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Anticancer Agent Cyclophosphamide Disturbs Mice Spermatogenesis

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The anticancer agent cyclophosphamide (CY) is widely used in chemotherapy regimes. It causes severe side effects on many rapidly proliferating cells including germinal cells. In the present study the effect of CY on mouse spermatogenesis was investigated. Sexually mature BALB/c mice were injected i.p. with 200 mg/kg CY applied in a single dose or as 10 doses of 20 mg/kg each. Blood samples and pieces of testes were taken on the 1, 3, 7 and 15 days after the last treatment and preceded for RIA, autoradiographic and ultrastructural study. The obtained results showed impairment of testicular function expressed in decline of mitotic activity of spermatogonial cells, ultrastructural alterations of different germ cells, disruption of acrosome formation and increase of testosterone level, restored in 15 days after the treatment. In conclusion our results demonstrate that CY caused temporary interference of normal male reproductive system, but in high dose chronic treatment, dysfunction might be permanent.

Key words: cyclophosphamide, spermatogenesis, electron microscopy.

Introduction

CY is an alkylating and antimitotic drug widely used in anticancer therapy. It is known that CY produce serious side effects especially on male reproductive system expressed as diminished sperm number and sperm abnormalities [6] . In addition CY decreases the number of mitoticaly diving germ cells (spermatogonia) [3] and damages spermatocytes. Apoptosis is one of the cell death mechanisms, responsible for the elimination of damaged cells, triggered by cytotoxic drugs as cytostatics [5]. Many studies recently have shown that the heat shock proteins play critical roles in modulating the apoptotic cascades. Two main pathways of apoptosis have been described: intrinsic and extrinsic. The intrinsic pathway involves loss of mitochondria membrane potential in response to death signals, leading to permeabilization of the outer membrane, cytochrom c and the effector caspase-3 [9]. The extrinsic pathway transduces death signals through the binding of extra cellular death ligands to their cell surface receptors and activation of procaspase 8 and caspase 3 [1]. Little is known about the action of CY on not rapidly proliferating testicular Leydig cells. We have shown prolonged inhibition of thyroxin synthesis accompanied with anormal ultrastructure of thyrocytes after acute or chronic administration of 200 mg/kg CY [7]. In the present paper we focussed on the effect of CY on mice testicular morphology and ultrastructure after acute or chronic treatment.

Material and Methods

Forty-eight sexually mature male BALB/c mice were used. CY (Germed, Germany) was given i.p. as a single dose of 200 mg/kg b.w. or as 10 doses of 20 mg/kg each, three times weekly. Control groups were treated with saline. On the 1st, 3rd, 7th and 15th day after the last treatment the serum levels of testosterone were measured radioimmunologically. Pieces of testes were fixed in 2,5% glutaraldehyde, postfixed in OsO_4 and embedded in Durcupan. The observations were made on Opton 109 electron microscope.

Results

The application of 200 mg/kg CY in acute experiment did not change the serum testosterone concentration while it was elevated significantly on the 1st, 3rd and 7th day after the 10 doses of 20 mg/kg (data not shown). In 15 days testosterone value was in the norm. On the 3rd day after application of 200mg/kg CY a strong decrease in the number of spermatogonia was registered (Fig. 1). Majority of seminiferous tubules showed normal appearance. In some the contacts between germ and Sertoli cells were disturbed. In some germ cells vacuolization of mitochondria was seen (Fig. 2A). Among spermatocytes those with normal ultrastructure were predominant. In parallel to the intact spermatids at different stages of spermiogenesis, round spermatids with disturbed acrosome formation (Fig. 2B) or disruption of acrosomal membrane in elongated spermatids (Fig. 2C) was seen. The bulk of Sertoli and Leydig cells showed normal structure. Disruption

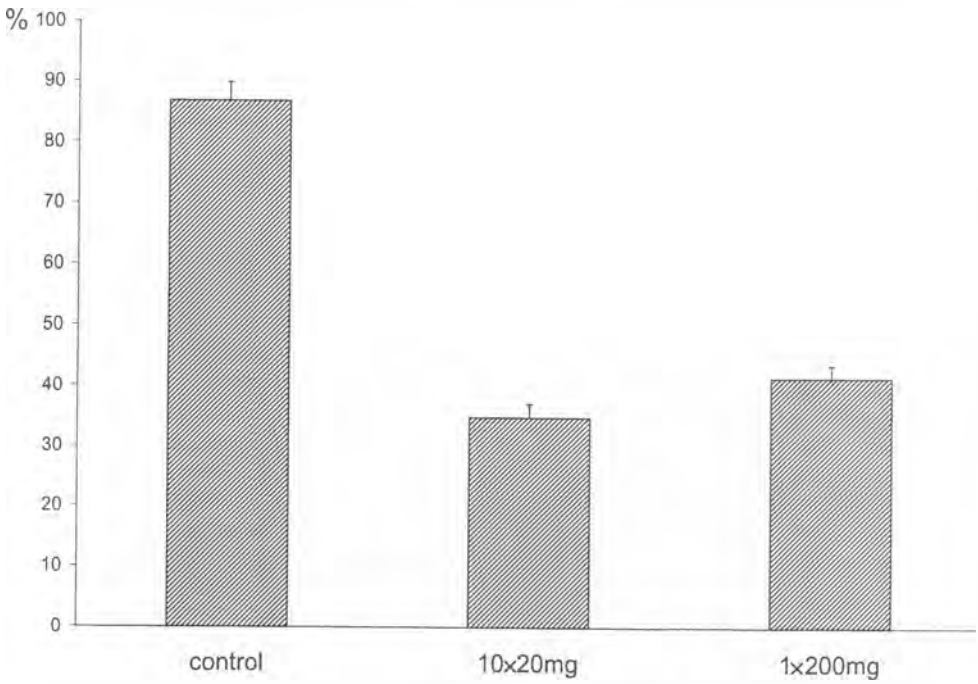

Fig. 1. Percentage of proliferating spermatogonia after acute or chronic treatment with cyclophosphamide

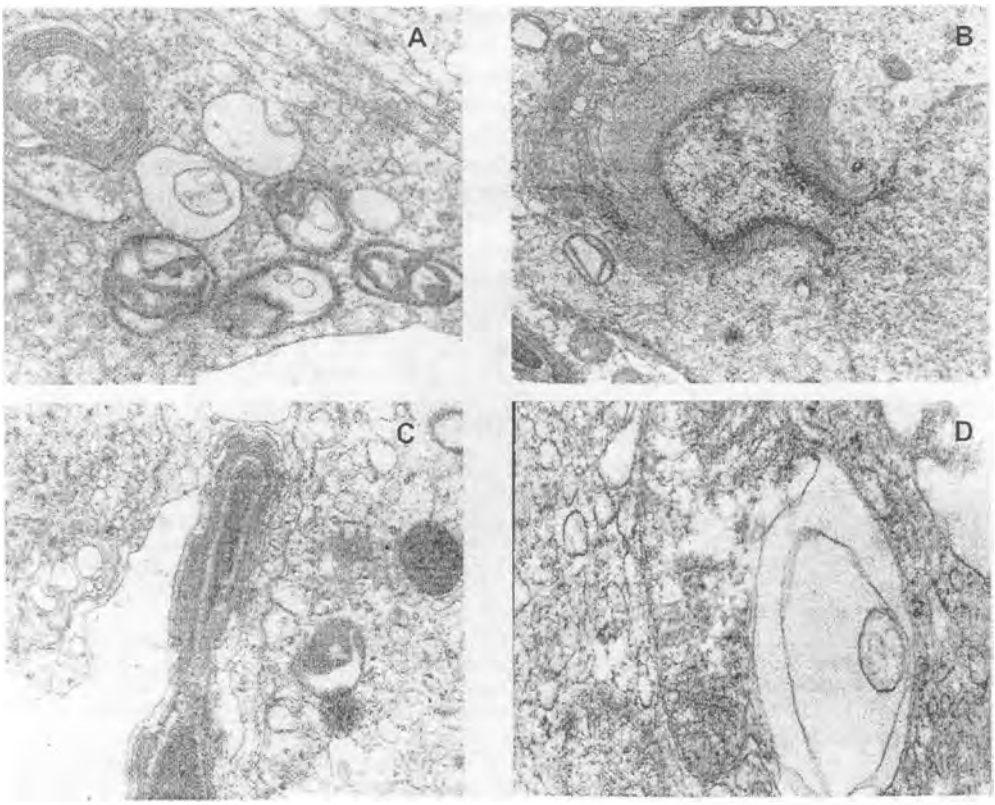

Fig. 2. Ultrastructural alterations of different testicular cell populations after treatment with cyclophpsphamide: A – Spermatogonia: vacuolization of cytoplasme and mitochondria, × 30 000; B – Round spermatid: abnormal acrosome formation. × 20 000; C – Elongated spermatid with acrosomal membrane disruption, × 12 000; D – Leydig cell: vacuolization of cytoplasme and mitochondria, × 50 000

of mitochondrial membranes and destruction of cristae were observed in some Leydig cells (Fig. 2D). On the 3rd day after injection of 10 doses 20mg/kg CY the number of spermatogonia was more decreased in comparison with the acute experiment (Fig. 1). The ultrastructure of germ and somatic cells in mouse testis at all intervals was more affected, expressed with dilatations of endoplasmic reticulum, vacuolization of the cytoplasm and abnormal acrosome formation (data not shown).

Discussion

Our results confirm the data about the testicular failure in mice caused by the anticancer agent CY [8]. The most sensitive cells in mouse testis are proliferating spermatogonia as well as spermatids in different stages of maturation. Sertoli cells are damaged to a lesser extent while Leydig cells are less affected. It is known that spermatogonia are the most sensitive to many cytotoxic insults and therefore can serve as indicators of hazard to stem spermatogonia [4]. Treatment with 200mg/kg has shown sharp decline in total sperm counts in mouse epididymis and decrease of fertilization ability of spermatozoa [2]. Multiple CY injections resulted significant temporary elevation of serum testosterone. It is known that testosterone is a final result of variety of processes invol-

ving its synthesis, consumption and degradation. This result can be a consequence of spermatogenesis impairment and failure in metabolic control mechanisms including hypothalamo-pituitary-gonadal axis. Profound changes in mitochondrial ultrastructure in germ and some somatic testicular cells probably is a sign of intrinsic death pathway of apoptosis [1]. The permeabilization of mitochondrial membrane triggers release of pro-death molecules like cytochrome c and Smac/Diablo into the cytoplasm and leads to formation a functional apoptosomes. The next steps of signal transduction leads to cell death by activation the efector caspase-3 [9].

Our results demonstrate that CY caused temporary interference of normal male reproductive system, but in high dose treatment, dysfunction might be permanent.

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