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Short paper

Standardization of therapeutic, urinary gonadotrophins: An update on the use and availability of International Standards for Menotrophin

Jackie Ferguson [a,*], Jason Hockley [b], Richard Tiplady [a], Chris Burns [a]

[a] Endocrinology Section, Biotherapeutics Group, National Institute of Biological Standards and Control, Blanche Lane, South Mimms, Potters Bar, Hertfordshire EN6 3QG, UK
[b] Biostatistics Group, National Institute of Biological Standards and Control, Blanche Lane, South Mimms, Potters Bar, Hertfordshire EN6 3QG, UK

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ABSTRACT

The potencies of therapeutic preparations of gonadotrophins of human, urinary origin, which comprise a heterogenous mix of isoforms with follicle-stimulating hormone (FSH) and luteinizing hormone (LH) bioactivities, are standardized by WHO International Standards (IS). We report here, the evaluation, through an international collaborative study, of a candidate preparation, coded 10/286, to replace the 4th IS, 98/704, for human, urinary FSH and LH (Menotrophin) which has been used for many years for the potency assignment of therapeutic preparations using bioassays. The mean FSH and LH bioactivities of 10/286, determined by *in vivo* bioassays in terms of 98/704, were 183 IU per ampoule (95% confidence limits 165–202) and 177 IU per ampoule (95% confidence limits 159–197), respectively.

1. Introduction

Preparations of human menopausal gonadotrophins (Menotrophin) of urinary origin, containing follicle-stimulating hormone (FSH) and luteinizing hormone (LH) bioactivities have been used since the 1960s, to treat infertility in women presenting with anovulation resulting from hypothalamic or pituitary dysfunction [1]. Menotrophins are also widely used in assisted reproductive technologies, and are considered a cost-effective approach to achieving controlled superovulation for oocyte retrieval [2]. Menotrophin products have developed from early, crude urinary extracts to the high-purity versions which are now available. Urinary-derived preparations of purified FSH and recombinant preparations of FSH and LH, produced in Chinese hamster ovary cell lines, provide alternative treatments to stimulate ovulation.

LH and FSH are heterodimeric glycoproteins with three or four N-linked glycosylation sites, that are secreted by the anterior pituitary gland as a complex mix of isoforms which vary in the size and structure of the attached oligosaccharide. The *in vivo* bioactivity of a preparation of Menotrophin is highly influenced by the isoform composition. Less acidic isoforms have a greater specific activity than acidic, sialylated forms but are rapidly cleared from the body through hepatic mechanisms. Sialylated isoforms exhibit longer half-lives but have a decreased response at a cellular level [3]. The isoform composition varies during puberty, the menstrual cycle and menopause and with conditions of steroid hormone imbalance such as polycystic ovarian syndrome. In conditions of low circulating oestrogen, such as in the post-menopausal women who provide the urinary source material for Menotrophin, there is an increased prevalence of sialylated isoforms [4,5]. However, the exact isoform composition of a therapeutic, gonadotrophin preparation, of either human or recombinant origin, will depend on both the isoform composition of the source material and the purification procedures applied during the manufacturing process which may select for particular isoform species.

This structural complexity, coupled with the need to ensure effective hormonal treatment with minimal risk of overstimulation, which can lead to ovarian hyperstimulation syndrome, means there is a requirement for standardization in the assignment of potency to therapeutic preparations of Menotrophins. This is achieved through the provision of WHO International Standards (IS) which for Menotrophins, define the International Units (IU) of FSH and LH bioactivities. We describe here, the assessment of a candidate preparation in a multi-laboratory study in order to establish the 5th WHO IS for human, urinary FSH and LH bioactivities calibrated in terms of the 4th IS, 98/704 [6], by *in vivo* bioassay.

Abbreviations: FSH, follicle-stimulating hormone; IRP, International Reference Preparation; IS, International Standard; IU, International Unit; LH, luteinizing hormone.

* Corresponding author. Tel.: +44 (0) 1707 641135.
E-mail addresses: Jackie.Ferguson@nibsc.org (J. Ferguson), Jason.Hockley@nibsc.org (J. Hockley), Richard.Tiplady@nibsc.org (R. Tiplady), Chris.Burns@nibsc.org (C. Burns).

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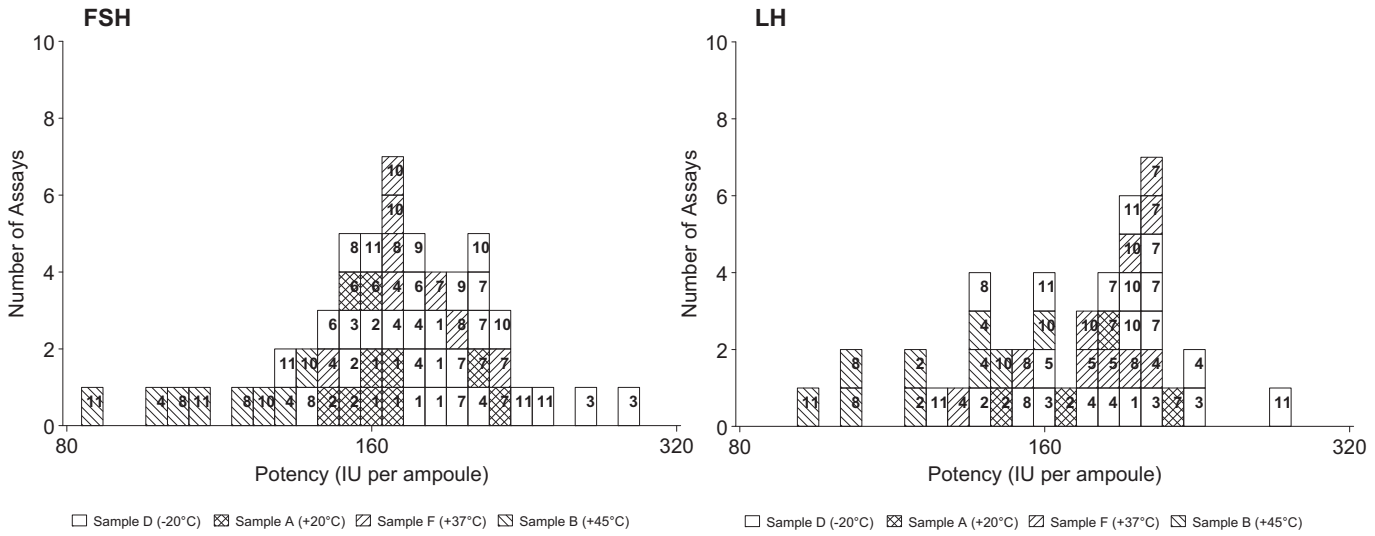

Fig. 1. Potency estimates of the FSH and LH bioactivities in IU per ampoule, calculated using $\log_{10}$ (organ weight) as the assay response relative to the 4th IS, 98/704, of the candidate preparation, 10/286 stored at −20 °C, (open boxes) and accelerated thermal degradation samples of 10/286 stored at +20 °C, +37 °C and +45 °C for 6 months (hatched boxes, as indicated). The potencies assigned to 10/286 were calculated from the samples stored at −20 °C only. Each box represents an individual potency determination by a collaborating laboratory, designated numerically. Note: Laboratory 2 estimates were calculated using organ weight as the assay response due to assay invalidity when using logarithmic transformation.

2. Methods

2.1. Preparation of the candidate standard, 10/286

Bulk preparations of highly purified, human, urinary Menotrophin were generously donated to the WHO by Instituto Massone S.A., Argentina (Batch No. 3626365910) and IBSA Institut Biochimique S.A., Switzerland (Batch No. WHO 01/2010). Combined, these preparations provided approximately 260 mg protein with approximately 2900 IU/mg of FSH bioactivity and 3123 IU/mg LH bioactivity reported by the manufacturers. The material was formulated with 0.2% (w/v) human plasma albumin and 0.5% (w/v) lactose and 1.0 ml was dispensed into glass ampoules, lyophilized and sealed according to WHO guidelines for the preparation, characterization and establishment of international and other standards and reference reagents for biological substances [7]. Ampoules, coded 10/286, were stored at −20 °C in the dark.

2.2. Bioassay methods employed for the calibration of 10/286

A worldwide collaborative study involving eleven expert laboratories in ten countries was performed in order to calibrate 10/286 in terms of the 4th IS, 98/704 using FSH bioassays based on the bioassay described by Steelman and Pohley [8] and/or LH bioassays based on the bioassay described by Van Hell, Matthijsen and Overbeek [9] and as described in the Pharmacopoeial monographs for Menotrophin. Thermally-accelerated degradation samples of 10/286, stored for 6 months at +20 °C, +37 °C and +45 °C, were also included in the collaborative study to allow a prediction of long term stability. All samples were coded and the laboratories were identified by number.

2.3. Statistical evaluation of the candidate standard

Potency estimates for the candidate standard, 10/286, were calculated relative to 98/704 by fitting a parallel-line model comparing assay response to log concentration [10]. Assay validity was assessed by analysis of variance with non-linearity and non-parallelism considered significant at the 1% level ($p < 0.01$). An in-house program [11] was used to determine any outlier responses and assess homogeneity of variance across treatment groups. Any outliers were omitted from calculation of relative potency. Laboratory means were calculated as weighted geometric means except in cases where the individual assay estimates were found to be heterogeneous ($p < 0.1$ in χ^2 test for homogeneity) and a semi-weighted geometric mean was calculated. Overall means were calculated as the unweighted geometric mean of laboratory means. Variability between laboratories has been expressed using geometric coefficients of variation ($GCV = \{10^s - 1\} \times 100\%$ where s is the standard deviation of the $\log_{10}$-transformed potency estimates). The stability of 10/286 stored at −20 °C was predicted from the relative bioactivities of the thermally accelerated degradation samples by assuming a linear relationship between the log of the degradation rate and the reciprocal of absolute temperature [12,13].

3. Results

3.1. Characterization of the candidate standard, 10/286

The candidate standard comprised a batch of ampoules coded 10/286 with a mean fill weight of 1.0081 g ($n = 166$; CV 0.18%), a mean dry weight of 0.0082 g (CV 3.77%), a residual moisture content of 2.84% (CV 22.12%) and a mean oxygen content of 0.21% (CV 53.96%). Quality analysis of the candidate ampoules confirmed that the mean fill weight, mean dry mass and mean oxygen head space were within the expected values. The mean residual moisture content of the candidate standard, 10/286, was higher than expected (2.84% (CV 22.12%)). This has been observed previously with glycoprotein hormone preparations in our laboratory and although stability was not affected, further bioassays of accelerated thermal degradation samples of 10/286 will be performed. Eleven laboratories contributed bioassay data providing 30 individual estimates of FSH potency from a total of 23 bioassays and 26 individual estimates for LH potency from a total of 19 bioassays. All laboratories provided organ weight data and all but one also provided the final body weight.

3.2. Assignment of potency

Analysis of heterogeneity of variance ($p < 0.05$ in Bartlett's test) demonstrated that $\log_{10}$ (organ weight) provided better agreement with the variance homogeneity required for parallel-line analysis

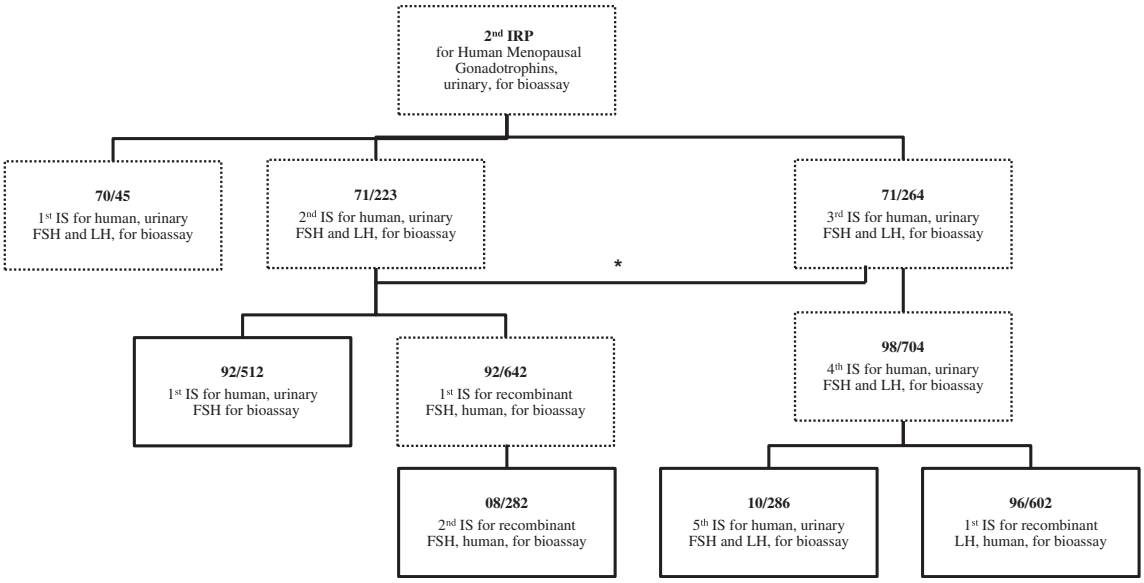

Fig. 2. The origins of WHO International Standards for the standardization by bioassay, of FSH and LH bioactivities. Standards which are currently available from NIBSC are shown in solid boxes. *During the collaborative study to calibrate the 1st IS for human, urinary FSH for bioassay and the 1st IS for recombinant, human FSH for bioassay (92/512 and 92/642) in terms of the 2nd IS for human, urinary FSH and LH for bioassay (71/223), stocks of 71/223 were exhausted and were replaced with the 3rd IS, 71/264 which had been shown previously to be equivalent to 71/223 in FSH and LH bioactivities.

than untransformed assay responses. This response also allowed inclusion of data from all laboratories. The majority of assays allowed statistically valid estimates of relative potency to be calculated, although some samples were excluded from further analysis due to significant non-linearity or non-parallelism, or a lack of significant dose-response. Using $\log_{10}$ (organ weight), the geometric mean potency estimate was determined as 183 IU per ampoule ($n = 10$; 95% confidence limits 165–202; GCV 15%) for FSH bioactivity and 177 IU per ampoule ($n = 9$; 95% confidence limits 159–197; GCV 15%) for LH bioactivity (Fig. 1).

A number of laboratories also performed assays on the accelerated thermal degradation samples of 10/286 (Fig. 1). The decrease in FSH bioactivity through storage at elevated temperatures allowed the prediction of a 0.001% loss of FSH bioactivity per year, suggesting that the material will exhibit long term stability at the recommended storage temperature of −20 °C. However, LH bioactivity did not show a consistent trend with storage temperature, thereby preventing determination of the predicted stability at −20 °C.

4. Discussion

International Standards enable the assignment of potency to biological medicines by comparison to an internationally-available reference preparation in a biological assay system. A challenge when replacing an IS is to maintain continuity of unitage while ensuring that the composition of the new IS provides a like-for-like comparison with the samples to be measured. Donations to WHO by manufacturers of Menotrophin products have provided International Reference Preparations (IRP) and International Standards (IS) since the 1960s when International Units of human, urinary FSH and LH bioactivities were first assigned to the 2nd IRP for Human Menopausal Gonadotrophins of urinary origin [14]. Subsequently, the 1st, 2nd and 3rd IS (70/45, 71/223 and 71/264) [15] were calibrated directly in terms of the 2nd IRP and the 4th IS, 98/704, in terms of the 3rd IS, 71/264 (Fig. 2).

The 2nd IRP for Human Menopausal Gonadotrophins of urinary origin was also used to calibrate by *in vivo* bioassays, the 1st and 2nd IRP for human pituitary FSH and LH, 69/104 and 78/549. These were established to standardize immunoassays to detect and quantify FSH and LH in patient samples. However, with the increasing purity of pituitary preparations and the introduction of recombinant preparations of FSH and LH, a discontinuity of unitage between bioactivity and immunoreactivity became apparent [16–19]. Thus, separate IS are provided for the standardization of bioassays and immunoassays.

The exhaustion of stocks of the 4th IS for Menotrophins demonstrates the continued clinical use of urinary-derived, gonadotrophin preparations which cannot be calibrated by physicochemical methods alone. Ensuring the continued standardization of this product and reflecting the high-purity versions now available, we report here the establishment of the 5th IS for human urinary FSH and LH bioactivity, calibrated in terms of the 4th IS, 98/704. Coded 10/286, the 5th IS has an assigned potency of 183 IU FSH bioactivity and 177 IU LH bioactivity per ampoule [20]. This material is now available from the National Institute of Biological Standards and Control (www.nibsc.org).

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