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Excretion of estrogens, catecholestrogens and phytoestrogens in carriers of BRCA1 gene mutations: effects of metformin

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BRCA1 gene mutation is associated with a combination of excessive aromatase activity/expression, predominantly estrogen receptor-negative phenotypes of tumors, and only scarce information about estrogen contents in body fluids. In the present work, isotope dilution capillary gas chromatography/mass spectrometry was used to study urinary excretion of estrogens, their catechol metabolites, and phytoestrogens in 22 women (11 with BCRA1 gene mutations and 11 without these mutations) in average 5.1±0.4 years after surgery for breast cancer. BCRA1 mutation carriers (including 3 premenopausal females) compared with respective controls showed significantly higher urinary estradiol and estrone excretion and a trend to an increased 2-OH-E2 excretion. In the subgroup of untreated postmenopausal women, BCRA1 mutation carriers showed a trend to increased estradiol and estrone excretion and to a higher value of the mean levels of all estrogen metabolites tested. The treatment after the baseline laboratory investigation of 6 women from postmenopausal group with the antidiabetic biguanide metformin for 3 months was associated with decreases in the excretion rates of 4-hydroxyestradiol, 2-methoxyestradiol, and 16-epiestriol and did not influence phytoestrogen excretion. The decrease in 2-methoxyestrogen excretion was more consistent in women without BCRA1 mutations than in BCRA1 mutation carriers. The data suggest the possibility that aromatase complex activation in BCRA1 mutation carriers is combined with increases in both, estrogen metabolism into catecholestrogens and their inactivation by methoxylation, and that metformin may affect both of these pathways.

Key words: *breast cancer, estrogen metabolism, BRCA1 gene, biguanides, metformin*

Mutations of the breast cancer gene-1 (BRCA1) occur approximately in 1 of 800 Caucasian women (from 1:500 to 1:2500), except for some specific populations [1]. Over their lives, about 40-80% of the carriers of such mutations develop breast cancer, and about 3-5% of all breast cancer cases are associated with BCRA1 mutations [2]. Significant differences are known to exist between the prevalence of the BCRA1-associated and hereditary forms of breast cancer, the latter occurring at least 4-5 times more often, as well as between some of their endocrine manifestations [3, 4]. These differences gradually bring the endocrinological aspects of BCRA1 mutation occurence into the focus of current attention [5, 6, 7, 8, 9].

The specific endocrine features of BCRA1 mutation bearing are based on several factors, the most prominent being insulin-like growth factor receptor and aromatase hyperexpression [10, 11, 12] and the predominance of the estrogen receptor-negative tumor phenotype [13, 14]. There are only few data about blood estrogen levels in BCRA1 mutation carriers [5] and phytoestrogens content remain virtually unstudied (despite their known important 'interactions' with BCRA1 [15, 16]). The urinary excretion of estrogens and some catecholestrogen fractions was studied only in women who had breast cancer patients among their blood relatives, and no special attention was paid to BCRA1 mutation bearing [17, 18].

On the other hand, the recently emerged new wave of interest towards the antidiabetic biguanide metformin in oncological endocrinology and cancer area in general [19, 20, 21, 22, 23] attracts attention to the fact that this agent can not only influence the insulin/IGF-1 system (which is suggested to be taken into account in the cases of BCRA1 mutation bearing [23, 24]) but also can modify aromatase activity and estrogenic signal transduction [25, 26, 27]. These data seem important in view of the fact that classic estrogens may be converted into carcinogenic catecholestrogens [28, 29] and, therefore, there is the need for drugs able to limit this conversion or to influence estrogen and phytoestrogen metabolism/accumulation in the body in other ways.

In summary, the objectives of the present pilot study were to compare the specific features of urinary estrogens, catecholestrogens, and phytoestrogens excretion in women who bear BCRA1 mutation and to check whether metformin treatment may be associated with any changes in the parameters studied.

Patients and methods

Subjects. The two main groups of the present study comprised 11 breast cancer patients bearing a BCRA1 mutation (BCRA1$^+$), predominantly of the 5282insC insertion type [30], and 11 breast cancer patients with no BCRA1 mutations (BCRA1$^-$). The mean ages in the groups were 49,6±2,8 and 56,4±3,0 years, respectively (p = 0,14), 19 patients being postmenopausal. Surgery for breast cancer was performed, on average, 4,2±0,6 and 5,9±0,3 years, respectively, prior to the investigation. At the moment of investigation, the patients presented no complaints, and no objective signs of breast cancer were found. In the BCRA1$^+$ group, three women were premenopausal: two patients retained their normal menstrual cycles, and they were examined on the cycle day 22; one patient was amenorrheic (58 days after the last menses). Two other patients have got adjuvant hormonal therapy: one with letrozole (aromatase inhibitor) and one with tamoxifen (antiestrogen). In the BCRA$^-$ group, one patient was treated with tamoxifen. The rest of the patients in both groups received no treatment for one year at least. This information was taken into account in the below analysis of data.

Treatment with metformin. The antidiabetic drug metformin was prescribed after the baseline laboratory investigation at a dose of 1.0 to 1.5 g/day for 3 months to six patients aged 58,7±4,4 years. Three of them were in the BCRA1$^+$ and three in the BCRA1$^-$ group, and no one showed signs of retained menstrual cycle. This and all others aspects of the study were approved by the Local Ethic Committee.

Urine collection. The morning portions of urine were collected into plastic bottles. No special diet was prescribed. Urine samples supplemented with ascorbic acid (about 0,1-0,2%) were stored frozen at -20°C, and when all of the samples were collected (including the ones collected on the next day after the end of metformin treatment), they were transported in dry ice to the analytical laboratory in Helsinki. Creatinine concentrations were measured to make correction for possible diurnal changes in urine volume.

Determination of estrogen and phytoestrogen fractions. Urinary estrogen and phytoestrogen profile determination method based on isotope dilution capillary gas chromatography/mass spectrometry was used. The details of the method, which provides data about 15 estrogen metabolites (including 4 catecholestrogens) and 11 phytoestrogens (4 lignans and 7 isoflavones), are given elsewhere [31]. The following estrogen fractions were measured: estrone (E1), estradiol (E2), estriol (E3), 2-hydroxyestrone (2-OHE1), 2-hydroxyestradiol (2-OHE2), 4-hydroxyestrone (4-OHE1), 4-hydroxyestradiol

Table 1. Excretion of estrogen and various estrogen metabolites (nmol/mol creatinine, M ± m) with urine in females of BRCA1$^+$ and BRCA1$^-$ groups

Group	Fractions													
	E2	E1	2metE1	16αOHE1	15αOHE1	16βOHE1	16oxoE2	2metE2	2OHE1	4OHE1	2OHE2	4OHE2	17epiE3	16epiE3	E3	Sum of averages
BRCA1+, all (n=11)	1,17± 0,18[a]	3,07± 0,60[b]	1,01± 0,47	0,90± 0,35	0,11± 0,04	0,19± 0,14	0,78± 0,24	0,25± 0,07	2,84± 0,80[c]	0,77± 0,21	0,25± 0,09	0,04± 0,02	0,04± 0,02	0,07± 0,02	1,78± 0,50	13,27
BRCA1-, all (n=11)	0,70± 0,6[a]	1,42± 0,13[b]	0,10± 0,07	0,39± 0,20	0,05± 0,02	0	0,69± 0,11	0,25± 0,06	1,40± 0,35[c]	0,78± 0,26	0,08± 0,03	0,05± 0,02	0,01± 0,01	0,05± 0,01	1,06± 0,27	7,03
BRCA1+, MP (n=6)	1,08± 0,23[d]	2,56± 0,73[e]	1,39± 0,78	1,08± 0,54	0,10± 0,06	0,22± 0,22	0,59± 0,18	0,24± 0,11	2,35± 1,02	0,69± 0,23	0,21± 0,09	0,04± 0,03	0,04± 0,03	0,06± 0,02	1,44± 0,62	12,09
BRCA1-, MP(n=10)	0,72± 0,06[d]	1,40± 0,15[e]	0,11± 0,08	0,43± 0,22	0,05± 0,02	0	0,70± 0,12	0,26± 0,07	1,48± 0,40	0,82± 0,28	0,08± 0,03	0,05± 0,02	0,01± 0,01	0,05± 0,01	1,14± 0,29	7,30

Notes and abbreviations: all – all studied patients; MP – postmenopausal group without any treatment;
E2 – estradiol, E1 – estrone, 2metE1 – 2-methoxyestrone, 16αOHE1 – 16-alpha-hydroxyestrone, 15αOHE1 – 15-alpha-hydroxyestrone, 16βOHE1 – 16-beta-hydroxyestrone, 16oxoE2 – 16-oxo-estradiol, 2metE2 – 2-methoxyestradiol, 2OHE1 – 2-hydroxyestrone, 4OHE1 – 4-hydroxyestrone, 2OHE2 – 2-hydroxyestradiol, 4OHE2 – 4-hydroxyestradiol, 17epiE3 – 17-epiestriol, 16epiE3 – 16-epiestriol, E3 – estriol
[a] p 0,02; [b] p 0,01; [c] p 0,07; [d] p 0,09; [e] p 0,07

(4-OHE2), 2-methoxyestrone (2-MOE1), 2-methoxyestradiol (2-MOE2), 16-oxoestradiol (16-oxoE2), 16-alpha-hydroxyestrone (16**α**OHE1), 16-beta-hydroxyestrone (16**β**OHE1), 15-alpha-hydroxyestrone (15**α**OHE1), 16-epiestriol (16-epiE3) and 17-epiestriol (17-epiE3). Among studied phytoestrogens were: enterodiol (End), enterolactone (Enl), matairesinol (Mat), secoisolariciresinol (Seco), daidzein (Da), genistein (Gen), O-desmethylangolensin (O-Dma), equol (Eq), dihydrodaidzein (DHDa), dihydrogenistein (DHGe), and glycitein (Gly).

Statistical analysis. Group means ± standard errors and catecholestrogen/estrogen ratios were calculated. The differences between the means were assessed with *t*-test using the software package SigmaPlot for Windows (SPSS Inc., Chicago, IL, USA).

Results

The data obtained in this study show that, in women bearing BCRA1 mutations compared with the control group, urinary estradiol and estrone excretion is significantly higher, and 2-OH-E2 excretion tends to be increased. Trend to the increment in 2-methoxyestrone excretion is revealed too (Table 1). A roughly similar pattern including an increased sum of the means of all examined parameters is evident when the analysis is limited to the untreated postmenopausal women (the lower panel of Table 1).

Phytoestrogen excretion assessed by the sum of the averages of their separate fractions was higher in the BCRA1$^+$ group; however, solely the increase in enterodiol excretion was significant (p=0.04), and the difference disappeared when only postmenopausal women were included in the analysis (Table 2).

Metformin administration for 3 months to six postmenopausal females was associated with decreases in excretion rates of 4-hydroxyestradiol, 2-methoxyestradiol, and 16-epiestriol (Table 3); the effect related to 2-methoxyestradiol excretion being more pronounced in BCRA$^-$ women (n = 3; 0,40±0,11 nmol/mol creatinine before metformin *vs* 0,11±0,03 after metformin; p=0,07) than in BCRA$^+$ women (n = 3; 0,52±0,05 *vs* 0,40±0,06; p=0,19). The selectivity of the decrease in 4-hydroxyestradiol compared with other catecholestrogen excretion rates upon metformin administration is confirmed by changes in the ratios of the excretion rates of these estrogen metabolites to the excretion rates of the respective classical estrogens, i.e., only the ratio 4-OHE2/E2 showed a noticeable trend to decrease (see Table 3). No changes were found in phytoestrogen excretion rates upon metformin administration (data not shown).

Discussion and conclusions

In women with undefined BRCA1 mutation status who had breast cancer patients among their blood relatives, morning urine samples were found to feature some decrease in estradiol, 2-OHE1, and 16**α**-OHE1 and no change in the 2-OHE1/16**α**-OHE1 ratio (17). According to other observations, changes in these parameters (decrease or increase) were associated, with women, having the presence of certain allelic polymorphisms of genes implicated in estrogen metabolism, such as CYP1B1, COMT, and CYP17 (18). No differences in blood estradiol were found when healthy women bearing BCRA1 mutations were compared with women from families with no such mutations (5).

With all that, in an analysis of the results obtained in the present work, one needs, first of all, to consider any confound-

Table 2. Excretion of phytoestrogens (nmol/mol creatinine, M ± m) in females belonging to BRCA1$^+$ and BRCA1$^-$ groups

Group	Fractions											
	End	Enl	Mat	Seco	Da	Gen	O-Dma	Eq	DHDa	DHGe	Gly	Sum of averages
BRCA1+, all (n=11)	21,77± 4,59[a]	0,23± 0,05	2,45± 0,19	21,51± 2,93	117,55± 64,50	48,55± 30,51	15,68± 7,71	7,71± 1,70	6,44± 4,78	10,99± 7,51	58,62± 41,63	311,50
BRCA1-, all (n=11)	10,69± 2,39[a]	0,25± 0,07	2,17± 0,33	25,95± 8,85	23,57± 9,89	9,79± 4,56	6,59± 2,34	7,19± 1,62	2,24± 1,61	0,39± 0,17	3,47± 1,55	92,30
BRCA1+, MP (n=6)	18,63± 7,27	0,20± 0,05	2,21± 0,16	18,40± 3,57	31,18± 12,31	20,22± 10,73	16,80± 12,15	8,40± 3,02	0	0,40± 0,23	4,82± 2,80	121,26
BRCA1-, MP(n=10)	10,84± 2,64	0,27± 0,07	2,17± 0,36	24,32± 9,62	25,25± 10,77	10,66± 4,94	7,08± 0,12	7,28± 1,79	2,47± 1,76	0,43± 0,19	3,63± 1,71	94,40

Notes and abbreviations: all – all studied patients; MP – postmenopausal group without any treatment;
End – enterodiol*, Enl – enterolactone*, Mat – matairesinol*, Seco – secoisolariciresinol*, Da – daidzein, Gen – genistein, O-Dma – O-desmethylangolensin, Eq – equol, DHDa – dihydrodaidzein, DHGe – dihydrogenistein, Gly – glycitein
* lignans; not marked – isoflavones
[a] p 0,04

Table 3. Excretion of estrogens and various estrogen metabolites (nmol/mol creatinine, M ± m) and some catecholestrogens/estrogens ratios before and after 3-month metformin course

Fractions and ratios	Before metformin (n=6)	After metformin (n=6)	p
E2	0,99±0,18	0,72±0,14	
E1	1,78±0,46	1,81±0,57	
2metE1	0,81±0,53	0,39±0,39	
16αOHE1	0,50±0,31	0	
15αOHE1	0,05±0,02	0,04±0,02	
16βOHE1	0,22±0,22	0	
16oxoE2	0,50±0,15	0,20±0,13	
2metE2	0,46±0,06	0,26±0,07	0,051
2OHE1	2,12±0,57	1,34±0,60	
4OHE1	0,60±0,23	1,05±0,37	
2OHE2	0,18±0,07	0,10±0,08	
4OHE2	0,07±0,04	0	0,07
17epiE3	0,04±0,02	0	
16epiE3	0,07±0,02	0,03±0,01	0,09
E3	0,90±0,27	0,74±0,21	
Sum of averages	9,29	6,68	
2-OHE2/E2	0,20±0,08	0,12±0,09	
2-OHE1/E1	1,90±1,00	0,68±0,16	
4-OHE2/E2	0,07±0,03	0	0,055
4-OHE1/E1	0,43±0,18	0,56±0,09	

Notes: see Table 1 for abbreviations

ing factors that can bias the final conclusions. Obviously, there is no reason to consider tumor effects, because the study subjects were in good health even by objective criteria, and significant time periods elapsed from surgery to the present investigation in both study groups, BCRA1$^+$ and BCRA1$^-$.

Mean ages were not significantly different in the compared groups. Nevertheless, the BCRA1$^+$ group included three premenopausal women. This observation and the fact that three other women (two in the BRCA1$^+$ and one in the BRCA1$^-$ group) continued therapy with the aromatase inhibitor letrozole or the antiestrogen tamoxifen may underlie possible biases in the results that relate, in particular, to estrogenic metabolites [32, 33, 34]. Therefore, the subgroup of postmenopausal untreated women was analyzed separately, and it was just the type of patients who were chosen for metformin administration.

The data on estrogen and various estrogen metabolites excretion suggest that, even after the above confounding factors are eliminated, the group of BCRA1$^+$ women shows a trend towards increase in some fractions, notably estradiol and estrone, in the amount of all means, and in 2-hydroxyestrogens (Table 1). In considering these results one should mind that the "replacement" of wild type BCRA1 by its mutated form is believed to be associated with an increased aromatase/estrogen synthetase activity [8, 11, 12] and, also, indicates the possibility of the involvement of the genotoxic mechanisms in the neoplastic transformation of estrogen-responsive tissues [7, 35, 36]. Therefore, the fact that the administration of antidiabetic agent metformin (which in some cell systems inhibits aromatase activity [25, 26] and can modify estrogenic signal transduction [27]) is associated, as the present study shows, with a trend to a decreased excretion of most carcinogenic and genotoxic catecholestrogen [37, 38] 4-hydroxyestradiol, may be among explanations of the reduction in the incidence of certain malignant neoplasms in patients treated with this drug [39, 40].

These conclusions, one would think, are incompatible with the observation that metformin distinctly decreased the excretion of 2-metoxyestradiol as well [Table 3], both in BCRA1$^+$ and, especially, in BCRA1$^-$ women. This estrogenic metabolite, as was demonstrated, is capable to inhibit angiogenesis and tumor growth [41]. In addition, some data suggest that metformin can directly enhance angiogenesis, the effect being associated with the AMP kinase activating action of metformin and being predominantly observed in estrogen receptor-negative neoplasms [42], to which the BCRA1 mutation-associated breast cancer relates [13, 14]. Therefore, the reasonability of using this medicine in such cases warrants additional examination.

The present study failed to reveal any specificity in phytoestrogens excretion (including the cases of metformin administration), except for an increased enterodiol excretion in BCRA1$^+$ women when no correction for confounding factors, such as the menopausal status, is made (Table 2). The possibility is not ruled out that, in this case, the age-related factors (absence of menopause in 3 patients) are more important than BCRA1 mutation. However, to the best of our knowledge, data about age-associated changes in lignans and isoflavones excretion are lacking, and it is likely that the differences found are the result of some uncontrolled nutritional factors.

In summary, the present work suggests that the trend to the increased urinary excretion of some estrogens in women who bear BCRA1 mutations results from the previously reported [8, 11, 12] activation of aromatase complex in such women, which is subsequently associated with the increased generation of estrogen metabolites including 2-hydroxylated derivatives. Some authors do not qualify 2-hydroxyestrone and 2-hydroxyestradiol as carcinogenic [29, 37]; however, there are observations (43) that are not quite consistent with this view. On the other hand, the presence of a BCRA1 mutation may be associated not only with the generation of catecholestrogens but, also, with tendency to an increased excretion of their methoxylated metabolites (Table 1). Therefore, although further studies are needed to reproduce these results, the influence of metformin on mentioned reactions of estrogen metabolism should be taken into account when often reported its antitumor effects [see 19, 20, 21, 22, 23, 39, 40] are considered.

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