Intensive Cytotoxic Chemotherapy

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It must be readily admitted that intensive cytotoxic chemotherapy is more the product of necessity than of virtue. Clearly, we are still at the very beginning of drug therapy for cancer, and the limitations of our present methods are grave. Intensive cytotoxic chemotherapy can be a hazardous exercise in 'brinkmanship', and to a problem of still unplumbed depth and remarkable delicacy it brings a philosophy that is hardly more refined than that of the blitzkrieg. Cytotoxic agents are crude and dangerous tools that more readily heighten than diminish human suffering.

Yet one of the functions of the clinician is to make the most effective use of the measures available. If anti-cancer drugs lack, in their primary actions, the ability to discriminate between the target cell population and vital normal cell populations, the clinician can attempt to achieve that discrimination by the mode of administration. We are still learning how best to use agents that have been available for ten to twenty years. The obvious variables are dosage, timing, route of administration, drug combinations, and the use of antagonists. But knowledge of human physiology and immunology, the patho-physiology of tumours, and the standard techniques of acute clinical medicine add substantially to the potentialities of therapeutic agents. The agents themselves may be crude but there is no need for ineptitude in their application. For some patients with otherwise rapidly fatal neoplasms, intensive therapy offers the best hope of prolongation of useful life, and in some cases cure is no longer a pious hope.

It is not fitting that the clinician should assume the Churchillian splendour of 'give us the tools and we will finish the job'. The clinician has an increasing responsibility to use situations where chemotherapy is effective to extend our knowledge of malignant processes, to improve methods for detecting and locating small tumour masses as well as to improve therapeutic methods. It can be said that clinical medicine has already contributed to recent progress in cancer therapy and to knowledge of basic mechanisms. The pace of its contributions might well be quickened by wider recognition of its potential.
HISTORICAL PERSPECTIVES

Until the mid 1950s it was widely held that tumours were the product of rapid and uncontrolled cell proliferation. Were this so, the antimitotic approach to cancer might have proved more immediately successful. Up to that time, antimitotics tended to be used either in small daily doses over long periods, or in short sharp attacks that were rarely repeated. Valuable results were obtained in the chronic leukaeemias, transient remissions occurred in the acute leukaeemias, and a limited range of solid tumours showed some temporary reduction in volume. But the overall results were disappointing. Evidence also began to accumulate that cell proliferation is not as fast in tumours as it is in the normal cell renewal populations of such vital tissues as the intestinal crypts and the haemopoietic system. Moreover, there was no evidence of selective uptake of antimitotic agents by tumour cells, so that the credibility of the antimitotic approach became questionable.

Then in 1956 came the report by Li et al. that folic acid antagonists had a profound effect on trophoblastic tumours. Since these tumours tend to metastasise at an early stage and, since the effect of folic acid antagonists subsequently proved curative, it gradually became clear that the long-sought goal of a chemical cure of disseminated cancer had at last been achieved, if only in some instances of a type of cancer uncommon in European populations.

This success had the additional aspect that trophoblastic tumours present unusual opportunities for the study of human cancer. One of these is their production of chorionic gonadotrophin (HCG) which can be used as an index of viable tumour cell mass. The ability to measure the response of the tumour, even with the bioassay methods then employed, enabled Hertz and his colleagues to observe that methotrexate was more effective when used intermittently in high dosage than it was when used in prolonged low dosage schedules.

Inevitably these observations resulted in a new surge of activity in the field of chemotherapy and in the scientific study of cell proliferation kinetics. In the USA, funding for chemotherapeutic activities was boosted and the screening programme for new agents was enlarged. Individual agents and combinations of agents that came through the screening programme for clinical evaluation were tested more rigorously. Although many variations have evolved, the intensive use of cytotoxic agents has continued to prove rather more effective than daily low dosage schedules when profound rather than palliative effects are sought. The general pattern has remained one of alternating treatment periods and rest periods with the periodicity ranging in duration from a few hours to about a week or more, depending on the agent or combination of agents used.
PROBLEMS OF SCALE

Tumours kill by reason of the volume they occupy in the general or in the local sense. As clinicians we are used to thinking primarily in terms of tumour volume. Yet we have to be prepared constantly to change gear between the phenomena of tumour masses and the phenomena of the tumour cell.

The objective of therapy is, of course, to reduce a large population of tumour cells to a small population, preferably to less than one cell, and to do this without a comparable reduction in any population of normal cells. The initial target population is likely to be in the range $10^9$–$10^{12}$ cells, depending on the type of tumour and its total volume (Collins et al., 1956; Skipper et al., 1964). If we reduce the problem to its simplest terms and consider an average tumour of $10^{10}$ cells, then elementary calculations indicate that if 90 per cent of the surviving cells are eliminated by each of a series of courses of treatment, the last cell should be eliminated with the eleventh course. Each course of this model (Fig. 1) is measured from the beginning of one treatment phase to the beginning of the next, and so includes the intervening rest phase. In practice, a complete treatment-rest phase cycle that results in such a high rate of cell kill is unlikely to total less than 14 days and, therefore, even with highly effective treatment, elimination of a modest sized population would take almost six months. This is, incidentally, close to the average time taken to eliminate metastasised choriocarcinomas with chemotherapy.

Fig. 1. Schematic representation of the reduction of a tumour cell population during a series of alternating treatment and rest periods.
When we examine the problem a little more closely, we find that the tumour cell population starts to increase again during each rest phase. If the population is reduced to 90 per cent of its initial size after a complete treatment-rest phase cycle, the reduction achieved during the treatment phase itself has to exceed 90 per cent by an amount equal to the re-growth of the tumour population that occurs in the rest phase. This may require the number of cells killed in the treatment phase to be 99 per cent or more of the starting population. A kill rate of this order cannot be achieved by antimitotic action alone unless the fraction of cells entering mitosis during the treatment phase amounts to, or exceeds, 99 per cent of the population’s starting size. It is probably exceptional for the fraction of the cells in a human tumour that is engaged in mitotic processes, that is the growth fraction, to be of this magnitude.

*Cell Losses by Other Mechanisms*

Most tumour cell populations, like normal cell renewal populations, undergo losses from a variety of different mechanisms, and these, added to the cells killed by antimitotic action, may bring the fraction dying to a high level. The tumours most susceptible to chemotherapy are indeed characterised by high rates of ‘spontaneous’ cell loss (Bagshawe, 1969, 1970). These losses do not reach the rate found in normal steady-state cell renewal populations where additions equal losses, but the more closely a tumour approaches this ‘steady state’, the more readily it is reduced. The role of ‘spontaneous’ cell death in tumour response is not widely recognised, although the idea that the chemotherapeutic attack can be supported by an immune response is generally accepted. Immune mechanisms form only one of the several mechanisms contributing to cell death in a tumour population.

Perhaps the point is illustrated best with a simplified example. After a given period of time a population of cells increases from 100 to 101. This could result from the addition of one cell by one mitosis with no intercurrent cell deaths. It could also result from 100 mitoses with 99 intercurrent cell deaths. Both populations would be ‘growing’ at the same rate, but antimitotic action would have very different effects. In the same period of time, antimitotics would reduce the first population to 99 cells but the second population would be reduced to zero provided all the original 100 cells divide.

It is therefore useful to define our objective in another way; namely, to make total cell losses exceed total cell additions in the tumour population for a period of time long enough to eliminate that population. We are thus reminded that therapeutic agents have to be considered not only for their direct effects on the tumour but also for their indirect effects on the ability of host mechanisms to restrain tumour growth.
**Constant Fractional Kill**

Another aspect of this model is that whereas the first course of treatment reduces the population by $9 \times 10^9$ cells, the effective kill falls stepwise with each course, and the last course kills only one or two cells. In other words, the fraction of the total cells killed by each course of treatment tends to be constant but not the absolute number. The concept of 'constant fractional kill' was first elaborated by Skipper *et al.* (1964) on the basis of observations with the murine L. 1210 leukaemia system, and it is interesting that choriocarcinoma, the only human tumour where the process of reducing the cell population has been monitored so far, provides evidence that this principle often appears to operate here (Bagshawe, 1969).

Deviations from constant fractional kill result from alteration with time of the growth fraction of the population and from various other factors. Its significance is, of course, the great length of time required to eliminate tumour populations that are far below the size likely to produce clinical effects. The difference between clinically advanced disease and complete clinical remission is generally a matter of only one to three logarithms in cell numbers. When clinical remission has been achieved, the journey down to zero cells has

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![Table of cell counts](image)

Fig. 2. A scale of estimated cell numbers at critical stages in the growth and regression of tumours.
hardly begun, for there are still another 8 to 11 logarithms of cells to be eliminated (Fig. 2).

TUMOUR INDEX SUBSTANCES
It is often said that a major handicap in the diagnosis and management of malignant disease is the lack of an indicator that would tell us what is happening to the tumour cell mass. This is undoubtedly so, but curiously little interest has been paid to those substances that can provide such information for specific tumours. A universal ‘cancer indicator’ seems highly improbable, but we can now anticipate a wide range of substances being used for the detection, clinical management, and follow-up of an increasing range of tumours. For instance, there are the intestinal tract carcino-embryonic antigen described by Gold and Freedman (1965), the placental alkaline phospahse isoenzyme described by Stolbach et al. (1969), the myeloma immunoglobulins and foetoprotein, in addition to a wide range of so-called ectopically produced polypeptide hormones. As tumour index substances each of these is likely to have substantial imperfections. Nevertheless, their limitations as index substances can be defined and it is reasonable to expect that they will provide useful information similar to that provided by chorionic gonadotrophin for trophoblastic tumours.

The physical properties of such tumour index substances and the specificity, sensitivity, and reproducibility of the methods used in their measurement greatly influence the interpretation of the data they provide. Each substance has to be studied in detail from its point of synthesis within the cell through its secretory, metabolic, and excretory phases. After more than forty years of quite intensive study of HCG, basic data relevant to its use as a tumour index substance is still being collected.

Only one of the many aspects of HCG as a tumour index substance can be referred to here. One vital issue is how closely it reflects changes in the tumour cell population. There is evidence from a number of approaches that the amount of hormone secreted by the tumour cells is closely reflected in the urinary excretion rate, but the low clearance rate of the hormone introduces a lag effect between changes in the cell population and changes in its concentration in body fluids. As expected, the urinary output of HCG falls during the treatment phase and increases again during the rest phase. However, with a rapidly responding tumour the excretion rate falls exponentially through both treatment and rest phases, especially if the treatment phases are kept close together. However, the fall and rise effect expected from the model system is seen quite well with slowly responding or non-responding tumours (Fig. 3).
Fig. 3. Urinary gonadotrophin excretion during chemotherapy of choriocarcinoma.

(a) The low renal clearance of the hormone damps down the fluctuations so that in patient K.H. the rate of fall approaches an exponential pattern, whereas in the more slowly responding case, H.J., the fluctuations are evident but not synchronous with the periods of treatment and rest.

(b) When chemotherapy fails to produce a progressive reduction in the tumour cell mass the reduction achieved during the treatment phase is still seen but is compensated during the rest phase, as seen in this patient.
The cross reaction between HCG and LH still imposes a limit to the minimum size of detectable tumour but it is possible to recognise and remove surgically tumours with a viable cell mass of only 1–2 mm³.

**SELECTION OF DRUGS FOR COMBINATION THERAPY**

There is little evidence to suggest that present-day cytotoxic drugs, used in sub-lethal dosage in vivo, kill cells other than those engaged in mitotic processes. Antimetabolites and alkylating agents exert profound effects on those populations with a high rate of cell turnover, the so-called cell renewal populations. There are many differences between the agents, and they are not all equally effective in arresting cell division in particular populations, but the mitotic rate in each tissue presumably constitutes the limiting rate at which antimitotic mediated cell kill can be achieved.

It is currently the vogue to speak of those agents that interfere with DNA reduplication as ‘cell cycle specific agents’ and those that damage cells at any phase of the cell cycle, such as alkylating agents, as ‘non-cycle specific agents’. There is good evidence for the validity of this distinction in so far as the direct chemical action of the agent on the cell is concerned. However, the unfortunate phraseology has, it seems, given birth to the idea that by combining an agent from each category something approaching continuous antimitotic action can be achieved. Fortunately, this is not so, for if it were, it would inevitably prove fatal. Thus, although alkylating agents may cross-link DNA at any stage in the cell cycle, the damage is repaired in time (Roberts et al., 1968) and, in general, cells entering mitosis soon after alkylation fail to produce viable daughter cells but those whose entry is delayed long enough divide successfully.

There may, however, be good reasons for combining an antimetabolite with an alkylating agent or for any other combination of agents. If resistance to one agent is known to develop by a mechanism that can be effectively blocked by another agent, their combination may be useful. It was this principle, used somewhat hopefully, that led us to combine methotrexate and 6-mercaptopurine in the treatment of trophoblastic tumours. The results obtained were better than those achieved with methotrexate alone and, indeed, it has been difficult to improve on this combination.

A more general principle in selecting agents for combination therapy lies in the fortunate fact that they are not equally damaging to different normal tissues. We do not yet know what causes these differences, although they probably result from a variety of factors and, not least perhaps, from different patterns of cell renewal. Ideal drugs for combination would damage the target tissue but their other effects would not overlap (see Fig. 4).

Another example of drug combination is the use of methotrexate with
folic acid in systemic as opposed to local infusion therapy. Clearly the results obtained when a cytotoxic agent is used together with its own antagonist depend critically on the dosage schedule employed. It has proved possible to achieve sustained remission in about 50 per cent of patients with gestational
trophoblastic tumours with this combination and to do so with virtually no subjective toxicity. The regimen used is one of continuous intravascular infusion of methotrexate at a rate of 0.5 mg/kg/day. This is generally continued for seven days and, in the absence of folic acid, this dose would be fatal to most patients. Folinic acid 0.1 mg/kg is given every 12 hours by intramuscular or oral route throughout the period of methotrexate administration. One benefit of the combined regimen is a wider safety margin than with methotrexate alone, although toxicity may still arise if renal function is impaired. The ability of the folic acid to protect normal cell populations without giving equivalent protection to the tumour cells is most vividly seen in alimentary tract mucosae. In a comparable regimen in mice the intense damage to the jejunal villi produced by continuous exposure to methotrexate is reduced by intermittent administration of folic acid (Bagshawe and Rawlins—to be published). The number of successfully dividing crypt cells, although reduced, is able to maintain the functional integrity of the mucosae and, thus, the consequences that attend disruption of the mucosa are avoided.

It is not yet clear why it is possible to discriminate in this way between a rapidly proliferating normal cell population and rapidly proliferating trophoblastic tumour cells. There are, in fact, several possible mechanisms about which one can speculate. The DNA synthesis (S) phase in trophoblastic tumour cells tends to be much longer than that in intestinal crypt populations. A pulse of folic acid long enough to allow DNA synthesis to be completed in some intestinal cells may be inadequate for most of the trophoblastic tumour cells in S phase. This then is one example of a quantitative difference between tumour and normal cell populations that can be used advantageously even with non-specific agents. Undoubtedly there are other differences of a broadly similar nature that have not yet been fully exploited.
Drug Resistance
Using intensive chemotherapy, and in some cases immunotherapy, radiotherapy, and surgery, about three quarters of the patients with choriocarcinoma and all those with invasive moles can be brought into remission. Many of these patients subsequently have normal children. But just as this tumour indicated the potential of intensive antimitotic chemotherapy so also does it indicate the limitations.

A wide variety of agents is effective in reducing the size of trophoblastic tumours, when used as the first agent. Yet in a significant number of patients drugs fail to eliminate these tumours. Antibiotic resistance by micro-organisms as a result of the classical mechanisms of adaptation and selection has long been demonstrated. Similar forms of resistance have been induced in experimental tumour systems, and this has fostered the notion that the mammalian cancer cell, like certain micro-organisms, has unlimited powers of adaptation. Yet such resistance at a cellular level in human tumours has rarely been demonstrated, and cells from clinically ‘resistant’ trophoblastic tumours have proved as sensitive when tested in vitro as those from sensitive tumours (Hertz, 1967). There is also the inconsistent observation that choriocarcinomas that have become resistant to methotrexate and actinomycin-D are usually resistant to other agents to which they have not been exposed.

Thus, there are good reasons for questioning the assumption that drug resistance by tumours is analogous to antibiotic resistance by micro-organisms. Several aspects of cell resistance suggest that it is a highly complex phenomenon and one clinicians can help to unravel.

References
Bagshawe, K. D. (1969) Choriocarcinoma. The clinical biology of the trophoblast and its tumours. London: Edward Arnold.
Bagshawe, K. D. (1970) Tumour Growth and Curability. The Scientific Basis of Medicine Annual Reviews 1970. p. 89.
Collins, V. P., Leoffler, R. K. and Tivey, H. (1956) Amer. J. Roentgenol., 76, 988.
Gold, P. and Freedman, S. O. (1965) J. Exp. Med., 121, 439.
Hertz, R. (1967) in Choriocarcinoma: Transactions of a Conference of the International Union against Cancer. Berlin: Springer-Verlag, p. 26.
Li, M. C., Hertz, R. and Spencer, D. B. (1956) Proc. Soc. exp. Biol., (N.Y.) 93, 361.
Roberts, J. J., Crathorne, A. R. and Brent, J. P. (1968) Nature (Lond.), 218, 970.
Skipper, H. E., Schabel, F. M. and Wilcox, W. S. (1964) Cancer Chemother. Rep., 35, 1.
Stolbach, L. L., Krant, M. J. and Fishman, W. H. (1969) New Engl. J. Med., 281, 757.