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Perspectives and Commentaries

Priming: Theory or Fact?

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PRIMING: THEORY OR FACT?

SINCE the advent of cancer chemotherapy, its narrow therapeutic ratio has been the main limitation to its use and success. A variety of approaches have been contemplated to overcome this problem.

Cancer chemotherapists have a problem somewhat similar to the gunmaker who has to deal with enemy tanks. When tanks first became girded with armorplate of ever-increasing thicknesses, the gunmaker built even heavier guns to fire even heavier shots to punch through the armor. At one point, the guns got so heavy and so expensive that this approach became impractical. Similarly, the cancer chemotherapist in an attempt to fight against cancers untouched by small doses, gave even larger doses of his drugs until the approach could be used only by either a small number of highly experienced teams or if the malignancy was so 'thin skinned' as to be amenable to that approach.

Our gunmaker, instead of using brute force, has since resorted to cunning, to a more elaborate use of physical laws to create a hollow charge, thus avoiding the use of heavy guns. Similarly, designers of chemotherapeutic agents and of drug combinations have tried to apply some basic scientific information about the drugs they employ to enhance their results.

Coming from this select group of 'basic science assisted' designs are a number of drug combinations previously tested in animal models, some of which were very good indeed, whereas others have been gracefully forgotten. Another approach consisted of alternating combinations of drugs. Although the idea works very well as a computer model, the clinical application has required more knowledge of drug efficacy and less practical limitations than has usually been available. Rare is the situation in which two or more drug combinations are available, both of which are equivalent in their ability to kill the same logarithmic fraction of cancer cells and are not cross-resistant. A third approach is to reduce the bone marrow and gastrointestinal toxicity secondary to large doses of chemotherapy by a preliminary and appropriately-timed dose of cytocidal drug without inducing the same degree of protection to the cancerous growth.

In the sixties, two groups of clinicians noted that a small dose of merophan protected against a subsequent high dose of the same drug and that colchicine and vinca alkaloids protected against subsequent whole-body irradiation [1, 2]. The main proponents of this approach have expanded their large animal research by investigating cyclophosphamide priming before carboplatin [3]. This last agent, an analog of cisplatin, has demonstrated activity both in animal tumors and in an expanding circle of human tumors. This research group demonstrated that a priming dose of intraperitoneal cyclophosphamide given 1 or 2 days before a lethal dose of carboplatin reduced animal mortality by 80–100%. It also raised the number of pluripotent hematopoietic stem cells (CFU) to an amount similar to the one seen with cyclophosphamide given alone. It eliminated entirely the post-chemotherapy leukopenia and anemia, while

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reducing and shortening the chemotherapy-induced thrombopenia. Conversely, priming with cyclophosphamide did not interfere with the cancericidal activity of carboplatin but appeared to act as a synergistic agent.

Many unanswered questions are raised by this study. The tissue distribution of carboplatin on days 1 and 4 is no different between control and primed animals, except for increased carboplatin concentration in skin, muscles, and kidney, although this could be a matter of temporal sampling. It is unfortunate that similar data on concentration were not obtained for the tumor. Similarly, blood–urea nitrogen was higher and kidney weight was smaller in primed animals; this could be explained by the additive renal toxicity of the 2 agents in combination. The authors do not explain, either, how 2 cytocidal agents managed to raise the white cell count in these animals.

In general, proliferating cells are more sensitive to cancer chemotherapeutic agents than are nonproliferating cells. This applies not only to hematopoietic cells but also to cells of the gastrointestinal epithelium. This differential effect can be demonstrated only if a proper time interval between drug administration and the assay is chosen. The best time would be at the completion of all cell killing but before the remaining cells have started to proliferate. As a corollary, a second dose of chemotherapy could enhance cytotoxicity if given during a period of increased renewal of the stem cell population.

The laboratory evaluation of a cytotoxic insult will vary, depending on the technique used and the time factor [4]. A single dose of cyclophosphamide will reduce DNA synthesis of bone marrow cells with a nadir at 12 hr, and a return to control value by 36 hr, followed by an overshoot. Cells of the gastrointestinal mucosa follows a similar pattern, whereas the DNA synthesis of the tumor remains depressed for 6–8 days. If one examines the peripheral white cell count and bone marrow cellularity levels, nadirs will be reached later than DNA synthesis—36 hr in the case of white cells, 3½ days for bone marrow. The toxic reactions from 2 separated doses of cyclophosphamide are better related to the DNA synthesis than to the clinical tests of white cell count and bone marrow cellularity. The toxicity of the second dose will be the smallest if given at the nadir of DNA synthesis, i.e. at 12 hr and the greatest at the peak of DA synthesis, i.e. at 3 days.

A somewhat similar situation should apply to the clinical situation [5]. Short-term cell cultures of human bone marrow done at varying times after administration of 1 g/m^2 of cyclophosphamide show a nadir of 30% within 3 hr and last about 1 week to overshoot base line value beyond day 18.

Millar *et al.* have done many animal studies to further define the use of priming. A single injection of cyclophosphamide can protect most tumor-free mice from a lethal dose of busulphan if given 1 or 2 days before busulphan [6]. The white cell counts follow a time course similar to that seen if cyclophosphamide had been given alone.

A similar phenomenon occurred if a single injection of cyclophosphamide was used as a priming agent before a lethal dose of gamma-irradiation [7]. Cyclophosphamide also induced a rapid recovery of the blood leukocyte count as compared with irradiation given alone. However, irradiation could not act as a priming agent to a subsequent dose of irradiation and neither did busulphan.

The concept of priming was then extended to idea of protecting the intestinal epithelium against a large dose of melphalan [8]. Other agents, such as cytosine arabinoside, methotrexate, or low-dose melphalan, were also able to act as priming agents [9]. Again, there was a much more rapid recovery of marrow stem cells and peripheral leukocytes. In an animal tumor model, pretreatment with cyclophosphamide or melphalan did not protect the tumor against the challenge agent but, rather had a further cancericidal effect [10].

Thymectomized, immunodepressed mice bearing human oat cell xenografts were able to tolerate a larger dose of cyclophosphamide after priming than if nonprimed [11]. Priming did not reduce the tumor kill of the large challenge dose but did allow a larger one to be given. This improved the therapeutic index because of reduced toxicity to the normal tissues. It was also determined that the priming did not alter the pharmacokinetic behavior of the challenge drug.

The mechanism of priming remains in doubt; however, a modification in the pharmacokinetic behavior of the challenge drug has been ruled out. Also, the induction of repair enzymes has not been demonstrated. The induction of one or more circulating factors by the priming drug to stimulate the stem cells of rapidly dividing tissue has been suggested by the results of some experiments. However, this would make the bone marrow and gastrointestinal epithelium even more susceptible to damage by the challenging dose. The theory of circulating factors has been given support by their isolation, synthesis, and better definition of their cell targets. Another explanation is that the priming dose shuts down the stem cell compartments, thereby allowing a greater tolerance to the subsequent exposure to the challenging dose, a theory exactly opposed to the previous one.

The clinical applications of priming have not been very successful. Ettinger *et al.* administered high-dose cyclophosphamide for small cell bronchogenic carcinoma divided into 2 daily doses, on

either days 1 and 2 or on days 1 and 8 for 1 cycle [12]. There was no difference in the incidence of remission. The day 1 and day 8 schedule was less toxic than day 1 and day 2 schedule for limited disease presentation in cases of thrombocytopenia only.

Hedley *et al.* treated 7 melanoma patients with 500 mg of cyclophosphamide, 7 days before administering 140 mg/m^2 of melphalan [13]. The white cell count nadir was similar to that of 4 patients treated with 60–125 mg/m^2 melphalan alone. However, recovery of leukocytes was faster after day 18.

Harland *et al.* treated small cell bronchogenic carcinoma with cyclophosphamide, methotrexate, and vincristine every 4 weeks, but half of the patients recieved 600 mg/m^2 of cyclophosphamide 1 week before [14]. There was no difference between these 2 groups of patients in response rates, median duration of response, median duration of survival, or peripheral leukocyte count.

Spitzer *et al.* treated 36 patients with bronchogenic carcinoma of various histologies by giving melphalan at 20–40 mg/m^2 [15]. The patients then received the same regimen, 4 weeks later, preceded by cyclophosphamide, 300 mg/m^2, 1 week before melphalan. Therapeutic results were disappointing, with no objective remissions. Cyclophosphamide priming did not modify the nadir level of leukocytes or platelets.

Selby *et al.* (personel communication) evaluated the chromium edetic acid (EDTA) absorption test as a measurement of degree of intestinal permeability in patients treated with 200–220 mg/m^2 of melphalan with or without a priming dose of cyclophosphamide (300–400 mg/m^2, 1 week earlier). Priming reduced the degree of intestinal permeability in a significant manner, although the overall effect is modest.

At this moment, priming remains an interesting, but poorly understood, laboratory curiosity. Its application in the clinic is dependent upon the clarification of a number of details: (1) the amount of the priming dose, (2) the best drug to use for priming, (3) the best dosage of a single agent or combination of agents used for challenge, and (4) most important, the time interval between the priming and challenging doses. Most authors have used a fixed interval of 7 days instead of testing a number of different time intervals. Conceivably, a much shorter interval might be preferable to this.

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