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Commentary

Human Tumor Clonogenic Assay: What is New?

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(A COMMENT ON: Neumann HA, Herrmann DB, Fiebig HH, Engelhardt R. Treatment of human clonogenic tumor cells and bone marrow progenitor cells with bleomycin and peplomycin under 40.5°C hyperthermia *in vitro*. *Eur J Cancer Clin Oncol* 1989, **25**, 99–104.)

ACTIVE chemotherapeutic agents are rare, provide only palliation in most advanced solid tumors and are responsible for a limiting dose-dependent toxicity in normal cells. Therefore it is important to develop new active and less toxic drugs for the treatment of cancer. The classical murine based screening program for new drugs, used by the NCI over the last three decades, has been rather disappointing and effort has been put into developing analogs to known active drugs with the hope of finding either a wider and different range of activity or a lower toxicologic profile with respect to the parent compounds. Improving the results achieved with available active drugs can be performed by combination therapies, or by associations with potentiating agents or with other therapeutic modalities such as irradiation, immunotherapy or hyperthermia.

The prediction of individual response to chemotherapy is a perpetual challenge in cancer. Clonogenic and non-clonogenic *in vitro* chemosensitivity assays using fresh tumor biopsies from investigated patients have been very accurate in such predictions, particularly when the tumors were resistant to drugs [1]. However, the routine use of such assays has historically been limited by the poor growth of these fresh tumors yielding only few successful and interpretable results. Fortunately, improved culture methods developed over the last few years seem to have overcome this problem [2–4]. Starting from the observation that drugs inactive in the murine screening model were cytotoxic *in vitro* against fresh tumors [5], the NCI is currently investigating a new *in vitro* screening program described as 'disease oriented', based on the use of panels of cell lines representing different tumor types [6]. The main advantage of using cell lines over fresh tumors is that they provide a potentially unlimited amount of material to work with and that they are easy to handle and to store. However, the biological behavior and the chemosensitivity of permanent cell lines, since they represent through their culture conditions selected populations of tumor cells, may be representative neither of the chemosensitivity of fresh tumors with the same histologies, nor even of the fresh tumors from which they were derived [7].

When dealing with *in vitro* chemosensitivity assays, the drug concentration used is an important issue. Most of the drugs that do not require bioactivation can be cytotoxic *in vitro* at a randomly chosen and maybe inadequately high concentration. One way of avoiding false positive results is to use fractions of peak plasma concentrations achieved *in vivo*. However, these values are sometimes highly variable from one publication to another and this information is missing for investigational drugs. Since most chemotherapeutic agents produce a clinical limiting myelotoxicity, many investigators, following a concept pioneered by Hug *et al.* [8], derive from experiments performed *in vitro* on human marrow cells a concentration range to test against tumor cells. This defines an *in vitro* 'therapeutic index' for drugs. The clinical implications of such a model have recently been discussed by Ajani *et al.* [4], and we recently developed a similar system using murine permanent hematopoietic progenitor cells [9].

Bleomycins (bleomycin A and B) are a part of many curative chemotherapeutic regimens, particularly in the management of lymphomas and germinal tumors. Using permanent tumor cell lines, Hahn

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et al. previously showed that the cytotoxicity of these drugs could only be enhanced *in vitro* by high temperatures (above 42°C) [10]. With the fresh tumor model, Neumann *et al.* demonstrated that even a mild temperature of 40.5°C, easily achievable in the clinic for the treatment of disseminated diseases, could potentiate the *in vitro* effect of these drugs [11]. In a recent issue of this journal, these authors compared results achieved with mild hyperthermia associated with bleomycins and one analog, peplomycin, against fresh tumor biopsies and xenografts, using a clonogenic assay. Peplomycin has been selected for clinical trials on the basis of preclinical studies that proved similar to better toxicity against different tumor models and less pulmonary fibrosis in animal models when compared to the parent compound [12]. They also studied the effect of drugs with or without hyperthermia on human marrow cells taken as reference.

The main criticism one could raise against this article concerns some aspects of the methodology described by the authors. The technique of tumor culture used is easier to perform than classical bilayer methods [1], but yields poor cloning efficiencies (number of evaluable tumor colonies per cells seeded) as compared to the improved techniques mentioned above.

Nevertheless, this article discloses fundamental aspects of *in vitro* thermochemosensitivity of cells, likely to be tested in the clinic.

The most important finding was that 2 h of mild hyperthermia had no adverse effect *per se* on the marrow cells, nor did it potentiate the toxicity of chemotherapeutic agents on these normal cells *in vitro*. If this could be verified by clinical studies, a higher therapeutic index could be achieved *in vivo* with the combination of total body, mild hyperthermia and drugs.

When comparing bleomycin and peplomycin toxicities against tumor cells and the enhancing effect of mild hyperthermia, it is important to notice that the tumor types used *in vitro* (melanoma, lung carcinoma, myosarcoma, gall bladder carcinoma), are all known to be refractory to chemotherapy in the clinic. One could postulate since chemosensitivity results were not shown that these tumors were also, on the average, very resistant to drugs *in vitro*. If this was true, it is unlikely that hyperthermia could enhance the weak cytotoxicity of drugs in most of the cases. This could explain why only 3/13 (23%) tumors exhibited a hyperthermic enhancement. This reasoning is strengthened by results of a similar published study using an active drug (cisplatin), against a sensitive tumor (ovarian carcinoma), where 43% of the tumors exhibited a thermal enhancement of drug cytotoxicity *in vitro* [13]. The authors reported that peplomycin was in half of the cases more active than bleomycin. Based on the published figures of the three cases that showed a thermal enhancement, the IC_{50} (drug concentration achieving 50% inhibition of colony growth) of peplomycin was 10-fold lower than that of bleomycin. On the other hand, on marrow cells, bleomycin appears to be 10 times more cytotoxic than peplomycin. This means that, at least in these three cases and in agreement with preclinical studies, peplomycin has an *in vitro* therapeutic index much higher (almost 2 log) than the parent compound. Although exhibiting different comparative toxicities on individual tumors, both drugs were enhanced in the same tumors. This might reflect similar mechanisms of action and of thermopotentiation.

Another important finding in the article is that in one tumor, drug cytotoxicity was inhibited by hyperthermia. Similar occasional cases have been published by the same authors and by others [13, 14].

If all these conclusions could be validated by prospective clinical trials, this model could be very useful for screening and comparing drugs and analogs that could be selected for clinical mild hyperthermia–chemotherapeutic studies. On a more individual basis, this method could differentiate three categories of patients: in the first category, mild hyperthermia might potentiate the beneficial effect of chemotherapy. One might guess that in known sensitive tumors and with active drugs, this would be the case in 40–50% of the patients, whereas in resistant tumors, only 20–30% of them would exhibit a beneficial effect with the association of hyperthermia and chemotherapy. The second category would consist of patients in whom mild hyperthermia does not potentiate the effect of drugs and, finally, the third category would be represented by patients in whom mild hyperthermia might exhibit an adverse effect on drug cytotoxicity.

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