Urol. Res. 9, 29-32 (1981)

Urological Research

Effects of LH and Oestrogen on the Growth of Feminising Testicular Tumours in Mice

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Accepted: August 20, 1980

Summary. Testicular tumours in mice with testicular feminisation (tfm/y) are made up of masses of large Leydig and lipid-laden cells, surrounded by darkly-stained smaller Leydig cells. The activity of 3 β-hydroxysteroid dehydrogenase (3 β-HSDH) is more apparent in the small cells of the peripheral than in the larger cells of the central part of the tumour. Oestrogen alone can stimulate the incorporation of thymidine into deoxyribonucleic acid (DNA) by cultured cells but not the activity of 3β-HSDH. On the other hand, luteinizing hormone (LH) promotes activity of the enzyme, but not the synthesis of DNA. Together, they have a greater effect on DNA synthesis and enzyme activity than if present separately. It seems that in tfm/y mice gonadotrophin is necessary for the proper action of oestrogen in control of testicular tumour growth.

Key words: Testicular feminisation (tfm/y), Testicular tumour, LH-Oestrogen.

Testicular feminisation, an inherited form of male pseudohermaphroditism (tfm/y), is a syndrome characterized by the presence of small cryptorchid testes in a phenotypic female. Mice with this syndrome exhibit an end organ insensitivity to androgens. This is manifested by a failure of male sexual differentiation (13). The primary genetic defect is believed to be a deficiency of androgen receptor proteins in the target organs (1, 3). In the cryptorchid testes of adult tfm/y mice, spermatogenesis and cytodifferentiation of Leydig cells are impaired. The synthesis of testosterone is decreased due to defective activity of 17 β-hydroxysteroid dehydrogenase; however, activities of 3 β-HSDH and other oxidative enzymes are increased (2, 7). In rodents and humans, individuals with tfm/y tend to develop testicular tumours (5, 6, 17). It was the purpose of this study to investigate the effect of oestrogen and gonadotrophin on the function and growth of testicular tumours which occur spontaneously in tfm/y mice. It was anticipated that the results of this study would advance our understanding of the cause, treatment and prevention of testicular disorders and would provide better insight into the pathogenesis of testicular feminisation in humans.

MATERIALS AND METHODS

Tfm/y tumour-bearing male mice weighing 45 g were obtained from a Colony maintained at the University of Oklahoma Health Sciences Center. For light microscopy, testicular tumours from tfm/y mice were fixed in Bouin's solution, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin. For histochemistry, tumours were placed on block holders and frozen quickly in a cryostat maintained at -20°C. Sections were cut at 5 μm and mounted on glass slides. Sections and cultured cells on glass slides were processed to reveal the activity of 3 β-HSDH, using dehydroepiandrosterone as a substrate. The samples were incubated for 45 min in the proper medium according to the method described by Wattenberg (18). Control incubations were carried out concurrently in a similar medium without substrate. The degree of enzyme activity in the tumour cells was rated subjectively from 0 (no formazan deposit) to 3+(maximum reaction).

For cell culture, tfm/y testicular tumours (mostly central parts) were removed aseptically, washed with Puck's saline, minced to 0.5 mm^3 pieces and washed twice with the same saline. The minced tissues were incubated in 0.25%

0300-5623/81/0009/0029/$ 01.00

trypsin with 0.02% EDTA (pH 7.2) at 4°C with constant stirring for 30 min. After incubation the cells were obtained by low-speed centrifugation. Fresh cold trypsin solution was added and trypsinisation continued for an additional 2 h. The resulting cell suspension was filtered through sterile cheese cloth to remove fibrous elements and undispersed tissues. Dissociated cells were collected by low-speed centrifugation. The collected cells were resuspended in Eagle's MEM, counted for viability using a 1% solution of trypan blue, and plated at 1×10^5 cells per culture flask in 10 ml of the same culture medium, supplemented with 10% fetal calf serum. The flasks were then incubated at 37°C for 3 days in the absence of hormone to eliminate any interference by endogenous hormones. Thereafter, cultures of cells in the absence (control) or presence of hormones (5 µg/ml of LH or 5 µg/ml of LH or 5 µg/ml of estradiol-17 β or both) were carried out. All cultures, experimental (with hormone) and control (without hormone) were fed at three day intervals and incubation was continued for an additional 3 weeks. The cultured cells with or without hormone were studied for the rate of DNA-synthesis, and for activity of 3 β - HSDH histochemically and biochemically.

For the activity of 3 β-HSDH, the cultured cells on glass slides were examined histochemically as described above. The conversion of pregnenolone to progesterone indicates the activity of 3 β-HSDH (15). To demonstrate the same enzyme activity biochemically, cultured cells were homogenised and microsomal fractions were prepared (16). Aliquots of microsomal fraction were suspended and incubated in Dulbecco's phosphate buffered saline, pH 7.4, containing glucose (10 mM) and pregnenolone-7-^{3}H (0.5 µCi, 0.5 µM) for 90 min at 37°C. Reactions were stopped by the addition of acetone. Twenty µg of unlabelled pregnenolone and progesterone as carrier and ^{14}C-steroid for recovery were added to each sample. After centrifuging at 20,000 g for 15 min, the supernatants were decanted and evaporated with nitrogen to give a concentrated aqueous phase. Steroids were then extracted and purified by thin layer chromatography (4). The radioactivity was counted in a Packard Tri-Carb liquid scintillation spectrometer.

For DNA synthesis, cultured cells were washed twice in 5 ml of calcium-magnesium-free Hank's balanced salt solution (HBSS), collected after detachment with trypsin-EDTA solution, and incubated at 37°C for 4 h in 3 ml of Eagle's MEM containing 10% fetal calf serum and 1 µCi/ml of thymidine (methyl-^{3}H). The incubate also contained 5 µg/ml of LH or 5 µg/ml of oestradiol-17 β or both, except for the control. After incubation the cells were washed twice with 2 ml of ice-cold HBSS and then homogenised in 2 ml of distilled water. Aliquots of 100 µl were taken, to which 2 ml of 10% trichloroacetic acid (TCA) was added. Samples were vortexed and placed on ice for 10 min. The precipitates were collected on 2.4 cm Whatman GF/A glass fibre discs with the aid of suction. The discs were washed twice with 2 ml of 5% TCA and absolute ethanol, and assayed for radioactivity in 10 ml of PPO-POPOP-toluene scintillation fluid in a liquid scintillation counter.

Protein content of a fifty µl aliquot of the homogenate was determined by the method of Lowry et al. (12), using bovine serum albumin as standard. The rate of incorporation of tritiated thymidine into DNA was expressed in terms of dpm/mg protein.

RESULTS

The tfm/y mouse testicular tumours appeared as masses of light and dark cells. Cells in the more central part included larger lightly-stained Leydig cells along with lipid-laden degenerating cells, fibroblast-like cells and macrophages (Fig. 1). Many of the Leydig cells were highly vacuolated and showed degenerative changes.

The 3 β-HSDH was more active in the peripheral part than in the central part of the tumours (Fig. 2, 3). This enzyme activity in the peripheral part of the tumours was similar to that of the non-tumorous tfm/y testes. LH alone stimulated the activity of 3 β-HSDH in cultured tumour cells but higher activity of these enzymes was observed when LH was treated along with oestrogen (Table 1). Increased conversion of pregnenolone to progesterone ($P < 0.001$) also indicated the enhanced activity of 3 β-HSDH in the presence of LH or both LH and oestrogen (Table 1).

Effects of LH and oestrogen singly or in combination on DNA synthesis by cultured tumour cells are shown in Table 1. No significant effect of LH on the incorporation of thymidine into DNA was noted ($P > 0.1$). However, cells from oestrogen treated groups showed an increased capacity for DNA synthesis ($P < 0.001$). Furthermore, the combination of LH and oestrogen was more effective ($P < 0.01$) than oestrogen only in synthesising DNA from thymidine.

DISCUSSION

Testicular tumours occur spontaneously in mice with testicular feminisation (5). Similar tumours occur in male pseudohermaphrodite rats (17) and patients with testicular feminisation (6, 14). Considerable information exists on the incidence and formation of testicular tumours in different genetic, hormonal and environmental influences, yet their true aetiology still remains obscure.

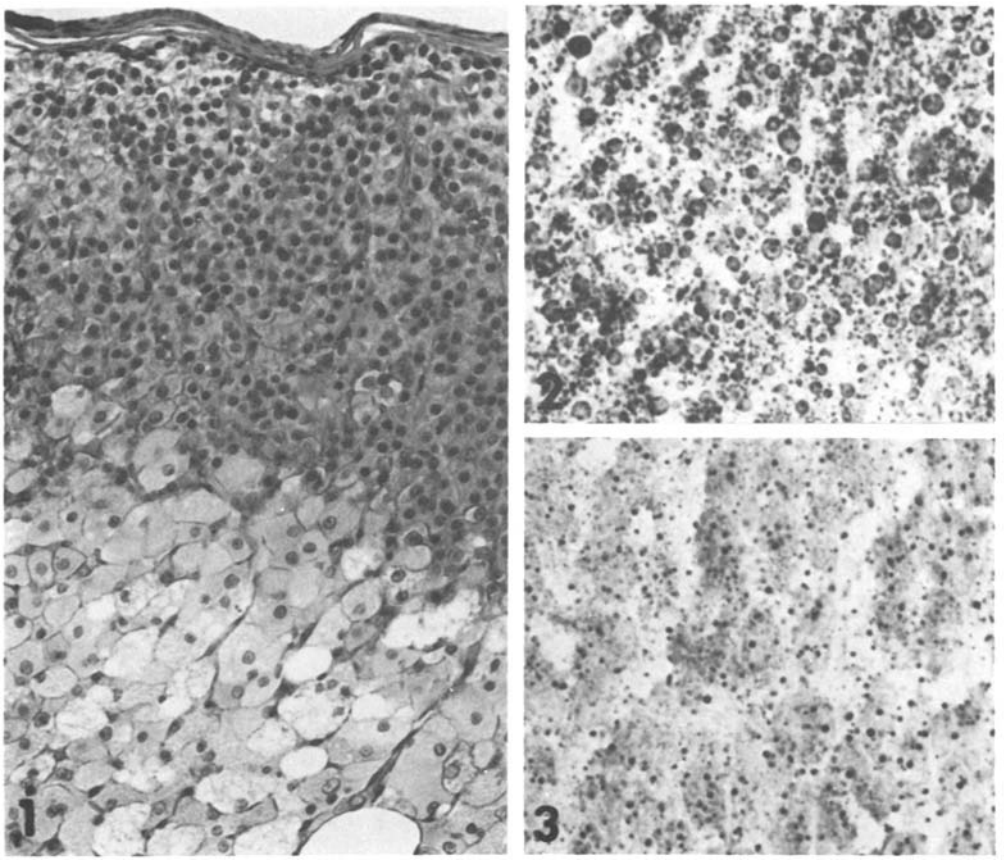

Fig. 1. Light micrograph of testicular tumour from tfm/y mice exhibiting darkly-stained small cells and lightly-stained large cells. Histochemical reaction for 3β-HSDH shows increased activity in the peripheral part (Fig. 2) as compared to that in the central part of the tumour (Fig. 3). Fig. 1, H&E, x 225; Figs. 2-3, x 200

Table 1. Effects of LH and oestrogen on DNA synthesis and activity of 3β-HSDH in cultured mouse testicular tumour cells

Treatment group	DNA Synthesis ($1x10^4$ dpm/mg protein)	Histochemical 3β-HSDH	Microsomal 3β-HSDH ($1x10^4$ dpm-P[a]/mg protein)
Control	1.2 ± 0.19[b]	+[c]	3.5 ± 0.52
LH	1.3 ± 0.17[d]	++	7.1 ± 0.84[e]
Oestrogen	2.7 ± 0.41[e]	+	2.9 ± 0.43[d]
LH + Oestrogen	3.9 ± 0.58[e, f]	+++	12.7 ± 1.85[e, f]

[a] Progesterone formation from pregnenolone; P = Progesterone

[b] Mean ± Standard error

[c] Activity of 3β-HSDH per tumour cell was rated from O (no formazan deposit) to 3+(maximum reaction)

[d] Differences from control ($P > 0.1$)

[e] Differences from control ($P < 0.001$)

[f] Differences from oestrogen alone ($P < 0.01$)

The tfm/y mouse testicular tumour cells retain the characteristic function of tfm/y Leydig cells to produce steroid hormones (5), however, the degree to which they are produced varies from one part of the tumour to another. In the peripheral part of the tumour, 3β-HSDH activity in the Leydig cells is on a par with that in the non-tumorous testes, but in these cells from the centre of the tumour, it is greatly reduced (2). This enzyme activity is not altered in cultured

cells under the stimulation of oestrogen alone, but its activity is increased by LH alone. It appears that LH along with oestrogen is capable of further enhancing the activity of this steroid enzyme.

The growth-promoting effect of hormones on tumour cells in culture was measured by the synthesis of DNA. Although LH alone fails to stimulate the DNA synthesis by cultured tfm/y testicular tumour cells, thier synthetic capacity is increased 2-fold by oestrogen stimulation over the control value. Furthermore, the combination of LH and oestrogen is even more effective than oestrogen alone in stimulating the incorporation of thymidine into DNA of the tumour. This suggests the possibility that in tfm/y mice such a steroid hormone does act as a potent stimulator for the growth in the presence of gonadotrophin. Effects of oestrogen and gonadotrophin on the development and growth of testicular tumours has also been reported in other rodent species (8-11). Oestrogen administered to BALB/c mice not only induces the formation of their testicular tumours, but also markedly increases the incidence of such tumours in cryptorchids (8-11). Administration of LH to this strain of mice, however, fails to cause testicular tumours to develop (8).

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