

EFFECTS OF 4-CHLOROTESTOSTERONE ACETATE ON THE PHAGOCYtic ACTIVITY OF HUMAN MONOCYTES RESULTS OF A DOUBLE-BLIND TRIAL

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SUMMARY

A comparative trial on 4-chlorotestosterone acetate and placebo was conducted in humans by the double-blind technique. The effects of the drug were tested by measuring the phagocytic activity of blood monocytes *in vitro* for colloidal carbon. Monocytes from patients treated with 4-chlorotestosterone acetate displayed a phagocytic power significantly higher than that of monocytes from patients treated with the placebo. Such an increased phagocytic activity is discussed in relation to cell mechanisms and their rôle in anti-infective defence.

Following the reports of Nicol & Bilbey (1960), Nicol *et al.* (1963) and Umehara *et al.* (1965), investigators of our group became interested in the effects of anabolic hormones on human reticulo-endothelial cell activity. Fiori *et al.* (1969), using a modification of Rebuck's skin-window technique (Magliulo *et al.*, 1967), demonstrated that in man testosterone propionate and the less virilizing derivative, 4-chlorotestosterone acetate, stimulate the ingestion of colloidal carbon particles by human skin-window macrophages.

In this paper we report comparative studies on the phagocytic activity of blood monocytes from humans treated either with 4-chlorotestosterone acetate or with a placebo according to the double-blind technique.

The object of the study was to determine whether enhancement of phagocytic activity by 4-chlorotestosterone acetate took place and to attempt elucidation of the possible mechanisms of such an action.

MATERIALS AND METHODS

The study was planned according to the double-blind technique. The placebo and the drug under investigation were used in identical capsule form, each capsule containing 25 mg of substance. The drug was 4-chlorotestosterone acetate, 4-CTA (4-chloro-4-androsten-17-beta ol-3-one-17-beta acetate) kindly supplied by Farmitalia, Milan.

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The allocation of the drug or of the placebo to each patient was made by means of a table of random numbers. Each capsule of placebo contained: lactose, 165 mg; Mg stearate, 5 mg. A total of twenty individuals (ten for the placebo and ten for the hormone) were treated.

Each patient was given two capsules per day for 10 days. Before and after therapy monocytes were isolated from peripheral blood by the dextran-albumin flotation technique of Bennett & Cohn (1966). Monocytes were then suspended in a medium composed of TC 199 (Difco) 80% and human serum 20% at a final concentration of 1×10^6 cells per ml. The cell suspension was distributed in Leighton tubes with cover slips at a volume of 1 ml per tube. Tubes were incubated at 37°C for 2 hr in order to allow monocytes to adhere to the cover slips. Then the culture medium was substituted with a fresh one to which 0.01 ml of a suspension of carbon in 2% gelatin was added. The tubes were then kept at 37°C for a further period of 1 hr, after which the cover slips were washed in TC 199, rapidly air-dried and stained by May-Grunwald-Giemsa. Microscopic examination was performed with an optical microscope equipped with an oil-immersion objective. The intensity of phagocytosis was measured by counting, for each individual, 1000 monocytes to which were allotted an arbitrary score according to the intensity of carbon ingestion:

- Grade 0: no carbon in the cells or presence of carbon in a quantity less than that present in the background.
- Grade +: fine granulations evenly dispersed in the cell in a quantity higher than that in the background.
- Grade ++: coarse granules and aggregates still leaving free one-third of cell surface.
- Grade +++: coarse granules and aggregates covering more than two-thirds of cell surface.

The difference in phagocytic activity found between patients treated with 4-CTA and the individuals receiving the placebo was examined using the analysis of variance. In order to assess the possibility of reclassifying the patients according to the degree of phagocytic activity, a test of discriminant function was applied to the differences observed using the four above-mentioned degrees of phagocytosis as discriminating parameters.

RESULTS

In Fig. 1 the morphological aspects of human monocytes cultivated *in vitro* in the presence of a suspension of colloidal carbon are illustrated. The cells are clearly seen to have ingested particles of carbon and are classifiable in accordance with the degrees of activity previously mentioned.

Table 1 presents the extent of carbon particle phagocytosis in the cells of patients before and after treatment with the placebo. As may be seen, no marked differences were observed in phagocytic activity between patients before and after treatment with placebo. In Table 2 the data collected in the patients treated with 4-CTA are reported. These figures are presented as histograms in Fig. 2. A consistent decrease in '0' and '+' cells was observed, whereas the frequencies of phagocytic cells in grades ++ and +++ appears markedly increased after 4-CTA treatment.

The analysis of variance applied to differences in phagocytic activity observed after treatment with the placebo and 4-CTA showed that the differences between the mean percentage

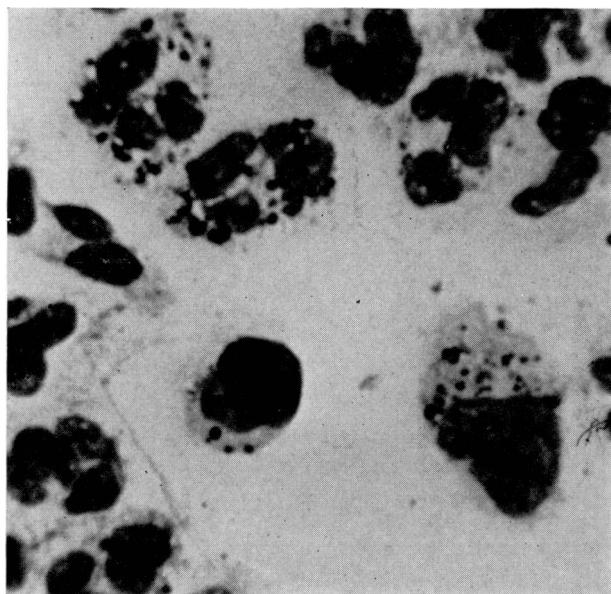


FIG. 1. Morphological aspects of carbon phagocytosis.

of cells showing phagocytosis in the various grades for the two treatments is statistically significant (grade 0: $P < 0.001$; grade +: $P < 0.01$; grade ++: $P < 0.05$; grade +++: $P < 0.01$). In order to establish whether the differences in the phagocytic activity values accurately reflect the effects produced by the different treatments, a discriminatory analysis applied to differences between the pre- and post-treatment state, by considering the four

TABLE 1. Number of cells per thousand ingesting carbon particles at four progressively increasing degrees of activity in patients before and after treatment with placebo

| Patient | Before treatment | | | | After treatment | | | |
|---------|------------------|-------|-------|-------|-----------------|-------|-------|-------|
| | 0 | + | ++ | +++ | 0 | + | ++ | +++ |
| 1 | 95 | 142 | 638 | 125 | 108 | 167 | 572 | 163 |
| 3 | 84 | 192 | 579 | 145 | 77 | 203 | 582 | 138 |
| 6 | 182 | 164 | 572 | 82 | 175 | 173 | 573 | 79 |
| 7 | 264 | 133 | 520 | 83 | 311 | 148 | 441 | 100 |
| 11 | 54 | 464 | 384 | 98 | 76 | 502 | 310 | 112 |
| 12 | 133 | 367 | 378 | 122 | 141 | 365 | 360 | 134 |
| 14 | 140 | 252 | 543 | 65 | 136 | 324 | 458 | 82 |
| 15 | 98 | 142 | 570 | 190 | 121 | 138 | 520 | 221 |
| 19 | 88 | 241 | 572 | 99 | 92 | 258 | 540 | 110 |
| 20 | 110 | 210 | 580 | 100 | 108 | 267 | 510 | 115 |
| Average | 124.8 | 230.7 | 533.6 | 110.9 | 134.5 | 254.5 | 486.6 | 124.4 |

TABLE 2. Number of cells per thousand ingesting carbon particles at four progressively increasing degrees of activity in patients before and after treatment with 4-chlorotestosterone acetate

| Patient | Before treatment | | | | After treatment | | | |
|---------|------------------|-------|-------|-------|-----------------|-------|-------|-------|
| | 0 | + | ++ | +++ | 0 | + | ++ | +++ |
| 2 | 42 | 419 | 451 | 88 | 38 | 274 | 578 | 110 |
| 4 | 170 | 523 | 208 | 99 | 82 | 361 | 403 | 154 |
| 5 | 141 | 205 | 419 | 73 | 82 | 103 | 640 | 175 |
| 8 | 120 | 133 | 602 | 145 | 54 | 76 | 547 | 323 |
| 9 | 210 | 154 | 558 | 78 | 63 | 98 | 625 | 214 |
| 10 | 76 | 488 | 324 | 112 | 42 | 140 | 570 | 248 |
| 13 | 178 | 240 | 460 | 122 | 110 | 191 | 502 | 197 |
| 16 | 205 | 209 | 498 | 88 | 83 | 157 | 550 | 210 |
| 17 | 188 | 87 | 610 | 215 | 62 | 78 | 540 | 320 |
| 18 | 84 | 329 | 407 | 180 | 76 | 70 | 624 | 230 |
| Average | 141.4 | 278.7 | 453.7 | 120.0 | 69.2 | 154.8 | 557.9 | 218.1 |

grades of phagocytic activity as discriminant criteria, was carried out. The discriminant function obtained appears to be highly significant and gives the following scheme:

| | | | |
|---------|---|---------|---------|
| | | A | B |
| Reality | { | 4-CTA | 10 0 |
| | | Placebo | 0 10 |

It therefore seems correct to conclude that the criteria adopted in this analysis accurately reflect the differences caused by the treatments.

DISCUSSION

The test of carbon particle uptake by human monocytes *in vitro* was satisfactory for the assessment of phagocytic activity in the two groups of patients, one of them being treated with a placebo and the other with the anabolic agent, 4-chlorotestosterone acetate. Human blood monocytes from both groups of patients in this study displayed consistent phagocytic activity before treatment. However, whereas treatment with placebo did not modify this activity, treatment with 4-chlorotestosterone acetate induced a significant increase in the number of cells engulfing carbon and in the quantity of carbon particles phagocytosed by single cells.

That this kind of action is not confined to blood monocytes is proved by the analogous findings of Fiori *et al.* (1969) in human skin-window macrophages.

The previous work of Nicol & Bilbey (1960) and Nicol *et al.* (1963) in mice did not provide any evidence that testosterone might modify the phagocytic activity of reticulo-endothelial cells, tested by means of the carbon blood clearance test. However, more recently Umehara *et al.* (1965) showed that testosterone propionate and 4-chlorotestosterone acetate can markedly stimulate the blood clearance of heterologous red cells injected intra-

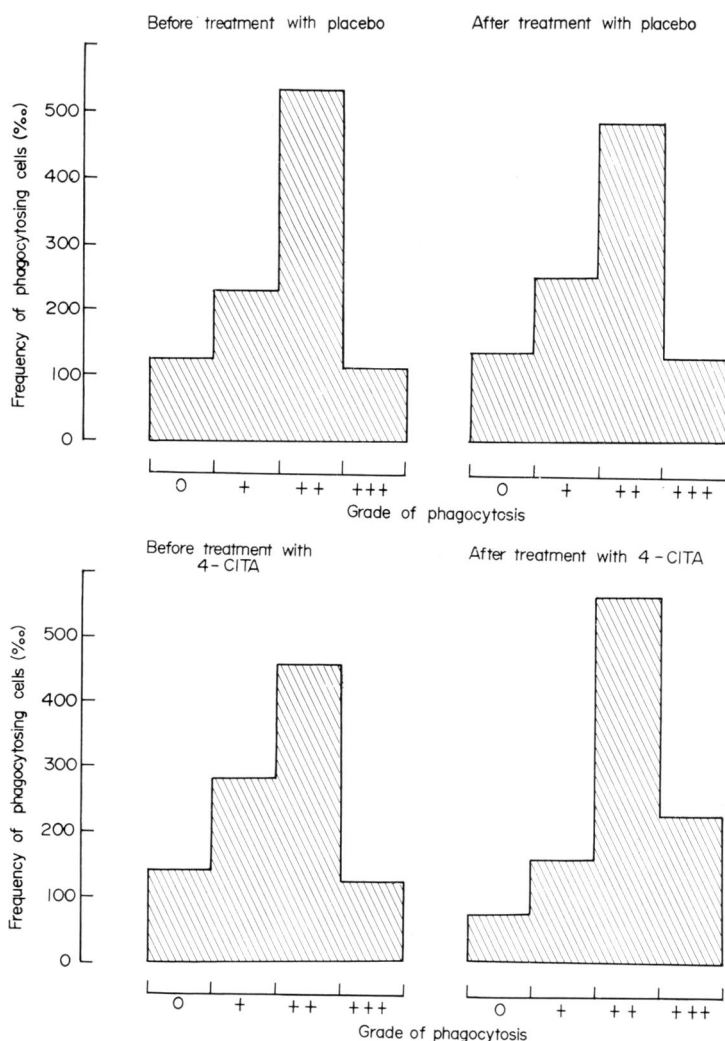


FIG. 2. Frequency of cells in different degrees of phagocytosis in control and in treated patients.

venously in rabbits. Furthermore 4-chlorotestosterone acetate exerted a protective effect against the inhibiting action of cortisone on reticulo-endothelial system phagocytic activity.

We have no evidence that the increased phagocytic activity induced in our patients by 4-chlorotestosterone acetate is connected with the anabolic activity of the drug, though such a possibility would be most interesting.

Umehara *et al.* (1965) prompted the hypothesis that in rabbits 4-chlorotestosterone acetate could work by way of an unspecified mechanism in the spleen. However, if a central activity were necessary, we would not discard the idea that an action might be exerted on bone marrow, the organ which, according to Volkman & Gowans (1965), is probably the source of macrophage precursors. Another possibility is that 4-chlorotestosterone acetate

might activate macrophages peripherally in an as yet unknown manner. A clue to the problem could be provided by the evidence recently obtained that certain agents that increase non-specific resistance to infection are associated with an increased capacity on the part of macrophages to ingest, and in some cases to inactivate, specific micro-organisms. This macrophagic activation appears well correlated with the intracellular content of lysosomal enzymes, the increase in number of dense bodies and in cytoplasmic mass (Cohn, 1968).

It may be that 4-chlorotestosterone acetate induces similar changes in human macrophages, favouring the maturation of hyperphagocytic cells. Such an effect might be due to increased protein synthesis at the level of the rough endoplasmic reticulum and the free ribosomes.

Our findings may provide an explanation for the activity of anabolic hormones in some experimental infections. According to Ghione (1958) and Ghione & Turolla (1963), anabolic hormones exert a protective action against endotoxins. Furthermore, Tolentino, Terragna & Iannuzzi (1961, 1966) and Terragna & Iannuzzi (1963) demonstrated that anabolic steroids potentiate antibody production, non-specific resistance and the opsonizing and bactericidal properties of serum.

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