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*Commentary*

# Cytological Evaluation of the Effectiveness of Cytostatic Therapy in Leukemia

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(A COMMENT ON: CLARE MG, TAYLOR JN, BLAIN E, JONES WG. The quantitation of sister chromatid exchanges in lymphocytes of cancer patients at intervals after cytotoxic chemotherapy. *Eur J Cancer Clin Oncol* 1983, **19**, 1509–1515.)

DURING the past 10 yr considerable progress has been observed in the treatment of acute leukemias (AL). In childhood acute lymphoblastic leukemia (ALL) remission induction is achieved in more than 80% of the cases; conversely, it is about 50% or less in adult acute non-lymphocytic leukemia (ANLL) [1]. Recently the role of bone marrow transplantation in the cure of ANLL of adults has been emphasized, but at the present time its wide application is being hindered by the age limit of the leukemic patients as well as by the limited number of HLA-matched donors [2]. Therefore chemotherapy remains the therapy of choice for most of our leukemia patients.

Advances in chemotherapy may come from the development of new anticancer agents and the better use of the existing ones. Since it is a recognized fact that new combinations of existing antileukemic drugs can lead to a high complete remission rate in adults [3], there is renewed interest in the prediction of drug response in AL. The stratification of the patients according to prognostic categories is based on cytomorphology, cytochemistry, cell surface markers and cytogenetics, but it is clear that AL cells have a different sensitivity to the different drugs and this is not exclusively related to the different cytological and cytochemical subtype characteristics [4] or pretreatment factors (age, the presence of Auer rods, temperature, liver size, history of an antecedent hematologic disorder etc.) [5]. This supports the necessity to individualize the treatment in acute leukemias.

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Various systems to study the drug sensitivity of the leukemic cells are in experimental use, including the tumor stem cell assay [6] and the labeling index perturbation test [7], but at the present time they do not offer a guideline to alternate therapy. The shortcomings of these tests were recently critically reviewed [1].

Since almost all the antineoplastic agents currently used in the treatment of malignancies primarily attack the DNA, the study of the DNA–cytostatic interaction in cancer patients is an alternative approach to the problem. The recent development of the sister chromatid exchange (SCE) technique introduced by Latt [8], which was further investigated by Perry and Wolff [9], allows a rapid cytological analysis of the DNA damage caused by various mutagens [10] as well as the detection of the characteristics of the DNA–cytostatic interaction in man [11].

Clare *et al.* stress the possibility of using the SCE technique to tailor chemotherapy of malignant disorders in an individualized fashion in a paper appearing in this issue of the journal [12]. The method so far has proved to be a valuable tool to detect the clastogenic effects of various S-phase-dependent cytostatic agents in man; furthermore, it seems to have potentialities for better determination of the syndromes in which a DNA repair alteration exists. Some cytostatics, especially those alkylating the DNA or cross-linking with it, cause a long-lasting elevation in the frequencies of SCE in the peripheral blood lymphocytes, whereas other agents such as the spindle poisons or base analogs do not induce SCEs or perhaps do so for a very short period of

time [11]. Thus the action of the cytostatics can be characterized by the alteration of the SCE. Since there are considerable inter-individual differences in the effect, it is logical to try to use the SCE technique to characterize the DNA–cytostatic interaction in patients under treatment or during the pretreatment period. The DNA–cytostatic interaction is of crucial importance for the effectiveness of the cytostatic therapy; therefore the SCE technique seems to be potentially suitable as a prediction test of therapeutical sensitivity. Clare *et al.* [12] show that the exposure of lymphocyte cultures *in vitro* to plasma containing a drug from the same patient obtained immediately after its administration and at various intervals after beginning of chemotherapy results in a dose-dependent SCE elevation. In this case the alteration of the frequencies of the SCEs is clearly due to the active metabolites of cyclophosphamide present in the treated plasma at various intervals after the treatment.

Previously, Kram *et al.* [13] concluded that the SCE elevation caused by mitomycin C *in vitro* in mouse is comparable to that caused by the same dose of cytostatic *in vivo*; therefore the SCE elevation can be used to calculate unknown doses of cytostatics active *in vivo*. In an attempt to mimic the *in vivo* situation as much as possible with regard to the availability of active metabolites at therapeutical concentrations, we found that the SCE level in phytohemagglutinin-stimulated lymphocytes of ara-C-treated patients was in correlation with the clinical response of the patients to the given cytostatic. Plasma taken from leukemic patients after ara-C administration at short intervals (10, 30, 90 and 120 min) induced SCE elevations with varying inter-individual differences. Patients whose treated plasma was able to induce an SCE elevation 90 min after the administration of ara-C showed a more favorable response to the therapy than those who failed to do this. The presence of active plasmatic metabolites of ara-C actually available for the biotransformation of the cells should be determined by the differences in the individual metabolism of the drug, and the SCE levels reflect this biological event [11].

Two important aspects of these investigations must be stressed: (i) blood samples taken from tumor patients during the cytostatic therapy will give the so-called treated plasma in which metabolites of the cytostatic(s) are present and reflect the characteristics of human metabolism and the dynamics of degradation (pharmacokinetics) of the drug(s). Consequently, (ii) a dose–response relationship between the dose of the administered cytostatic and/or the active metabolites and the SCE level may be expected.

Therefore the SCE assay in its premature form may be suitable to reveal inter-individual differences of drug metabolism by determining the changes in the SCE level with regard to the effective concentration of the active metabolites of the cytostatic(s) in the plasma which readily reacts with the DNA.

However, there are problems: (i) it has to be kept in mind that, in treated patients, the alteration of the frequency of the SCE in the peripheral blood lymphocytes will also be influenced by damage in the DNA of the cells caused by the preceding courses of cytostatic treatment; and (ii) in its present form the test system will not give any information on the sensitivity of the leukemic or tumor cells to the cytostatics. For this reason the leukemic cells or the cells from solid tumors should be cultured, which may make the technique somewhat more difficult.

The individualization of the treatment for the patients is a great challenge for all those who are involved in the chemotherapy of leukemic patients. The prediction of drug response is not only to select drugs or combinations to which the patient's malignant cells are extremely sensitive but also to detemine the patients with potential adverse reactions to certain drugs. It has been established that ataxia telangiectasia patients with lymphoma or ALL are abnormally sensitive to irradiation, spindle poisons and bleomycin [14]. Heterozygotes for this condition, amounting to about 2% of the whole population [15], may behave similarly. Thus a selection of these patients even before the beginning of cytostatic therapy would be desirable. Perhaps unexpected marked differences in the SCE response may point in this direction, as has been demonstrated in the *in vitro* conditions [14].

The great advantage of the SCE assay is that it takes into account a major variable of at least some cancer chemotherapeutic agents, i.e. the biotransformation of the active metabolites which may occur *in vivo* before entry into the tumor cells. Thus, by sampling the patients at various intervals after the initiation of the cytostatic therapy, the alteration of the SCE level may reflect an *in vivo* situation, i.e. the complexity of the DNA–cytostatic interaction. The SCE assay in the lymphocyte system is readily available for this purpose and seems easy to perform. The assay is ready for evaluation in 3 days and requires about 8 hr of work by a technician. The culturing time can be shortened by maintaining successive lymphocyte cultures to which the patient's treated plasma is added during the last 24 hr, the time needed for the SCE phenomenon to arise. However, further clinical and experimental trials

are needed to evaluate the usefulness of the SCE technique in predicting drug response.

It is most obvious, however, that no test available at the present time will satisfy our needs to predict a therapeutic response. Still, the reasonable combination of cytological tests such as the SCE assay and the labeling index perturbation or stem cell assay may establish two important aspects of the cytostatic action in man, i.e. the pharmacokinetics of the active metabolites of cytostatics in an individual and the mode of interaction with the DNA of the test lymphocytes and tumor cells. Whichever system is used, however, one point must be kept in mind: the *in vitro* drug sensitivity tests deal at best only with the capability of the cytostatic drug(s) to eliminate the leukemic cells *in vivo*. The prediction of the outcome of remission induction therapy must be based on the integration of the clinical prognostic factors and drug sensitivity data, which will probably be offered by different *in vitro* tests complementary to each other.

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