Long-term administration of vitamin A and the process of spermatogenesis

I.A. Sadek[1] and M.H. Abdul-Mohsen[2]

العلاقة بين تعاطي الفيتامين "أ" مدة طويلة وبين تكوُّن الحيوانات المنوية

إسماعيل أحمد صادق ومريم حسين عبد المحسن

خلاصة: تمت دراسة تأثير الرتينويدات على تكوُّن الحيوانات المنوية في ذكور فئران الجربوع، واستعمل لهذا الغرض الفحص المجهري الضوئي والإلكتروني. فأعطي للفئران إما 13 – حمض الرتينويك المقترن، أو أسيتات الرتينول، لمدة ستة أسابيع، وقورنت النتائج مع مجموعة شاهدة. ولقد وجد أن 13 – حمض الرتينويك المقترن، أدى إلى التوقف التام تقريباً لتكوّن الحيوانات المنوية، وأحدث تغيرات في هيولى (سيتوبلازم) خلايا لايديغ. ولم تشاهد أية تغيرات في خصيات الحيوانات التي أعطيت أسيتات الرتينول بالمقارنة بخصيات الحيوانات الشاهدة، بعد فحصها بالمجهر الضوئي. ولكن اتضح أنها أدت إلى تغيرات ملحوظة بالبنية المستدقة في خلايا لايديغ. وبعد 12 أسبوعاً من توقف المعالجة اختفت التغيرات التي لوحظت. لذلك ينبغي توخي الحذر لدى إعطاء الرتنويدات مع الطعام للوقاية من السرطان.

ABSTRACT The effect of retinoids on spermatogenesis in adult male gerbils (*Gerbillus cheesemani*) was studied using light and electron microscopy. Treatment with either 13-cis-retinoic acid or retinol acetate was given for 6 weeks and their effects were compared with controls. It was found that 13-cis-retinoic acid induced almost complete cessation of spermatogenesis and produced alterations in the cytoplasm of Leydig cells. No differences were seen in the testis of animals treated with retinol acetate compared with controls using light microscopy but it appeared to produce noticeable ultrastructural changes in Leydig cells. The changes observed were reversed 12 weeks after stopping treatment. Caution should be exercised regarding the use of dietary retinoids in the prevention of cancer.

L'administration à long terme de vitamine A et le processus de la spermatogenèse

RESUME L'effet des rétinoïdes sur la spermatogenèse chez des gerbilles mâles adultes (*Gerbillus cheesemani*) a été étudié au microscope classique et au microscope électronique. Un traitement avec la 13-cis trétinoïne ou l'acétate de rétinol a été administré pendant 6 semaines et leur effet a été comparé avec des témoins. On a constaté que la 13-cis trétinoïne induisait un arrêt quasi-total de la spermatogenèse et produisait des changements dans le cytoplasme des cellules interstitielles des testicules. On n'a pas trouvé de différences dans les testicules des animaux traités avec l'acétate de rétinol par rapport aux témoins à l'examen au microscope classique mais il est apparu que cette substance a produit des changements ultrastructuraux visibles dans les cellules interstitielles des testicules. Les changements observés ont été inversés 12 semaines après l'arrêt du traitement. Il y a lieu d'être prudent en ce qui concerne l'utilisation de rétinoïdes en compléments alimentaires dans la prévention du cancer.

[1]*Department of Zoology, Faculty of Science, Alexandria University, Alexandria, Egypt.*
[2]*Department of Zoology, Faculty of Science, Kuwait University, Kuwait.*
Received: 07/07/98; accepted: 17/09/98

Introduction

Vitamin A (retinol, retinoids) and beta-carotene (provitamin A) are used in experimental, clinical and epidemiological studies for cancer chemoprevention and treatment [*1,2*]. Some studies on the effect of retinoids on carcinogenesis have, however, given apparently contradictory results. For example, retinoids have been found to increase the frequency of cancer in some studies on experimental animals [*3*].

Sterility is a common side-effect in men treated with chemotherapeutic drugs [*4*]. Degeneration of the seminiferous tubules and reduction of the germinal epithelium have been observed in cases of vitamin A deficiency in rats and rhesus monkeys [*5*]. On the other hand, hypervitaminosis A has been found to cause testicular degeneration and to delay spermatogenesis in the rat testis [*6*]. It has also been shown that vitamin A acid suppresses spermatogenesis in toads [*7*]. However, the exact role of vitamin A in the process of spermatogenesis is still unknown.

Retinol acetate and 13-cis-retinoic acid have undergone clinical examination for the treatment of a number of pathologies, in particular certain neoplastic [*8*] and integumentary [*9*] disorders. To date there is no information on the effect of 13-cis-retinoic acid or retinol acetate on the process of spermatogenesis in male *Gerbillus cheesemani*. Our study was designed to investigate the effect of vitamin A treatment on the process of spermatogenesis in order to shed more light on its mechanism of action. Light and electron microscopy were used with special reference to the structure of Leydig cells.

Materials and methods

Adult male gerbils (*G. cheesemani*), weighing 30–50 g, were obtained from a regular supplier in Kuwait City. The animals were housed in individual cages under controlled lighting conditions (14 hours light and 10 hours dark) and temperatures of 20–22 °C. They had unlimited access to food (Dixon laboratory chow) and water. The animals were divided into 4 groups of 30 gerbils each.

- Group I: animals injected intraperitoneally with 6 mg 13-cis-retinoic acid dissolved in 0.2 ml olive oil per 50 g body weight, 3 times per week for 6 weeks.
- Group II: animals injected intraperitoneally with 6 mg retinol acetate dissolved in 0.2 ml olive oil per 50 g body weight, 3 times per week for 6 weeks.
- Group III: animals injected intraperitoneally with 0.2 ml olive oil per 50 g body weight, 3 times per week for six weeks.
- Group IV: control animals, which received no treatment.

Retinol acetate and 13-cis-retinoic acid and olive oil were purchased from Sigma Chemical Company, St Louis, USA.

After 6 weeks of treatment, 15 animals from each group were killed and their testes examined histologically, and 12 weeks after withdrawal of treatment, the remaining 15 animals in each group were similarly treated. The testes were fixed in Bouin's fluid, embedded in paraplast and sectioned at 5 µm. The sections were stained with haematoxylin and counter-stained with eosin.

For electron microscopy, whole testes from each of the four groups were removed and placed in 2.5% glutaraldehyde fixative (pH 7.4) for 3 hours. Tissue cubes were

then rinsed in three changes of 0.1 M sodium cacodylate buffer (pH 7.4) for 1 hour and post-fixed for 2 hours in 2% osmium tetroxide in the same buffer. They were dehydrated in a graded ethanol series, cleared in several changes of propylene oxide, embedded in Epon 812 and incubated at 60 °C for 24 hours. After embedding, the tissue blocks were cut with glass knives using a Reichert OmU3 ultramicrotome. Selected ultrathin sections were stained with uranyl acetate and lead citrate. Sections were examined with an Opton EM9 electron microscope.

Results

Administration of 13-cis-retinoic acid induced nearly complete cessation of spermatogenesis and degenerative changes in most of the seminiferous tubules (Figure 1) compared with the controls (Figure 2). The diameter of the seminiferous tubules decreased, resulting in enlargement of interstitial spaces compared with normal testis.

Sections of the testis from animals treated with retinol acetate showed almost normally shaped seminiferous tubules, except for a slight difference in their diameter (Figure 3).

In animals treated with olive oil, the seminiferous tubules were comparable to the normal testis except for a slight change in their diameter (Figure 4).

The tubules appeared to recover 12 weeks after withdrawing retinoic acid, retinol acetate or olive oil. Seminiferous tubules regained their normal diameter and spermatogenesis in all groups. However, some damaged tubules could still be observed in the retinoic acid group.

Several alterations were observed in the cytoplasm of Leydig cells treated with ret-

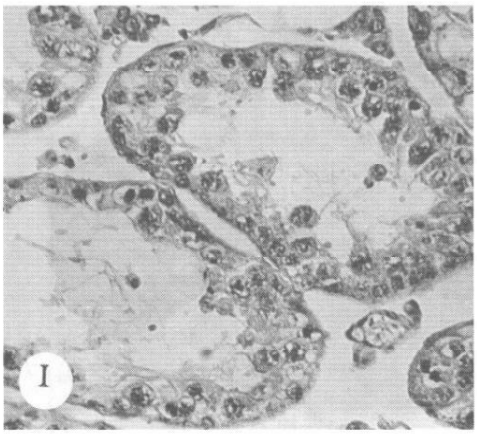

Figure 1 **Light micrograph of transverse section of the testis of *G. cheesemani* treated with retinoic acid for 6 weeks, showing almost complete cessation of spermatogenesis and degenerative changes in most of the seminiferous tubules with reduced diameter and wide interstitial spaces in between (H&E × 400)**

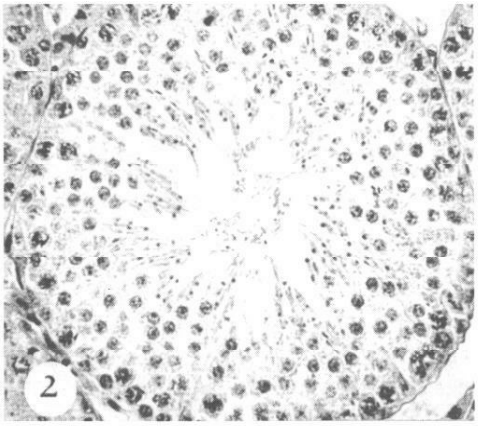

Figure 2 **Light micrograph of transverse section of the testis of control *G. cheesemani* which received no treatment. Note the regular seminiferous tubules with all developmental stages of spermatogenesis and interstitial spaces among tubules (H&E × 400)**

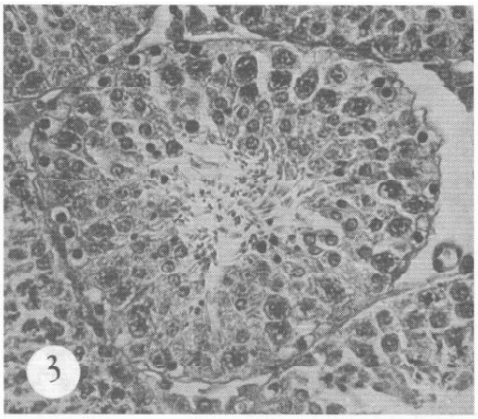

Figure 3 **Light micrograph of transverse section of the testis of *G. cheesemani* treated with retinol acetate for 6 weeks. Note the reduced diameter of the seminiferous tubules (H&E × 400)**

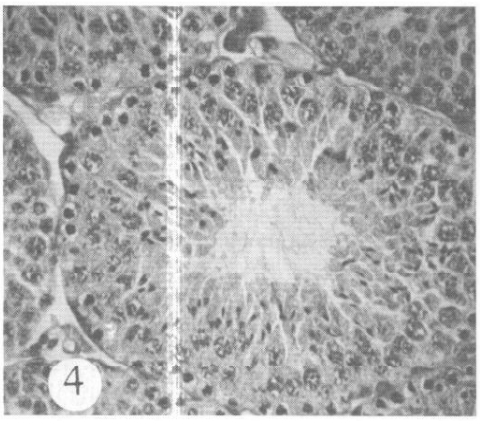

Figure 4 **Light micrograph of transverse section of testis of *G. cheesemani* treated with olive oil for 6 weeks. Note the normal shape of the seminiferous tubules with all spermatogonial stages and interstitial spaces in between (H&E × 400)**

inoic acid (Figure 5). Mitochondria appeared with lamellar cristae, and some showed disruption of the inner membrane and a vaculated matrix ending with hypertrophied mitochondria. The cytoplasm contained dilated hypertrophied cisternae of smooth endoplastic reticulum (SER) and rough endoplastic reticulum (RER).

Noticeable ultrastructural changes were observed in the cytoplasm of Leydig cells treated with retinol acetate (Figure 6). The mitochondria exhibited few lamellar cristae, but showed irregular membranes and a vaculated matrix. Large amounts of cisternae of RER and SER were observed in the cytoplasm of these treated cells.

The ultrastructure of Leydig cells treated with olive oil was normal (Figure 7) compared with the control (Figure 8).

Leydig cells recovered 12 weeks after drug withdrawal in all groups; however, slightly vaculated mitochondria were observed in the retinoic acid group.

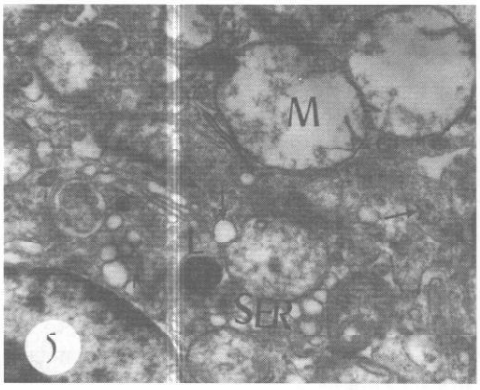

Figure 5 **Electron microscopy of part of two Leydig cells of the testis of *G. cheesemani* treated with retinoic acid for 6 weeks. The mitochondria (M) appear hypertrophied with indistinct membranes, few cristae and a vaculated matrix. Note the different stages of association between mitochondria and SER (←). In some cases the mitochondria appear to be adjacent to each other while in other cases they appear to be enveloped by the SER. Abundant cisternae of SER (↞) are present in the cytoplasm (× 9500)**

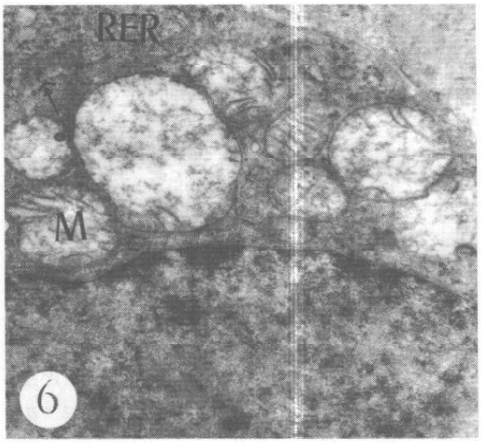

Figure 6 **Electron microscopy of part of Leydig cells of the testis of *G. cheesemani* treated with retinol acetate for 6 weeks, showing some altered mitochondria (M) with indistinct outer membranes, a vaculated matrix and few cristae. RER and clusters of ribosomes (←) are present (× 9500)**

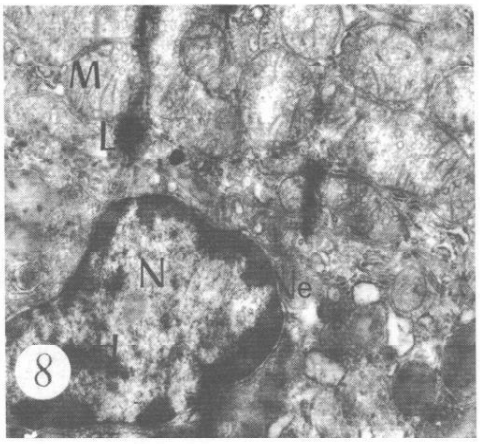

Figure 8 **Electron microscopy of part of Leydig cells of the control testis of *G. cheesemani*, showing an oval nucleus (N), with a regular nuclear envelope (Ne) and peripheral heterochromatin (H). Mitochondria (M), cisternae of SER and lysosomes (L) can be seen (× 9500)**

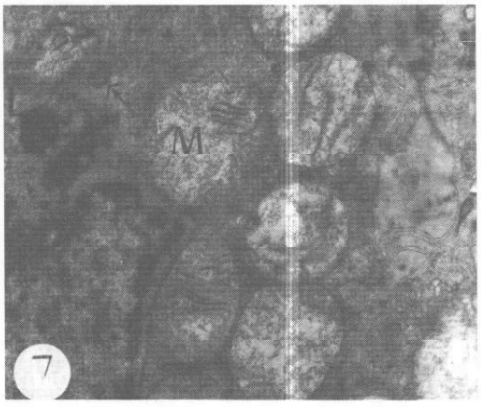

Figure 7 **Electron microscopy of part of Leydig cells of the testis of *G. cheesemani* treated with olive oil for 6 weeks. Note the presence of mitochondria (M) with few cristae, a light matrix and indistinct outer and inner membranes. Clusters of free ribosomes (←) and dense lysosomes (L) are indicated (× 9500)**

Discussion

Our results show that long-term administration of vitamin A acid or vitamin A alcohol has an effect on the process of spermatogenesis in the male gerbil.

In mammals it is well known that spermatogenesis is totally dependent on testosterone, but exactly what level of testosterone produced by the Leydig cells is needed and how it acts upon the Sertoli and peritubular cells of the seminiferous tubules to drive spermatogenesis is still unknown [*10*]. Our results give evidence that the administration of vitamin A acid or vitamin A alcohol affects the process of spermatogenesis in the male gerbil. The association of 13-cis-retinoic acid treatment of the male gerbil with the inhibition of spermatogenesis and degeneration of the mitochondria in Leydig cells may reflect a

decrease in the testosterone level. Ewing and Zirkin [*11*] suggested that cholesterol from the metabolically active pool might be transported into the mitochondria where the cholesterol-side-chain-cleavage enzyme converts it to pregnenolone. Pregnenolone is then transported into the SER where it is converted into testosterone. Cessation of spermatogenesis has been reported in the presence of low serum testosterone levels in male rats [*12,13*]. The level of testosterone also decreased in plasma samples from male New Zealand rabbits treated with 13-cis-retinoic acid, while in plasma, luteinizing hormone (LH) and follicle stimulating hormone (FSH) concentrations increased [*14*]. This elevation may reflect an increase in gonadotropin-releasing hormone (GnRH). The effect of GnRH in the cessation of spermatogenesis in male gerbils cannot be excluded since GnRH sites in the Leydig cells of the rat testis have been demonstrated [*15,16*].

The damage caused by vitamin A treatment in the testis of the male gerbil can be corrected by withdrawal of the drug for at least 12 weeks. However, there are a number of important questions to be answered before we can confidently make recommendations on the use of dietary retinoids in the prevention of cancer. It is worth mentioning that retinoic acid and several analogs have been found to promote skin tumours in mice when administered topically or as a dietary supplement [*17*].

References

1. Lupulescu A. The role of vitamins A, beta-carotene, E and C in cancer cell biology. *International journal for vitamin and nutrition research*, 1994, 64:3–14.
2. Hill DL, Grubbe CJ. Retinoids and cancer prevention. *Annual review of nutrition*, 1992, 12:161–81.
3. Kulesz-Martin M, Blumenson L, Lisafeld B. Retinoic acid enhancement of an early step in the transformation of mouse epidermal cells *in vitro*. *Carcinogenesis*, 1986, 7:1425–9.
4. Meistrich ML et al. Damaging effects of fourteen chemotherapeutic drugs on mouse testis cells. *Cancer research*, 1982, 42:122–31.
5. O'Toole BA et al. Vitamin A deficiency and reproduction in rhesus monkeys. *Journal of nutrition*, 1974, 104:1513–24.
6. Lopes RA et al. Effect of hypervitaminosis A on the testis of the rat during lactation. *International journal for vitamin and nutrition research*, 1974, 44:159–66.
7. Sadek IA, Sabry I. Effect of vitamin A acid on the testis of the Egyptian toad. *Folia morphologica*, 1986, 34:141–4.
8. Moon RC, McCoromick DL. Inhibition of mammary carcinogenesis by retinoids. *Journal of the American Academy of Dermatology*, 1982, 6:809–14.
9. Elias PM, Williams ML. Retinoids, cancer and the skin. *Archives of dermatology*, 1981, 117:160–80.
10. Sharpe RM. Paracrine control of the testis. *Clinics in endocrinology and metabolism*, 1986, 15:185–207.
11. Ewing LL, Zirkin BR. Leydig cell structure and steroidogenic function. *Recent progress in hormone research*, 1983, 39:599–635.
12. Krueger PM, Hodgen GD, Sherine RJ. New evidence for the role of the Sertoli

cells and spermatogonia in feedback control of FSH secretion in male rats. *Endocrinology*, 1974, 95:955–62.

13. Rich KA, de Kretser DM. Effect of differing degrees of destruction of the rat seminiferous epithelium on levels of serum follicle stimulating hormone and androgen protein. *Endocrinology*, 1977, 101:959–68.

14. Sadek IA et al. Effect of 13-cis-retinoic acid on plasma levels of FSH, LH and testosterone in male New Zealand rabbits. *Journal of nutrition, growth cancer*, 1986, 3:117–20.

15. Clayton RN et al. Direct inhibition of testicular function by gonadotropin-releasing hormone: mediation by specific gonadotropin-releasing hormone receptors in interstitial cells. *Proceedings of the National Academy of Sciences USA*, 1980, 77:4459–63.

16. Quinn PG, Payne AH. Oxygen-mediated damage of microsomal cytochrome P-450 enzymes in cultured Leydig cells. Role of steroidogenic desensitization. *Journal of biological chemistry*, 1984, 259:4130–5.

17. McCormick DL, Bagg BJ, Hultin TA. Comparative activity of dietary or topical exposure to three retinoids in the promotion of skin tumor induction in mice. *Cancer research*, 1987, 47:5989–93.

As most countries have ongoing national health-related research activities, particularly in universities and research institutes, and the results of such research are not always shared with decision-makers at the national level or with scientists in other countries of the Region, the Regional Office has been keen to strengthen national research focal points to achieve this target and is also making every possible effort to ensure the publication of the results of these studies through national and regional health journals and periodicals.

Source: The work of WHO in the Eastern Mediterranean Region. Annual Report of the Regional Director 1 January–3 December 1997. *Pages 16–17.*