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Letter to the editor

Pharmacokinetics in experimental and clinical chemotherapy

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Sir,

In his recent excellent editorial Moore [1] described the principal difficulty arising from the use by experimental chemotherapists of assays developed originally to determine the respone of tissues to ionising radiation. In radiobiology the dose absorbed by a unit mass of tissue bears a constant relationship to the amount of energy directed at it. Thus the experimental radiobiologist or radiotherapist can deliver the requisite dose to the target tissue very accurately with minimal heterogeneity of distribution. With chemotherapy, by contrast, the distribution of dose is governed not by the laws of physics but by pharmacokinetics. Because of the numerous factors involved in absorption, distribution, metabolism and excretion it is difficult to predict concentrations of drugs and metabolites in tissues and cells, and considerable heterogeneity can exist both between and within tissues [2]. Differential response is therefore as likely to result from unequal drug distribution as from disparities in target sensitivity and repair.

As emphasised in the timely review by Powis [3], despite the explosion in the pharmacokinetic literature over the last twenty years there have been few attempts to explore in a quantitative fashion the relationship between measured drug or metabolite concentrations and biological response: in other words to relate pharmacokinetics to pharmacodynamics. This criticism applies not only to studies in patients and experimental animals, but also to most experiments in cell culture where concentrations of drugs and metabolites are rarely measured in the medium let alone the treated cells. In vitro 'pharmacokinetic' studies have shown that conclusions regarding the comparative sensitivity to different nitrosoureas can be markedly altered in the light of data concerning drug decay in the medium and cellular uptake [4]. Likewise the comparative resistance of the innermost cells of multicellular spheroids to adriamycin can be explained, at least in part, by the inability of this drug to penetrate beyond the first few cell layers [5].

Recent studies from this laboratory can be used to illustrate how the measurement of tissue drug concentrations can facilitate interpretation of in vivo tumour response data, including effects of route of administration of the type cited by Moore [2]. When the two drugs are administered IP to mice bearing solid tumours, single large doses of the nitroimidazole misonidazole are able to increase response to the nitrosourea CCNU to a greater extent in tumour than in dose-limiting normal tissues [6]. In experiments where tissue nitrosourea concentrations were measured by HPLC alongside tumour regrowth delay, the enhanced response could be accounted for entirely by the increased peak drug levels in tumour, an effect which was not seen in the bone marrow or gut [7]. When the same combination was used but with CCNU given orally, however, misonidazole reduced tumour response to the nitrosourea [8]. This initially perplexing result was explained by parallel pharmacokinetic studies which demonstrated a complex interaction, involving a reduction in peak nitosourea concentrations by misonidazole. These experiments also demonstrated that, compared to IP CCNU, oral CCNU gave a twofold greater antitumour activity but was 1.5-fold less toxic in terms of animal lethality. The former was attributed to the higher peak tumour concentrations seen with the oral route. The reason for the latter remained unclear, but a change in dose-limiting normal tissue was indicated and differential drug distribution was suspected.

Although pharmacokineticists have tended for ease to work mainly on circulating drug levels, tissue concentrations are clearly more relevant and can be determined without great difficulty using such techniques as HPLC, GLC, mass spectrometry, atomic absorption spectrometry etc. Non-invasive techniques such as NMR and PET are now being used to measure regional distribution of drugs in experimental and human tumours. Such studies, particularly in man, will undoubtedly provide important data to help interpret biological responses to treatment. This has now become an accepted part of radiosensitizer development at both the preclinical and clinical level [9] and will become increasingly important in the development of other agents.

A limitation of all of the analytical techniques mentioned above is that they generate concentration data averaged over a small region of tissue. Clearly, the ultimate resolving power would allow drug concentrations to be measured in individual cells. A combination of appropriate staining techniques, fluorescence-activated cell sorting and clonogenic assay should in the future allow us to ask questions about the relationship between pharmacokinetics and pharmacodynamics at the level of individual cells. The most simple application is to those drugs with native fluorescence. Thus Durand [10] has demonstrated a correlation between intracellular drug level and survival for cells from mutlicellular spheroids which were sorted on

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the basis of their adriamycin fluorescence. The same group have recently demonstrated the potential for in vivo use [11]. Mice were injected with the fluorescent DNA stain Hoechst 33342 which, like adriamycin, penetrates only a few cell layers. Thus, disaggregated tumour cells could be sorted into populations at different distances from blood vessels. They did not use this technique to assay response to the Hoechst dye itself, but to show that cells far away from vessels were comparatively resistant to adriamcin and X-rays [11]. An alternative approach would be to sort treated cells on the basis of response to fluorescent probes such as polyclonal or monoclonal antibodies, e.g. to bromodeoxyuridine [12] or cisplatinum-DNA adducts [13], or DNA stains recognising bound drug or chromatin changes induced by binding or damage (Smith PJ, personal communication).

Novel techniques of this type are currently under experimental development. In the meantime relatively straightforward assays for plasma and tissue should be used more widely to aid understanding of experimental chemotherapy data in vitro and in vivo as well as to facilitate the interpretation of clinical toxicity and response data.

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