, whereas [^{44}AANA^{47}]-RANTES rapidly enters the bloodstream and is readily detectable in the serum as early as 30 min post-dose. However, the ELISA does not allow distinction between RANTES and its variant (Johnson et al., 2004).

Surface Enhanced Laser Desorption Ionization time-of-flight mass spectrometry (SELDI-TOF-MS) enables multiple protein analyses on a single experimental platform, combining selective protein capture with sensitive and quantitative mass spectrometric analysis. The experimentally measured mass of the captured molecules allows for the accurate detection of multiple variants of a given protein in a single assay (Rossi et al., 2006). Also, nonspecific antibody cross-reactivity, that might be present in an ELISA, is eliminated using the SELDI, as only the peaks with the accurate molecular mass are quantified. Furthermore for capture from biological samples, polyclonal antibodies are most suitable, and moreover are relatively rapidly produced. We therefore used this technology to distinguish between wild type RANTES and the [^{44}AANA^{47}]-RANTES variant.

Using serum samples from mice dosed with [^{44}AANA^{47}]-RANTES analyses by ELISA and SELDI technology yielded similar pharmacokinetic profiles. Additionally, as well as demonstrating that SELDI may be used as an alternative to ELISA, we show by changes in mass that the protein is processed *in vivo*. Here we show that not only was the protein truncated at the amino terminus to produce first the 3–68 form, and subsequently a further truncation produced the 4–68 form, but also that on exposure to serum, the protein was rapidly oxidized. It has been previously demonstrated that chemokines are vulnerable to a variety of proteolytic processing activities (Struyf et al., 1998; Proost et al., 2001; Overall et al., 2002).

Thus determination of the plasma exposure of candidate protein therapeutics proteins by SELDI will not only rapidly provide information on the PK properties of the protein, but can also provide precious information on *in vivo* processing as well as biochemical modifications such as oxidation – information that is crucial for the development of protein therapeutics.

2. Materials and methods

2.1. Instrumentation

SELDI-TOF-MS, Surface Enhanced Laser Desorption Ionization time-of-flight mass spectrometry a technology supplied by Ciphergen Biosystems (Bruenner et al., 1996; Davies et al., 1999) was performed using a Biology System IIc (Ciphergen Biosystems Inc., Fremont, CA).