

ORAL HORMONAL CONTRACEPTION - THE INFLUENCE ON HUMAN GENOME AND LIPID STATUS

Dragan Loncar

Micronucleus test is the method which is used in discovering of chromosome aberrations in the cells exposed to the effects of chemical mutagens. The cell which suffered the DNA damage cytologically fits to extranuclear bodies in cytoplasm, which represent the chromosomal fragments, the whole chromosomes or the groups of chromosomes. These cytoplasmic masses look like small cores, micronuclei, and their size varies depending on the size of chromosome fragments or the number of chromosomes in the cytoplasm.

The aim of the paper was to examine the influence of monophased, combined, low-dosed contraceptive pills which contain ethinyl-estradiol and gestoden on the micronucleus frequency in human lymphocytes of peripheral blood and their influence on the lipid status of the users.

We examined 30 patients older than the age of 18. All the patients took part in the research in order to prevent unwanted pregnancy. The existence of cardiovascular, endocrinological, neurological and malignant diseases was excluded by using clinical examinations, biochemical, hematological and additional researches.

After the therapy of 20 µg of ethinyl-estradiol and 75 µg of gestoden in the period of six consecutive menstrual cycles in order to prevent unwanted pregnancy, there was not statistically significant change of micronucleus frequency in lymphocytes of peripheral blood in the cases of patients which took part in the research ($p > 0,05$).

The therapy significantly increases the value of cholesterol, low-density lipoproteins (LDL) and triglycerides in the blood of the patients ($p < 0,05$).

The therapy can be applied along with the regular gynecological controls in order to prevent unwanted pregnancy. *Acta Medica Medianae 2007;46(1):11-16.*

Key words: *gestodene, ethinylestradiol, micronuclei, genotoxicity, lipoproteins*

Department of Obstetrics and Gynecology, Medical Center and Clinic in Kragujevac

Contact: Dragan Loncar
Department of Obstetrics and Gynecology, Medical Center and Clinic
34000 Kragujevac
Phone.: 064/6168999,
E-mail dloncar@ptt.yu

Introduction

Voluntary and temporary contraception can be indicated and wanted for any of the following reasons: socio-economic, medical, eugenic and personal reasons. The basic concept of contemporary prenatal care – the right of every newborn infant is to be born physically, mentally and emotionally healthy – begins with family planning. The family planning in our country is the right and obligation of the parents which can fulfill their wish for posterity by different methods and means of fertility regulation. During the past few years, a great number of studies which deal with different aspects of mutagenesis have been performed. This was the result of greater knowledge about this problem and the result of

technical accomplishments in the tissue culture and chromosome preparations. Because of that, the research on types of exogenous agents was conducted, for example – radiation, viruses and chemicals which can cause chromosomal damage. Today, men are exposed to a great number of agents. Concerning the genetic defects, the chemicals mentioned above are potentially more dangerous than radiation. It is important to determine the mutagenic frequency of cells in genetical defects. The use of oral contraception in order to prevent unwanted pregnancy emphasizes the problem, because these medicines were continually consumed for longer period. The mechanism of influence of these agents has not been completely explained, and it is very important to explore the long-term genetic effects of oral contraceptives. From the text above, we conclude that we should precisely determine the scope and the type of damage which appear in genetic material after the use of medicines through carefully planned and controlled studies. During the research we used monophased, low-dosed, combined contraceptives for oral use, in which the estrogen component is ethinyl-estradiol, and the progesterone component is gestoden.

Gestoden

In comparison with other progesterones of the third generation, gestoden has chemical structure and effects that are very similar to natural progesterone. It has stronger progestational effect and inhibits the ovulation with fewer doses than dezogestrel or norgestimat. Besides, gestoden has an outstanding anti-estrogenic effect, which can be useful, because it is opposite to unwanted metabolic effects which are connected with estrogens. Gestoden is a 100% bioavailable, and opposite to dezogestrel and norgestimat, it does not have to be metabolized in active form. This pharmacologic characteristic of gestoden enables its easier detecting in the blood, so we have reduced risk of its possible subdosing or overdosing.

Estrogen component

Ethinyl-estradiol was synthesized in 1938. It was the first synthetic, steroidal, orally active estrogen. However, the synthetic estrogen which was firstly used for contraception was mestranol (3-methyl ester ethinyl-estradiol) which metabolizes in ethinyl-estradiol in order to become biologically active in the organism.

Micronucleus test (according to FENECH- MORLEY)

Micronucleus test is a method used to discover chromosomal aberrations in the cells exposed to the influence of chemical mutagens. A cell with damaged DNA cytologically responds by appearance of extra nuclear corpuscles in the cytoplasm, which represent chromosomal fragments, whole chromosomes, or groups of chromosomes. These cytoplasmic masses look like little nuclei and their size varies depending on the size of the chromosome fragment or the number of chromosomes in the cytoplasm (1). The test is used as a rapid test for clastogene agents i.e. agents that cause the breach on chromosomes and for chemical agents capable of causing damage at the level of cell division. (2,3).

Fenech and Morley (1985) introduced a modification of the test suggesting CB method (Cytokinesis-block method). CB-MN test analyses those cells that had only one division. The main task was how to block the cells in this stage. This was achieved by adding Cytochalasin B (Cyt-B) to cultivated cells before the first mitosis which blocks cytokinesis by stopping the actin filaments of the cortical region of the plasma membrane, while the nucleus division takes place without being disturbed (4). This is how binuclear lymphocytes are formed.

Aims

The aim of the paper was to examine the influence of monophased, combined, low-dosed contraceptive pills which contain ethinyl-estradiol and gestoden on the micronucleus frequency

in human lymphocytes of peripheral blood and their influence on the lipid status of the users.

Material and methods

By using the farsighted study of the chosen patients, we examined the effects of using oral, combined, low-dosed contraceptives on the micronucleus frequency in human lymphocytes of peripheral blood and on biochemical parameters.

We examined 30 patients older than the age of 18. The oldest patient was 30 years old and the youngest was 20 years old. None of the patients used the hormonal contraception in the period of 12 months before the beginning of the study. All the patients took part in the research in order to prevent unwanted pregnancy. The existence of cardiovascular, endocrinological, neurological and malignant diseases was excluded by using clinical examinations, biochemical, hematological and additional researches. All the patients were colpocytologically examined.

We have done all the analyses in the Department of Obstetrics and Gynecology in Kragujevac and in biochemical laboratory of KBC Kragujevac.

Results

Table 1. Shows the results of micronucleus frequency in lymphoblast before the therapy

Ordinal number	Micronucleus frequency	Number of bn- c	Number of MN/1000bn-c [#]
1	9	1000	9
2	6	1000	6
3	6	1000	6
4	3	1000	3
5	7	1000	7
6	12	1000	12
7	6	1000	6
8	4	1000	4
9	4	1000	4
10	8	455	17
11	3	1000	3
12	11	1000	11
13	6	1000	6
14	1	1000	1
15	12	1000	12
16	4	576	7
17	11	1000	11
18	3	235	12
19	6	1000	6
20	3	1000	3
21	15	1000	15
22	3	1000	3
23	6	1000	6
24	7	1000	7
25	5	1000	5
26	16	1000	16
27	4	1000	4
28	8	1000	8
29	6	1000	6
30	6	1000	6

bn-c binucleate cells

Table 1 shows the results of micronucleus frequency in lymphoblast before the therapy. Defining the micronucleus frequency is done by analysis of 1000 binucleate cells of each patient, except in the cases of the 10th, 16th and 18th patient. In this example, the average number of binucleate cells with micronuclei (MN) is 7, 40 with the range of variation from 1 to 16. In the cases of 8 patients (26, 66%), we noticed 6 binucleate cells which contain MN. The average number of analyzed binucleate cells of each patient is 942, 20.

Table 2. Shows the results of micronucleus frequency in lymphoblast after the therapy

Ordinal number	Micronucleus frequency	Number of bn-c [#]	Number of MN/1000 bn-c [#]
1	8	1000	8
2	7	1000	7
3	5	1000	5
4	6	1000	6
5	9	1000	9
6	13	1000	13
7	5	1000	5
8	5	1000	5
9	6	1000	6
10	14	1000	14
11	5	1000	5
12	4	1000	4
13	6	1000	6
14	2	1000	2
15	10	1000	10
16	6	1000	6
17	7	1000	7
18	7	1000	7
19	9	1000	9
20	5	1000	5
21	6	436	13
22	4	1000	4
23	5	1000	5
24	8	1000	8
25	6	1000	6
26	16	1000	16
27	5	1000	5
28	10	1000	10
29	7	1000	7
30	8	1000	8

bn-c binucleate cells

Defining the micronucleus frequency was done by analyzing 1000 binucleate cells of each patient except in the case of the 21st patient. The average number of analyzed binucleate cells of each patient is 981,20. The average number of micronuclei is 7,37 with the range of variation from 2 to 16. In cases of 7 (23,33%) patients, the average number of MN is 5.

Table 3. Statistical review of the difference of average values of micronucleus frequency before and after the therapy

	Mn1	Mn2
X average	7,40	7,36
SD	4,11	3,23
N		30
Difference		0,03
SD difference		2,37
t		0,08
Degree of freedom		29
p		0,94

Table 4. Presentation of distribution of the lipid status parameters after completion of therapy

Ordinal number.	Hol	Ldl	Hdl	Trg
1	5,3	2,7	2,2	1,00
2	4,7	3,1	2,8	0,90
3	5,7	3,0	2,4	0,80
4	5,7	3,7	2,9	0,90
5	4,5	2,6	3,0	0,90
6	4,2	1,9	2,6	1,30
7	5,3	2,5	3,0	1,70
8	4,9	2,9	2,7	1,80
9	4,9	3,0	3,4	1,40
10	3,6	2,6	3,1	0,90
11	4,3	3,2	3,7	1,60
12	5,7	0,9	4,0	1,85
13	4,7	2,3	1,9	1,00
14	4,9	1,7	1,9	1,40
15	4,6	0,9	3,2	1,40
16	4,8	2,3	3,2	0,60
17	4,0	1,9	2,9	1,70
18	4,3	2,0	2,8	1,30
19	4,3	2,3	3,5	1,20
20	4,1	2,2	2,7	1,10
21	4,9	2,4	3,6	1,10
22	6,6	1,3	4,6	1,60
23	4,2	2,8	1,1	1,30
24	4,3	2,3	3,8	1,50
25	5,7	2,3	3,6	1,00
26	4,2	2,9	3,5	1,70
27	4,2	3,2	4,6	1,60
28	4,3	2,4	3,7	1,70
29	4,0	3,8	3,3	0,80
30	4,8	2,5	3,3	1,10

*legend Hol-cholesterol;
Ldl-lipoproteins low density;
Hdl- lipoproteins high density;
Trg- triglycerides

Table 5. Presentation of distribution of the lipid status parameters before completion of therapy

Br	Hol	Ldl	Hdl	Trg
1	5,0	2,9	2,5	1,60
2	5,3	3,3	2,5	1,10
3	6,0	3,2	2,7	1,20
4	5,5	3,7	2,5	1,20
5	4,9	2,8	3,3	1,10
6	4,1	3,0	3,6	1,20
7	5,6	2,9	3,4	1,80
8	5,6	3,0	2,9	1,70
9	5,1	3,4	3,6	1,60
10	5,0	3,6	3,9	1,30
11	4,6	3,6	3,7	1,20
12	5,3	1,1	3,8	1,50
13	4,9	2,7	2,2	1,30
14	5,0	2,1	2,7	1,80
15	4,3	2,4	2,9	1,60
16	4,0	3,4	2,7	1,30
17	4,3	2,2	3,0	1,90
18	5,0	2,4	3,0	1,60
19	4,9	2,9	3,4	1,40
20	4,3	2,6	3,9	1,60
21	20	2,4	3,5	1,20
22	6,4	2,0	3,0	3,10
23	5,0	2,1	3,1	2,20
24	5,3	2,7	3,3	1,30
25	4,6	2,7	3,7	1,40
26	5,5	2,9	3,4	1,70
27	4,1	2,3	2,8	1,80
28	4,6	3,0	4,0	1,50
29	5,1	3,4	2,2	1,80
30	5,5	3,0	3,9	1,90

*legend Hol-cholesterol;
Ldl-lipoproteins low density;
Hdl- lipoproteins high density;
Trg- triglycerides

Table 6. Statistical review of the differences between average values of concentration of cholesterol before and after the therapy

	Hol I	Hol II
X average	4,72	4,97
SD	0,66	0,58
N		30
Difference		-0,24
SD difference		0,59
t		-2,26
Degree of freedom		29
p		0,03

Hol-holesterol

Table 7. Statistical review of the differences between average values of high- density lipoproteins (HDL) before and after the therapy

	LDL I	LDL II
X average	2,45	2,79
SD	0,69	0,57
N		30
Difference		-0,34
SD difference		0,88
t		-3,77
Degree of freedom		29
p		0,0007

LDL - lipoproteini male gustine

Table 8. Statistical review of the differences between average values of low- density lipoproteins (LDL) before and after the therapy

	LDL I	LDL II
X average	3,10	3,10
SD	0,75	0,75
N		30
Difference		-0,70
SD difference		0,75
t		-2,26
Degree of freedom		29
p		0,61

HDL – high density lipoproteins

Table 9 Statistical review of the differences between average values of triglycerides before and after the therapy

	Trg I	Trg II
X average	1,27	1,56
SD	0,35	0,40
N		30
Difference		-0,29
SD difference		0,40
t		-3,98
Degree of freedom		29
p		0,0004

Trg- triglycerids

Discussion

The population of sexually active women uses the oral hormonal contraceptives. According to the epidemiological data their usage is especially popular among women that did not have deliveries. During the last three decades many researches of the influence of hormonal contraceptive pills on metabolism, cardiovascular system and psychological behavior of the patients have been conducted. Low-dosed contraceptives reduced the risk of complications which appeared during the use of earlier high- dosed contraceptive pills. The small number of studies about the way that oral hormonal contraceptives

affect the human genome has been published. Both components, estrogen and progesterone, after joining with cytosolic or nucleic receptor, join with wanted DNA cells (5,6). Data from the literature point to a need for new researches about the influence of combined oral contraceptives on the quality of chromosomal material of embryo and fetus which appeared in the period after using these medicines.

Micronucleus test is the method which is used in discovering chromosome aberrations in the cells which are exposed to the effects of chemical mutagens (7). The cell which suffered the DNA damage, cytologically fits to extranuclear bodies in cytoplasm, which represent the chromosomal fragments, the whole chromosomes or the groups of chromosomes. These cytoplasmic masses look like small cores, micronuclei, and their size varies depending on the size of chromosome fragments or the number of chromosomes which are in the cytoplasm.

This method means stopping the cell division (blockade of cytokinesis) while the division of the core is in progress. This effect is achieved by cultivating the cells in medium to which Cytohalasin B is added. That's how binucleate cells originate. The number of micronuclei is determined by using 1000 binucleate cells. It is considered that the normal value is less than 12 micronuclei on 1000 binucleate cells. We think that chemical factors which cause the mutagen response up to that number of micronuclei on 1000 binucleate cells are not significant in the process of mutagenesis (8).

Sixty analyses were done by using CB micronucleus test and by cultivating lymphocytes of peripheral blood of 30 women. The average value of MN test before the therapy with 20 µg of ethinyl- estradiol and 75 µg of gestoden during the six consecutive cycles was $7,40 \pm 4,11$, with outstanding individual variability of micronucleus frequency (from MN 1 to MN 16/1000) in the examined sample. The existing variability of micronucleus frequency can be explained by different life and working conditions, by different nourishment, earlier therapeutic procedures, habits etc. Mentioned authors think that only smoking can affect the variability rate of MN by causing 25% of increase, while the age and the sex did not have the influence. It was noticed that using alcohol and vegetarian food can increase the micronucleus frequency which was induced by smoking. In our example, smoking was not the mutagen agent, because we did not include patients with such habit during our study from the obvious reasons. At the beginning of the research, out of 30 patients, 6 (20%) of them had 12 and more micronuclei on 1000 binucleate cells. That value is considered to be the upper limit of physiological value. After therapeutic treatment, micronucleus frequency/1000 binucleate cells did not increase. You can find the studies in the literature which show that if you treat different people with the same dose of mutagen, the level of chromosomal damage is different, because of the different individual sensitivity. Exactly the individual sensitivity causes the

differences in MN response in vivo conditions, because there are differences in metabolic activation of mutagens and the efficiency of DNA benchmark. This was explained as a result of induction of chromosomal benchmark mechanism, so the authors marked this phenomenon as adaptive answer. The adaptive answer is the phenomenon in which cells that were exposed to a low-dosed agents, which damage the DNA (conditional treatment), later develop the bigger resistance in exposure to a higher dose (provoking treatment). The aim of our further research was to discover the influence of applied therapy on biochemical parameters (9). By analyzing the lipid and lipoprotein status of the patients during the research, we noticed that there was statistically significant increase of the average value of concentration of cholesterol, low-density lipoproteins (LDL) and fraction of triglycerides. High-density lipoproteins (HDL) did not show statistically significant increase after the research (10,11). After the therapy, the average value of the cholesterol is $4,97 \pm 0,58$ g/L, and if we compare it with the value before the therapy we shall see that there is statistically significant increase of $p < 0,05$. Low-density lipoproteins (LDL) with the average value of $2,79 \pm 0,57$ after the therapy, and the triglycerides with the average value of $1,56 \pm 0,40$ also showed the increase, comparing with the value before the therapy, of $p < 0,05$. This effect of OHKC is expected, because the earlier results speak in favor of our study results. In the case of taking OHKC, changes that occur in the lipid status are based on the effect of estrogen component which increases the levels of LDL and triglycerides, while the level of HDL is reducing (12). OHKC which contains low doses of estrogens has incomparably better influence on the lipid status (13). Hypertriglyceride, as an occurrence for itself, is a significant index of high risk for thrombosis of blood vessels. The conclusion of many already published studies is that LDL preventively acts as antithrombotic factor if it follows the increase of triglycerides (14).

Conclusion

Based on the results obtained in this prospective, *in vivo* study, we can conclude the following:

1. After the therapy of 20 µg of ethinyl-estradiol and 75 µg of gestoden in the period of six consecutive menstrual cycles in order to prevent unwanted pregnancy, there was not statistically significant change of micronucleus frequency in lymphocytes of peripheral blood in the cases of patients which took part in the research ($p > 0,05$).

2. The therapy significantly increases the value of cholesterol, low-density lipoproteins (LDL) and triglycerides in the blood of the patients ($p < 0,05$).

3. The therapy can be applied along with the regular control of the gynecologist in order to prevent unwanted pregnancy.

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ORALNA HORMONSKA KONTRACENCIJA - EFEKAT NA HUMANI GENOM I LIPIDNI STATUS

Dragan Lončar

Mikronukleus test je metoda koja se koristi za otkrivanje hromozomskih aberacija u ćelijama koje su izložene dejstvu hemijskih mutagena. Ćelija koja je pretrpela oštećenje DNK citološki odgovara pojavom ekstranuklearnih telašca u citoplazmi, koja predstavljaju hromozomske fragmente, cele hromozome ili grupe hromozoma. Ove citoplazmatične mase izgledaju kao mala jedra, mikronukleusi, a njihova veličina varira u zavisnosti od veličine fragmenta hromozoma ili broja hromozoma koji se nalazi u citoplazmi.

Cilj rada bio je ispitivanje uticaja monofaznih, kombinovanih, niskodoznih kontraceptivnih tableta koje sadrže etinil estradiol i gestoden na frekvencu mikronukleusa u humanim limfocitima periferne krvi i na biohemijske parametre lipidnog profila.

Ispitano je 30 bolesnica starijih od 18 godina. Sve ispitanice su uključene u ispitivanje iz razloga sprečavanja nastanka neželjene trudnoće. Konsultativnim kliničkim pregledima, biohemijskim, hematološkim i dopunskim ispitivanjima isključeno je postojanje kardiovaskularnih, endokrinoloških, neuroloških i malignih oboljenja.

Nakon završene terapije sa 20 µg etinil- estradiola i 75 µg gestodena, u trajanju od šest uzastopnih menstruacionih ciklusa, u cilju sprečavanja nastanka neželjene trudnoće, nije došlo do statistički značajne promene frekvence mikronukleusa u limfocitima periferne krvi kod bolesnica uključenih u ispitivanje ($p > 0,05$).

Terapija značajno povećava vrednosti holesterola, lipoproteina male gustine (LDL) i triglicerida u krvi ispitanica ($p < 0,05$).

Terapija se može primenjivati uz redovnu kontrolu nadležnog ginekologa u cilju sprečavanja nastanka neželjene trudnoće. *Acta Medica Medianae* 2007;46(1):11-16.

Ključne reči: gestoden, etinil estradiol, mikronukleus, genotoksičnost, lipoproteini,