Reproductive Effects Of human Interferon-Alpha-2b Administration on Male Albino Mice Testes.An Experimental Study.

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Abstract

Background: Recombinant human interferon alpha (rh-IFN-α) is used therapeutically in malignant disorders and chronic hepatitis. The phenotypic effects of this drug at the structural levels on testicular tissue were hardly ever addressed. Hence, this work was designed in adult male albino mice to study the phenotypic effects of rh-INF-α-2b on testicular tissue as well as assessing its effects on serum testosterone and gonadotropins levels.

Objective: This research was planned to through light on the effects of interferon-alpha-2b (IFN-alpha-2b) on the hypothalamic-pituitary-testicular (HPT) axis of the adult male albino mice.

Design: Experimental study.

Setting: National Hepatology and Tropical Medicine Research Institute (NHTMRI). The study was conducted from November (2004) to February (2005).

Materials and methods: Thirty sexually mature male mice were divided into three groups (10 mice in each group), namely: the control, the experimental and the recovery groups. Mice in the experimental and recovery groups were administered recombinant human interferon alpha intraperitoneally at a dose of 3000 U / mouse weekly for 12 weeks in a volume of 1.0-microliter isotonic normal saline, then animals in the recovery group were left to recover for a further period of two months. At the end of the experiment, serum concentrations of gonadotropins and testosterone were measured and then all animals were then sacrificed to study histopathologically the possible effects of interferon on the testicular tissue.

Results: rh-IFN-α-2b induced remarkable decline in the serum levels of both follicle stimulating hormone (FSH) and luteinizing hormone (LH) in mice of the experimental group compared to the corresponding control and mice of recovery group. At the same time, testosterone was moderately increased in the experimental group, and then returned to its normal levels within 2 months after cessation of treatment. Histopathologically, in the experimental group, there were focal thickening of the basement membrane, degenerative changes and clumping of the germinal epithelial cells in the center of seminiferous tubules, partial desquamation of the germinal epithelium from basement membrane, reduction in the germ cell height, partial arrest of maturation and increased number of Sertoli cells. Increased number of Leydig's cells and hypervascularity were detected in the interstitial spaces. In the recovery group, there was lessening of the germ cell hypoplasia manifested by restoration of spermatogenic cells and accidental disruption in the basement membrane. Most of the spermatogenic and Sertoli cells restored their polarity, height and maturation.

Conclusion: our results suggest that rh-INF-α-2b temporally affects the hypothalamic-pituitary-testicular axis (HPT), both centrally and peripherally (at the testicular level), through the lessening of FSH, LH, raise of testosterone serum levels and direct phenotypic effect on the testicular tissue.

Introduction

Interferons (IFNs) are potent biological response modifiers. In addition to their antiviral activity, IFNs exert various biological effects, including inhibition of cell growth, activation of the immune system and induction of messenger RNA (mRNA) synthesis of various genes (Kalvakolanu and Borden, 1996). Three major classes of IFNs have been identified. The leukocyte IFNs are designated IFNα and IFNγ, fibroblast IFN is designated IFNß, and it has an effect on reproductive functions. Treatment of mice with IFN-γ results in altered germinal epithelium and decreases spermatogenesis (Natwar *et al*, 1995 and Bussiere *et al*., 1996). Male mice that are transgenic for IFNs display alteration of the sperma-togenic process and became sterile (Hekman *et al*., 1988 and Iwakura *et al*., 1988). IFN gamma also inhibits gonadal steroidogenesis in both vivo and in vitro (Orava *et al*., 1985a, b, Orava *et al*., 1989 and Meikle *et al*., 1992). However, the mechanism by which the INF-γ inhibits Leydig's cells steroidogenesis remains unclear. In cultured cells, the somatic epithelial Sertoli cells and the peritubular myoid cells, as well as gem cells produce IFNα. In contrast, IFN- γ has been shown to be produced by early spermatids but not somatic cells (Dejucq *et al*., 1995). Mammalian sperm cell has expression of IFN-alpha and IFN-gamma receptors, which seem to develop during sperma-togenesis in the testes. These findings may have implications in male infertility and antisperm contraceptive vaccine develo-pment (Naz *et al*., 2000). Targeted gene mutagenesis studies suggest that these IFNs can alter the development of testicular germ cells. In transgenic mice overexpressing either the IFNα or IFNß gene, the process of normal germ cell development (spermatogenesis) is disrup-ted with concomitant destruction of germ cells (Hekman *et al.,* 1988 and Iwakura *et al*., 1988). IFN-α administration in healthy men reduced serum concentration of both testosterone and free androgen index (Corssmit *et al*., 2000). Natwar *et al.* (1995) noticed that IFN – γ induced pronounced deleterious effect on the testicular tissue of rats leading to desquamation of the germinal epithelium, reduction in the germinal cell height and tubular diameter and significant decrease in the number of Sertoli cells.

Whereas the effects of interferons on a large number of biological systems have been thoroughly investigated, yet few controlled studies regarding their effects on the reproductive system are available. Moreover, most of the work in literature, concerning effects of interferons addresses the hormonal, and more generally the homeostatic effects of these drugs. The phenotypic effects of these drugs at the structural levels on testicular tissue were seldom addressed. Hence, this work was designed in adult male albino mice to study the phenotypic effects of rh-INF-α-2b on testicular tissue as well as assessing its effects on serum testosterone and gonadotropins levels.

Materials and methods

The study was conducted from November 2004 to February 2005 in National Hepatology and Tropical Medicine Research Institute (NHTMRI).

Study Animals: This study was performed on thirty sexually mature male Swiss albino mice. They were about 6 – 7 weeks old, average weight 20 – 25 grams. Animals were allowed to stabilize for a minimum of 6 days within our facility before treatment. Food and water were allowed ad libitum and they were housed at room temperature. The animals were randomly divided into three groups; each comprised 10 mice, the control group, the experimental and the recovery groups.

1-The control group.
2-The experimental group were injected with interferon alpha-2b.
3-The recovery group received the same regime as experimental group and was then left for further two months after drug cessation to study the revers-ibility of the possible testicular effects induced by interferon.

Study drug :(Interferon) Interferon-alpha-2b was purchased from Nile Company for pharmaceuticals and chemical industries.

Mode of administration: The drug was administered once weekly for 12 inject-ions. Each mouse in the experimental and recovery groups received 3000 units weekly via intraperitoneal injection, in a volume of 1.0 micro liter isotonic normal saline according to Maria *et al*. (2002).

Hormonal assay: Serum testosterone and gonadotropins (FSH & LH) were detected trice: first prior to study, second at the end of the study and finally at the end of the recovery period. Blood samples were drawn via an indwelling cannula in the tail artery. Serum was separated and stored at -20°C until estimation. Serum levels of testosterone were measured by radioimm-unoassay as described by Abraham (1975). The levels of FSH & LH in the serum were also determined using radioimm-unoassay as previously described by Billiar *et al*. (1989).

Light microscopic study: At the end of the trial, all animals were sacrificed to study the possible effects of interferon on testicular tissue. The testes were removed, fixed in bouin's fluid and processed for paraffin blocks. Sections were cut at a thickness of about 3μ. The sections were mounted on slides and stained with Hematoxylin and Eosin. It is noteworthy to mention that the same technique of fixation of the specimens was followed strictly to ensure avoidance of fixation artifacts that might interfere with the histological appearance of examined sections.

Image analysis: average spermatogenic cell count per seminiferous tubule, average tubular dimensions and average interstitial cell of Leydig per interstitial space in all studied groups were detected using a Quantimet 500+ image analyzer (Leica, UK). Sections or areas with artifacts were omitted from such analysis. A minimum of 20 seminiferous tubule and 20 interstitial spaces were examined for each testis.

Statistical analysis: Data were collected, coded and analyzed by statistical package for Social Science (SPSS) software version (12) under windows XP and EpiInfo version 6 program. For each measured data (hormonal profile data, average dimensions of seminiferous tubules, average number of interstitial cells of Leydig per interstitial space and average number of spermatogenic cells per seminiferous tubule), means, standard deviations and standard errors were obtained from results of the studied groups. Analytical and statistical comp-arative study of the recorded data were done using independent groups't-test for samples which used for comparison of means of the matching variables; one tailed test for significance was used. P-value < 0.05 was considered statistically significant and a p –value < 0.01 was considered statistically highly significant (Bryman and Cramer, 1999).

Results

Hormonal profile:

Serum Testosterone level (Table 1):
The concentration of serum testosterone level showed a remarkable increase in the experimental group compared to the corresponding control by the end of the experimental period (0.71±0.199 compared to 0.503±0.176). The difference between these two groups was about to reach the level of statistical significance (p = 0.0115). This was followed by a decline at the end of the recovery period, yet it failed to reach the corresponding control level. Such change was not statistically significant (p = 0.4925).

Serum FSH level (Table 1):
The concentration of serum follicle stimulating hormone (FSH) level showed a remarkable decline in the experimental group compared to the corresponding control by the end of the experimental period (0.50±0.147 compared to 17.5± 2.003). The difference between these two groups was highly significant (p = 0.000). This was followed by a rise at the end of the recovery period, yet it failed to reach the corresponding control level. Such change was statistically significant (p = 0.000).

Serum LH level (Table 1):
The concentration of serum luteinizing hormone (LH) in mice of the experimental group showed a remarkable decline in the experimental group comp-

ared to the corresponding control by the end of the experimental period (0.100±0.026 compared to 7.5±1.55). The difference between these two groups was highly significant (p = 0.000). This was followed by a rise at the end of the recovery period, yet it failed to reach the corresponding control level. Such change was statistically significant (p = 0.000).

Light microscopic study and image analysis:
Control group:

Histological examination of testicular sections of control mice revealed normal appearance of seminiferous tubules with normal interstitial tissue between them and intact abundant interstitial cells of Leydig (Fig.2). The average perimeter, length, width, and diameter of the seminiferous tubules were 6.038±2.25, 2.04±0.99, 1.18±0.31 and 1.18±0.31 mm respectively (Magnification 320X).

Experimental group:

Histological examination of testicular sections of mice in the experimental group, revealed both qualitative and quantitative changes, observed by the end of the experimental period:

Quantitative changes:

There was mild germ cell hypoplasia and the average number of spermatogenic cells per tubule was much lower compared to corresponding controls (601.2273 ±301.4512 compared to 950±25.3). This difference was found to be statistically significant (p = 0.0151) (Table 3).

The average number of interstitial Leydig's cells per interstitial space clearly increased to 20±12.5 compared to 16±11.2 in corresponding control (Table 4). This difference was not statistically significant (p=0.29). The average diameter of the seminiferous tubules was much lesser compared to corresponding control (1.18±0.13 compared to 1.59 ±0.19). This difference was found to be highly statistically significant (p = 0.000) (Table 2). This was followed by an increase at the end of the recovery period to nearly reach the corresponding control level (p = 0.183).

Qualitative changes:

Interferon administration induced deleterious effects on the testicular tissue of mice of the experimental group, in the form of focal thickening of the basement membrane (1/10), degenerative changes and clumping of the germinal epithelial cells in the center of seminiferous tubules (2/10) (Fig. 3 &4) partial desquamation of the germinal epithelium from basement membrane (3/10) (Fig. 7 &8). In addition, there was reduction in the germ cell height (2/10), partial arrest of maturation (3/10) (Fig. 5&6) and increased number of Sertoli cells (5/10). Increased number of Leydig's cells (6/10) (Fig. 9) and hypervascularity (2/10) were detected in the interstitial spaces. There was a significant difference in the tubular diameter between the experimental and corresponding control group.

Recovery group:
Quantitative changes:

lessening of the germ cell hypoplasia manifested by restoration of some spermatogenic cells within the tubules, however partial maturation arrest was still observed in many tubules. The average number of spermatogenic cells per tubule was still lower compared to corresponding controls (635.5±301.4512 compared to 950±25.3). This difference was found to be statistically significant (p = 0.0151) (Table 3). The average number of Leydig's cells per tubule was 18±12.2 compared to 16±11.2 in corresponding control (Table 4). This difference was not statistically significant (p = 0.59).

Qualitative changes:

Although basement membrane defects were less than the experimental group, yet focal disrupted areas could still be observed in some tubules (1/10). Most of the spermatogenic and Sertoli cells restored their polarity, height and maturation (Fig. 10). Leydig's cells restored their normal configuration and number. Their was no significant differences between tubular diameter and germ cell height in comparison with the corresponding control group.

Table (1): Values of FSH, LH, and testosterone among the experimental group (group II) and the recovery group (group III).

Groups	Testosterone (pg/ml)			FSH (mIU/ml)			LH (mIU/ml)		
	Mean±SD	SE	P value (Two-tailed)	Mean ±SD	SE	P value (Two-tailed)	Mean±SD	SE	P value (Two-tailed)
Experimental	0.71±0.199	0.063		0.50±0.147	0.047		0.100±0.026	0.01	
Corresponding control	0.503±0.176	0.056	0.023*	17.5±2.003	0.634	0.000**	7.5±1.55	0.49	0.000**
Recovery	0.5015±0.177	0.056		1.4±0.29	0.092		2.8±0.84	0.27	
Corresponding control	0.503±0.176	0.056	0.985	17.5±2.003	0.634	0.000**	7.5±1.55	0.49	0.000**

SD = Standard Deviation
SE = Standard Error
*   = Significant (two tailed) at P< 0.05
** = Highly Significant (two tailed) at P< 0.01

Table (2): Average dimensions of seminiferous tubules in the testes of male albino mice in the experimental and recovery groups compared to the corresponding controls (Magnification 320X).

Groups	Perimeter (mm)			Length (mm)			Width (mm)			Diameter (mm)		
	Mean±SD	SE	P value (Two-tailed)	Mean±SD	SE	P value (Two-tailed)	Mean±SD	SE	P value (Two-tailed)	Mean±SD	SE	P value (Two-tailed)
Experimental	6.038 ±2.25	0.218		2.40 ±0.99	0.095		1.18 ±0.31	0.029		1.18 ±0.31	0.03	
Corresponding control	5.53 ±0.95	0.299	0.18	2.07 ±0.04	0.127	0.048*	1.31 ±0.215	0.07	0.206	1.59 ±0.194	0.06	0.000**
Recovery	5.11 ±2.35	0.29		1.89 ±0.84	0.106		1.05 ±0.33	0.04		1.44 ±0.51	0.06	
Corresponding control	5.53 ±0.95	0.30	0.586	2.07 ±0.4	0.127	0.284	1.31 ±0.215	0.07	0.019*	1.59 ±0.194	0.06	0..366

*   = Significant (two tailed) at P< 0.05
** = Highly Significant (two tailed) at P< 0.01

Table (3): Average number of spermatogenic cells per seminiferous tubule in the testes of male albino mice in the experimental and recovery groups compared to the corresponding controls.

Groups	Average number of spermatogenic cells / tubule Mean ± SD	P value (Two tailed)
Experimental	601.2273±301.45124	0.0302*
Corresponding control	950 ±25.3	
Recovery	635.5±241.79975	0.022*
Corresponding control	950 ±25.3	

* Significant (two-tailed) at P< 0.05

Table (4): Average number of interstitial cells of Leydig per interstitial space in the testes of male albino mice in the experimental and recovery groups compared to the corresponding controls.

| Groups	Average number of Leydig's cells / space Mean ± SD	P value (Two tailed)
Experimental	20±12.5	0.29
Corresponding control	16 ±11.2	
Recovery	18 ±12.2	0.59
Corresponding control	16 ±11.2	

SD = Standard Deviation

Fig.1: A magnified sample of image analysis of the first field of slid 1 in the recovery group.
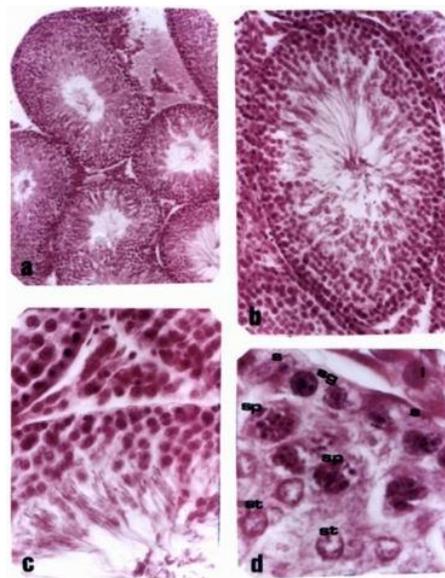
Fig 2: Photomicrographs of Hx and E stained sections of testes of control male albino mice showing:
a) Normal structure of seminiferous tubules with normal interstitial spacing between them (x 100)
b) Normal appearance of seminiferous epithelium at different stages of the cycle (x 200)
c) Normal cellular and vascular appearance of interstitial tissue enclosed between the seminiferous tubules with intactness of the basement membrane (x400)
d) Regularity of the chromatin pattern of spermatogenic cells with more or less vesicular nuclei (x 1000).
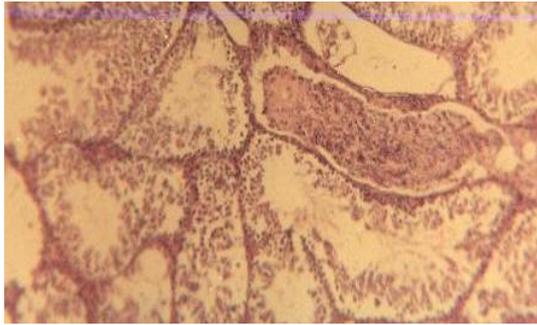
Fig 3: Section in mice testis of the experimental group showing degenerative changes and clumping of germ cells in the center of the seminiferous tubules (H&E x200).
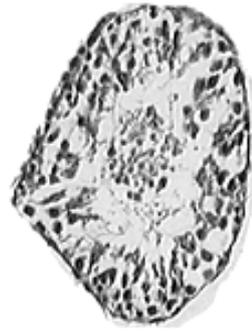
Fig 6: Section in mice seminiferous tubule of the experimental group showing germ cell hypoplasia, arrest of maturation and clumping of the spermatogenic cells in the center of the tubules (H&E x200).
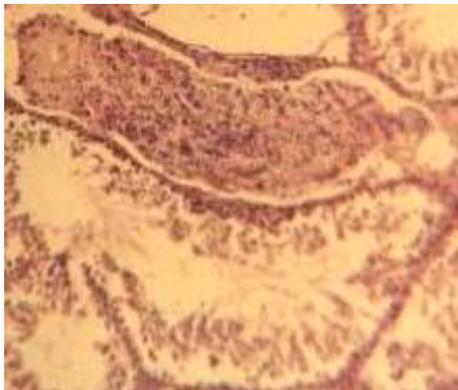
Fig 4: A higher magnification of Fig 3.
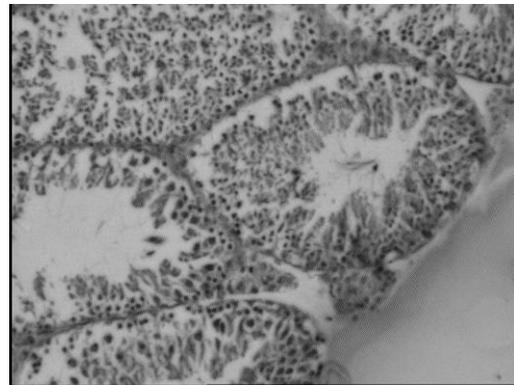
Fig 7: Section in mice testis of the experimental group showing desquamation of the germinal epithelium from the basement membrane (H&E x200).
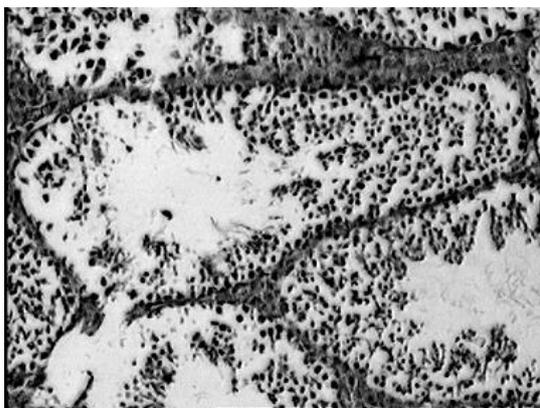
Fig 5: Section in mice testis of the experimental group showing germ cell hypoplasia and arrest of maturation (H&E x200).
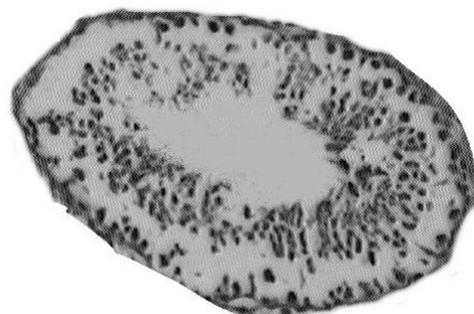

Fig 8: Section in mice seminiferous tubule of the experimental group showing desquamation of the germinal epithelium from the basement membrane (H&E x200).
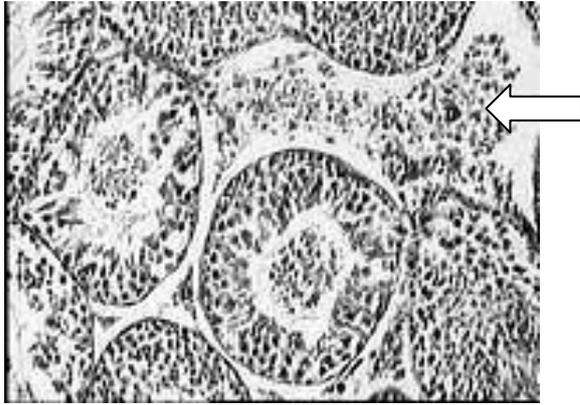
Fig 9: Section in mice testis of the experimental group showing increased no. of Leydig's cells (H&E x200).
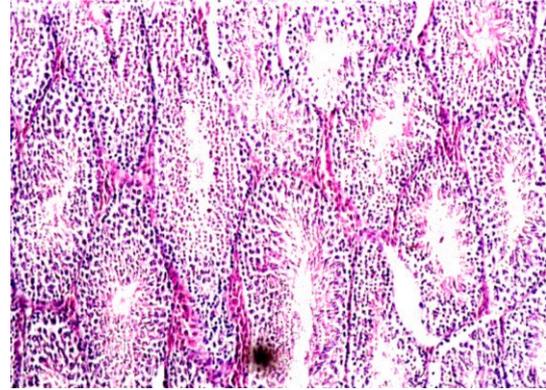
Fig 10: Section in mice testis of the recovery group Showing restoration of configuration and structure of seminiferous tubules and interstitial tissue (H&E x200).

Discussion

Testicular cytokines represent recently identified local signaling molecules that apparently affect spermatogenesis in a legend-specific manner. Interferons (IFNs) are a family of secreted polypeptides that are divided into three main categories: IFNα and IFNß (type I IFNs), initially described as the product of virus-infected leukocytes and fibroblasts, and IFN gamma (γ) (type II IFN), produced when lymphocytes and natural killer cells are stimulated by antigenic or mitogenic substances (Blakwill, 1989). Mammalian cells into which the human interferon beta gene has been introduced produce interferon beta-1a. Interferon beta-1b is made by bacterial fermentation of a strain of Escherichia coli that is a genetically engineered plasmid containing the gene for human interferon beta (Healthdigest.org, 2003). The mammalian sperm cell has expression of IFN-alpha and IFN-gamma receptors, which seem to develop during spermatogenesis in the testes (Naz *et al*, 2000). In cultured cells, the somatic epithelial Sertoli cells and the peritubular myoid cells, as well as germ cells produce IFNα. In contrast, IFN γ has been shown to be produced by early spermatids but not somatic cells (Dejucq *et al*, 1995). Interferon- γ (IFN- γ) is an immunomodulating cytokine that has profound effects on reproductive function. IFN - γ inhibits steroidogenesis both in vivo and in vitro. The mechanism by which IFN - γ inhibits Leydig's cell steroidogenesis remains unclear. In 2003 Chen and co-workers proposed that one possible mechanism of inhibition of Leydig's cell steroidogenesis by IFN – γ, is that it down regulate the expression of glucose transport 8 (GLUT8), which results in decreased glucose uptake by Leydig's cells and inhibition of testosterone production. On the other hand the effect of human gamma interferon on the testicular histology of mice was studied by Natwar *et al*., 1995, and they found with increasing doses of IFN – γ pronounced deleterious effect on the testis leading to desquamation of the germinal epithelium, reduction in the germinal cell height and tubular diameter, significant decrease in the number of Sertoli cells, stage-7 spermatids and stage-16 spermatozoa. Opposing to this, Hibi *et al*., (2004), confirmed that alpha-interferon might improve testicular spermatogenesis and increase epididymal sperm concentration in the rat. Corssmit *et al*., (2000), performed a saline-controlled crossover study in six healthy men, sequentially measuring the

serum concentrations of gonadotropins, testost-erone, the free androgen index and sex hormone-binding globulin (SHBG) after a bolus subcutaneous injection of recombi-nant human interferon alpha and they found that rh-IFN-alpha induced a sustained decrease of both testosterone and free androgen index, whereas concentra-tions of LH, FSH, and SHBG were not different between the studies, so they concluded that rh-IFN-alpha affects the hypothalamic-pituitary-testicular axis at the testicular level. On contrary to this Barreca *et la*., (1993) studied the effect of long-term treatment with recombinant interferon-alpha 2b (IFN-alpha 2b) on luteinizing hormone (LH), testosterone, free testosterone, and sex hormone-binding globulin (SHBG) in 7 male patie-nts suffering from chronic viral hepatitis and they reported that IFN-alpha 2b was not responsible for any measurable imbalance in male sex hormones. In a therapeutic trial performed on four patients with bilateral mumps orchitis, Erpenbach and Derschum (1991) observed that systemic treatment with interferon-alpha-2b appears to be highly effective in preventing sterility and testicular atrophy in patients with bilateral mumps orchitis. Interferon A has been studied for its effect on fertility in Macaca mulatta (Rhesus monkeys). Non-pregnant rhesus females treated with Roferon-A at doses of 5 and 25 MIU/kg/day have shown menstrual cycle irregularities, including prolonged or shortened menstrual periods and erratic bleeding; these cycles were considered to be anovulatory on the basis that reduced progesterone levels were noted and that expected increases in preovulatory estrogen and luteinizing hormones were not observed. These monkeys returned to a normal menstrual rhythm following discontinuation of treatment (HIV and Hepatitis.com, (2004). Fujisawa *et al*., (1998) investigated the role of interferon alpha and gamma in the seminal plasma on spermatogenesis. The levels of interferon alpha and gamma were determined in the seminal plasma of 101 males including normozoospermic, oligozoospermic and azoospermic men. The correlation between such levels and clinical parameter

(seminogram and serum hormone levels) was evaluated. The three groups did not differ as to the level of interferon gamma. No significant correlations were observed between the levels of interferon alpha or gamma in seminal plasma and the serum levels of follicle stimulating hormone (FSH), luteinizing hormone (LH), testost-erone, prolactin, or estradiol. Theses results suggest that the level of interferon alpha in the seminal plasma may be related to sperm production. Our results are not consistent with that reported by Corssmit *et al*., (2000) who found that rh-INF-alpha induced a sustained decrease of both testosterone and free androgen index, whereas concentrations of LH, FSH, and SHBG were not affected in healthy men after a bolus subcutaneous injection of recombinant human interferon alpha. In 1985 a & b, Orava and co-workers docu-mented that Human leukocyte interferon inhibits human chorionic gonadotropin stimulated testosterone production by porcine Leydig's cells in culture and the site of interferon action on hCG-stimulated testosterone production in primary cultures of porcine Leydig's cells is located distal to cAMP formation. Also in consistent with our results, Hibi et al, 2004 reported that Alpha-IFN might improve testicular spermatogenesis and increase the epidid-ymal sperm concentration in the rat. These promising results with alpha-IFN may pave the way for a new approach to treating male infertility. The present work confirmed that interferon has a stimulant effect on testosterone secretion, yet after cessation of the drug, normal testosterone levels were nearly achieved in recovery group by the end of the recovery period. This increase in testosterone secretion could be mediated either centrally through inhibition of luteinizing hormone (LH) or peripherally through the action of interferon at the testicular level. The significant changes in LH and FSH and serum testosterone among the experi-mental group, could exclude the exclusive peripheral (direct testicular) effect of rh-INF-α-2b on the gametogenic function of the testis in our study. Restoration of normal testosterone levels by the end of the recovery period may point to the

possibility of a reversible suppressive effect of Alpha-IFN on the level of this hormone.

The effect of interferon on fertility was pronounced and acutely inflicted. Histological sections of animals in the experimental group exhibited both quantitative and qualitative damaging effects. The average number of spermatogenic cells per tubule was much lower compared to corresponding controls. Such change was found to be statistically significant (p=0.0151). Despite the recovery observed in weaned animals, the average spermatogenic cells number per tubule was still about 2/3 of the normal control counterparts. Such sustained reduction was also statistically significant (p = 0.011). Qualitatively, the toxic effects of interferon on spermatogenic cells in the experimental animals were marked and involved both the spermatogenic cells and the basement membrane. These qualitative damaging impacts of interferon were markedly reduced in the recovery animals as manifested by lessening of the germ cell hypoplasia and accidental disruption in the basement membrane.

Histologically, Leydig cell changes paralleled testosterone level changes in the experimental group. The average number of interstitial Leydig's cells per interstitial space clearly increased to 20±12.5 compared to 16±11.2 in corresponding Such Leydig's cell changes could explain the early findings of Brown and Dobs (2002), concerning the increase of gonadal steroid and inhibition of hypothalamic-pituitary axis since Leydig's cell stimulation causes increase in testosterone production with subsequent release of a negative feedback mechanism on such axis.

The present work emphasizes the transitory adverse effects of interferon on the testis and highlighted such effects on fertility.

Conclusion

Our results suggest that rh-INF-α-2b temporally affects the hypothalamic-pituitary-testicular axis (HPT) both centrally and peripherally (at the testicular level), through the lessening of FSH, LH, raise of testosterone serum levels and direct phenotypic effect on the testicular tissue.

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تأثير عقار الانترفيرون على الإخصاب في فئران التجارب

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يعتبـر عقـار الانترفيـرون مـن البروتينـات التـي لـها نشـاط مضـاد لانتشـار الفيروسات ومضاد للسرطان ونشاط محفز لجهاز المناعة. كمـا أن لـه خاصية منع التليف التي تساعد في علاج مرضى الكبد المصابين بفيروس (سي) و (بي). ويتم تحضير الانترفيرون كعقار بالهندسـة الو راثية. وقد تم تخطيط هذا البحث لإلقاء الضوء على الآثار المحتملة لهذا العقار على نسيج الخصية وكذلك على مستويات هرمونـات الخصوبة فـي الـدم وذلك باستخدام ذكـور الفئـران البيضـاء كحيوانـات تجارب. وقد تم تقسيم الفئران إلى ثلاث مجموعات كل مجموعة 10 فئران وهى المجموعة الضابطة, المجموعة الاختبارية أعطيت عقار الانترفيرون (3000 وحدة في الغشاء البريتونى/ أسبوعيا لمدة 12 أسابيع و المجموعـة الناقهة أعطيت عقار الانترفيرون– ألفا-2ب ثم تركت فترة شهرين كـاملين للنقاهة من أثار الانترفيرون قبل التضحية بها, وذلك لدراسة إستدامة التغيرات المحتملة للعقار. في نهاية التجربـة تم الحصول على أنسجة الخصيتين الخاصة بالفئران وحضرت منها عينات للفحص الهستوباثولوجى بـالمجهر الضوئي العادي وذلك بعد التضحية بـالفئران . بالإضافة إلى ذلك أيضا تم تحديد مستوى وظائف هرمونات الجونادوتروبين والتيستيرون في فئران المجموعات الثلاثة وذلك قبل وبعد إجراء التجربة. ولقد بينت الدراسة أن عقار الانترفيرون– ألفا-2ب نتج عنه ارتفاع مستوى هرمون التيستيرون في الدم, في نفس الوقت الذي انخفض فيه مستوى هرمونات الجونادوتروبين. كما نتج عنه أبضا زيادة في عدد الخلايا البينية (المنتجة لهرمون التيستيرون) وتغيرات عديدة في الخلايا المنتجة للحيوانات المنوية. وقد أسفرت النتائج عن أن الانترفيرون – ألفا-2ب لـه تأثير **مؤقت** على خصوبة الفئران وذلك عن طريق التأثير المركزي على هرمونات الجونـادوتروبين والتيستوسـتيرون وكـذلك التـأثير المباشـر علـى نسـيج الخصـيتين.