INTRODUCTION TO CHEMOTHERAPY AND ANTINEOPLASTIC PHARMACOLOGY IN BREAST CANCER

A. ONNIS, A. GRAZIOTTIN,
M. VELASCO, M. MARCHETTI

Institute of Gynecologic and Obstetric Clinic, University of Padua (Italy)

SUMMARY

Tumor cell biology and growth-kinetics of human breast cancer are reviewed with reference to the different action mechanisms of main antitumor drugs. Particular emphasis is given to pharmacokinetics, dosage, mode of administration, effectiveness and side-effects of drugs most commonly used in breast cancer.

The importance of tumor Estrogen and Progesterone Receptor assay for the therapy planning is considered and a short review of estrogens, androgens, progestins, antiestrogens, corticoids and aminogluthetimide is presented.

The rationale for combined chemotherapy and/or hormonotherapy in breast cancer is finally discussed.

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The first results of our chemotherapy experience in gynecologic oncology were published in 1963 ([1, 2]) and a great deal of clinical experience has been made over the following years ([3, 4, 5]). The result is a range of observations, analyses and conclusions, still absolutely valid, on which the present antiblastic chemotherapy relies as far as its theoretical concepts and practical procedures are concerned.

Various individual, clinical, pharmacologic and biologic conditions govern the application of the chemotherapy and influence its effectiveness, which must obviously primarily take the tumour biologic aspect into account. Indeed, rational chemotherapy and hormonotherapy must carefully consider the tumour biologic features. The first step is identifying the caracteristics of the cell population, which grows steadily with reduced or no sensitivity to reproduction controls ([1, 6]). However, the growth-kinetics is not exponential because not all the tumour cells multiply (reproducing and increasing share in Go) and some of them are lost (by necrosis, desquamation etc.).

The tumour growth does not merely depend on the clonal multiplication but rather on the difference between that and the number of lost cells ([7]). Solid tumours have a Gompertz-type, not an exponential growth-kinetics. According to Gompertz, the growth speed keeps constant over a given period of time (doubling time). But this doubling time tends to increase alongside with the tumour age and, consequently, its mass ([8]). The tumour growth-increase thus becomes slower in time ([6]).

The medical therapy (chemotherapy, hormonotherapy) must slow down, stabilize or even make the tumour growth rate negative by increasing the sterile share or prolonging the Go phase. Prolonging the Go phase entails medium-term negative consequences by producing a quiescent cell area where subsequent therapeutical cycles bear little effect.

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On the other hand, any intervention to reduce the tumour mass (surgical, radiating, chemotherapeutical) alters Gompertz kinetics and the tumour tends to revert to an exponential growth-kinetics. Many of the quiescent cells re-enter the phase and start multiplying again. The increase in the mitotic (sensitive) cell share favours the effectiveness of a subsequent chemotherapeutical intervention ([9, 10]).

It is also worth recalling that radiotherapeutical and pharmacologic interventions always follow a primary kinetics. Each treatment cycle destroies a given percentage, not an absolute number of cells, indipendent of the population size (that is: the same dose is necessary to reduce the number of cells from 1 billion to 1 million and from 1 thousand to 1).

Radiant or chemotherapeutical interventions can only reduce, not eliminate tumours. The lower the number of cells, the greater the chances to reduce the tumour to so tiny a cell population to be destroied by the host's own factors, like immunity ([11]). The therapy effectiveness and duration depend on many factors, including the residual tumour growth-kinetics and the host's immunitary capability. The latter is however often lowered by chemotherapy and radiotherapy. The immunodepressive action of cytostatics basically stems from their toxicity for the immunocytes. This causes shortening of the latter's average life and inhibition of the delaied reactivity produced by phlogistic agents that are of particular importance in the defence against cancer.

However, the immunodepression is conditioned not only by the kind of drug but also, and particularly, by the therapeutical pattern (time/dose) ([12]). The reduction is sharper with low doses administered over a certain period than with high, single doses. Chronic administrations of oncochemotherapeutical drugs must therefore be avoided to the utmost as, in time, they compromize the immunocompetent systems ([3]).

These reflections and our clinical and test experience in the use of antitumoural chemotherapeutical drugs highlight the need for effective doses spread over a given period (to avoid the risk of single doses approaching the lethal dose) and a comprehensive therapeutical scheme envisaging the combination of different cytostatics ([3, 4, 5]).

As breast cancer (even its clinically early forms) is diagnosed when it has already become a systemic disease, the antitumoural medical therapy (chemotherapy, hormonotherapy) and the traditional locoregional therapies (surgery and radiotherapy) must obviously mutually integrate. On the basis of our 20-year clinical experience ([1, 2, 8, 13, 51]) we support an integrated medical therapy mainly relying on a removal, antagonism or supply hormonotherapy in early forms, and on the administration of cytostatics, associated both among themselves and with hormonotherapies, in advanced forms ([5, 14, 51]). The pharmacologic action of the chemotherapeutical agents is their interfering with different phases of the cell cycle ([12, 15]). When the cell is in Go, it is in a stationary, non-reproductive phase corresponding, in normal tissues, to the own production of a given tissue, different according to the DNA specifically expressed areas.

The reproductive cycle is re-entered through the G_1 phase (pre-synthetic, in which the cell synthetizes RNA and Proteins) preparing for phase S (Synthetic) entailing the synthesis of acidic proteins, histones and DNA through a process of semiconservative duplication.

In the G_2 phase (post-synthetic) the cell carries on preparing RNA and proteins some of which are particularly important to the formation of the mitotic spindle and the beginning of the prophase of the real mitosis.

Clearly, chemotherapy mainly acts in the S phase, when the DNA is synthetized, although between two subsequent

mitoses the cell actually undergoes continuing renewal by active cytoplasmatic proteic synthesis ([15]).

This explanation highlights the rational approach to the use of chemotherapeutical substances. According to their action mechanisms and pharmacologic features, they can be divided as follows: Alkilating or radiomimetic agents; Antimetabolites; Alkaloides; Antiproliferative antibiotics; Hormones.

The alkilating agents produce punctiform changes in the genetic code and act on the pre-formed DNA (they are not specific of the S phase); if bivalent, they can change the melting point between the two DNA chaines, their action thus appearing at the time of mitosis ([15]).

The antimetabolites, interfering with the nucleous acids (DNA, RNA) synthesis, specifically in the S phase, inhibit the cell growth and reduplication ([3]). These substances are accepted by the cell mebolic processes thanks to their similarity to real metabolites. But, once enclosed in the DNA or RNA they obtain their effect thanks to their dissimilarity with the normal metabolites ([3, 16]). The most evident consequences are either punctiform changes or enzymatic inactivation. Among alkaloids, those obtained from the periwinkle (Vincristine and Vinblastine) are of major interest today. They act in the M phase blocking the function of the mitotic spindle proteins.

Antibiotics include drugs obtained through bacterial synthesis, like Adriamycin, Actinomycin D, Bleomycin, Daunomycin, Mitomycin-C and Mitramycin. They all block the protein synthesis in that, by combining with the DNA, they prevent the synthesis of the messenger RNA and consequently all processes relying on it.

The most promising drugs in breast carcinoma chemotherapy are today cyclophosphamide, thio-tepa, methotrexate, 5-phloroacil, vincristine and adriamycin.

The early monochemotherapeutical experience has evolved into polychemotherapy that should satisfy the following requirements:

1) each drug must be able to act individually too;

3) the various drugs must follow different action mechanisms;

3) their phase-specificity must vary;

4) the pharmacologic action must improve without increasing toxicity, thus improving the therapeutical quotient ([1, 17, 51]).

The various drugs action mechanisms must be taken into account in coosingh polychemotherapeutical associations. The results so far reported in the literature vary very widely, probably owing to:

- inadequate patients selection;
- unprecise staging;
- differing treatment timing;
- differing evaluation of the response quality and duration as well as of the post-treatment life quality ([16]).

The use of chemotherapeutical agents also entails many negative aspects, including:

a) not always favourable indications of advisability or therapeutical quotient (relation between LD_{50}/ID_{90}, that is between toxicity for the organism and tumour inhibition) ([1, 6]);

b) development of drug-resistant cell clones (due to rectifying mechanisms, like in the case of alkilating agents ([1, 3]).

c) failure of some drugs to reach the tumour cells (large tumour masses, particular seats like the encephalon) ([1]);

d) immunitary depression with particular regard to the delaied immunitary response ([1, 3, 4, 5]).

The present trend in the use of antineoplastic drugs in breast cancer shows two main ways of intervention:

- precautional (in case of operable tumours to supplement surgery);
- palliative (in advanced cancer with systemic dissemination).

Our experience, in agreement with the literature, shows that precautional mo-

nochemotherapy is particularly effective in patients presenting minimum residual disease, that is a low number of cells, provided the administration is not too long, so as to avoid jeopardizing the patient's immunitary defence ([1, 3, 18, 19, 20, 21]). In the monochemotherapy the following drugs are particularly effective on breast carcinoma: Adriamycin, Mecloretamine, Cyclophosphamide, Methotrexate, Thio-tepa, Mitomycin-C, 5-phlorouracil. The response rate ranges between 25% and 35%, with a 38% peak for antineoplastic antibiotics that have proven to be the most effective (Adriamycin, Mitomycin-C).

Polychemotherapy offers better prospects for both the disease-free period and survival ([22]).

Bonadonna *et al.* ([23, 24]) suggested the precautional use of ploychemothepy in oeprable breast cancers, in Italy.

The polychemotherapeutical administration scheme envisages: Cyclophosphamide (100 mg/m^2 p.o. from the 1st to the 14th day); Methotrexate (40 mg/m^2) i.v. on the 1st and 8th day); 5-phlorouracil (600 mg/m^2 i.v. on the 1st and 8th day) with a 2-week rest beween the various cycles. The results are statistically significant for women in pre-menopause.

A palliative treatment protocol is at present followed throughout Italy which includes various different combinations: CMF in doses as above described; CMFVP, that is adding vincristine and prednisone (30 mg/^{2}m p.o. between the 1st and 14th day); AV (Adriamycin and Vincristine); CAF (Cyclophosphamide, Adriamycin, 5-phlorouracil); AC (Adriamycin, Cyclophosphamide) (FONCAM) ([25]).

The Adriamycin-Cyclophosphamide association has proven the most effective in the management of disseminated carcinomas. Polychemotherapy reduces the metastases for a limited period only (6-18 months) and in no more than 85% of the patients. At three years from the outset of the palliative polychemotherapy the disease management is achieved in 15% of the patients only, and at 5 years the survival rate is below 5% ([26]).

We support the use of a precautional medical therapy as the disease is already systemic in its early clinical stage too. But in these cases we prefer an hormonotherapy that present less serious side-effects than cytostatic chemotherapy, which we would rather mainly use in advanced forms.

About 1/2 of women's and 1/3 of men's tumours originate from tissues whose proliferation and differentiation equilibrium is sensitive to the central and/or peripheral changes of the hormonal control ([27]).

The natural history of the hormonosensitive neoplasias is highly conditioned by hormones. Beside their "initiation" action, that has already been demonstrated in some particular tumours, they play an important role in the 'promotion' and 'progress', that is in determining and maintaining biochemical conditions of continuing proliferation, often associated with the appearance of malignant clones.

The endocrine environment can be altered by supplying or removing hormonal stimuli to manage proliferation and differentiation. However, the cell dependence on the hormonal regulation progressively lowers as the tumour anaplasia increases. The hormonotherapy can have a good biologic basis in the hormonal receptors, which provide criteria for selection, therapeutical indications and prognosis. Hormonosensitive cells (target cells) host receptors for many steroids and polypeptide hormones, located in the cytoplasm or the membrane, respectively. Oestrogen receptors have appeared as the most important in breast carcinoma. Depending on the quantity of receptors, (fentomoles/mg of protein) the tumour is referred to as positive receptor (R+), negative (R−) or borderline. For instance, Mac-Farlane distinguishes among tumours, according to oestradiol, ER+ (10

fmoles/mg), ER borderline (3-10 fmoles/mg), ER— (0-3) fmoles/mg).

The assay of progesteron receptors (PR) provides further differentiation of the tumours eligible for hormonotherapy.

A precise correlation between the tumour ER absolute levels and the response to the hormonal treatment, though claimed by some Authors ([28]), has not yet been univocally demonstrated. In fact, other Authors refer to tumours of differing degrees of hormonal dependence ([29]).

From 48% ([30]) to 55% ([31]), with a maximum of 60% ([32]) of ER+ breast tumours are reported to respond to hormonal treatment. Conversely, ER— tumours reported percentages are very low: 16% ([30]); 8% ([31]); 9% ([32]).

Our experience does not yet allow us to report percentages which demand sufficiently large and homogeneous groups of patients and adequate periods of observation to be reliable. We can nevertheless say that the protocols of pharmacologic and hormonal polytherapy have achieved significant and valid results in ER+ tumours, even in some advanced forms that had escaped the therapeutical control of other pharmacologic protocols of traditional therapies. The results are promising in ER+ PgR+ tumours.

The methods to evaluate the hormonal receptors and the widening of the studies on the neoplastic cell biology have triggered an extremely interesting and promising phase of experimental and clinico-practical analysis aiming at casting light on the cell proliferation and differentiation profiles' metabolism and hormonal regulation ([33]).

Hence, the present hormonotherapy protocols in breast cancer, following two guidelines:

– supply hormonotherapy (administration of natural or synthetic substances);

– removal hormonotherapy (surgical, radiologic or chemical suppression of the function of some endocrine glandes).

The following main mechanisms have been suggested to explain the action of the endocrinotherapy:

– removal m.: the ablation of ovary, suprarenal and hypophysis deprives the tumour of its oestrogen supply. This would explain why the response to the ablation endocrinotherapy is correlated with the tumour level of oestrogen receptors;

– antagonism m.: androgens and anti-oestrogens compete with oestrogens for the receptor sites, thus inhibiting the oestrogen-dependent cell prolipheration;

– maturation m.: high doses of oestrogens or progesteron cause greater differention in some cases of breast carcinoma (like in endometrial carcinoma);

– immunologic m.: high doses of oestrogens increase the activity of the reticulo-endothelial system on the tumour.

In the oestrogen chemotherapy with dietil-stilbestrol (15 mg/d), etinilestradiol (1-3 mg/d), conjugated natural oestrogens (15 mg/d) in three administrations), about 30% of the women in post-menopause respond to the treatment and the average remission lasts 12-16 months, up to 5 years occasionally.

This therapy achieves its maximum effect in patients in menopause with slowly developing tumours and metastases to soft tissues and lungs.

Side-effects must be taken into account and managed.

In case of relapse, about 30% of the previously responsive patients show tumour regression after the complete suspension of the treatment, this withdrawal regression has however a limited duration ([34]).

On the other hand, pre-menopause patients presenting rapidly developing tumours with negative oestrogen receptors and metastases to the liver and the central nervous system, respond rarely.

Antioestrogens (Clomiphene, Nafoxidine, Tamoxifene) are substances – not necessarily steroidal – which can decrease

the oestrogen specific uptake by target tissues, both *in vivo* and *in vitro*.

The main therapeutical interest of these susbtances is their ability to dislocate the 17-β estradiol.

Antioestrogens compete with oestrogens for the oestrogen receptor sites producing a compound which is rather inadequate to stimulate the cell growth [35]. The initial action of Tamoxifen on the 17-β estradiol is probably the same as that of the hormone. But the former rapidly loses its ability to resist a high receptor concentration in the cytoplasm. The initial decrease in the cytoplasmatic receptor sites, due to the common nuclear translocation of oestrogens and Tamoxifen, in the case of Tamoxifen is not followed by resaturation of the cytoplasmatic receptors, as it is in 17-β estradiol.

The initial nuclear translocation by Tamoxifen is probably responsible for the initial oestrogenic effect of this substance. The subsequent depletion of plasma 17-β estradiol receptors prevent any further oestrogenic action. This could explain the antitumoral effect of Tamoxifen and other similar compounds [36].

The effects of the antioestrogens also include the blockage of prolactin secretion through receptor competition in the hypothalamus [37].

Antioestrogens are reported to obtain favourable results in 30% of the ER+ advanced cases, with moderate complications.

- Citrated clomiphene (100-250 mg/die/os)
- Nafoxidin (180-270 mg/os)
- Tamoxifen (20-40 mg/die/os)

are commonly used.

Nafoxidin and Tamoxifen have the same effectiveness.

The response rate varies between 22% and 45% with a duration of 9-12 months on the average, occasionally reaching 4 years. Unlike Nafoxidin, Tamoxifen is virtually free from major toxicity.

Androgens too can still be of some interest: testosteron propionate has now been replaced by phlossimesteron (30 mg/die p.o.) and nandrolone (50-100 mg, intramuscular once a week). 10-25% of the patients presenting disseminated cancer and mainly bone involvement respond favourably. In 70-80% of the patients, pain due to bone metastases is also successfully controlled [38].

30% of the post-menopause patients, previously responsive to the oestrogen treatment, respond favourably to a subsequent administration of androgens. However, significant side-effects have been observed: hypercalcemic crises, colostatic icterus, virilization, acne, libido increase. Conversely, there is a favourable effect on mood, with feeling of euphoria and well-being.

Some patients dislike the virilization process to such an extent that the treatment becomes worse than the disease [39].

Progestinics are particularly significant in the current hormonotherapeutical protocols: acetated nor-ethisteron (60 mg/d in three administrations) and acetated medroxy-progesteron (in doses varying according to the various Authors: 1-2 mg/d intramuscular or 0,1 g p.o. three times a week). The different doses might entail different action mechanisms. The response, with objective regression, is observed in 30% of the post-menopause patients presenting positive oestrogen receptors and metastases to soft tissues.

In the absence of liver metastases progestinics prove useful in the treatment of post-menopause patients presenting progression of the disease after Tamoxifen [40]. Responses last 7 months on the average.

Side-effects, that are negligible with low doses, grow more serious with higher doses: hepatotoxicity reaching even colostatic icterus, thrombophilia, facies cushingoide.

Recently the use of Danazole (300-600 mg/d in three times) has been suggested in

the treatment of post-menopause patients presenting disseminated cancer ([41]). Regression lasts about 7 months and occurs in 27% of the patients taking this drug for at least 6 weeks.

There are moderate side-effects including nausea, lethargy and hot flushes. However, the use of this drug needs further consideration.

The removal hormonotherapy can be based on the following scheme of surgical or pharmacologic interventions:

– Surgical or radiant castration, with similar clinical results ([42]). We favour surgical castration which ensures a much more rapid lowering of hormonal levels than radiant castration. Castration is the elective adjuvant treatment given its circumscribed side-effects and the duration of remission.

The response is more favourable in pre-menopause patients with ER+ and at an early stage of the disease (precautional removal hormonotherapy).

In advanced tumours (bone metastases, to soft tissues or to lymph-nodes) the average favourable response, lasting 9-16 months, is about 29%, ranging from 25% to 35% (palliative ablation endocrinotherapy) ([43]).

Suprarenectomy and hypophysectomy have now become obsolete in surgery, owing to the possibility of less damaging and equally effective pharmacologic intervention. However, both surgical and pharmacologic suprarenectomy and hypophysectomy are indicated in patients who have favourably responded to previous hormonal therapies. The literature reports remissions up to 1-2 years in 35% of the cases.

However, surgical suprarenectomy entails high morbidity (20-40%) and mortality (12-25%) ([44]) and the need for substitutive treatments with gluco or mineralcorticoides (in personalized doses to prevent addisonian crises).

Hypophysectomy gives equal results with a far lower morbidity and mortality (1.2-7%) ([44]).

Chemical suprarenectomy can be obtained by using glucocorticoides or, more recently, aminoglutethimide.

In early cases the glucocorticoide treatment (desametazone 1-1.5 mg/die p.o.) must precautionally start at one month from the ovariectomy to inhibit the suprarenal oestrogen production.

In advanced cases, glucocorticoides (prednisone 200 mg/d or desametazone 12-16 mg/die) obtain remission in 24-48% of the cases, for 1-3 months (palliative therapy) ([45, 46]).

Today aminoglutethimide tends to replace the glucocorticoides. The latter are still- first-choice drugs in the treatment of particular conditions, like hypercalcemia (either spontaneous or iatrogenic) following oestrogen or androgen treatments in patients presenting bone metastases; in endocranial hypertension due to cerebral metastases or in respiratory insufficiecy due to neoplastic lymphangitis ([40, 47, 48]).

The inhibition of the suprarenal steroidogenesis by aminoglutethimide, incidentally discovered, is of great interest. Originally aminoglutethimide was used as anticonvulsant and often caused addisonian crises.

Aminoglutethimide blocks the desmolase enzyme which converts colesterol into pregnenolone, thus blocking the subsequent steroidogenesis and causing what is .known as 'medical suprarenectomy'. Furthermore aminoglutethimide acts on oestrogen production through an extra-suprarenal effect.

The administration of aminoglutethimide increases androstendione plasma levels while estrone and estradiol plasma levels simultaneously decrease ([49]). This effect is ascribed to the blockage of the microsomial aromatases by aminoglutethimide; which causes the peripheral inhibition of the estrone and androstendione aromatization.

Increased estrone metabolism and decreased androstendione clearance are less likely.

Originally, the administration of aminiglutethimide was associated with 0.75 mg/die of desametazone. The result was unsatisfactory suprarenal inhibition due to the pharmacologic interaction of aminoglutethimide and desametazone that speeded up metabolism and reduced the availability of desametazone.

Conversely, hydrocortisone and acetated cortisone have shown no interference with aminoglutethimide. They must therefore be associated with the latter to prevent addisonian crises. The current therapeutical scheme envisages the association of 1 mg/die of aminoglutethimide in 4 fractionated doses; acetated cortisone (25 mgx 2/die) and phlorocortisone (0.1 mg every second day) ([41, 50]).

Aminoglutethimide obtains complete or partial responses in 30-35% of the cases, for 12-18 months on the average, in postmenopause patients, presenting ER+ tumours, even though with metastases to soft tissues. It is also successful in bone pain. Its side-effects are lethargia (47%), cutaneous rushes (20%), nausea (14%), ataxv (8%) ([41]).

Chemical suprarenectomy by aminoglutethimide in reversible and causes fewer and less serious side-effects than glucocorticoides.

With regard to the role of chemotherapy in breast carcinoma we can draw the following conclusions:

a) In case of apparently localized neoplasia, the therapy to supplement the surgical intervention must be chosen according to the lymph-node and receptor conditions. In N−ER+cases (more favourable prognosis) the surgical therapy must be supplemented by removal (ovariectomy) and supply (MAP+Tamoxifen) hormonotherapies.

In N−ER− cases, given the greater aggressiveness of ER− neoplasias, the surgical therapy must be supplemented by a polychemotherapy which, according to our experience, should follow the CMF or AC scheme (Adriamycin + Cyclophosphamide).

In N+ER+ cases we supplement the surgical therapy by a combined polychemotherapeutico-hormonal treatment (ovariectomy-MAP-Tamoxyphene-Aminoglutethimide-CMP or AD) in cycles.

N+ER− patients (most unfavourable prognosis) need an aggressive antitumour polypharmacotherapy.

It is worth recalling here that breast cancer must today be treated in a multidisciplinary way, not just with often useless mutilating interventions. Surgeon, radiotherapist, chemotherapist, endocrinologist and clinical pathologist must co-operate. Breast cancer can no longer be treated in centers that cannot thoroughly study the receptor conditions.

When the tumour receptor situation is known, whether in pre- or post-menopause age, we believe it advisable to undertake precautionary removal hormonotherapy (ovariectomy), given that 1 out of 3 N− patients too show systemic relapse at 10 years.

In case of already disseminated cancer, in ER+/PgR+ and ER+/PgR− patients we advocate combined hormonal and pharmacologic polychemotherapy, in cycles, according to the explained principles and protocols.

In ER−/PgR− cases, cycles of pharmacologic treatment using the reported associations are the only possible palliative therapy.

When the analysis of the metastasis receptors is not possible, the main metastatic seat can provide indications. The primary involvement of bones and soft tissues anticipates a good response to the hormonal treatment.

On the other hand, visceral metastases are more frequent in aggressive, hormonoresistent forms, and therefore demand chemotherapy.

In conclusion, a rational chemotherapy relying on precise pharmacologic and hormonal bases can be a valid therapeutical instrument (in association with the traditional surgical and radiant therapies), as, in practice, this neoplasia is always a systemic disease.

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