IMMUNOSUPPRESSION IN IRRADIATED BREAST CANCER PATIENTS: IN VITRO EFFECT OF CYCLOOXYGENASE INHIBITORS*

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LOCAL radiation therapy is widely used as an adjuvant to surgery in the treatment of primary breast cancer. Prospective randomized trials have shown that this treatment prevents the development of local metastases, but does not seem to reduce the development of distant metastases or prolong survival of the patients. Since the radiation therapy may cause a profound

*Presented as part of a *Symposium on Combination Therapies: New and Emerging Uses for Cyclooxygenase Inhibitors, Calcium Blockers, and Biological Response Modifiers on Immunity*, held by the Section on Medicine of the New York Academy of Medicine and the George Washington University School of Medicine and Health Sciences at the Essex House, New York, N.Y. June 2 and 3, 1988, and supported in part by an educational grant-in-aid from The Aspirin Foundation of America, Inc.

This work was supported by the Cancer Society in Stockholm and the Swedish Medical Research Council (03X-5914 and 03P-6396)

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lymphopenia, we investigated in detail the changes in the immune system that occur after irradiation for breast cancer. In addition, we wanted to examine if there is any relationship between prognosis of the patients and the extent of irradiation-induced immunosuppression.

CHANGES IN THE IMMUNE SYSTEM

Our studies confirmed that local irradiation therapy for breast cancer (45 Gy) causes a severe lymphopenia with reductions of both T- and non-T-lymphocytes.[1] The latter cell population was depressed to the highest relative extent but its recovery was more rapid, within six months.[1] Recovery of the T-cell population appeared to proceed much more slowly, and recent studies have shown that it is still incomplete 10-11 years after irradiation therapy. A closer analysis of the T-cell population, using monoclonal antibodies directed against different subsets, has revealed that the prolonged T-lymphopenia is due to an extremely slow repopulation of T-helper cells.[2] The T-suppressor subset, however, exhibited a comparatively rapid recovery.

Several lymphocyte functions, measured on a cell-for-cell basis in vitro, were also found to be changed after irradiation. Pokeweed mitogen-induced production of immunoglobulins (Ig), in particular IgM, was sharply reduced after irradiation[1] as well as mitogenic responses of lymphocytes to polyclonal mitogens and specific antigens such as purified protein derivative of tuberculin (PPD) and allogenic cells.[1]

Impaired lymphoproliferative responses observed following irradiation were found to be due to nonspecific suppressor cells with characteristics of monocytes, suggesting that radiation-activated monocytes become nonspecifically immunosuppressive.[3,4] We could also demonstrate that irradiation therapy increased the oxidative metabolism of the individual monocytes as measured by capacity to reduce nitroblue-tetrazolium.[5]

Since activated monocytes are known to produce a number of arachidonic acid metabolites of both the cyclooxygenase and lipoxygenase pathways, we examined in more detail the role of monocytes in immunosuppression after irradiation and to determine whether this suppression could be explained by an increased biosynthesis of prostaglandins. Some of these are known to be strongly immunosuppressive. These in vitro studies showed that an inhibitor of cyclooxygenase, indomethacin, could partly inhibit the radiation-induced suppression of mitogen responses. A similar effect was observed when silica was added to the cultures.[6] Thus, addition of 1.0 μg of indomethacin per ml or of silica could restore the phytohemagglutinin and the PPD-response by approximately 0.25 $\log_{10}$ units.[6] This strongly suggests that the suppressive

activity in this experimental system is, to some extent, mediated by monocytes and prostaglandins. Results could be interpreted in two ways: either the biosynthesis of immunosuppressive prostaglandins by monocytes is increased after irradiation therapy or the sensitivity of the lymphocytes to prostaglandins is increased.

To test the latter possibility, the sensitivity of the phytohemagglutinin response of blood lymphocytes to different concentrations of prostaglandins E_2 and D_2 was examined in 15 patients with breast cancer before and up to six months after local radiation therapy (45 Gy). The results demonstrated that the sensitivity of the response was not significantly changed after treatment, suggesting that the immunosuppression observed after irradiation is at least partly due to an increased production of prostaglandins rather than an increased prostaglandin-sensitivity of lymphocytes.[7] The conclusion drawn from these studies is that local radiation therapy for breast cancer not only causes a long-standing lymphopenia but also activates monocytes to increased biosynthesis of prostaglandins which suppress immunological responses of T-cells. For this reason, we screened the effects of some cyclooxygenase products on different lymphocyte functions. It was demonstrated that prostaglandin D_2 reduced the expression of CD8-antigen (suppressor/cytotoxic phenotype) and of Fc-receptors for IgG. Prostaglandins A_2, D_2 and E_2 inhibited the phytohemagglutinin response in vitro. Natural killer cell-activity was reduced by prostaglandins D_2 and E_2.[8]

Experiments were performed to examine more systematically the effects of various inhibitors of cyclooxygenase on the mitogenic responses of nonpurified lymphocyte preparations to different concentrations of phytohemagglutinin. Although the results varied substantially between different tests, our general conclusion was that all the inhibitors tested, i.e., indomethacin, meclofenamic acid, and lysin-mono-acetyl salicylate may, to varying extents, increase the mitogen responses of lymphocytes from control individuals when present in the cultures at concentrations ranging from 10^{-7} to 10^{-5}M.[9] This stimulatory effect, most frequently observed in the presence of indomethacin and meclofenamic acid, occurred at all phytohemagglutinin-concentrations employed (0.1-3.0%). On a relative basis, however, the increases were most pronounced at suboptimally stimulatory concentrations.

To examine the role of monocytes, experiments were performed in which nonpurified and purified lymphocyte preparations from the same donor were stimulated with 0.3% of phytohemagglutinin (a suboptimal concentration) in the presence of various inhibitors. The following conclusions could be drawn from these experiments. Responses varied substantially between different

donors but within each experiment stimulations were always highest with purified lymphocyte preparations. The presence of inhibitors of arachidonic acid metabolism could, to varying degrees, increase mitogen responses of nonpurified lymphocyte preparations. Such an increase was not clearly observed when preparations of purified lymphocytes were used.[9] This suggested that monocytes and not lymphocytes were the main producers of immunosuppressive metabolites of arachidonic acid. However, we cannot exclude a role for thrombocytes in this context, as the cell preparations were contaminated to some degree by thrombocytes.

Relationship between Prognosis and Immunological Reactivity after Radiation Therapy

During the years 1971-1974 we examined the phytohemagglutinin and PPD responses of blood lymphocytes from 114 breast cancer patients who received local irradiation therapy (45Gy) as an adjuvant to surgery. They were included in a prospective randomized trial aiming at determining the clinical value of pre- or postoperative irradiation. Mitogen responses of blood lymphocytes were determined before radiation therapy and at various time intervals after its completion. After a clinical follow-up period of 10-13 years, we analysed for any relationship between mitogen responses and survival of the patients. There was no detectable association between the initial phytohemagglutinin and PPD-reactivity and survival of the patients.[10] On the other hand, mortality was significantly higher for patients with low postirradiation reactivity. Furthermore, patients with the most rapid recovery of these responses had better survival. The prognostic relevance of lymphocyte reactivity after irradiation could not be explained by clinical stage.[10]

A comparison between the observed and "expected" number of deaths for patients with stimulation values higher and lower than the median value, respectively, demonstrated that mortality was significantly higher for patients with lower reactivity at completion of radiotherapy. The difference between the two groups of patients was statistically significant up to eight years after radiotherapy. The same trend was noted for the whole period of observation, although the differences were no longer significant. However, patients with higher PPD reactivity six to 10 months after radiotherapy had a significantly higher survival rate during the whole observation period, which was not the case with phytohemagglutinin reactivity.

The prognostic relevance of PPD reactivity at completion of radiotherapy could be demonstrated both for patients with involved axillary lymph nodes and those without. On the other hand, PPD reactivity at six to 10 months after

postoperative irradiation was negatively correlated to the initial local extension of the tumor (clinical stage) ($P = 0.02$). To further elucidate the relation of PPD and phytohemagglutinin reactivity to prognosis regardless of the clinical stage, a log-rank analysis of survival was performed in which both preoperatively and postoperatively treated patients with low and high PPD reactivity at the completion of radiation were stratified for the stage. This analysis demonstrated a higher survival for patients with higher PPD reactivity during the first 8 years of the observation period ($P = 0.047$) also after clinical stage has been considered. The same trend, although not significant, was observed for the prognostic relevance of phytohemagglutinin reactivity ($P = 0.08$).

Conclusions and Hypothesis

Adjuvant local irradiation therapy for breast cancer is of clinical value since it reduces the development of regional recurrences. However, it does not seem significantly to improve survival of the patients. This may be because the treatment sharply reduced the size and several functions of the lymphocyte pool of the body, i.e., the natural immune defense against residual cancer cells may be weakened. If true, the beneficial effects of the radiation treatment may be overshadowed by its detrimental effects on the immune system. We believe that the clinical value of the treatment can be improved if the radiation-induced suppression of lymphocyte responses, which correlate to survival, are brought down to a minimum. Since this can be achieved in vitro with cyclooxygenase inhibitors it is possible that this can also be achieved in vivo with such drugs.

Goal of our Work

It would be interesting to launch a prospective randomized trial in which patients with operable breast cancer receive an inhibitor of prostaglandin synthesis or placebo tablets during radiation therapy. Clinical follow-up of the patients will reveal if there are any significant differences in disease free and overall survival of the two patient groups.

Choice of Drugs and Dose Schedule

There are several drugs such as meclofenamic acid, acetylsalicylic acid, aspisol, indomethacin, and diclofenac sodium that inhibit prostaglandin synthesis. To choose the most suitable drug to be used in such a study, we compared various inhibitors of cyclooxygenase inhibitors with respect to their capacity to revert a radiation-induced suppression of mitogen response.

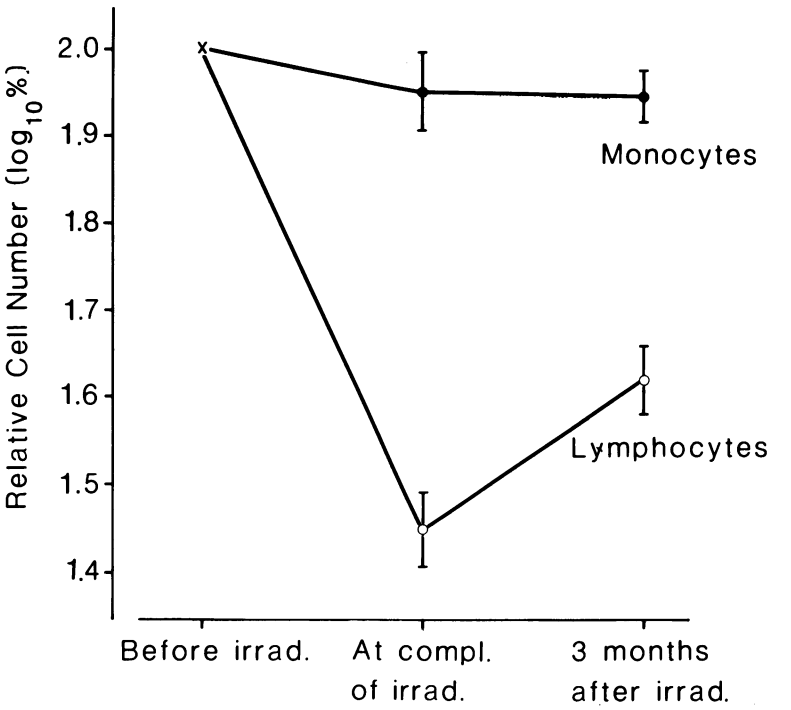

Fig. 1. Lymphocyte and monocyte counts in irradiated breast cancer patients

Twenty consecutive female patients with breast cancer treated postoperatively with local irradiation therapy (45 Gy) served as lymphocyte donors in this study. The lymphocytes decreased markedly following radiotherapy and recovered only partially three months after treatment (Figure 1). There was no change in the patients' monocyte counts (Figure 1). The relative phytohemagglutinin response of patients' lymphocytes was likewise reduced in a pronounced manner and only partially restituted three months after irradiation (Figure 2). These results were in complete agreement with our previous findings mentioned earlier in this report. The relative response of patients' lymphocytes before the irradiation treatment could be substantially elevated by all cyclooxygenase inhibitors tested, i.e., diclofenac Na, meclofenamic acid, aspisol, and indomethacin as compared to ethanol which

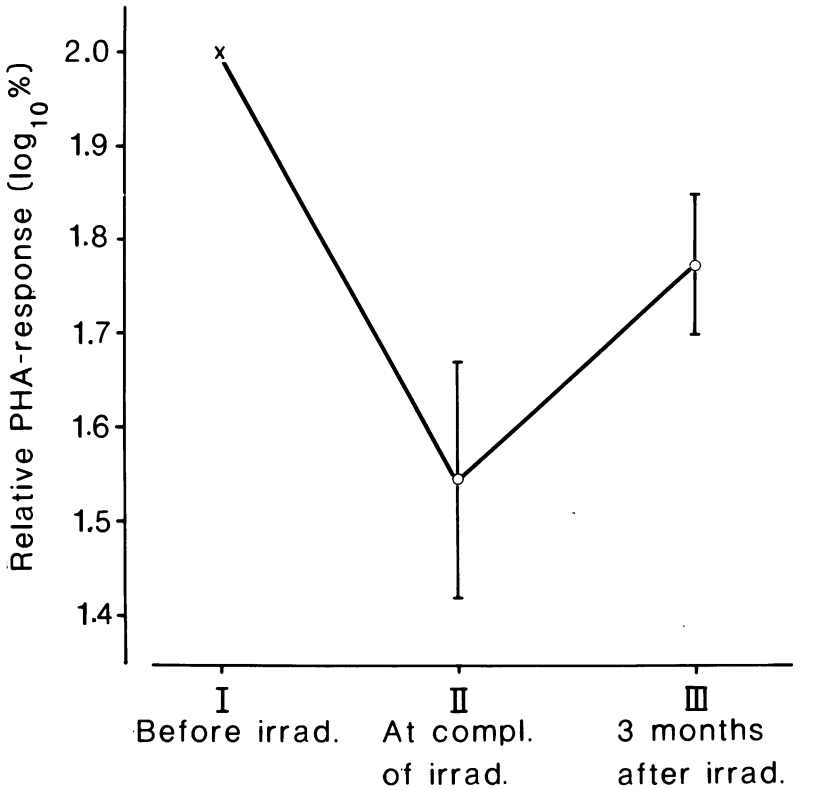

Fig. 2. Relative phytohemagglutinin reactivity in irradiated breast cancer patients

was used as solvent for some of these substances (Figure 3). Diclofenac Na followed by indomethacin were most potent in lowest molar concentrations, and aspisol was active only in rather high doses.

When examining lymphocytes at completion of irradiation the effects were the same although more pronounced (Figure 3). Also in these experiments diclofenac Na and indomethacin in low concentrations increased the relative phytohemagglutinin reactivity to the highest degree. At three months after irradiation the results were very much the same (Figure 3).

The conclusion reached as a consequence of the above study was that diclofenac Na in the first place and indomethacin in the second should be examined in vivo in irradiated breast cancer patients. However, it cannot at present be excluded that also meclofenamic acid and aspisol should be tested. Another four inhibitors are currently being tested in the same system. The patients will receive daily oral doses of the drug during the entire treatment period. Some patients will continue treatment with the inhibitor another two

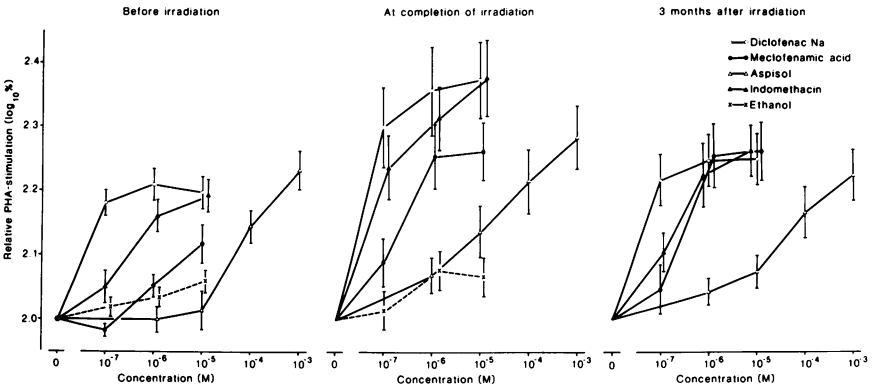

Fig. 3. Phytohemagglutinin response of lymphocytes from irradiated breast cancer patients in the presence of varying concentrations of cyclooxygenase inhibitors

months, i.e., during the early recovery phase of the lymphocyte population. The doses of the drug will be adjusted according to the results of the following laboratory tests, which will be performed before, during, and after irradiation.

The results of these studies will be of primary interest for the design of our prospective randomized trial in breast cancer. The tests will guide us in the choice of inhibitor, dose and dose schedules and time period for treatment.

SUMMARY

We have documented in previous studies that local irradiation therapy for breast cancer caused severe lymphopenia with reduction of both T and non-T lymphocytes. Non-T cells were relatively more depressed but recovered within six months. The recovery of T cells, on the other hand, remained incomplete 10-11 years after irradiation. Several lymphocyte functions were also severely impaired. An association was found between prognosis and postirradiation mitogen reactivity of lymphocytes from these patients. Mortality up to eight years after irradiation was significantly higher in patients with low postirradiation phytohemagglutinin and PPD reactivity. The radiation induced decrease in mitogenic response seemed mainly to be caused by immunosuppressive monocytes, which suggests that the underlying mechanism might be mediated by increased production of prostaglandins by monocytes. For this reason we examined the effect of some cyclooxygenase products on different lymphocyte functions and found that prostaglandins A_2, D_2, and E_2 inhibited phytohemagglutinin response in vitro. Natural killer cell activity was also reduced by prostaglandins D_2 and E_2.

The next step was to examine various inhibitors of cyclooxygenase in respect to their capacity to revert irradiation-induced suppression of in vitro mitogen response in lymphocytes from breast cancer patients. It was demonstrated that Diclofenac Na (Voltaren), Meclofenamic acid, Indomethacin, and lysin-mono-acetylsalicylate (Aspisol) could enhance mitogen responses both before and after radiation therapy. This effect was most pronounced at completion of irradiation. On a molar basis, Diclofenac Na was most effective followed by Indomethacin, Meclofenamic acid, and lysin-mono-acetylsalicylate.

The clinically beneficial effects of irradiation might be overshadowed by its effects on the immune system. If true, the value of treatment could be improved if radiation-induced suppression of lymphocyte response, which correlates inversely to survival, is reduced. Since such an effect can be achieved in these patients with cyclooxygenase inhibitors in vitro it is possible that it can be achieved also in vivo.

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