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EFFECT OF INFRARED LASER IRRADIATION ON THE TESTICULAR INTERTUBULAR TISSUE OF THE ADULT MICE

(With 1 Table and 10 Figures)

By

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تأثير أشعة الليزر تحت الحمراء على النسيج البيني لخصية الفئران البالغة

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تم إجراء هذه الدراسة على عدد ثلاثون فأرا قسمت إلى ثلاث مجموعات. أعتبرت المجموعة الأولى كمجموعة ضابطة بينما عولجت المجموعة الثانية بالليزر لمدة خمسة عشر يوما أما المجموعة الثالثة فعولجت بالليزر لمدة خمسة عشر يوما ثم تركت مدة مساوية. هذا وأخذت عينات من خصى المجموعات الثلاث وفحصت بالمجهر الضوئى والإلكترونى لدراسة تأثير الليزر على نسيجها البينى. لم يكن هناك تأثير معنوى على النسبة الحجمية للنسيج البيني للمجموعات الثلاث. على أنه كان هناك تأثير واضح على الخصائص الشكلية لخلايا ليدج البينية حيث أدى إستخدام الليزر إلى زيادة في نشاط هذه الخلايا والذى تمثل في كبر حجم وتطور بعض عضيات الخلية خاصة الميتوكوندريا والشبكة الإندوبلازمية الملساء وهى التي تقوم بإنتاج هرمون الذكورة. أما المجموعة التى تركت خمسة عشر يوما بعد إيقاف إستخدام الليزر فقد أظهرت هبوطا واضحا في نشاط خلايا ليدج. نستخلص من هذه الدراسة أن الزيادة في نشاط خلايا ليدج والناتجة عن الإشعاع بالليزر لا يستمر طويلا بعد إيقاف الإشعاع حيث يحدث بعد ذلك هبوط واضح في نشاط هذه الخلايا.

SUMMARY

The work was carried out on 30 adult mice to investigate the effect of infrared laser irradiation on the testicular intertubular tissue. The animals were divided randomly into 3 groups, 10 animals each. Group I is the

control (was not exposed to irradiation), group II was exposed to laser irradiation for 15 days and examined immediately after the last day of irradiation and group III was exposed to laser irradiation and examined 15-day after the last day of irradiation. Samples from the testis of all animals under study were examined light and electron microscopically. There was no significant effect on the volume percentage of the intertubular tissue due to laser irradiation. Morphologically, the Leydig cells display features of increased steroidogenic activity. These features are represented mainly by the presence of well-developed mitochondria and smooth endoplasmic reticulum, the two important organelles involved in the process of testosterone secretion. The group III, which was examined 15 days after irradiation, showed morphological signs of aborted activity. In conclusion, the increase in the Leydig cells activity induced by Laser irradiation is not a long-lasting effect, but is declined after stop of irradiation. Whether this decline in the activity is reversible or not is questionable and needs further investigation.

Key words: *Laser irradiation, testicular intertubular tissue, mice.*

INTRODUCTION

Laser irradiation as a tool of medical therapy is increasingly used in the last decade. Laser irradiation has been reported to increase metabolic activity (Passarella, Casamassima and Molinaria, 1982; Belkin & Schwartz, 1994 and Williams, Banniste, Berry, Collins, Dyson, Dussek and Ferguson, 1995). Laser irradiation also enhances cell proliferation (Karu, 1988). Increased mitotic division in rabbit cornea (Chentsova, Ukhaneva, Mozherenkov and Karachenko, 1985), skin (Tocco, Kaouel, Galindo and Aubert, 1986) and fibroblasts (Lubart, Wollman, Friedman, Rochkind and Laulicht, 1992) were reported to be due to laser irradiation. Moreover, laser irradiation was recorded to enhance the growth curve pattern of cartilage (El-Habiby, 1990) and the growth and differentation of cerebellar cortex (El-Meligy, 1993).

Mainster (1984) mentioned that the effect of laser irradiation depends on energetic and optical characteristics of radiation (wave-length, intensity, frequency of the beam emission and time of exposure). Moreover, the type of laser employed and the type of the tissue are important factors that control the effect of laser (Van der Zypen, Frankhauser, Bebie and Marshall, 1979 and Tsang, Yew and hui, 1986). The precise dosage, however, depends on the specific laser/tissue combination (Belkin and Schwartz 1994).

It is well known that, in all mammals, testosterone secreted by the Leydig cells is essential for normal spermatogenesis and fertility (Sharpe, Maddocks and Kerr, 1990 and Guyton, 1996). According to Celani, Grandi and Gilioli (1987), Leydig cells respond to laser irradiation by increasing their steroidogenic activity. The low energy laser radiation increases significantly the cyclic adenosine monophosphate (AMP) production, which could be related to the direct effect of laser rays on mitochondrial activity. Salet, Moreno and Vinzens (1979) and Berns, Aist, Edwards, Strahs, Girton, McNell, Rattenser, Kitzes, Hammer-Wilson, Liaw, Siemens, Koonce and Peterson (1981) reported that the laser light energy, trapped in the mitochondria, may directly convert adenosine diphosphate (ADP) into adenosine triphosphate (ATP), which in turn may be utilized for cyclic AMP production.

The aim of the present work is to visualize the effect of laser irradiation on the intertubular tissue of the adult mice. This is also a preliminary trial to clarify the benefits and hazards of applying infrared laser on the testis in cases of gonadal hypofunction.

MATERIALS and METHODS

This study was conducted on 30 adult male mice. They were housed at 30±5°C in the normal daily light and darkness cycle. They were fed with normal mice chow and water ad-libitum. The animals were divided randomly into 3 groups, 10 animals each. Group I was the control group (was not exposed to irradiation), group II was exposed to laser irradiation and examined immediately after the last day of irradiation and group III was exposed to laser irradiation and examined 15-day after the last day of irradiation.

The animals of groups II & III were spot irradiated over the inferior pole of the right testis. Laser probe was held perpendicular to the inferior pole of the testis to minimize the energy loss due to beam reflection. The animals were immobilized during irradiation to confirm the concentration of the beam on the testis. The laser beam was applied, for 2 minutes, once a day for 15 days to the animals of both groups II & III. The daily radiation dose applied to each animal was 1.92 J/cm^2. The cumulative doses used being therefore 28.8 J/cm^2.

After euthanasia, small cubes of the testicular tissue were fixed in a 5% gluteraldehyde in sodium coccodylate buffer for 24 hours. The samples then washed in sodium coccodylate buffer (7.2 pH) and post

fixed in osmium tetra-oxide, dehydrated in ascending grades of ethanol and embedded in ERL. Semithin sections were stained with toluidine blue and examined by light microscope. Ultrathin sections were mounted on cupper grids, stained with uranyl acetate and lead citrate (Reynolds, 1963) and examined with electron microscope.

The volume percentage of the intertubular tissue in the whole testis as well as the volume percentage of the Leydig cells in the intertubular tissue were measured using image analysis system (Leica Q500). Results were subjected to analysis of variance using general linear model procedure (SAS, 1987).

RESULTS

Morphometric findings:

The volume percentage of the intertubular tissue displays slight insignificant difference between the three groups (Table1 and Figs. 1-3). It constitutes 6.02% in the control, increases slightly in group II (7.36%) and decreases again in group III (6.82%). The volume percentage of the Leydig cells in the intertubular tissue records moderate and significant difference (Table1) being the highest in laser treated (41.94%) followed by control (36.45%) and lastly the rehabilitated group (32.45%).

Table 1: Volume percentages (Vv) of the intertubular tissue in the whole testis and volume percentages of the Leydig cells in the intertubular tissue.

Group	Vv of intertubular tissue	Vv of Leydig cells
Group I (Control)	6.02 ± 1.07^{a}	36.73 ± 7.16^{b}
Group II	7.36 ± 1.27^{a}	41.94 ± 3.18^{c}
Group III	6.82 ± 1.40^{a}	32.45 ± 5.64^{d}

Values with different superscript letters in the same column differ significantly ($P < 0.01$)

Morphological findings:

In the control group, the Leydig cells are variable in shape and size (Fig.4). The nuclei are oval or rounded. The cytoplasm demonstrates moderate amount of small and medium sized fat droplets. On the electron microscopic level (Fig.8), the nucleus has irregular outline with two nucleoli and peripheral chromatin patches. The cytoplasm is filled mainly with smooth endoplasmic reticulum and mitochondria. The mitochondria are rounded, oval or elongated in shape with tubular cristae.

In group II, the Leydig cells display features of increased activity (Figs.5, 6 & 9). The nuclei are large and vesicular with few chromatin contents and prominent nucleolus. The Leydig cells nuclei have few chromatin aggregations in comparison to control group. The most striking feature is the large number of large rounded, oval or elongated mitochondria. They have well-developed tubular cristae in comparison to those of the control group. Many fat droplets are scattered between the well-developed mitochondria and the large images of the smooth endoplasmic reticulum. Aggregations of rough endoplasmic reticulum as well as many free ribosomes are also seen in the cytoplasm. The outer surface of the cells presents few numbers of microvilli that contact those of adjacent cells.

In group III (Figs. 7 & 10), the Leydig cells show features of aborted activity. The cytoplasm contains numerous small-sized fat droplets and vacuoles. Some cells are completely filled with many variably sized vacuoles and lipid droplets and seem to be in way of degeneration. At the level of the electron microscope, many vacuoles containing depress of cell organelles are obviously seen in addition to aggregations of lysosomes. Mitochondria with sloughed cristae and vacuolations are also seen. Well-developed Golgi apparatus, with peripheral vacuoles inside, is also seen.

DISCUSSION

In the three studied groups, the intertubular tissue occupies a comparatively smaller part in the testicular tissue (an average of 7% of the testicular parenchyma). The volume percentage of the intertubular tissue varies insignificantly in the three groups. The slight increase, reported in group II, seems to be due to the slight expansion of the Leydig cells.

The morphology of Leydig cells of the control group is similar to that described by Belt and Cavazos (1970) and Cavicchia & Moviglia (1982). In group II, the Leydig cells display features of increased metabolic activity. In agreement with Williams et al. (1995), the vesicular nuclei with less chromatin content, observed in group II is a sign of increased metabolic activity.

The well-developed mitochondria with numerous tubular cristae in the Leydig cells of group II, in comparison to the control (group I), proves that the mitochondia are target organelles to the laser irradiation. Semilar observation was reported in the mice semineferous epithelium

after laser irradiation (Abd El-Hakim, 1999). In the same concern, Celani et al. (1987) studied the effect of infrared laser and helium-neon laser on mice Leydig cells and observed a significant increase in the cyclic AMP production. Similar observations were reported by Passarella et al. (1984) in the rat liver cells. They recorded increased ATP synthesis in rat liver mitochondria exposed to helium-neon laser *in vivo*. Fawcett (1993) considered the mitochondria to be of great metabolic significance as they produce energy from the oxidative breakdown of large molecules and carrying out various other chemical processes. They are the principal sites of a number of enzyme systems; particularly the oxidative phosphorylation associated with the tricarboxylic acid (Kreb's cycle) and the cytochrome electron transport sequences of respiration. They are also the sites of energy as they break ATP by an unusual chemical mechanism.

In the present study the cytoplasm of the Leydig cells of group II shows expansion of area occupied by the smooth endoplasmic reticulum, which appears enclosing fat droplets. Similar observations were reported by Celani, Gilioli, Fano, Montanini and Marrama (1984). They reported an increase in the secretory activity of the Leydig cells in the experimental animals. In the same respect, Celani et al. (1987) recorded an in vitro increase in the testosterone production by mouse Leydig cells following laser irradiation. Since the smooth endoplasmic reticulum is associated with synthesis of lipids, cholesterol and other steroids in addition to other metabolic processes (Dorrington & Khan, 1993 and Russell, 1993), the increase of these organelles could reflect an increase in the secretory activity of the Leydig cells (Zayed, Hifny, Abou-Elmagd and Wrobel, 1995).

The cytoplasm of the Leydig cells of group II shows numerous free ribosomes. These elements are associated with increased cellular activity as mentioned by Kemali, Delfino and Casale (1981); Abergel, Lyons, Dwyer and Uitto (1985) and Williams et al. (1995). Olban, Wachowicz, Koter and Bryszewska (1998) on studying the effect of laser irradiation on platelet function reported that low power infrared laser irradiation induces platelet secretory process and the release of substances stored in the specific granules.

Most of the Leydig cells of group III show features of aborted activity despite the elapse of two weeks after irradiation. The cytoplasmic organelles are still numerous but mitochondria with sloughed cristae are commonly seen. This could lead to a suggestion that the increased activity of Leydig cells due to laser irradiation is not long-

lasting and is followed by decreased activity 15 days after stop of irradiation. Whether this effect is reversible or not is questionable and needs further investigation. These findings disagree with that noticed by Porras, Bermudez, Parrado, Pelaez, Vidal and Perez-de-Vargas (1986); Bermudez, Carrasco, Diaz and Perez-de-Vargas (1991) and Bermudez et al. (1993) who recorded a delayed effect of laser irradiation which, may persist up to one seminiferous epithelial cycle.

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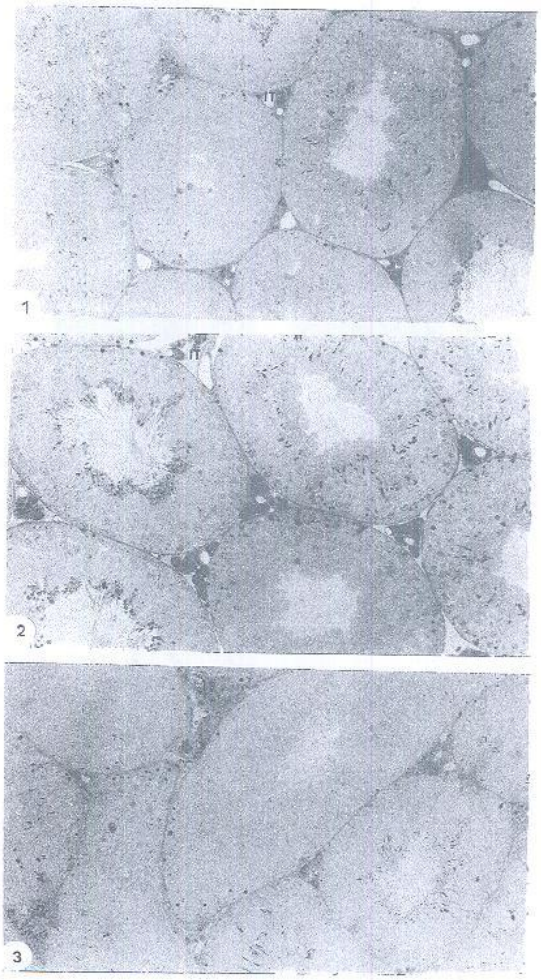
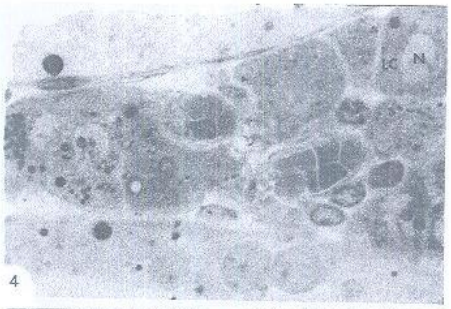
LEGENDS

Figs. 1-3: Semithin sections stained with toluidine blue of group I (Fig.1), group II (Fig. 2) and group III (Fig.3) showing an overview of testicular parenchyma. The intertubular tissue (IT) constitutes a relatively small proportion of the testicular parenchyma. X 160.

Figs. 4-7: Semithin sections stained with toluidine blue of group I (Fig. 4), group II (Fig. 5,6) and group III (Fig.7) showing the general picture of the Leydig cells. In group II (Fig. 5,6), the Leydig cells (LC) have large vesicular nuclei (N) and large number of small fat droplets. In group III (Fig.7), Many Leydig cells are filled with aggregations of fat droplets (FD) and vacuoles (V). X 1000.

Figs. 8-10: Electron micrographs of group I (Fig. 8), group II (Fig.9) and group III (Fig.10). Abbreviations: Nucleus (N), Mitochondria (M), smooth endoplasmic reticulum (SER), rough endoplasmic reticulum (RER), Golgi apparatus (G). Notice the numerous mitochondria with well-developed tubular cristae in the group II in comparisn to the other two groups. X 6700.





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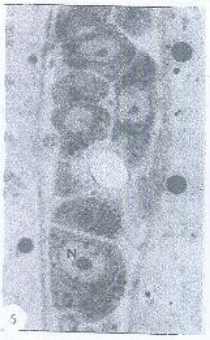

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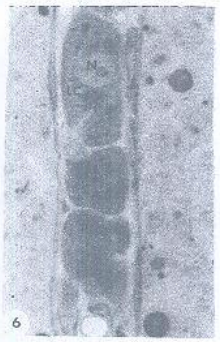

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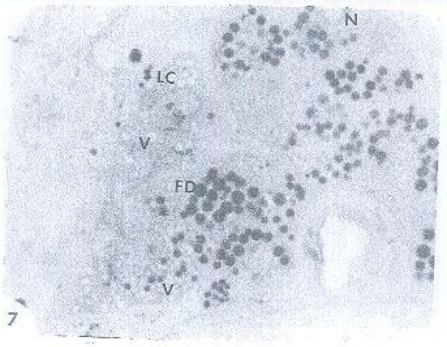

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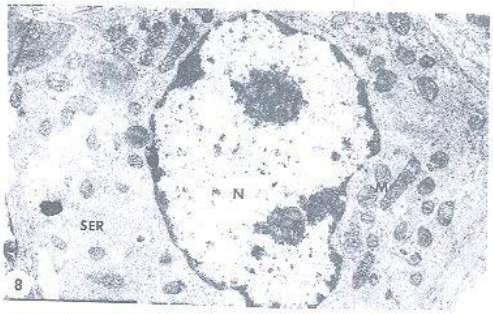
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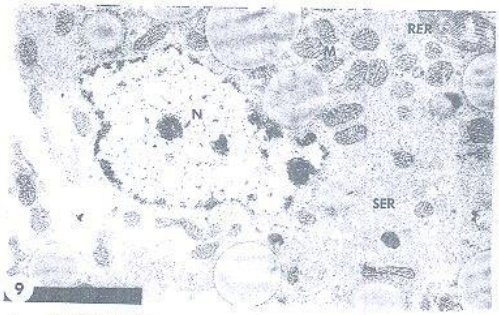
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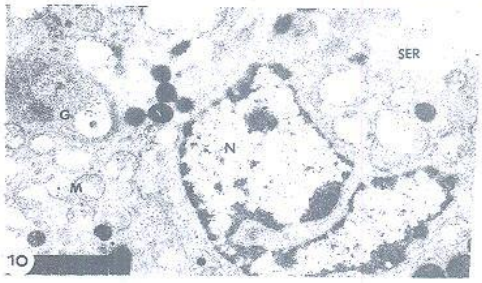
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