

The effects of cyclophosphamide on the prolactin cells of the normal rat

María S. Poblador, Carmen Rojas, Antonio Raya, Joaquín Quiralte, José A. Casares and José Luis Lancho-Alonso

Department of Morphological Sciences, Human Anatomy-Neuroanatomy Section, Faculty of Medicine, University of Córdoba, Spain

Summary. Cyclophosphamide administered at doses of 400 mg/m²/5 days with sacrifice two days later, and 200 mg/m²/5 days with a 21-day break and a further five days of treatment, with sacrifice two days later, provokes similar effects.

Functional activity is less marked in untreated and control animals than in treated ones, as the hormone data shows.

But the ultrastructure of PRL cells in treated animals indicates the existence of clear hormone synthesis activity, evident in the fusion and clustering of granules at differing stages of maturity, etc.

Key words: Rat, PRL cells, Cyclophosphamide, Ultrastructure

Introduction

Cyclophosphamide (CPA) is an alkylating agent which has as carrier a phosphorated ring which must be released in the liver through the action of oxidase and phosphoramidase in order for the drug to act (Brock, 1958; Busch and Lane, 1967; Brock, 1968; Doerge, 1977).

The exact mechanism through which cyclophosphamide exerts its effect is not known, but most authors agree that it affects the G₂- and S-phases of the cell-cycle.

Cyclophosphamide has various toxic effects, which include alopecia, nausea, vomiting, leukopaenia and to a lesser degree thrombocytopenia, mucous ulcerations, brief spells of dizziness, transverse striations in the fingernails, increased skin pigmentation, pulmonary fibrosis, liver poisoning and facial abrasions (Silverstein et al., 1984; Watson et al., 1985).

Cyclophosphamide, administered either alone or in conjunction with other drugs, has proved an effective treatment

in different types of tumour (disseminated Hodgkin's disease and non-Hodkin lymphomas, acute leukaemia, cancer of the breast and ovaries, myeloma, etc.).

In view of the paucity of literature available in this specific area, it is felt that the results obtained in this experiment to assess the effect of cyclophosphamide on prolactin cells may be of interest.

Materials and methods

47 adult male Wistar rats of 250 g. mean weight received intraperitoneal injections of cyclophosphamide *i. p.* injections providing virtually the same diffusion as intravenous inoculation (Ohnuma et al., 1974).

A further group of 8 normal male rats of similar weight and kept in the same environmental and dietary conditions as experimental animals were used as untreated controls.

All animals were housed in the same condition: a standard chow diet, light cycle of 12 hours, temperature ranging between 20-23°C, constant humidity and «ad libitum» water supply; the appropriate hygienic and sanitary measures were taken and animals were housed in individual isolated cages.

Cyclophosphamide was administered in two different programmes:

1. 400 mg/m²/5 days cyclophosphamide, animals sacrificed after 2 days.

2. 200 mg/m²/5 days cyclophosphamide, a 21-day break followed by a further five days treatment, animals being sacrificed 2 days later.

Animals were grouped into the following batches:

Batch Ia: 7 animals treated with 400 mg/m²/5 days. The 3 control animals received an equivalent dosage of vehicle.

Batch Ib: Animals treated with 200 mg/m²/5 days and a subsequent 21-day break, followed by a second cycle of treatment. 3 control animals received an appropriate volume of vehicle.

Batch II: Rats treated with 400 mg/m²/5 days cyclophosphamide.

Batch III: Rats treated with 200 mg/m²/5 days, with a

second cycle of treatment after a 21-day break. Animals were sacrificed two days later, i.e. 33 days after beginning treatment (as was the case with Batch Ib).

Control animals in Batches II and III (4 in each batch) received the appropriate quantity of vehicle.

CPA dosage was proportional to the human dosage proposed by De Vita et al. (1976) and Chabner and Myers (1984). The equivalent animal dose was obtained by multiplying the human dose by the conversion factor of 7 proposed by the National Cancer Institute (NCI), and by converting mg/kg weight into mg/m² surface area, multiplying the former by factor 37, as recommended also by the NCI.

All animals were killed by decapitation without anaesthetic. The hypophysis was extracted after opening the cranial cavity and separating the meningeal covering.

Roughly 45 seconds elapsed between the death of the animal and removal of the hypophysis.

Samples were fixed in 2% glutaraldehyde in phosphate buffer at pH 7.4, postfixed in 1% osmium tetroxide and subsequently contrasted with 0.5% uranyl acetate prior to embedding in araldite. 500-700 Å slices were cut from the blocks thus obtained, using an LKB microtome. Sections were again contrasted for 4 minutes with 4% uranyl acetate and for 5 minutes with lead citrate.

A Jeol CX 200 and Phillips EM 300 electron microscope were used for ultrastructural analysis.

Electron micrographs were obtained using Agfa-Gevaert Scientia plates, and micrograph positives were always made with the same degree of enlargement.

For animals in Batches Ia, Ib, II and III, serum prolactin levels were determined using the R.I.A. double-antibody technique, and mean values plotted on a graph using a Plotter 74-75 A connected to a Hewlett-Packard computer.

Results

One of the most important initial findings was that in rats from batches Ia and II (400 mg/m²/5 days), the cytology of control animals differed visibly from that of treated animals.

Ultrastructural findings in some cells clearly indicated the presence of activity, evident in the low number of secretory granules (Fig. 1), and in the fusion and clustering of granules at various stages of maturity around well-developed Golgi apparatus, and numerous free ribosomes.

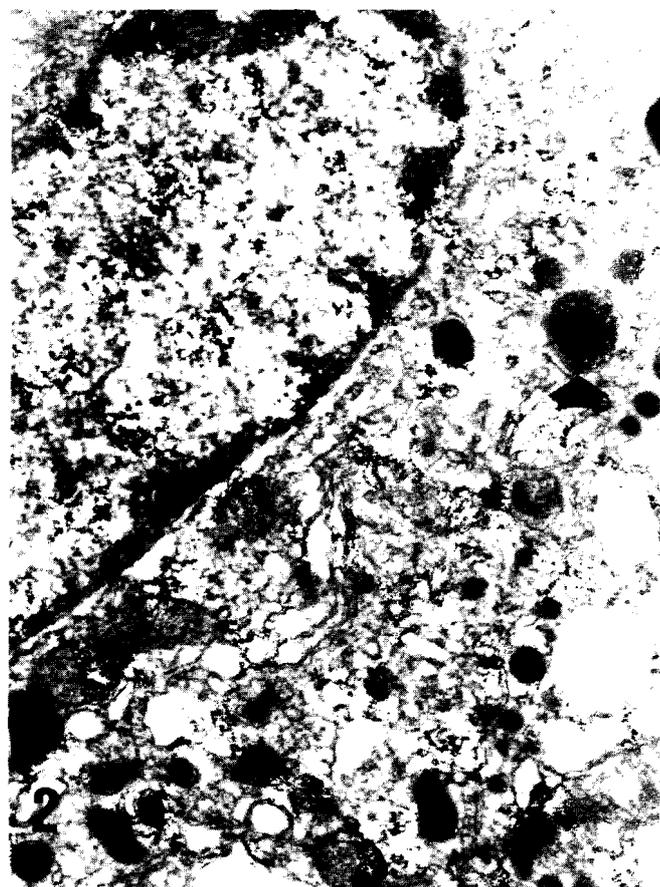
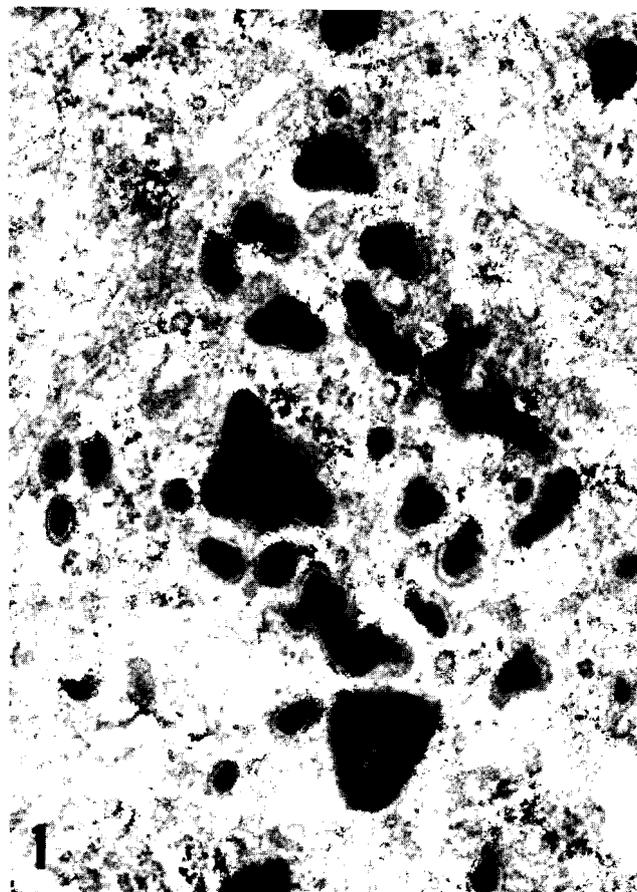
Internal condensation was observed in some granules in these prolactin cells, and this made it possible to differentiate two distinct areas of such granules, as shown in the lower part of micrograph in Fig. 2 (◊).

Another, rather surprising, finding for this cell type is shown in Fig. 3: a fragment of prolactin cell totally surrounded by the cytoplasmic processes of a follicle cell containing a large amount of highly electron-dense material, making definition difficult.

Prolactin cells of treated animals from batches Ib and III

Fig. 1. Left rostral section of the adenohypophyseal pars distalis of a treated animal (400 mg/m²). Negative × 14,000

Fig. 2. Right rostral section of the adenohypophyseal pars distalis of a treated animal (400 mg/m²). Negative × 12,000



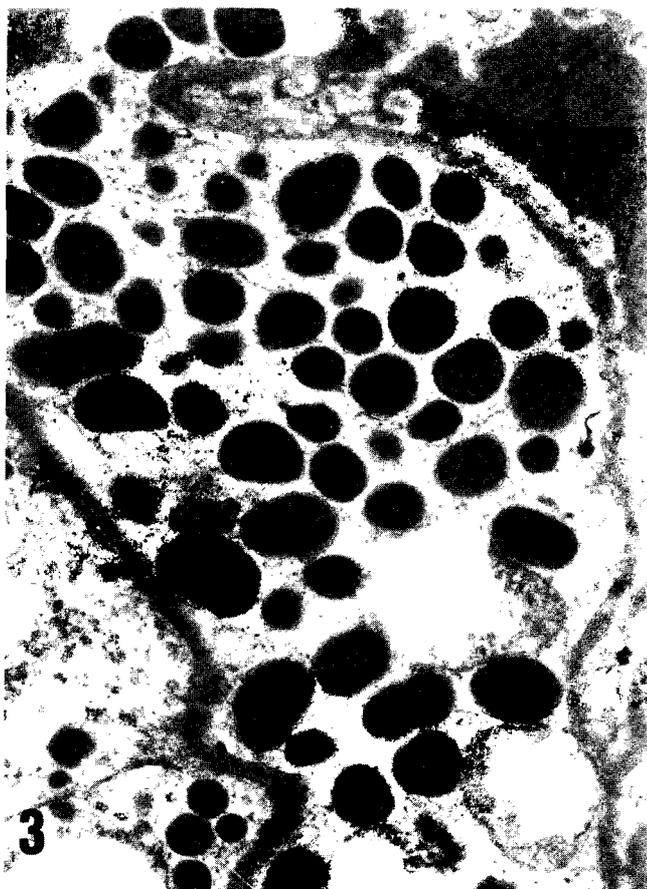


Fig. 3. Left intermediate section of the adenohypophyseal pars distalis of a treated animal (400 mg/m²). Negative \times 7,200

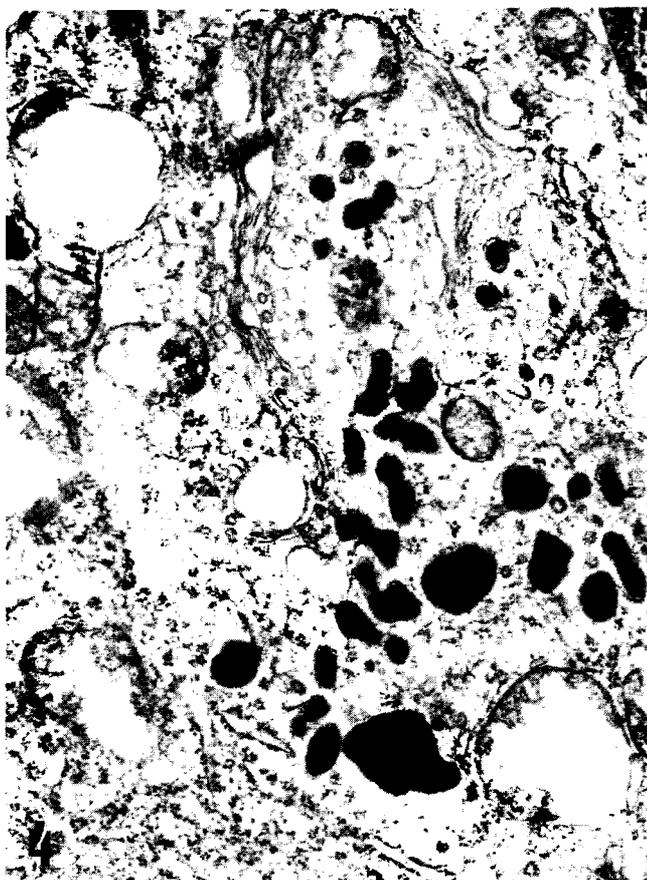


Fig. 4. Left caudal section of the adenohypophyseal pars distalis of a treated animal (200 mg/m²). Negative \times 10,000

(200 mg/m²/5 days, 21-day break and a further 5-day cycle of cyclophosphamide treatment) are shown in Fig. 4. The ultrastructural appearance was virtually the same as in the previous batches (Ia and II), which suggested that apart from the scarcity of cell granules and some mitochondrial alterations, the ultrastructure was similar in all respects to that of control animals. Attention should, however, be drawn to the large number of polysomes evident in Fig. 4, and to the abundant rough endoplasmic reticulum visible in the top left of the micrograph.

Mean serum PRL levels, both in animals receiving 400 mg/m² (batches Ia, II) and in those receiving 200 mg/m² (batches Ib, III) are shown in Fig. 5.

Mean values obtained for treated and control animals were 6.43 ng/ml and 14.10 ng/ml, respectively, in animals receiving 400 mg/m², as shown on the right of Fig. 5, and 6.09 ng/ml and 13.70 ng/ml, respectively, for those receiving 200 mg/m², as shown on the left of the same figure.

Discussion

As has already been pointed out in the Introduction, the exact mechanism by which cyclophosphamide exerts its effects is not fully known, but it can be confirmed that the main effect is exerted on DNA, blocking cell division and leading to cell death (Brooke, 1964; Mathé et al., 1975).

It may be assumed that adenohypophysis pars distalis cells, at the moment of receiving the first dose of cyclophosphamide, are able to remain in repose indefinitely, without changing to an active state of division; when stimulated, however they can be brought back into the cycle.

It should be borne in mind that the immunosuppressive properties of cyclophosphamide can give rise to leukopaenia and other adverse effects.

Moreover, as Kovacs and Steimberg, (1972) have pointed out, cyclophosphamide elicits three different reactions in mice: sudden death due to the injection itself, death through lymphopaenia after repeated high doses, and a chronic wasting syndrome accompanied by overinfection after administration of moderately high dosages on daily basis.

In our view, no significant differences exist with regard to either ultrastructural data or serum PRL levels using R.I.A. between animals receiving 400 mg/m² and those receiving 200 mg/m².

The ultrastructure and blood hormone levels observed suggest a discrete cell activation and discrete hormone release.

Even when serum prolactin levels were fairly high, they were lower in treated animals than in controls in both programmes of treatment; these results differ from those obtained by Watson et al. (1985) in studies of the long-term effects of cyclophosphamide on testicle function, which showed that blood androgen and prolactin levels in experimental patients were not significantly different from those of control patients.

Ciclophosphamide and prolactin cells

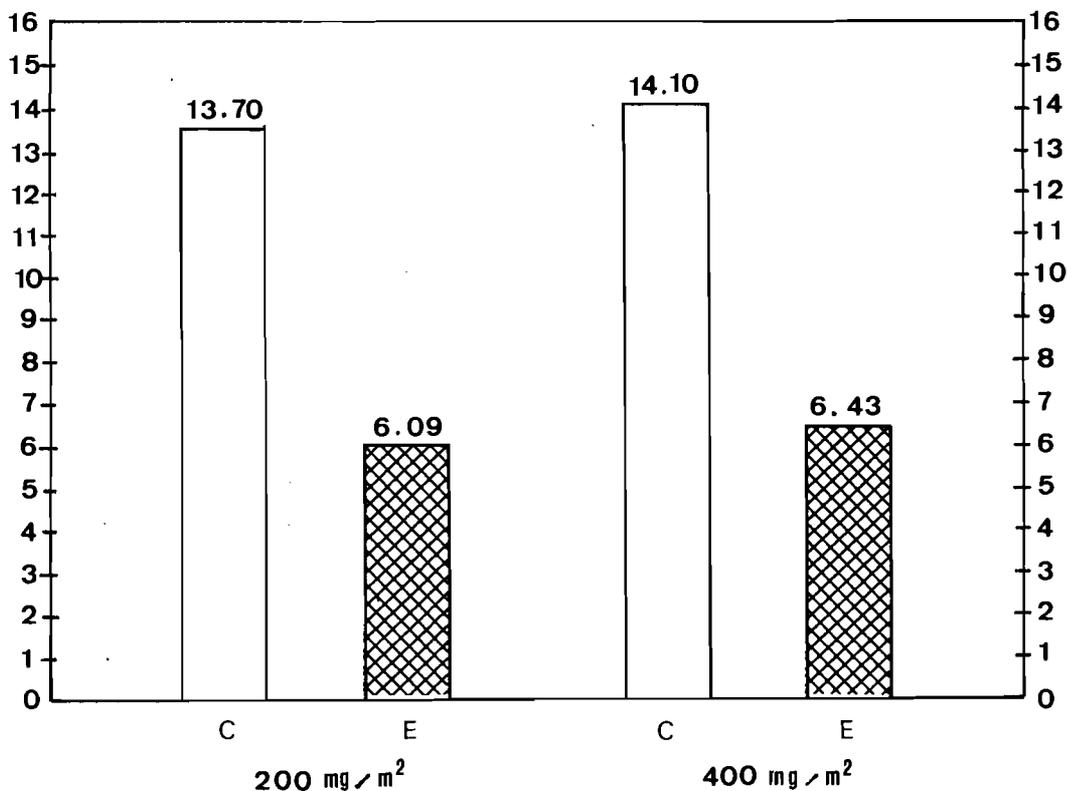


Fig. 5. Mean PRL values (ng/ml) in both treatment programmes. C: Controls. E: Treated.

As already mentioned, there can be no doubt that administration of cyclophosphamide at the dosages described for these experimental animals, apart from giving rise to the adverse effects already outlined above, cause a considerable general organic alteration, which may take the form of activation of the ACTH and PRL cells, or a general reaction in the whole animal organism, which must naturally effect the endocrine systems as a whole.

Rojas et al. (1984, 1986) in studies of the PRL cells of the normal rat treated with adriamycin, confirmed that PRL cells showed a functional synthesising and hormone-releasing activity readily identifiable through both ultrastructural and biochemical data (treated animals had higher amounts of serum prolactin than controls, the difference being more marked in the second week than in the first). Our results do not coincide with those of Rojas et al. (1984, 1986), since the drug used was different, and because prolactin activity is directly related to gonadotrophic activity. It should also be borne in mind that, due to a mechanism as yet no fully explained, the stressful situation implied by the experiment causes PRL production to either increase or fall.

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