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Poster Presentation

Analysis and characterization of different preparations of recombinant human follicle stimulating hormone (hFSH) and of its subunits

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Background

Human follicle stimulating hormone (hFSH), synthesized by the human pituitary gland, is a biologically active glycoprotein composed of two noncovalently bound α- and β- subunits and is critically involved in the maturation of ovarian follicles and in spermatogenesis. Considerable heterogeneity associated with different hFSH preparations has been reported, mainly related to the presence of different glycoforms [1]. The characterization of preparations of hFSH utilized as a therapeutic agent in reproductive medicine is therefore very important, especially considering that no specific monography has yet been published by the main pharmacopoeias.

In this work four hFSH preparations were analyzed, two of them being natural (pituitary- and urinary-derived) and the other two recombinant (CHO-derived). Studies were conducted to assess and compare hydrophobicity, molecular weight, charge heterogeneity and purity of the natural and recombinant heterodimeric preparations. These characteristics were examined by reversed-phase high performance liquid chromatography (RP-HPLC), matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF), isoelectric focusing and size-exclusion high performance liquid chromatography (HPSEC).

Results

RP-HPLC analysis indicated a significant difference ($p < 0.005$) between the retention time (t_R) of the pituitary and of the two recombinant FSH preparations. Urinary-derived hFSH was found more heterogeneous than the other three preparations. HPSEC analysis showed a significant difference ($p < 0.001$) between the t_R of the urinary preparation and that of the pituitary or of the recombinant preparations. Urinary-derived hFSH presented the lowest HPSEC t_R, in agreement with the highest molecular mass more accurately determined by MALDI-TOF mass spectrometry. The relative molecular mass (Mr) for the heterodimeric form of urinary, pituitary and recombinant hFSH preparations was 32527, 29176 and 28536 respectively.

An efficient subunit dissociation process (dissociation yield of 95%) was also set up by incubating pituitary- and CHO-derived FSH preparations with 3 M acetic acid, overnight, at 37°C. Yields of approximately 52% and 48% for the β and α subunit respectively were obtained via RP-HPLC, in agreement with theoretical yields based on the mass determined in this work via MALDI-TOF mass spectrometry (53% and 47%). The Mr of the individual subunits determined by this methodology for pituitary- and CHO-derived hFSH were respectively 14467 and 14082 for the α subunit and 16509 and 16067 for the β subunit. The urinary preparation presented a Mr of 15139 for the α subunit and of 17196 for the β subunit (see Table 1). All

Table 1: Relative molecular mass (Mr) of the heterodimer (α+β) and related subunits of different hFSH preparations, determined by Maldi-Tof mass spectrometry

			Heterodimer		
Preparation	α-subunit	β-subunit	Experimental	Calculated α+β	Calc/Exp
p-hFSH	**14467**	**16509**	**29176**	**30976**	**1.06**
r-hFSH	**14082**	**16067**	**28536**	**30149**	**1.06**
u-hFSH	**15139**	**17196**	**32527**	**32335**	**0.99**

Table 2: Retention times of heterodimeric hFSH before dissociation, of α-and β-subunits after dissociation and relative retention times (t_{RR}) of the α and β subunits with basis on heterodimeric hFSH, determined on RP-HPLC (n = 2).

SAMPLE	heterodimer t_R	β-subunit t_R	α-subunit t_R	β-subunit t_{RR}[a]	α-subunit t_{RR}[a]
p-hFSH	24.43 ± 0.156	26.98 ± 0.160	36.63 ± 0.198	1.104	1.499
r-hFSH Gonal F	25.19 ± 0.129	27.62 ± 0.235	38.86 ± 0.214	1.096	1.543
r-hFSH Puregon	25.29 ± 0.070	27.85 ± 0.131	38.16 ± 0.127	1.101	1.509

[a]t_{RR}, (relative retention time) = $t_R$subunit/$t_R$heterodimer

subunits, when analyzed on RP-HPLC, presented retention times significantly different from the retention time of the heterodimer ($p < 0.01$) and between them ($p < 0.001$). The mean relative retention times ($t_{RR} = t_R$ subunit/t_R heterodimer), though, were found highly constant, 1.100 ± 0.004 (CV = 0.4%) and 1.517 ± 0.023 (CV = 1.5%), respectively for the β- and α-subunit of the three preparations (see Table 2)

Conclusion

Different isoforms were observed, by RP-HPLC, in the analysis of hFSH preparations of different origins (CHO, urinary and pituitary-derived). While the recombinant and pituitary hFSH preparations presented one main peak, the urinary-derived hFSH presented two major isoforms, one of which was equivalent to the major form of the other preparations. The other form could be an oxidized form of FSH present in this urinary preparation in high amount, as reported [2]. The RP-HPLC characterization of the hFSH heterodimer and of individual subunits revealed differences in hydrophobicity in the following order: α-subunit > β-subunit > heterodimer. For the first time a quite satisfactory separation of the heterodimer from the dissociated β-subunit was attained.

Urinary-derived hFSH showed a higher Mr (11–14%) when compared with pituitary and recombinant hFSH, while pituitary hFSH showed a slightly higher Mr (~ 2%) in comparison with the recombinant preparation.

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