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Editorial

The Multiplication of Analogs, the Best Strategy for Rapid Extension of the Oncostatic Arsenal

How can They be Compared Experimentally?

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The best strategy for extension of the oncostatic arsenal depends on the development of analogs of the present effective agents.

Several analogs of an active family may have different toxicities [9, 25; Mori et al., unpublished data] and experimental and clinical tumors may present different primary [4, 11] or secondary resistance to different analogs [5, 16, 19]. Thus having two active analogs may be equivalent to having two families available.

Ideally, experimental pharmacologists should be able to provide the clinician with new analogs that have a higher antitumoral activity and or lower toxicity than the ones currently available, or which are not cross-resistant with them. But how can the oncostatic effects and the toxicity of analogs be compared experimentally?

A look at how experimentalists and clinicians compare analogs suggests that there are too many methods, all imperfect in varying degrees.

We have learned by experience that the in vivo experimental *screening* tests used routinely to assess the oncostatic potential of a drug do not give a very accurate prediction of its clinical usefulness. Every year only a few experimentally active drugs reach the point of clinical trials, and still less are shown to be active and useful in man. Furthermore, it has not been possible to predict the spectrum of activity of the drugs on human tumors experimentally [24].

In vitro tests are even more inadequate. There is no consensus as to what cell lines should be used. All permanent lines are derived from highly selected cell clone(s), which is (are) in no way representative of the original tumor cells. As far as the various compounds are concerned, their solubility is often different, so that investigators very often have to use different solvents. Furthermore, the in vitro method is not valid when the oncostatic drugs work via their metabolites, as it is often the case [4]. However, if the in vitro method is used not as the first step, but as the last experimental step after the collection of data in the in vivo study, it can be a remarkable method of testing the effect of each analog on each human tumor with the agar-colony technique described by Salmon [20].

The in vivo methods used for experimental *comparison* of analogs are also very much open to criticism, as most pharmacologists compare fractions of the LD_{30} or LD_{10} of the different compounds on murine tumors and on side effects. But this parameter does not seem at all satisfactory, as the LD_{30} and LD_{10} are due to all kinds of toxicity, especially some that can perfectly well be controlled by clinicians, e.g., short-term hemopoietic toxicities, which pose no problems since the patients can be cared for in aseptic environments [14, 21] and efficient antibiotics, white cells, and platelets can be administered [22].

Thus we thought that it would be more logical to compare analogs on the basis of their effect on a reference tumor target, rather than their effect on the host. It is essential to choose a tumor that is known to be sensitive to the series to be tested. In a study on Vinca alkaloids we chose P 388 [18], while for one on anthracyclines we used L 1210 leukemia [7, 8]. For our study on nitrosourea analogs we also chose L 1210 leukemia, which is the most frequently used tumor among those that are sensitive to this family [24].

We have compared the effects of a series of doses of each analog on these tumors, calculating the limits of the doses compared on the basis of the respective LD_{50}. We have established the correlation between dose and survival for different analogs, the ones available for some time and the three new ones [12].

At this point we should remark that, at least for L 1210 leukemia, one can only express the oncostatic results in terms of survival and not of the number of logs of killed cells [13]: we recognize that this last method can be the object of more scientific speculation, but for

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some oncostatics it may not be correct: i.e., we have observed that 124 mg cyclophosphamide/kg cures almost all mice with L 1210 leukemia, while 450 mg/kg cures only a few without the animals dying of the toxicity: the reason is that 400 mg/kg is immunosuppressive while 129 mg/kg is not [15].

In the study of the dose and survival prolongation correlation (DSPC), one may find a dose that is optimal for survival. This point in the curve represents the optimal dose for L 1210 leukemia ($OD_{L\ 1210}$). For some analogs there may not be only a single optimal dose, but a range of several doses [12]. This is known as the 'maximally efficient dose interval' (MEDI).

The ideal method for comparison of the oncostatic potential of analogs would be to study the dose-effect relationship for all analogs in all experimental tumors. If the effect of drugs on experimental transplanted tumors were predictive for human tumors, this considerable investment of time and money would be worthwhile, but this is obviously not the case [6].

A single dose should therefore be selected, and we find it logical to use the optimal dose for survival as the reference dose for comparison of the oncostatic effects of the analogs on other tumors. It is evidently not a perfect reference dose, but there is no perfect choice. And it is the most pragmatic one, as the chosen dose is known to be maximally effective for the compound in the conditions for which all 'parasitic' effects have the least chance to interfere.

Where there is an MEDI, we have arbitrarily chosen the median of the MEDI, a dose with the maximum efficacy and equally far from the point where the effect is not maximal and from the point where lethal toxicity begins to overcompensate the oncostatic effect.

Unfortunately, the toxicity of oncostatic agents is of multiple origin and has to be assessed separately for each. Some compounds have a peculiar toxicity, i.e., cardiotoxicity of anthracyclines [7, 8], pulmonary toxicity of bleomycin derivatives [19]. In such cases special techniques have to be developed to compare the toxicity of analogues.

Hematotoxicity is still the most frequent dose-limiting factor for oncostatic agents. Multiple techniques are used to compare the hematotoxicity of analogs. In vivo, blood counts can be monitored at different time intervals after single or repeated injections of the compound to the mouse. The development of more refined hematological techniques has made it possible to quantify the effects of a given drug on various hematopoietic precursors (CFU-S, CFU-C, BFU-E, CFU-E and even B- and T lymphocytic colonies and megacaryocytic colonies [Mori et al., unpublished data].

Some authors have even measured the hematotoxicity of drugs directly, after in vitro incubation with bone-marrow cells [1]. However, the extrapolation of these in vitro results to an in vivo situation is very hazardous.

The estimation of the extent of the hematopoietic effects of a drug also varies according to the technique used: as shown by Blackett et al. [2], the in vivo technique of splenic colonies described by Till and McCulloch [25], the agar colony [3], and the peritoneal diffusion chamber technique [10] may give different results on the hematological effect of a given drug. The effect of drugs may be different in various biological compartments. As we shall describe in the case of nitrosourea derivatives [Mori et al., unpublished data], the effect of drugs on hemopoietic precursors may differ widely according to the compartment being studied, bone marrow, spleen, or blood. Finally, there are also important differences between animal species, i.e., mouse, rat, human. The multiplicity of the hematological parameters may explain why the rare significant studies of the effects of drugs on hematopoiesis have generally been made by experimental hematologists. Very often the hematological effects of drugs have been poorly understood and the published results have not provided information of any use to clinicians.

Hence, the study of the hematotoxicity of drugs is rather complex and it is better to use a single dose to compare the various hematotoxic effects of a given drug than to use multiple doses of this drug in a single test, which is not representative of the hematological effects of the drug.

We are faced with the same problems of choosing the most suitable reference dose(s) for the comparative study of the other side effects of a drug: we have chosen either the $OD_{L\ 1210}$, when the DSPC curve shows a peak, or the minimal dose of the MEDI, which is perfectly logical as it has the lower chance of giving side effects with the maximum effect on the reference sensitive tumor [9].

It goes without saying that it is also necessary to compare analogs with respect to cross-resistance. This is a necessary study that must be set up systematically, because it is known that there may be no clinical cross-resistance of a neoplasia to a second analog for a tumor resistant to a first one. This was shown for human tumors such as acute lymphoid leukemia as regards vincristine and vindesine [16], and it might be the case for others as regards *cis*-platinum and malonatoplatinum [19]. In this case, the model used by Burchenal et al. [5], which requires the establishment of tumors made resistant to an analog by successive transfers under its treatment, is satisfactory and should be a model available in each laboratory for as many tumors as possible. We suggest that a bank of such resistant tumors should be set up.

In conclusion, we have tried here to substantiate the complexity and the imperfections of any comparative

study of analogs. We propose the use of new reference doses, such as the $OD_{L\ 1210}$ or minimum and median doses of the MEDI. We hope that a general discussion on this important matter of the various valid methods of comparing analogs of drugs will be organized, which will be more fruitful than the sterile disputes of those who try to impose their own criteria even if they do not correspond to biological and clinical reality and have failed to demonstrate their capacity to help the clinician by any experimental predictive value.

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