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MODULATION OF OESTROGEN EXCRETION PROFILES BY ADJUVANT CHEMOTHERAPY IN PRE- AND POSTMENOPAUSAL BREAST CANCER

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Summary—Modulation of steroid status by conventional chemotherapy was studied in 31 breast cancer patients receiving CMF and in 31 age-matched breast cancer patients without any therapy, taken as controls. This was achieved through the study of oestrogen excretion profiles using previously identified parameters and referring not only to classical but also to the "other", namely catechol and unusual, oestrogen metabolites. After CMF treatment the premenopausal patients exhibit a modified excretion pattern, mainly concerning a marked and significant reduction of classical oestrogens, as shown by pattern indices. Because there is evidence that oestriol metabolism is not markedly affected by CMF treatment, such a significant decrease in classical oestrogens must be attributed to the secretory function, presumably ovarian *ab origine*. To the contrary, after treatment, pattern indices show significantly higher median values in postmenopausal patients. Mean oestriol ratio values also display a significant increase, thus supporting the hypothesis that conventional cytotoxic drugs may act by enhancing oestrogen metabolic rates. In fact, the postmenopausal treated subgroup proved to have significantly higher excretion levels of most of the oestrogens considered to date. Surprisingly, $E_1 + E_1$-S fractions were strongly reduced in this subgroup and this leads to the suggestion of an increased steroid metabolic rate by CMF treatment. However, comparing 9 breast cancer patients, when having had both short-term and non-short-term CMF treatment, the effects on steroid excretion patterns appear to arise at an early stage.

INTRODUCTION

Numerous patients with breast cancer both in premenopause and in postmenopause are treated nowadays by conventional CMF chemotherapy [1], although uncertainties and controversies make it difficult to accept the routine use of this therapy [2]. Following treatment with cytotoxic drugs, ovarian disfunction or failure has been observed in a certain number of these patients in premenopause [3–8]. Consequently, it has been hypothesized that these drugs perform a secondary action, producing "via endocrine" a real form of chemical castration. In fact, after the first reported observation [9] of amenorrhea following cyclophosphamide (CY) it was demonstrated that developing germinal cells are affected by toxic action [10] and that CY can be toxic for human testicle [11], while Miller *et al.*[12] have shown that a loss of ovules causes functional ovarian failure. This was confirmed by Warne *et al.*[13] and hypothesized as a progressive rather than an all-or-nothing phenomenon. Subsequently, various authors have demonstrated a correlation with the age of ovarian disfunction [7, 14–17]. Recently, it has been proved that azoospermia may be produced after only one or two cycles of treatment [18] and that amenorrhea may appear after only 2–4 months of treatment with CMF [19].

All these studies, which dealt mainly with premenopausal breast cancer patients reported significantly low oestrogen plasma levels, mainly those of oestradiol and oestrone [4, 17, 19, 20], even if in some cases the oestradiol and oestrone plasma levels were found to be unchanged [21]. Only one report has evaluated excretion levels, but in this case also the study was restricted to the classical oestrogens $[E_1 + E_2 + E_3]$ [3]. In the few cases in which the investigation has been extended to postmenopausal patients, the plasma levels of these and other steroid hormones did not appear to be significantly changed [4, 19, 20]: for example, the levels of dehydroepiandrosterone sulphate (DHEA-S), a steroid metabolite of mainly adrenocortical origin, appeared to be unchanged after treatment. This gave rise to the suggestion that adrenocortical function was unaffected by conventional cytotoxic drugs [19]. Apart from the fact that this supposedly selective action by the cytotoxic drugs is somewhat difficult to understand, other extremely interesting points concerning this issue have recently emerged.

In fact, it has been observed that oestradiol impairs the antiproliferative effects of methotrexate (MTX) on cell lines such as MDA [22] and that the corticosteroids impair the antitumour action of CY in rats [23].

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Table 1. Modulation of steroid excretion profiles in B Ca patients

There is a physiological modulation:
PreM is marked by endogenous circalunar rhythms;
PM differs from PreM status because of E_3R, E_3 and classical oestrogen reduced excretion levels.
There is a pathological modulation:
in PM B Ca patients we observed significant hyperoestrogenism often concerning other, cathecol and minor oestrogens.
steroid patterns of B Ca patients show a bimodal behaviour above all in C/U ratio, E_3R and PI.
There is a pharmacological modulation:
hyperoestrogenism of B Ca patients might be significantly reduced following additive hormone (DES) therapy.

These last observations direct our attention towards further possible effects of interaction between endogeneous steroids and conventional chemotherapy: that is, the potential ability of both to interfere reciprocally on certain key aspects of their metabolic processes [24–26]. They stimulated our interest, therefore, in the study of the hormone status of breast cancer patients, undergoing CMF-treatment, by gas–liquid chromatography and mass spectrometry (GLC–MS) and by high-pressure liquid chromatography (HPLC), using parameters we had previously identified [27–30], and relating both to androgen (data not shown) and oestrogen excretion profiles. Our aims were:

(a) to evaluate the action of conventional chemotherapy (CMF) on steroid status, above all in postmenopausal patients;

(b) to assess modifications in steroid status in relation to the duration of CMF treatment, in both pre- and postmenopausal patients;

(c) to assess the different, if any, patients' responsiveness to CMF in relation to their steroid status.

SOME ASPECTS OF OESTROGEN PATTERNS AND METABOLISM

Three different fractions can be identified in oestrogen profiles; the first refers to the classical oestrogens E_1, E_2 and E_3, among which 16-epi-oestriol should also be included; the second refers to the cathecol-oestrogens, including their metoxy derivatives, whose levels and pathways are probably linked to the peripheral metabolism of particular target tissues and are probably interdependent with those of the classical oestrogens; the third, minor (unusual) oestrogens, are more heterogeneous, being characterized by the presence of a lot of metabolites, some of them having α-position bonds, e.g. 17-α-E_2, 11-dehydro-17-α-E_2, the stereoisomers of E_3 and 16-α-hydroxy-E_1, their biological activity being undoubtedly low and their biochemical significance not yet clear.

Our studies on steroid excretion patterns [27–33] of both breast (B) and endometrial (E) postmenopausal cancer (Ca) patients, evaluated by GLC–MS, showed up the following points (see also Table 1): (a) that there was a general significant increase in excretion levels of oestrogens [27], (b) that this hyperoestrogenism did not prove uniform for all patients [27–31], (c) that the introduction of particular parameters made it possible to evidence a bimodal expression of oestrogen profiles [30,31] concurrently with (d) a significant different probability of response to hormone therapy [30].

In patients which will respond to additive hormone therapy, with synthetic oestrogens (DES or HEX) at pharmacological doses [27, 30], we evidenced a significantly different behaviour of the two fractions, that of classical oestrogens and that of unusual metabolites, which, most interestingly, seemed to be regulated differently and, to some extent, independently of one another [33].

These results suggested different metabolic pathways in the different groups of patients, at least as regards steroids. When, however, excretion levels were compared with oestradiol and oestrone plasma levels, no significant correlation was found (unpublished data).

Many of the minor oestrogens, defined by us as unusual, are present in human urines only in very small amounts in normal conditions, and some of them suggest adrenal origin. These experimental findings, along with the already observed importance of oestrogen excretion profiles in setting up a discriminating function [30], make unusual metabolites a fraction of oestrogens worth studying for a more extensive comprehension of their metabolic processes.

We also wish to point out that in a previous study the pattern index (PI) was considered the best discriminating factor for distinguishing responder from non-responder B Ca patients [30].

The catechol oestrogens

Even in a few lines it is possible to point out the biochemical and endocrinological importance of catechol oestrogens. These particular oestrogens were long ago observed in human urine [34] and, although attention has only recently been paid to them, they are deserving of some particular remarks. The following points should be outlined: (a) they have been detected in brain and endocrine tissues [35]; (b) they have been suggested as possible control factors in the biological action of oestradiol [36]; (c) their control action in gonadotrophin and prolactin secretion has been observed to be agonistic to that of oestradiol [37]; (d) their role in stimulating and controlling prostaglandin synthesis has been demonstrated [38]; (e) their marked capability to compete with oestradiol for the specific receptor has been ascertained [39].

These and other experimental findings, largely reported in an excellent review [40], have led to the hypothesis that catechol oestrogens may perform a physiological action by acting as real antioestrogens.

Table 2. Nomenclature

Trivial name	Systematic name	Abbreviation
Estrone	1,3,5(10)-Estratrien-3-hydroxy-17 one	E_1
Estradiol-17β	1,3,5(10)-Estratrien-3-hydroxy-3,17β-diol	E_2
Estriol	1,3,5(10)-Estratrien-3-hydroxy-3,16α17β-triol	E_3
Estradiol-17α	1,3,5(10)-Estratrien-3-hydroxy-3,17α-diol	17αE_2
16α-Hydroxy-estrone	1,3,5(10)-Estratrien-3-hydroxy-3-16α-diol-17-one	16-α-OH-E_1
16-Epi-estriol	1,3,5(10)-Estratrien-3-hydroxy-3,16β,17β-triol	16-epi E_3
16-17-Epi-estriol	1,3,5(10)-Estratrien-3-hydroxy-3,16β,17α-triol	16-17-epi E_3
17-Epi-estriol	1,3,5(10)-Estratrien-3-hydroxy-3,16α17α-triol	17-epi-E_3
6-Keto-estrone	1,3,5(10)-Estratrien-3-hydroxy-3-ol-6,17-dione	6-keto-E_1
11Δ,17α-Estradiol	1,3,5(10)-Estratrien-3-hydroxy-11.dehydro-3,17α-diol	11Δ-17αE_2
-2-Hydroxy-estradiol	1,3,5(10)-Estratrien-3-hydroxy-2,3,17β-triol	2.OH-E_2
-2-Hydroxy-estrone	1,3,5(10)-Estratrien-3-hydroxy-2,3-dihydroxy-17-one	2-OH-E_1
-2-Hydroxy-estriol	1,3,5(10)-Estratrien-3-hydroxy-2,3,16α,17β-tetrol	2-OH-E_3
-4-Hydroxy-estradiol	1,3,5(10)-Estratrien-3-hydroxy-3,4,17β-triol	4-OH-E_2
-4-Hydroxy-estrone	1,3,5(10)-Estratrien-3-hydroxy-3,4-dihydroxy-17-one	4-OH-E_1
-4-Hydroxy-estriol	1,3,5(10)-Estratrien-3-hydroxy-3,4,16α17β-tetrol	4-OH-E_3
-2-Methoxy-estradiol	1,3,5(10)-Estratrien-3-hydroxy-2-metoxy-3,17β-diol	2-Me-E_2
-2-Methoxy-estrone	1,3,5(10)-Estratrien-3-hydroxy-2-metoxy-3-ol-17-one	2-ME-E_1
-2-Methoxy-estriol	1,3,5(10)-Estratrien-3-hydroxy-2-metoxy-3,16α17β-triol	2-Me-E_3

These data strongly suggest that looking only to the three classical oestrogens, including their plasma levels, may be misleading for the study of oestrogen metabolism in B Ca patients.

PATIENTS AND METHODOLOGY

These studies were carried out on different groups of breast cancer patients.

The first group comprised 31 patients treated with conventional CMF [1], with mean age 54.5 ± 10.0; of these, 9 were in premenopause, (PreM, mean age 44.1 ± 7.8) and 22 in postmenopause (PM, mean age 64.9 ± 2.3).

The second group comprised 31 B Ca patients (controls) age-matched with the first group (mean age $55\text{-}0 \pm 12.0$); of these, 15 were in PreM (mean age 43.3 ± 5.7) and 16 in PM (mean age 64.4 ± 5.9).

As one of the aims of the study was to compare the effects of short-term and non-short-term CMF therapy, two different subgroups were considered. The first comprised 10 patients who had received only two cycles of therapy, with mean age 48.7 ± 7.9 (Group A). The second (non-short-term; Group B) comprised 21 patients, with mean age 49.0 ± 7.9, who had received at least nine cycles of treatment. A third group (C) consisted of 39 clinically normal women in PM, mean age 61.1 ± 6.4.

It must be borne in mind that all the functions chosen to express the excretion levels of the various parameters were previously checked for the best fit in a normal population by Pearson's test and that the data for the CMF-treated breast cancer group were expressed as menopausal status weighted averages. B Ca patients on adjuvant chemotherapy (CMF) were given CY (600 mg/m^2), MTX (40 mg/m^2) and 5-fluorouracil (600 mg/m^2), every 21 days for 12 cycles.

The parameters chosen by us for the evaluation of steroid excretion profiles are: (a) excretion levels of classical oestrogens; (b) total oestrogens; (c) the oestriol ratio; (d) the ratio between classical metabolites and unusual metabolites (C/U); (e) the oestrogen–androgen ratio; and (f) the pattern index ($E_3R \times C/U = PI$) [27, 28, 30].

The excretion profiles of oestrogens, androgens and progestins were studied with GC and, when necessary, with MS, using the methods developed in our laboratory and previously reported [27–30]. The GC valuations are preceded by a series of preliminary purifications, by both acid and enzymatic hydrolysis using the methods of Brown and Beeling, by subsequent purifications in column chromatography (Gupta's method) and TLC and with various kinds of preparation of derivatives [27, 28]. GC was carried out with various and different stationary phases. MS was conducted in a GLC–MS unit, thus permitting MS analysis while the GC profile is being performed. For the HPLC study we followed the same approach, optimization of mobile phase, as previously used for corticosteroids analysis [32]. This approach consisted of optimizing the mobile phase by computer simulations using a personal computer (Olivetti M 20).

RESULTS

We carried out a preliminary examination of B Ca patients treated with CMF in order to assess the differences in steroid status modifications in the two different menopausal groups.

A striking observation is that the levels of E_1 (the sum of $E_1 + E_1$-S) show a marked decrease, though not significant, in PM as compared to PreM. Considering the ratios among selected oestrogens, the comparison between the PreM and PM CMF-treated patients shows that there are no significant differences in the three parameters studied, i.e. the oestriol ratio, the ratio of classical to unusual metabolites and the PI. These findings are certainly surprising, as in our previous studies PM is characterized by lower classical oestrogen excretion levels, lower E_3R and lower PI

Table 3. Comparison of classical and other oestrogens in CMF-treated and non-treated B Ca patients

Function measured (μg/24 h)	CMF-treated ($n = 31$)	Non-treated ($n = 31$)
√Classical	5.95 ± 1.02	4.66 ± 1.53 NSD
log others	3.39 ± 1.23	1.85 ± 1.01 NSD
log total	4.12 ± 0.12	3.46 ± 0.60 NSD

Menopausal status weighted averages; stat. cpr Student's t-test.

Table 5. Comparison of PIs between CMF-treated and non-treated PreM and PM B Ca patients

	CMF-treated		Non-treated	
PreM ($n = 9$)	0.93 (0.01–2.26)	$n = 15$	1.58 (0.25–8.25)	$P < 0.001$
PM ($n = 22$)	1.29 (0.20–2.65)	$n = 16$	0.27 (0.20–1.02)	$P < 0.0001$
	NSD		$P < 0.05$	

Median and (I.R.) Wilcoxon's test.

values than for PreM; moreover, it is common to find higher E_1 and UM excretion levels in PM.

This experimental postulate that we achieved made it possible for us to consider CMF-treated B Ca patients as a set, though considering menopausal status weighted averages, in order to carry out a more accurate statistical comparison between CMF-treated and untreated patients. The comparison between the oestrogen patterns of the latter two groups is shown in Tables 3, 4 and 6.

Table 3 refers to both classical and "other" oestrogens. It can be observed at once that the average excretion levels of the group of CMF-treated patients are higher than those of the untreated patients as regards both classical and other oestrogens, but this increase does not prove significant.

Table 4 shows the same comparison between the two groups but refers to the various components of the other oestrogens, i.e. catecholoestrogens and minor oestrogens. In this case too the average excretion levels prove considerably and significantly higher in the PM subgroup of CMF-treated patients. This increase concerns both the fractions of classical oestrogens and of other oestrogens, and among the latter seems to affect both the group of more polar oestrogens and that of the minor oestrogens. Hence these results do not seem to point to a particular effect on the excretion levels of particular fractions, but to a general and diffuse effect on the oestrogen metabolism.

If we consider the E_3R, we can observe that the CMF-treated PM subgroup of patients exhibits a significantly higher value than the mean value of untreated patients (1.61 ± 1.23 vs 0.46 ± 0.46, $P < 0.0001$). This finding suggests that the classical metabolic pathway of oestrogens is not negatively modified in the first group, and that the formation of the principal urinary metabolite of the oestrogens, i.e. oestriol, is, in some way, increasingly affected.

However, if we consider another of the ratios between selected metabolites of oestrogens, namely the PI (see Table 5), we can observe significantly different behaviour in the two menopausal subgroups of treated vs untreated patients. In the second group, i.e. untreated B Ca patients, the median value of this product of ratios decreases significantly in PM as compared to PreM, as expected on account of the reduction in both the E_3R and the C/U ratios. Quite to the contrary, this trend is not observed in the treated patients' subgroups.

Besides, on comparing the PreM subgroups of treated and untreated patients, the PI parameter exhibits significantly reduced values ($P < 0.001$) in treated patients. This finding suggests a reduction of oestrogen excretion rates in the treated patients, mainly of ovarian, rather than of any other origin, as is to be expected in PreM. Surprisingly, a marked and significant increase of the PI median value is related to the treated PM subgroup.

MODIFICATION OF OESTROGEN EXCRETION PATTERNS IN RELATION TO DURATION OF TREATMENT

In an attempt to study the possible correlation between modifications in oestrogen excretion patterns in treated patients and duration of therapy, two subgroups of patients were compared: (A) having received short-term treatment, i.e. only two CMF cycles; and (B) having received non-short-term treatment, at least nine CMF cycles. The results are shown in Table 6.

It appears evident that the excretion levels of total oestrogens practically overlap in these two groups

Table 4. Comparison of minor and cathecol oestrogens in CMF-treated and non-treated B Ca patients

Function measured (μg/24 h)	CMF-treated ($n = 31$)	Non-treated ($n = 31$)
log catechols	2.86 ± 0.92	1.19 ± 1.24*
log minor	2.38 ± 1.22	1.23 ± 1.02**

Menopausal status weighted averages.
*$P < 0.001$.
**$P < 0.0001$.

Table 6. Comparison of ratios, including unusual oestrogens, in selected CMF-treated patients having ⩽2 (A) and ⩾9 (B) cycles of therapy

Function measured (μg/24 h)	Group A ($n = 10$)	Group B ($n = 21$)
E_3R[a]	1.61 ± 1.22	1.60 ± 1.28 NSD
C/U	0.86 ± 0.59	1.02 ± 0.42 NSD
PI[a]	1.75 ± 1.50	2.31 ± 2.42 NSD

[a] × 10^{-1}.
Menopausal status weighted averages; $\bar{X} \pm$ SD Student's t-test.

and that the classical oestrogen levels are not significantly different. It is interesting to note that the minor oestrogen fraction tends to be slightly higher in the non-short-term group (B), though the increase is not significant, indicating a further increase of this fraction of oestrogens if CMF treatment is prolonged.

Even the values of the ratios (shown in Table 6) do not exhibit significant differences in these two groups; however, we note that the PI value in the non-short-term groups shows a particularly marked tendency to be higher but fails to achieve a significant difference, mainly because of higher standard deviations.

If we compare the subgroup of treated PM patients with PM advanced B Ca patients (Group C), it becomes evident that the sum of $E_1 + E_2 + E_3$ does not differ significantly from one group to another (while both appear significantly different from healthy controls). By contrast, the minor oestrogen fraction appears significantly higher in the group of patients treated with CMF, even compared to Group C, taken as controls (data not shown).

Hence the general picture which emerges suggests higher excretion levels in CMF-treated patients at an early stage of treatment, as indicated by the results of the comparison between the short-term and the non-short-term subgroups. These higher excretion levels seem to affect the various oestrogen fractions, though not to the same extent, bearing in mind that the study of PM patients shows that this increase is particularly marked in the minor oestrogen fraction and that within the latter various oestrogen metabolites seem to originate mainly from the adrenocortical function. Despite the limited number of cases there is still evidence of a bimodal expression of the steroid status, previously observed by us, both in the treated or untreated patient groups, reflected in the large standard deviations.

DISCUSSION

It has been established that conventional CMF treatment, may produce amenorrhea secondary to primary ovarian disfunction or failure and suggested that a secondary action of the cytotoxic drugs may occur "via endocrine" [12–14]. Nevertheless, to date very little attention has been directed to other aspects of possible alterations in steroid metabolism, following conventional chemotherapy (e.g. the relationship between free and bound hormones [25], modulation of carrier proteins or metabolic conversion rates), to consider only those factors which might have a marked effect on the hormone plasma levels.

Very recent studies have indicated the importance of further study of possible interactions between steroids and drugs [22–24, 26]. In fact, experimental evidence has shown how steroids interfere with the activity of cytotoxic drugs; for example, it has been observed that oestradiol has an adverse effect by causing a significant reduction in MTX antimetabolic and antiproliferative activity [22] in well-known cell lines, such as MDA-MB 436, derived from human breast cancer.

Another important factor is the observed ability of corticosteroids [23] to depress the action of CY, i.e. its antitumour effect, in mice. This adverse effect of corticosteroids is related to the enhanced metabolism of CY and might also be produced by the endogenous steroids. In this connection it might be mentioned that the first step in CY metabolism is the formation of 4-hydroxy-CY, mediated by microsomal enzyme activity linked to cytochrome P-450. The same activity is involved in the mono-oxygenation reaction of steroids and it is circadian rhythm-regulated by endogenous corticosteroids.

Although these data have been obtained from experimental models, they are particularly suggestive because, if confirmed in man, they would enrich to a certain extent the question of the interactions between steroid hormones and drugs. This was indeed one of the aims of our studies. In fact, these experimental results indicate the importance of the study of the steroid status, including its relation to possible interference by steroids with the effectiveness of drugs.

Many authors agree that there is experimental evidence of reduced oestradiol and oestrone plasma levels in PreM patients after CMF treatment [4, 17, 19, 20]. The studies we carried out indicate a considerable and significant drop in the PI values of treated patients in the PreM subgroup. Our results show a significant reduction in oestrogen excretion levels, at least in the case of the classical oestrogen, in premenopause, through the action of cytotoxic drugs. These results then, in complete agreement with previous observations by other authors [3–10, 12–17], confirm the impairment of ovarian function following CMF treatment. Our studies are also in complete agreement with the most recent observations concerning the early effects of cytotoxic drugs on both ovarian and testicular steroidogenesis [18, 19].

When the effects of short-term treatment are compared with those of longer-term treatment, our data indicate that there are no significant distinctions in the two subgroups. This suggests that modifications in patients' steroid status occur at an early stage. As regards PM patients, at least according to the indications of their oestradiol, oestrone and other steroid plasma levels, there was no evidence of any reduction in adrenocortical function, since they do not show any apparent alteration in their steroid status [19, 20] Experimental evidence, therefore, points to the conclusion that cytotoxic drugs act selectively on both ovarian and testicular steroidogenesis but not on adrenocortical or pituitary function. This is the case in both PreM and PM women. These conclusions have recently been confirmed by evaluations carried out on certain androgens such as androstenedione, DHEA, DHEA-S and testosterone, all especially

important in the biosynthesis of oestrogens. Their plasma levels were found to be insignificantly affected by CMF treatment.

In these studies which we carried out on B Ca patients undergoing CMF treatment, DHEA and DHEA-S excretion levels were found to be in agreement with previous observations, that is, they were reduced, though not to a significant extent, in comparison with excretion levels of untreated B Ca patients (data not shown). These last-mentioned data would seem to confirm that adrenocortical function is unaffected by chemotherapy.

It should not be forgotten, however, that the plasma levels of single hormones are the result of a homeostatic equilibrium, often regulated by other hormones as well, between their biosynthesis rate(s) and their metabolic conversion rate(s). This should be kept in mind when interpreting the reductions in E_1 and E_1-S excretion levels (both fractions were in this case worked out together) which we observed while comparing the two menopausal subgroups of CMF-treated breast cancer patients. Moreover, these values are significantly reduced in the subgroup of treated PM patients as compared with untreated PM patients and even reverse the previously observed trend in both breast cancer patients and endometrial cancer patients of significant increases in the excretion levels of this oestrogen fraction, particularly in PM [27, 28, 31]. One possible explanation, therefore, might be a faster metabolic conversion rate of $E_1 + E_1$-S in these treated patients. Such an explanation would be amply supported by the significant increases observed in the PM subgroup both in oestriol excretion levels and in the oestriol ratio.

Furthermore, in treated patients, there is a significant increase in the catecholoestrogen pool, above all in the PM subgroup. This increase is most evident in the fractions of 4-hydroxy metabolites, in particular 4-OH-E_1 and 4-OH-E_3, which might suggest that E_1 can be rapidly converted into 4-hydroxylated compounds. Therefore, the DHEA and DHEA-S excretion levels, which our observations showed to be reduced, though not significantly so, in postmenopause, as well as the unchanged levels observed by other authors [19, 20] in plasma androstenedione, DHEA, DHEA-S and testosterone, in PM patients treated with chemotherapy, might also be co-determined by an increased peripheral production of oestrogens, which may originate from these androgens.

On the basis of the plasma values of these steroids (data not shown) and the oestrogen and androgen excretion levels which we determined, it may be concluded that adrenocortical steroidogenesis is not imparied by the action of cytotoxic drugs, but at the same time another different action on the steroid metabolism cannot be ruled out. In fact, the oestrogen excretion levels in these PM patients appear to be significantly higher, after treatment with conventional chemotherapy, and this applies to all the different oestrogen fractions we considered: those of the classicals, the catechols and the unusual metabolites or minor oestrogens, although to varying degrees.

The effect produced by CMF treatment on steroids appears, therefore, to be a general one which might be related to: (a) reduced uptake by, or (b) higher metabolic rates in peripheral tissues (e.g. adipose and/or lymphatic). Nevertheless, in the presence of the significant increases observed in oestrogen excretion levels, which seem to be long-lasting, it would appear difficult to rule out an increase in steroid secretion by the adrenal cortex.

For this reason, a study conducted a few years ago [41] reported adrenocortical hyperplasia attributed to MTX in rats. In our studies, the levels of all the minor oestrogens (some of which, as already observed, are associated with adrenocortical hyperfunction) appear to be approximately twice as high, and this would suggest that adrenocortical function is affected.

Although B Ca tissue may produce oestrogens [42] it was shown that direct adrenocortical oestrogen secretion is substantial (much larger than adrenocortical A conversion [43]) and there is a predominantly adrenocortical contribution to PM oestrogen secretion [44].

Tissue of PM patients produced mainly E_1 [45], as yet observed. E_1 is synthesized in fact from adrenal A [46] and peripheral E_1 conversion from adrenocortical A occurs through a process catalysed by aromatase enzymes. This activity is about 3 times higher in PM than in PreM females [47]. Very recently it has been reported that E_1, but not E_2 levels, are significantly higher in patients on adjuvant CMF with recurrent disease, in spite of a significant incidence of amenorrhea and the limited incidence of obese patients in this group [48].

All these studies might through light on the controversy about use of the chemotherapy regimen sequentially to or in association with hormone therapy.

These indications need to be confirmed, above all in view of possible interference by the steroids with the effectiveness of chemotherapy. To this end, it is imperative to extend observations to a greater number of patients and analyse in a follow-up study their responsiveness to CMF in relation to their steroid status, because the bimodal expression we have already observed, particularly in PM, in the steroid patterns of these patients still appears to be marked.

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