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Opinion

Therapeutic Peptide Quantitation in Human Plasma: The Challenge of Develop an Adequate Strategy According to Pharmacokinetic Monitoring in Clinical Trials

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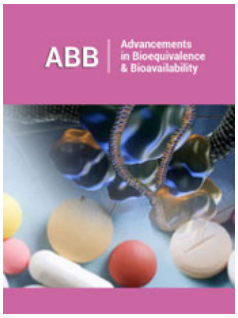

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Abstract

Mass spectrometry alone or in combination with liquid chromatography has become the analytical tool by choice to achieve therapeutic peptide quantitation in biological samples. Because of the challenge that entails to embrace more than 85% of the concentration time curve during pharmacokinetics studies, all strategies involving the peptide quantitation in biological fluids are still a difficult task and need to be tailored. We present here our recent experiences in the development and validation of customized bioanalytical methods applied to pharmacokinetic studies included in phase I clinical trials. All methodologies were optimized case by case for CIGB-500, CIGB-300 and CIGB-814 therapeutic peptide candidates developed at the Centre for Genetic Engineering and Biotechnology, from Havana (Cuba). The three bioanalytical methods were fully validated according to the FDA guidelines for industry. It was possible to obtain the PK profiles and main PK parameters for all of the assessed candidates.

Introduction

Because of their low oral bioavailability and propensity to be rapidly metabolized, peptides were considered as poor drug candidates [1]. However, the perspective is currently changing. With the improvement in peptide synthesis strategies, and the new approaches beyond its traditional design, peptide-based therapeutics are currently experiencing a renaissance in the global market [2]. The epidemic increase in clinical indications like obesity, type II diabetes along with complex diseases like cancer and autoimmune disorders, that can't be approached by traditional therapies, conduct to rethink in the use of peptide therapeutics as an attractive approach [3]. This market growth is mainly driven by the increasing research and development expenditure by pharmaceutical companies in the peptide industry leading to a rapid entry of multiple peptide-based drug in clinical trials [4].

One of the main goals of phase I clinical trials are the safety and pharmacokinetics assessment of the drug, so the development and validation of bioanalytical methods with this purpose became crucial to this stage. Talking of therapeutic peptides, it is also a huge challenge, because they are chemically and physically instable, prone to hydrolysis and oxidation and have a short half-life and fast elimination, what causes more than one inconvenience regarding peptide recovery from the biological sample and quantitation. Mass Spectrometry (MS) has impressive capabilities in terms of sensitivity, resolving power, mass accuracy and different scan-modes versatility. Either alone or in combination with liquid chromatography it is the analytical tool of choice for synthetic therapeutic peptide characterization [5,6,7]. Nevertheless, for peptide quantitation in human plasma or other biological sample, the design of the Internal Standard (IS) and the optimization of the sample processing and LC-MS analysis are also key elements for a successful outcome. In general, all strategies involving the peptide quantitation in biological fluids are challenging and need to be tailored.

To date, our experience in the development and validation of customized bioanalytical methods applied to pharmacokinetic studies of therapeutic peptide candidates, included in phase I clinical trials, its diverse. We briefly present here the bioanalytical approaches for the absolute quantitation of three therapeutic peptides, where different alternatives to the AQUA® methodology [8,9] were used. However, the design of the IS, sample processing and mass

spectrometry techniques were optimized case by case for CIGB-500, CIGB-300 and CIGB-814 peptide candidates. For instance, the IS for CIGB-500 [10] and CIGB-814 [11] were synthetic peptides labelled with stable isotopes (13C and/or 15N) in specific residues within the amino acids sequence, instead of IS for CIGB-300 that was a N-terminus acetylated peptide [12]. On the other hand, sample processing, mainly based on plasma proteins organic or acid precipitation was adapted according to the peptide recovery. In the particular case of CIGB-300, no liquid chromatography separation was needed before MS analysis by MALDI-TOF MS [5].

In the case of CIGB-500, also known as GHRP-6 [13], it was applied the LC-MS analysis with Simultaneous Ion Monitoring (SIM) in full scan mode. The cytoprotective effect of this therapeutic peptide was widely demonstrated in animal models of ischemia reperfusion [14,15], fact that strongly support the hypothesis of its use for the treatment of cardiovascular diseases [16]. Concerning bioanalytical method, the three Daltons mass shift between the analyte and its internal standard was sufficient for its absolute quantitation. Protein precipitation via acetone and positive full scan mode acquisition provided an adequate selectivity and sensitivity to analyse this peptide in human plasma. Besides the calibration range was from 5 up to 50 ng/mL, the dilution integrity was demonstrated. Sample stability evaluations showed that both the peptide and the IS were stable under the assayed conditions. Regarding the PK study, it was carried out after peptide single intravenous administration in nine men healthy volunteers (three in each dose level: 100, 200 and 400μg/kg of body weight). Confident results were obtained from the analysis of clinical samples that required dilution at the early monitored times. LLOQ was reached in all cases after 12h post-administration, so the method is sufficiently sensitive for modelling the pharmacokinetic profile of the peptide, as it includes more than 85% of $AUC_{0\rightarrow\infty}$. CIGB-500 exhibited a biphasic pharmacokinetic profile of plasma concentration vs. time, with a very fast distribution phase, as it is typical for peptide-based drugs. The clearance was very similar among the three dose levels, instead of C_{max} and AUC, that shown an important rise with the increment of the administered dose [17].

The CIGB-814, originally named as E18-3 APL1 or APL1 in preclinical experiments [18] is a novel therapeutic peptide candidate for Rheumatoid Arthritis (RA). It is an altered peptide ligand containing a novel CD4+T-cell epitope from human heat shock protein 60 that significantly inhibits the course of adjuvant induced arthritis (AA) in Lewis rats and collagen induced arthritis (CIA) in DBA/1 mice [19]. For CIGB-814 it was used the LC-MS analysis in Single Reaction Monitoring Mode (SRM). All parameters of the bioanalytical method developed for the absolute quantitation of this peptide in human plasma met the acceptance criteria according to FDA guidelines [20]. The analysis of clinical samples during the phase I clinical trial in RA patients, enabled the reliable estimation of the main PK parameters after single sub cutaneous administration (1,2.5 and 5mg doses), monitoring more than 85% of the total $AUC_{0\rightarrow\infty}$.

Although dose dependence was not established, there was a trend of the C_{max} and consequently the AUC, to increase when the dosages augmented. It was found that CIGB-814 was rapidly cleared from plasma, for all dose's levels, with an average clearance half-life of 0.74±0.34 hours. In terms of safety, this result is very promising for an immunomodulatory drug candidate, because long-lasting self-peripheral tolerance would probably require repeated doses. In general, the PK study in the phase I clinical trial, reinforce the therapeutic potential of CIGB-814 as immunomodulatory drug candidate [11]. On the other hand, for the pro-apoptotic CIGB-300 antitumor peptide, which mechanism implies CK2 phosphorylation impairment [21], the PK study was carried out in patients with solid tumours. In this case report, the bioanalytical method for peptide quantitation in plasma was achieved by MALDI-TOF MS analysis. The bioanalytical method was fully validated according to the FDA guidelines [20]. Despite the narrow range of the calibration curve (from 0.5 to7.5μg/ml), dilution integrity tests demonstrated that more concentrated samples could be successfully analysed.

Stability studies confirmed that both the peptide and its internal standard were stable under the assay conditions. The method was successfully applied to the analysis of CIGB-300 in clinical samples from pharmacokinetic study with this peptide after intravenous infusion (1^{st} and 5^{th} daily administration of 1.6mg/kg dose). Main pharmacokinetics parameters were not statistically different ($p>0.05$) to those obtained by a competitive ELISA analysis, except for Cmax and AUC that were significantly different due to the intrinsic divergence between the selectivity of both bioanalytical methods [22]. It has been confirmed through this paper that bioanalytical methods for therapeutic peptides based on mass spectrometry need to be customized depending mostly, but not only, on their physicochemical properties. It's also very important to consider the sample where the quantitation will take place, the route of administration as well as the adequate sensitivity to rich the proper limits of quantitation. In order to characterize adequately the PK of the drug substance during phase I clinical trials, it is crucial to embrace more than 85% of the AUC with therapeutic entity quantitation by the bioanalytical method previously developed and validated.

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Conflict of Interest

The funding for this work was fully provided by the Centre for Genetic Engineering and Biotechnology. There's not conflict of interest with the results were published.

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