Effect of a nonsteroidal gametic factor linked to DNA on senile cataract in man

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SUMMARY In view of the results obtained in animal experiments we have studied clinically the effects of administering partially purified gametic extracts to elderly patients with opacity of the lens. Out of 12 patients treated orally or parenterally 10 have shown, on subjective and objective evaluation, a significant increase in vision, a clearing of the opacity of the lens, and an improvement of general mental and physical conditions.

Many experimental data show that the male gamete contains one or more hydrosoluble nonsteroidal and nonhistone factor(s) of low molecular weight (1000–1500 daltons), probably strongly linked to nuclear chromatin, acting on transcriptional and translational processes in vitro and in vivo. This factor or factors appears to counteract also, temporally and partially, some dysfunctions normally occurring in the later part of mammalian life, the so-called senescence phenomena.1,3

The first experimental proof of this activity was reported by Fachini and Gianfranceschi senior in 1964.4 These authors achieved evident clearing of the opaque lens in the senile cataract of old dogs by administering parenterally either whole homologous semen or extracts of homologous and heterologous spermatozoa.

Senile cataract is in fact probably one of the more frequent structurally irreversible changes occurring in senescence, and it is a syndrome that, unlike other senile phenomena, may be evaluated almost semi-quantitatively. Further data on the same activity were reported by Maselli et al.5 in 1972 and recently by Lugaro et al.1 in a more extensive research carried out on 43 dogs. At the same time Casellato et al.2 have shown that the same active extracts can normalise in senescent rats the inducibility of some liver enzymes (NADPH-oxidase, tyrosine amino transferase, δ-aminolaevulinic acid synthetase, and also glucokinase), which activity appears to be inversely proportional to the age of the animal. As regards this aspect, Lugaro et al.6 have reported that the activity on the enzyme inducibility may be correlated in the aging animal with a significant increase in the rate of liver transcriptional and translational processes.

In view the results we obtained in dogs we decided to investigate this problem in human cataract by giving partially purified gametic extracts by mouth or parenterally.

Material and methods

Bovine spermatozoa were obtained, as described below, from total semen containing about 10⁶ cells/ml.

Extraction and purification of the gametic factor for parenteral use. The whole process of extraction and purification of the gametic factor was done as previously reported.1 We started not from a gamete homogenate in toto but from dried and lipid-free spermatozoa, isolated from bovine semen according to Borenfreund et al.6 with minor modifications.

The purified extract was ultrafiltered and passed again through a sterilising membrane (porosity 0.23 μm, Millipore), then lyophilised in the presence of glycine as substrate. The samples were sterile, protein-free, and pyrogen-free.

Extraction of the gametic factor for oral use. Dried and lipid-free spermatozoa isolated as described above were accurately homogenised with corindone in saline (50 mM sodium chloride), and the suspen-
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Table 1  Summary of the results obtained in 6 subjects after 21-day oral treatment (see text). First examination, at the end of treatment; second examination, 30–40 days later. In brackets are indicated the optical corrections employed to obtain these values of visual acuity

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Eye</th>
<th>Fundus</th>
<th>Lens opacities</th>
<th>Vision Before treatment</th>
<th>Vision 1st examination</th>
<th>Vision 2nd examination</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>80</td>
<td>R</td>
<td></td>
<td>Miopic chorioidosis</td>
<td>Corticonuclear</td>
<td>Counting fingers 3/50 (−20)</td>
<td>1/10 (−22)</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>82</td>
<td>R</td>
<td></td>
<td>Vascular sclerosis</td>
<td>Cortical</td>
<td>1/20 (−5)</td>
<td>2/10 (−4)</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>85</td>
<td>R</td>
<td></td>
<td>Diabetic retinopatia</td>
<td>Cortical and anterior subcapsular</td>
<td>1/20 (−5)</td>
<td>1/20 (−5)</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>85</td>
<td>R</td>
<td></td>
<td>Papillaris cupping</td>
<td>Nuclear</td>
<td>1/50 (−1−5)</td>
<td>1/50</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>88</td>
<td>R</td>
<td></td>
<td>Vascular sclerosis</td>
<td>Cortical and subcapsular</td>
<td>3/10 (−7)</td>
<td>3/10 (−7)</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>75</td>
<td>L</td>
<td></td>
<td>Vascular sclerosis</td>
<td>Cortical and subcapsular</td>
<td>1/20 (−5)</td>
<td>1/20 (−5)</td>
</tr>
</tbody>
</table>

sion centrifuged at 5000 g for 30 min. To one volume of supernatant was added one volume of 0.4 M ammonium acetate-ammonia buffer pH 9.5. After precipitation overnight in the cold with two volumes of 95% ethanol, and subsequent centrifugation at 13000 g for 30 min, the supernatant was evaporated to a small volume under vacuum at 30°–35°C. The concentrated solution, brought to pH 9.5 with dilute ammonia, was ultrafiltered through a membrane with a porosity less than 5 nm (Sartorius Membranfilter 12136, mol. wt. cut off 10000 daltons) and then lyophilised twice. A third lyophilisation was conducted after mixing with an inert compound (glycine).

Steroid content. The presence of free steroids or their conjugates in the extracts was excluded (before and after enzymatic digestion with glucuronidase and sulphatase) by radioimmunoassay for testosterone, 5α-dihydrotestosterone, progesterone, and 17β-estradiol (<5 pg for fraction equivalent to about 10⁶ spermatozoa or 1 ml of total semen.)

Treatment. The clinical assay was carried out in 16 elderly patients, all proposed for surgical operation, between 74 and 88 years old, suffering from monocular or bilateral senile cataract. Six patients were treated by mouth, 6 parenterally, and 4 with a placebo (glycine). In the case of oral administration the treatment lasted for 21 days with a quantity of the extract equivalent to about 10⁶ spermatozoa daily. In the case of parenteral administration, two intramuscular injections with a 12-h interval, each equivalent to the extract of 2.5×10⁶ spermatozoa/kg body weight, were given.

Orally treated subjects were submitted to a careful inspection of the eyes before the treatment, at the end (1st control), and 30–40 days later (2nd control). Subjects parenterally treated were examined before, 2–6 days after treatment (1st control), and 2–3 weeks later (2nd control).

The examination of the vision and refractory status with the pupil in normal condition, and on biomicro-
Photobiomicroscopy of the crystalline lens of the left eye opacified from senile cataract in patient 5 (see Table 1), before (A, vision 1/20) and after (B, vision 4/10) oral treatment (second examination).

Photobiomicroscopy of the crystalline lens of the left eye opacified from senile cataract in patient 1 (see Table 1), before (A, vision 3/50) and after (B, vision 2/10) oral treatment (second examination).

Results

Tables 1 and 2 show the results in treated patients. In the 6 treated by mouth the data (Table 1) show a variable increase in visual acuity, which is shown also by the objective evaluation of the lens. Patients 5 (Fig. 1) and 6 showed the best subjective and objective improvement. In patient 1 (Fig. 2) the increase in vision was satisfactory, as was the related clearing of the lens, particularly in the left eye. Patients 2 and 4 had less improvement, but it was significant. Patient 3 did not come back for the second examination, so any evaluation was impossible.

The vision of the 4 placebo-treated patients was unaltered.

As to the 6 parenterally treated subjects (Table 2), we observed evident improvement of the right eye vision in patient 4p. A slight increase in vision was observed also in patients 1p, 2p, and 3p, although in 2p the increase seemed to be transient; in fact it was no more appreciable at the second examination than earlier. Patients 5p and 6p did not come back for the second examination; patient 6p did not show any variation at the first examination.

Discussion

These findings show that the gametic factor can induce, at least in some subjects, an evident clearing of the initial opacities, chiefly in the cortical layer of the lens. The biomicroscopic observations show a lessening in the typical milky aspect of the lens and a break of the less thick opacities in the cortical layer.
It would seem, then, that cortical cataract may yield the best results from treatment, while nuclear or posterior subcapsular opacities generally show a minor clearing. In the latter case the partial improvement of vision can be referred only to the clearing of the cortical opacities.

At the same time it is possible to see an objective and a subjective improvement in general health conditions (wellbeing, muscular tone, mental alertness, etc). Among these 'side effects' of the therapy, it is interesting to underline the lessening, in number and intensity, of the so-called maculae cutaneae senilis, the brownish cutaneous spots which occur typically on the back of the hands in old people. As noted previously, we do not know the mechanism of this temporary correction. The results seem to reflect, in the individual and in the organs, what has already been widely observed in haematological, biochemical, cellular, and subcellular variables.

The active factor, which may be a peptide or a family of peptides of low molecular weight naturally linked to nuclear chromatin, exerts an inhibitory action when it is, conversely, in accelerated cyto-kinetic or cytodynamic conditions (for instance, in a young growing organism). This was shown in trials carried out on the δ-aminolaevulenic acid synthetase inducibility in prepuberal rats, where, in contrast to what takes place in aging rats, the inducibility is significantly decreased owing to a direct action in the transcriptional and translational processes.

This fact leads us to suggest that the considered factor(s) would act as general factor of homeostasis in more complex mechanisms which control the basal processes of gene expression.

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References
3. Lugaro G, Manera E, Casellato MM, Riboni L. A nonsteroidal gametic factor linked to DNA modulates δ-aminolaevulenic acid synthase inducibility acting on liver transcriptional and translational processes. Submitted for publication.