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BIOLOGICAL ACTIVITY DETERMINATION OF HUMAN MENOPAUSAL GONADOTROPIN IN WISTAR RATS

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ABSTRACT

Human menopausal gonadotropin (HMGs, or menotropin) are a combination of FSH and LH in 1:1 ratio i.e., extracted from urine obtained from post menopausal women.HCG contains large quantities of LH with little FSH it is secreted by placenta an extracted from the urine of pregnant women. HMG is a medication i.e., composed of FSH with LH and is used for stimulation of egg development in women who do not ovulate spontaneously, who ovulate extremely irregularly, or to increase the number of eggs developed in a single cycle in women who already ovulate. FSH is fundamentals for follicular recruitment in the first stages of folliculogenesis and is involved in physiological events that promote the growth of an appropriate pre ovulatory follicle in the last stages of folliculogenesis.

KEYWORDS: HMG, FSH, LH.

ABBREVATIONS

HMG - Human Menopausal Gonodotropin

FSH – Follicle Stimulating Hormone

LH – Luteinizing Hormone.

INTRODUCTION

Gonadotropins are glycoprotein hormones secreted by gonadotropic cells of the anterior pituitaryof vertebrates. This family includes the mammalian hormones follicle-stimulating

hormone(FSH) and luteinizing hormone (LH), the placental/chorionic gonadotropins, human chorionic gonadotropin (hCG) and equine chorionic gonadotropin (eCG), as well as at least two forms of fish gonadotropins. These hormones are central to the complex endocrine system that regulates normal growth, sexual development, and reproductive function. LH and FSH are secreted by the anterior pituitary gland, while HCG and ECG are secreted by the placenta in pregnant humans and mares, respectively. The gonadotropins act on the gonads, controlling gamete and sex hormone production. Gonadotropin is sometimes abbreviated Gn. The alternative spelling gonadotropins which inaccurately implies a nourishing mechanism is still sporadically used.

Urine of postmenopausal women reflects the hypergonadotropic state of menopause -levels of follicle stimulating hormone (FSH) and luteinizing hormone (LH) are high - and contain a mixture of these gonadotropins. Other protein substances may be present, including small amounts of human chorionic gonadotropin (HCG). In 1949 Piero Donini found a relatively simple method to extract gonadotropins from urine of postmenopausal women. Menotropins were successfully introduced into clinical use by Bruno Lunenfeld in 1961. While earlier menotropin medications contained FSH and LH at a 1:1 ratio, the recognition that it is FSH that is critical for follicle stimulation has led to development of newer preparations that contain a much higher FSH/LH ratio.

Human menopausal gonadotropin (hMG), which contains two gonadotropin components corresponding to the pituitary-related hormones, FSH and LH, was first successfully extracted from the urine of post-menopausal women in 1950. In 1953 hMG was shown to produce ovarian stimulation in female hypophysectomised infantile rats, and Leydig cell stimulation and full spermatogenesis in male hypophysectomised infantile rats. These experiments suggested that hMG would be useful in humans; however, to enable clinical testing, large-scale extraction and purification methods were required, in addition to an agreed standard to enable reproducibility. Furthermore, the starting dose for humans needed to be established.

FUNCTIONS OF HMG

Menotropin contains FSH and LH at 1:1 ratio. In combination with FSH, human menopausal gonadotropin (HMG)/menotropin is used to induce the super ovulation. Menotropins are used to produce multiple eggs during ovulation, in preparation for in-vitro fertilization. Menotropin preparations are designed for use in selected women where they stimulate the

ovaries to mature follicles, thus making them more fertile. They are administered by typically daily injection, intramuscularly or subcutaneously, for about ten days under close supervision to adjust dose and duration of therapy. They can also be used in hypogonadal men to stimulate sperm production.

Human menopausal Gonodotropin interacts with the LHCG receptor of the ovary and promotes the maintenance of the corpus luteum for the maternal recognition of pregnancy at the beginning of pregnancy. It has been hypothesized that hMG may be a placental link for the development of local maternal immunotolerance. For example. HMG-treated endometrial cells induce an increase in T cell apoptosis (dissolution of T cells).

These results suggest that hMG may be a link in the development of peritrophoblastic immune tolerance, and may facilitate the trophoblast invasion, which is known to expedite fetal development in the endometrium.

PRODUCTION

Menotropin (also called human menopausal gonadotropin or hmg) is a hormonally active medication for the treatment of fertility disturbances. Frequently the plural is used in the medication is a mixture of gonadotropins Menotropins are extracted from the urine of postmenopausal women. Menotropin may be prepared by suitable fractionation procedure followed by ion-exchange chromatography. It is prepared in conditions designed to minimize microbile contaminations and to be in compliance with the requirements of viral safety.

Menotropin has the property in females of stimulating growth and maturation of ovarian follicles and .In males of maintaining and stimulating testicular cells related to testosterone production and of being responsible for full development and maturation of spermatozoa in seminiferous tubules.

TEST PROCEDURE

The potency of menotropin with respect its follicle stimulating hormone activity is estimated by comparing its effect in enlarging the ovaries of Immature female rats with that of the standard preparation of human urinary FSH and human urinary LH under the condition of suitable of assay.

On the day of injection bring the cages to the respective place

Weigh the animals in each group and assign code number for each of two standard and test preparation.

Weigh the animals before Injections on day 1 and 5th day before kill the animals.

Draw 0.2ml of standard or test preparation into syringe

Assay

Assay for follicular stimulating hormone activity

Assay for luteinizing hormone activity

ASSAY

Potency, The potency of menotropin with respect to its follicle stimulating hormone activity is estimated by comparing its effect in enlarging the ovaries of immature female rats with that of the standard preparation oh human urinary FSH and human urinary LH under the condition of suitable method of assay.

The potency of menotropin with respect to its luteinizing hormone activity is estimated by comparing its effect in increasing the weight of the seminal vesicles or the prostate glands of immature FSH and urinary LH under the conditions of suitable method of assay.

Standard Preparation

The standard preparation is the International standard preparation of human urinary follicle stimulating hormone (FSH) and Luteinizing Hormone (LH), consisting of freeze-dried extract from the urine of post menopausal together with lactose or any other suitable preparation, the potency of which has been determined in relation to the international reference standard.

ASSAY OF FOLICULE STIMULATING HORMONE ACTIVITY

- Select female Wister rats, 19 to 28 days old and differing in age by not more than 3 days.
- Inject to each rat Subcutaneous into the dorsal area 0.2ml of solution.
- Repeat the procedure after 24 and 48 hours.
- About 24 hours the last injection, euthanize the rats and remove the ovaries.

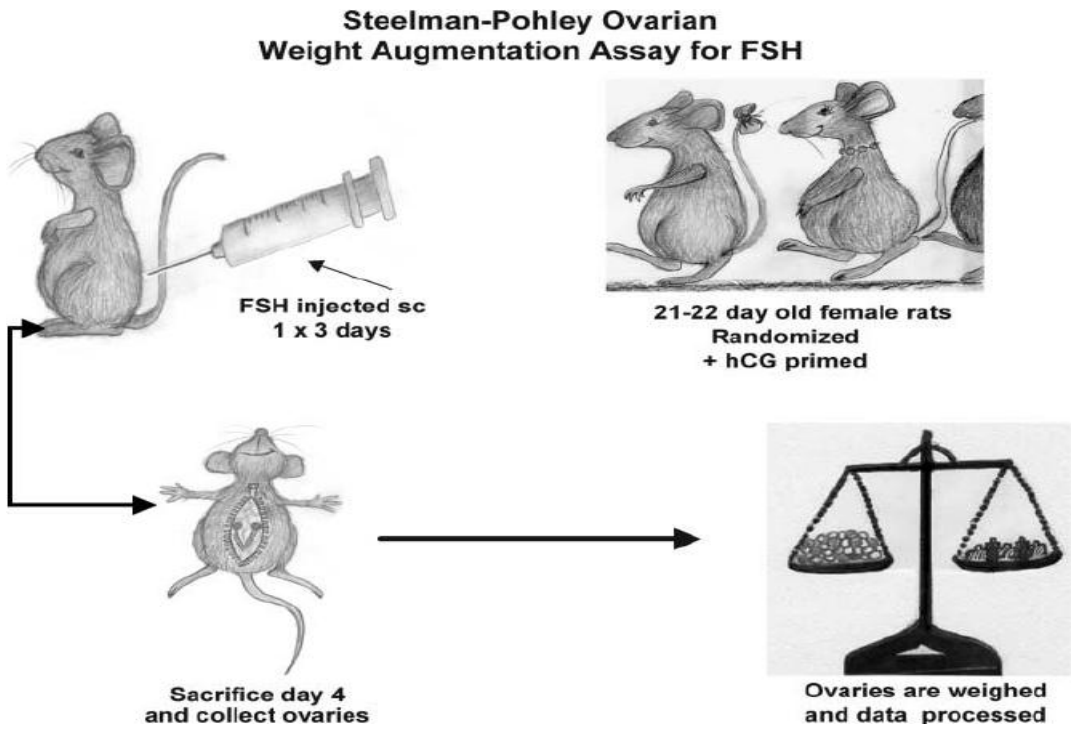
- Remove any extraneous the rat tissue and immediately weigh the ovaries from each animal.
- Record the combined weight of both ovaries from each rat.

ASSAY OF LUTEINIZING HORMONE ACTIVITY

- Select male wistar rats, 19 to 28 days old and differing in age by not more than 3 days.
- Inject to each rat Subcutaneous into the dorsal area 0.2ml of solution. And allocated to its group on 4 consecutive days at the same time each day.
- On the fifth day, about 24 hours after the last injection, euthanize the rats and remove the seminal vesicles or the prostate gland.

Remove any extraneous fluid and tissue and weigh immediately the seminal vesicles prostate glands.

In the current study we have used a conventional method for measuring the bioactivity of HMG which is first described by Steelman-Pohley which is also the mainstay of pharmacopiel monographs for the statutory determination of the potency of the urinary HMG preparations.



MATERIALS AND METHODS

ANIMAL HUSBANDRY

HOUSING

Animal room was cleaned before receiving of animals. During the study, the floor of the experimental room and work tops were swept and mopped with disinfectant solution every day. Cages were changed along with bedding material for weekly twice. Polypropylene cages with metal top grills were used for housing the animals. Three animals/sex/cage were housed during the entire study period.

IDENTIFICATION

Individual cage was identified with cage card. Acclimatization cage card was having details of study number, Test Item, species, strain, sex, animal number, acclimatization start and end dates. During treatment period, cage card was containing details of study number, test item, species, strain, sex, dose, animal number, date of treatment, date of necropsy. Individual animal was identified with tail marking method during entire study period.

BEDDING

Autoclaved corn cob was used as bedding material. Details and analysis report of bedding material used was incorporated in the raw data.

FEED & WATER

Conventional laboratory rodent diet supplied by approved vendor was offered ad libitum except, before dose administration animals were fasted overnight. Feed was provided for fasted animals after 3 hours of dose administration. Purified water was available ad libitum via drinking water bottles. Feed and water analysis reports were incorporated in raw data.

ENVINORMENTAL CONDITIONS

Animal holding room was maintained at controlled temperature and relative humidity of 20 to 25 °C and 50 to 60 % respectively throughout the study period. Throughout the study period 12 air changes were maintained in animals holding room. Artificial light was set to give a cycle of 12 hours light and 12 hours dark.

ACCLIMATIZATION

Animals were acclimatized for 7 days. Animal body weight were recorded at the time of receipt and on last day of acclimatization. During acclimatization period animals were

subjected for mortality check twice a day and cage side observations once a day. Detailed clinical observations were performed on day 7 of acclimatization.

EXPERIMENTAL DESIGN ON HMG - LH

Group No.	Group	Dose#	Conc.(IU/mL)	No. of Animals	Animal Strain/ Sex Of Animal	Animal b.wt/ Age
T1	Untreated Control	-	-	6 Animals	Wistar Rats/ Male	25 - 38 Grams/21 Days old animals
T2	Buffer Control	-	0.2 mL	6 Animals	Wistar Rats/ Male	25 - 38 Grams/21 Days old animals
T3	Workings Standard Low dose	8.75 IU	0.2mL/ Total dose per rat 7.0 IU	6 Animals	Wistar Rats/ Male	25 - 38 Grams/21 Days old animals
T4	Workings Standard Middle dose	17.5 IU	0.2mL/ Total dose per rat 14.0 IU	6 Animals	Wistar Rats/ Male	25 – 38 Grams/21 Days old animals
T5	Workings Standard High dose	35.0 IU	0.2mL/ Total dose per rat 28.0 IU	6 Animals	Wistar Rats/ Male	25 - 38 Grams/21 Days old animals
T6	Sample Low dose	8.75 IU	0.2mL/ Total dose per rat 7.0 IU	6 Animals	Wistar Rats/ Male	25 - 38 Grams/21 Days old animals
T7	Sample Middle dose	17.5 IU	0.2mL/ Total dose per rat 14.0 IU	6 Animals	Wistar Rats/ Male	25 - 38 Grams/21 Days old animals
T8	Sample High dose	35.0 IU	0.2mL/ Total dose per rat 28.0 IU	6 Animals	Wistar Rats/ Male	25 - 38 Grams/21 Days old animals

Conc.: Concentration, No.: Number, ml: milli liter, b.wt.: body weight

EXPERIMENTAL DESIGN ON HMG - FSH

Group No.	Group	Dose#	Conc.(IU/mL)	No. of Animals	Animal Strain/ Sex Of Animal	Animal b.wt/ Age
T1	Untreated Control	-	-	6 Animals	Wistar Rats/ Female	25 - 38 Grams/21 Days old animals
T2	Buffer Control	-	0.2 mL	6 Animals	Wistar Rats/ Female	25 - 38 Grams/21 Days old animals
T3	Workings Standard Low dose	2.5 IU	0.2mL/ Total dose per rat 1.5 IU	6 Animals	Wistar Rats/ Female	25 - 38 Grams/21 Days old animals
T4	Workings Standard Middle dose	5.0 IU	0.2mL/ Total dose per rat 3.0 IU	6 Animals	Wistar Rats/ Female	25 – 38 Grams/21 Days old animals
T5	Workings Standard High	10.0 IU	0.2mL/ Total dose per rat 6.0 IU	6 Animals	Wistar Rats/ Female	25 - 38 Grams/21 Days old animals

	dose					
T6	Sample Low dose	2.5 IU	0.2mL/ Total dose per rat 1.5 IU	6 Animals	Wistar Rats/ Female	25 - 38 Grams/21 Days old animals
T7	Sample Middle dose	5.0 IU	0.2mL/ Total dose per rat 3.0 IU	6 Animals	Wistar Rats/ Female	25 - 38 Grams/21 Days old animals
T8	Sample High dose	10.0 IU	0.2mL/ Total dose per rat 6.0 IU	6 Animals	Wistar Rats/ Female	25 - 38 Grams/21 Days old animals

CONCLUSION

In conclusion, the implemented assay for determination of biological activities of HMG, LH and FSH, relying on hormone receptor activation, offers a valuable alternative for evaluation of different hormone preparations. We have demonstrated high sensitivity and reproducibility of the assay for HMG, LH and FSH testing, which could potentially be used in clinical research and pharmaceutical industry.

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