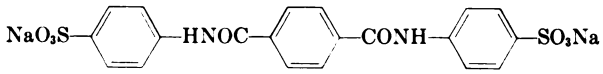
Informal Discussion on Toxicity of Anti-Leukemic Drugs

PREPARED BY JAMES F. HOLLAND

Roswell Park Memorial Institute, Buffalo, New York

The delayed toxicity seen in animals by Dr. Kensler is of the same sort Dr. Burchenal and co-workers found in patients with the pterephthalanilide NSC-53306[1]. Not until 42 days after the discontinuance of the drug in 1 patient did renal electrolyte resorption become impaired. Chloride, sodium, potassium, calcium, and magnesium then poured out in the urine, and the serum levels fell. Several other patients getting even smaller doses of NSC-53306 had delayed toxicity, chiefly electrolyte abnormalities without azotemia or tubular necrosis.

A search for compounds to potentiate or counteract the anti-leukemic effects of the terephthalanilides had occupied Burchenal, Hirt, Fischer and Balsiger. Monosulfonic and disulfonic acid derivatives synthesized by Hirt and Berchtold, which are somewhat structurally related to the terephthalanilides, are of interest.



NSC-55156

The simplest one has sulfonic acid groups in the terminal positions instead of imidazolin, tetrahydropyrimidine, or amidine groups of the terephthalanilides. They were looking for ways to prevent the acute toxicity of such compounds as NSC-57155 or NSC-67725 so they could give a complete course of therapy in a single dose for studies such as Dr. Skipper mentioned. Single-dose therapy might also be important in cattle trypanosomiasis, an area where certain of the terephthalanilides seem to have considerable promise.

Single doses of 100 mg/kg of NSC-57155 or 100 mg/kg of NSC-67725 (2 and 10 times the acutely lethal doses, respectively), when mixed before injection with 100 or 200 mg/kg of NSC-55156, lost their acute toxicity but retained their therapeutic effect in leukemia P815. Other doses eliminated chronic toxic effects but also anti-leukemic effects. The search continues for some combination of terephthalanilide and sulfonic or phosphoric acid analog in which the acute and chronic toxicity is diminished but the anti-leukemic activity remains unchanged.

DR. KENSLER recalled that polysulfonated Congo red and chlorazol fast pink could counteract the effects of curare in frogs. He cited data that showed that polysulfonates complexed with curare and thus scavenged molecules that had not been bound. Hopefully, a polyanion may be found that has a higher affinity for the phthalanilides than to the tissue components; it would thus be able to terminate, or selectively reduce, toxicity.

Dr. Kensler stated that pathologically the predominant renal lesion from terephthalanilide was necrosis and that there are some hepatic necrosis and fatty infiltration. A fuller description is available (1).

DR. CALABRESI then presented some results of azauridine treatment of psoriasis and mycosis fungoides. He made the point that if one seeks evidence of toxicity in an organ that is diseased, it may be obscured. This was a difficulty in assessing the clinical pharmacology of azauridine in leukemic patients. It has now become apparent in patients with psoriasis that there is some mild hemopoietic toxicity of azauridine.

In psoriasis the basal skin layer is very active and frequently shows increased mitosis. There is an indication of increased nucleic acid metabolism, as evidenced by high serum uric acid levels. There is hyperplasia of the cell population, and the end product is an immature cell that retains its nucleus. The keratin is abnormal.

Serial biopsies at 7, 14, and 21 days from the same patient treated with azauridine over this period showed substantial improvement of the histologic picture of psoriasis, practically back to normal. Clinical regression of psoriasis of the generalized plaque form and the exfoliative form, after short courses of azauridine treatment, was also demonstrated.

Mycosis fungoides, a skin disorder thought to be related to lymphoma, and certainly associated with leukemia in some instances, showed partial regression after azauridine therapy. Although activity in these diseases is not unique, since it is achieved with minimal toxicity to other normal cells, it may be an important point in terms of not losing hope of finding other agents that have selectivity.

DR. GOLDIN presented data on a single treatment with a terephthalanilide on the 7th day following leukemic inoculation, at half the optimal dose, followed by daily methotrexate. This gave a remarkable increase in the survival time of the animals. This exemplifies another way in which one could attempt to avoid undesirable toxicity of a drug; namely, give the drug for a shorter duration, in this instance only 1 treatment, and give a smaller dose in combination with a 2nd drug. He also presented data indicating that appropriate combination chemotherapy of mouse leukemia can enhance chemotherapy and can delay the origin of resistance.

DR. LATARJET made a brief presentation of a few experiments with irradiated virus, from which he deduced isogenous interference was possible. When a cell, tissue, or organ is infected *in vitro* or *in vivo* with 2 viruses, each of

[1] The following abbreviations are used: NSC-53306, terephthalanilide, 4',4''-di-2-imidazolin-2-ylamino, dihydrochloride; NSC-57155, terephthalamidine, N,N''-bis[*p*-(*N'*-methylamidino)phenyl], tetrahydrochloride; NSC-67725, quinolinium, 6,6'-(terephthaloyldiimino)bis[1-methyl]; NSC-55156; benzenesulfonic acid, 4,4'-(terephthaloyldiimino).

which ordinarily could grow in the absence of the other, an interference may be set up and one virus excludes or depresses the other one. A particular case of interference is that which is produced by the virus itself when it has been suitably irradiated.

With a certain dose of irradiation, one may decrease the infectivity of the virus but still leave a great deal of its interfering capacity. Most of the time the interfering capacity is much more radioresistant than the infectivity.

In 3 experiments cited, Dr. Latarjet reported a significant reduction in the incidence of "spontaneous" early injection of irradiated, inactivated leukemic extracts.

He stressed that there are many unknown factors, such as the state of the virus, its resistance to irradiation, the method of inoculation, the type of radiation, the means a virus has to reach cells where interference can take place. Thus it should not be surprising if some experiments are negative, and several were presented.

Dr. Latarjet summarized that 3 main complex possibilities exist to explain the positive data: (*a*) A competition existed at the cellular level between inactive particles and active virus, such as has been visualized with bacteriophage in the bacterium. The 2 particles would be competing for certain special sites of the cell required for virus multiplication, and if the cell does not differentiate between the particles, and the irradiated one comes first, then the other active virus would be excluded. (*b*) The 2nd possibility is an interference of a type linked to the capability of irradiated virus to induce synthesis of interferon in the cell. (*c*) An immunologic response is a possibility. Repeated injection of irradiated virus might produce an antibody reaction, such that the malignant cells, when they are formed, encounter an immunologic response that would eliminate them. For the time being, Dr. Latarjet favored an interference phenomenon.

DR. WELCH cited unpublished data of Dr. Richard Stewart that showed that neoplastic transformation of chick fibroblasts in culture following exposure to Rous virus can be profoundly suppressed by 5-iododeoxycytidine.

Rous virus, although an RNA (ribonucleic acid) virus, may actually initiate the synthesis of DNA (deoxyribonucleic acid) in some way, since 5-iododeoxycytidine has no other known role as an inhibitor except of DNA synthesis. Dr. Welch speculated that RNA viruses might involve some DNA pathways in neoplastic transformation and that chemotherapeutic agents such as 5-iododeoxycytidine and 5-iododeoxyuridine might be useful for RNA viral-induced neoplasia as well as DNA viral tumors.

DR. MODEST presented the question of the immunosuppressive properties of anti-leukemic agents. He and Dr. Richard Adams considered it possible that the wide variety of agents with activity against human leukemia may have some common explanation as far as mode of action is concerned. If one assumes a number of things, the story runs roughly this way: Assume a mutation to form the leukemic cell; assume the leukemic cell is antigenic, but in an abnormal manner. Assume that the leukemic cell depends for its survival on the presence of antigens on its cell surface, which are protected through the enhancement phenomenon by circulating antibodies. Next, because these antigenic sites are protected by circulating antibodies, they are not available to allow the cell to be destroyed by cellular antibody. If a differential inhibition of circulating antibody production over cellular antibody production occurs, from anti-leukemic or immunosuppressive agents, a mechanism exists whereby an anti-leukemic effect can be achieved.

DR. DAY postulated that antigenicity of lymphoblastic leukemic cells existed at the beginning but that the production of antisera that cause reaction with these new antigens is probably a homeostatic regulator permitting the survival of leukemic cells. Perhaps leukemia is due to the operation of antisera against newly formed antigens that otherwise would prevent the survival of the cells rather than cause initiation or continuation of leukemia. In the case of immunologic enhancement, therefore, Dr. Day suggested that there might be antibody coating on antigenic sites in cells; immunologic enhancement probably is very analogous in its effects to antigenic deletion. He stated that antisera probably, at least for the present, might be more deleterious in leukemic treatment than an effective drug. This is in contrast to the probable favorable action of cellular immune forces.

REFERENCES

1. Kensler, C. J. Chemotherapeutic Activity of Phthalanilide Derivatives. An Approach to Anticodic Therapy? Cancer Res., *23:* 1353–63, 1963.