Original research article

Assessment of the activity of an oral contraceptive on the levels of oxidative stress and changes in oxidative stress after co-treatment with two different types of physiological modulators with antioxidant action

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Abstract

Background: The aims of this research were to document the nature of oxidative stress (OS) while taking an estrogen/progestagen-combined oral contraceptive (OC) and to evaluate the action of two different products composed of a combination of antioxidant, vitamins and natural products in physiological quantity and classified as antioxidant/food supplement. For this reason, the two products are classified as physiological modulators (PM), able to restore the balance between antioxidants and reactive oxygen species in the organism.

Study Design: The Reactive Oxygen Metabolites-derived compound test, a photometric assay that measures the hydroperoxides levels in biological fluids, was used to determine the OS. OS was analyzed every 3 days (from \( t_1 \) to \( t_2 \)) for 28 days on 10 healthy volunteers during three successive OC treatment cycles with a contraceptive (Microgynon®: ethinylestradiol 50 mcg plus levonorgestrel 125 mcg). In the first cycle, the OC was administered by itself; in the successive two cycles, the OC was administered in association in an open crossover study with two different types of PMs with antioxidant action. The main difference in the composition of the two products is the presence/absence of catechins from green tea.

Results: With just OC treatment, all the volunteers showed an increase in the OS values from 240±22.3 (mean±SD) Carratelli Units. (normal value) up to values \( N_400 \) Carratelli Units (severe OS), then returned to normal when the OC therapy was suspended. The concomitant use of the two PMs showed that only the product containing green tea catechins was able to reduce the OS values, on average, by approximately 50% \( (t \text{ test } p<.05) \).

Conclusion: We conclude that to control the OS generated by OC, specific types of physiological modulators are needed.

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Keywords: Birth control; Oxidative stress; Physiological modulators

1. Introduction

The use of oral contraceptive (OC) has been shown to cause oxidative stress (OS) [1,2]. Nevertheless, an increase of the oxidation phenomena was also observed in healthy women during approximately two thirds of the menstrual cycle during the maturation phase of the ovarian follicle (estrogen phase) and in the subsequent phase of possible implantation (progestin phase) (internal data, U. Cornelli, Am J Physiol, in press).

Both the estrogens and the progestins stimulate the production of cellular energy, and both could therefore be oxidant producing, but the oxidative balance depends on how many of the stimuli allow the production of endogenous antioxidants.

Extensive experience conducted in vitro or ex vivo showed the antioxidant capacity of estrogens on lipoprotein, for example, causing a similar action to that of \( \alpha \)-tocopherol or \( \beta \)-carotene on lipid peroxidation [3,4].

Most of the experience gained [3,4] was in reality obtained using concentrations much greater than the physiological concentrations, on the order of \( 1–10 \text{ nmol/ml} \), corresponding to 273 and 2730 ng/ml, respectively. These concentrations are barely reached at the time of the midcycle estrogen peak and for limited periods of time; this is why it
is difficult to justify a protective antioxidant action by the low-density lipoprotein (LDL) [5,6].

The behavior may be similar to that which occurs during exertion [7], or rather an oxidation of the LDLs that, on a circulatory level, are more easily bound by the blood receptors and kept under control and partially "diminished" by the muscular antioxidant systems [7]. If, however, their quantity increases, since a sufficient antioxidant system does not exist, they are subject to vascular uptake (subendothelial) triggering the atherosclerotic inflammatory process. The subendothelial will in fact be subject to the action of myeloperoxidase [8,9].

This action could explain the reduction in cholesterol and LDL in premenopausal women; the phenomenon, interpreted as a greater efficiency of the LDL blood receptors, is attributed to an increased uptake of LDL as a result of their partially oxidized condition [10]. This condition normally does not exceed the minimum repairable oxidation limit, above which would trigger the macrophage reaction [10].

Any oral administration of estrogen/progestin generates a hematologic peak (increased hormones levels in plasma) in estrogens or progestins (depending on the type of OC), which is not on a physiological scale, and therefore, any "light and controllable" oxidation becomes OS.

The fact that OS is generated by an increase in the oxidized amount of LDL, or by an increase in the levels of hematic copper (Cu), or by an overregulation of the inducible nitric oxide synthase [1], or all of the above has by now been demonstrated [1,2]. The problem is how to control it.

The purposes of this research are to document the nature of OS while taking OC and to evaluate the action of two different products composed of associations of physiological modulators (PM) with antioxidant action.

PM is a definition given by Olson [11] in 1996 for antioxidant vitamins and for natural compounds "that affect physiological function and ultimately may influence the progression of chronic diseases."

2. Materials and methods

Ten healthy women undergoing OC treatment between the ages of 27 and 35 years were analyzed after a written and informed consent. The study also received an institutional review board approval from the ethics committee of the University of Chieti. The women did not undergo any treatment except for the OC treatment, which was to be used for at least 3 months. The OC used (Micogynon®) has 50 mcg ethinylestradiol and 125 mcg levonorgestrel. The OC was administered for 21 days, interrupted for 7 days and restarted the 29th day. The PM was administered daily for all the months, only during the second and third treatment cycle (month) with the same OC. Women who used dietary supplements of any type or had a body mass index >25 kg/m² were not included. The excessive consumption of alcohol (>3 alcohol units or AU; considering 1 AU=120 ml of wine or 330 ml of beer or 40 ml of spirits), tea (>3 cups/day), coffee (>3 cups/day) and chocolate (>50 g/day) were considered exclusion criteria.

2.1. Type of study

The experimental design took place over three successive treatment cycles with the same OC: the first cycle, without any PM integration, or the control cycle, was used to determine the nature of the OS in the 10 volunteers during the different days of the menstrual cycle under OC treatment (from t1 to t27), at 3-day intervals (see below); the second and third cycles in succession were used to evaluate the action of the two antioxidant PMs, following the crossover methods and performing the OS determinations at the same intervals as the control cycle.

During the first leg of the crossover, five women were treated with ARD Stenovit® and five with MF Templar® (see below) who were then switched in the second leg of the experiment. The initial assignment of one product or the other was done randomly by a computer.

There was no washout period between one leg and the other of the crossover.

2.2. Analytical determinations

The analysis of the OS condition was assessed on the basis of the Reactive Oxygen Metabolites-derived compounds (d-ROMs) test [12], which is used to determine the levels of hydroperoxide in the plasma (expression of lipid oxidation). The levels of hydroperoxides were quantified in Carratelli Units (U.CARR.; 1 U.CARR.=0,08 mg H₂O₂/100 ml).

The OC was ingested in the evening (9:00—11:00 PM) following the assumption of the PM (one after the 136 OC), and the test was performed in the morning between 8:00 and 9:30 AM with fasting since the evening before the examination. The blood was taken by pricking the finger and collecting 100 μL of blood in a heparinized microcuvette. The microcuvette was immediately centrifuged (2500 rpm for 1 min) in order to isolate the plasma on which the test is performed, within and no longer than 2 h from being drawn.

The first determination occurred at the start of the OC

Table 1

 Composition of ARD Stenovit®

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount (mg)</th>
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<tbody>
<tr>
<td>0.2% Selenium yeast</td>
<td>24.00</td>
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<tr>
<td>97.5% Protected vitamin C</td>
<td>30.77</td>
</tr>
<tr>
<td>40% Bioflavonoids from citrus fruits</td>
<td>75.00</td>
</tr>
<tr>
<td>Zinc pidolate</td>
<td>25.00</td>
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<tr>
<td>Coenzyme Q10</td>
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<tr>
<td>L-Cysteine hydrochloride</td>
<td>12.61</td>
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<tr>
<td>50% Vitamin E acetate</td>
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<tr>
<td>Pyridoxine hydrochloride</td>
<td>1.22</td>
</tr>
<tr>
<td>Vitamin A acetate 50,000 UI</td>
<td>0.7</td>
</tr>
<tr>
<td>10% β-carotene</td>
<td>0.5</td>
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2.1 Table 2  
2.2 Composition of MF Templar®  
2.3 Ingredients Amount (mg)  
2.4 0.2% Selenium yeast 24.00  
2.5 Pyridoxine hydrochloride 2.44  
2.6 α-Lipoic acid 10.00  
2.7 Coenzyme Q10 10.00  
2.8 10% β-carotene 0.5  
2.9 Extract of decaffeinated green tea with 60% catechin,* 150.00  

*a Epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG), and epicatechin (EC).

3. Results  

All the volunteers completed the experimental sessions, and no side effects of clinical importance were observed. The results of the d-ROMs test from the experimental sessions are reported in Table 3. After examination of the data, it was revealed that there is a significant increase in the average d-ROMs values in the control cycle, above the normal values (<300 U.CARR.), which on average increase more than 70% with respect to the base values. This increase is caused by the presence of OS, which already begins on the third day of therapy with OC. All the cases examined between t3 and t21 (period of taking the OC pill) were significantly above the normal. If you take into account that the OC is suspended on day t21, it seems logical that 3 days after suspension (t24), a recovery of the OS is noticed that ends at t27. In fact, none of the volunteers had OS at t27. Data relative to each of the two products examined were isolated from the second and third cycles under crossover conditions. From these data, we see that only the MF Templar® can positively change the OS conditions, while ARD Stenovit® had no action whatsoever. The MF Templar® reduced the OS by approximately 50%.

4. Discussion  

As can be seen from the analysis of the data, to be able to reduce the level of OS created by the use of the studied OC, the PM formulations with antioxidant action must have special characteristics. The combination found in ARD Stenovit®, even though it contains all the antioxidant types (membrane, circulatory, system and internal cell), is unable to modify the OS condition with an OC.

Based on previous experience, ARD Stenovit® has been shown to have an antioxidant action when given to both healthy volunteers [13] and in patients with peripheral...
vascular diseases [12]. This indicates that the OS generated by OC therapy has particular characteristics that must be specifically addressed.

Conversely, the PM combination in MF Templar® is able to substantially reduce the OS increase. This is considered to be attributed to the presence of catechin, which is able to work in summation or synergy with the other components and reduce oxidation more effectively with regard to OC treatment. Catechins are antioxidant PMs that belong to the flavonoid group. They are found in many plant species, but the most significant source in the human diet comes from the tea plant (Camelia sinensis). In fact, the dry tea leaf contains about 25% by weight of catechin. Its overall content can vary greatly with the type of plant, the place they grow, variations in light, season and altitude, and thus, a drink prepared at home can have a widely varying catechin content. Catechins are present in all types of tea, including white tea, green tea, black tea and oolong tea, but are also found in chocolate, fruit, vegetables, wine and many other plant species. Generally speaking, the catechins found in green tea have good bioavailability and are also distributed to the tissues.

MF Templar® contains 150 mg of these catechins from green tea. This type has been shown to have good in vitro antioxidant activity [14] as well as good experimental bioavailability [15], which allows, at least for epigallo-catechin-3-gallate, 8%–10% of the level in the blood to reach the tissues in the main organs (heart, brain, liver, uterus, etc.) [16].

Furthermore, the presence of lipoic acid and coenzyme Q10 in the formulation supports the mitochondrial action needed for the production of ATP. ATP is helpful for the regeneration of reducing equivalents like GSH, whose consumption is increased in relation to the level of estrogen/progestin.

With regard to the specificity of one antioxidant PM formulation compared with another, it can be stated that it is sufficiently clear that OS is compartmentalized, meaning that it is not identical in all parts of the body, both in terms of formation and in terms of antioxidant remedy. There are types of PM that are very specific to a tissue level, as, for example, squalene for the epidermis [17] or carnosine for cerebral tissue [18].

It therefore does not seem surprising that more specific “remedies” may also exist for OS caused by OC.

Not much is known, however, about the methods of OS production after OC treatment.

Other investigators have observed an increase in this condition [1,2]. There are many hypotheses about this increase, and it may depend on the reduced secretion of luteinizing hormone and follicle-stimulating hormone, which is the basis of the contraceptive action, or even on the reduction in the secretion of relaxin, which is produced by the corpus luteum.

One must also consider that the hormonal load caused by the OC is metabolized by the blood enzymes.

Both the progestins and the estrogens undergo conjuga-

tion processes (sulfates, glucuronates), which consume a great deal of energy. This energy removal can compromise the regulation of the immune system via glutathione, in particular the regeneration of the GSH starting with oxidized glutathione causing OS. This also occurs in the normal menstrual cycle [19] and could be worsened by treatment with OC, during which the exogenous supply of estrogen/progestin significantly increases the levels of these hormones above the physiological limits.

Clearly, we are still at the stage of understanding the inner mechanisms that regulate the progression of the oxidative balance, which for now are almost exclusively assessed indirectly, as in the case of hydroperoxides and all the other markers.

5. Discussion

Based on the measurement of the hydroperoxides, we observed that the use of OC therapy, at least for the type used, generates an OS condition. This condition cannot be modified by a common combination of antioxidant PM, but is affected by a type of product where the presence of catechin seems to be important.

Acknowledgments

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References


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