

REGIONAL PROTECTION IN CANCER CHEMOTHERAPY. I. INFUSIONS OF THYMIDINE INTO EXTERNAL CAROTID ARTERY OF PATIENTS RECEIVING SYSTEMIC 5-iodo-2'-DEOXYURIDINE *

By PAUL CALABRESI

(From the Departments of Medicine and Pharmacology, Yale University School of Medicine, New Haven, Conn.)

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The ability of an antimetabolite to interfere with the production of DNA is accompanied by manifestations of cellular toxicity in rapidly proliferating tissues. Attempts to achieve a more specific inhibition of rapidly dividing cells have focused attention on thymidine, since this pyrimidine nucleoside is required exclusively for the biosynthesis of mammalian DNA. Welch and Prusoff (1) recently have reviewed studies with a halogenated analog of thymidine, 5-iodo-2'-deoxyuridine (IUDR) (Figure 1), which demonstrate its ability to interfere with the utilization of thymidine for the synthesis of DNA. Although IUDR is capable of being incorporated into DNA (2, 3),

replacing up to approximately one-third of the thymidylic acid units of the total DNA polymers in a single cell replication (4), preferential utilization of thymidine for this purpose is 30 to 40 times as effective as that with equimolar amounts of IUDR (5). The competitive nature of this inhibition has been substantiated by experiments showing that, in mice, the cytotoxic effects of IUDR can be entirely prevented by the concomitant administration of thymidine (6).

Intravenously administered IUDR exerted mild antitumor effects in man, but only with dosages that produced stomatitis, alopecia, and leukopenia (7). Unfortunately, as with other antimetabolites acting on related sites, the best inhibitions of tumors occur at the expense of marked toxicity to rapidly proliferating normal cells (8-10), an effect that notably compromises their clinical usefulness in the management of patients with advanced neoplastic disease. Therefore, pending the demonstration of preferential metabolic pathways that may allow biochemical discrimination between normal and neoplastic cells, other methods must be sought to enhance the therapeutic efficacy of the available drugs.

The present study was designed to investigate the feasibility of protecting a region of the body by local infusion of thymidine during systemic administration of IUDR. A preliminary report has been presented (11).

MATERIALS AND METHODS

Clinical material. Fourteen patients with advanced metastatic neoplasms and without obvious bone marrow involvement were treated with IUDR as described below. Five of these patients had been included in previous toxicity studies (7). Three others succumbed to their disease before a sufficient period of observation had elapsed and were excluded from the tabulations. In every case there was need for palliation not obtainable

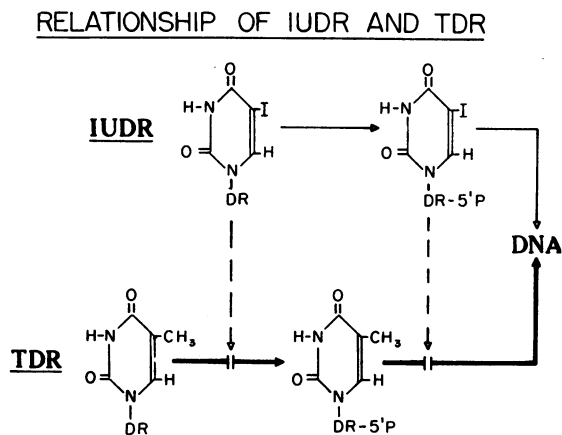


FIG. 1. SCHEMATIC REPRESENTATION OF THE RELATIONSHIP OF THYMIDINE (TDR) AND 5-iodo-2'-DEOXYURIDINE (IUDR) IN THE METABOLIC SEQUENCES LEADING TO THE BIOSYNTHESIS OF DNA. Deoxyribose is abbreviated as DR, and 5'P signifies the presence of a phosphate group on the 5-position of the deoxyribose. The heavy lines denote the normal, preferential pathway with the interruptions indicating probable sites of inhibition.

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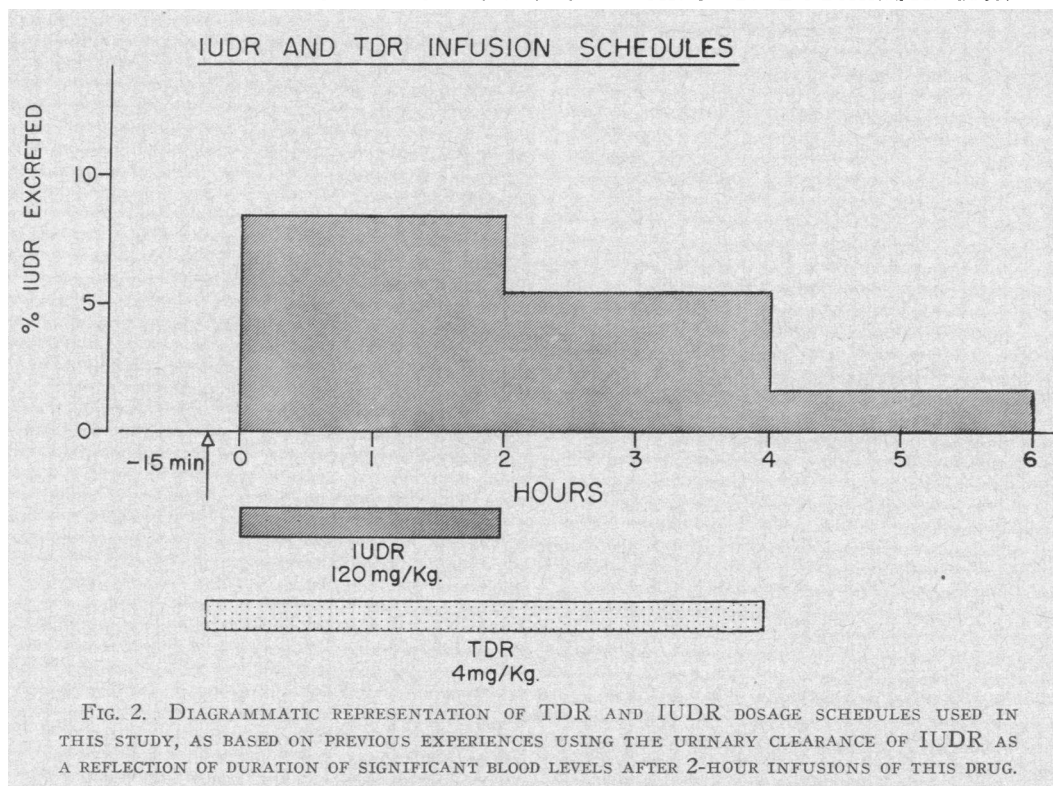


FIG. 2. DIAGRAMMATIC REPRESENTATION OF TDR AND IUDR DOSAGE SCHEDULES USED IN THIS STUDY, AS BASED ON PREVIOUS EXPERIENCES USING THE URINARY CLEARANCE OF IUDR AS A REFLECTION OF DURATION OF SIGNIFICANT BLOOD LEVELS AFTER 2-HOUR INFUSIONS OF THIS DRUG.

by surgery, irradiation, or standard drugs, and the patients requested investigative therapy for their disease.

Drugs and dosage schedules. Solutions of IUDR in concentrations of 5 to 8 mg per ml of 5 per cent glucose in water were adjusted to a pH of 8.6 [the compound is poorly soluble at pH 7.4 (1)] and prepared for infusion by sterile filtration. Intravenous doses of 110 to 120 mg per kg were administered during a period of 2 hours daily for 5 days. Solutions of thymidine in concentrations of 0.2 to 0.4 mg per ml of 5 per cent glucose in water were started 15 minutes before and continued for 2 hours after the completion of each 2-hour infusion of IUDR (Figure 2). The daily doses of thymidine, 4 mg per kg, were infused with a Sigmamotor pump (model TM-11) either into the external carotid artery (Figure 3) through a PE-90 polyethylene catheter (Clay-Adams, N. Y.) or into the bone marrow cavity of the posterior iliac crest (Figure 4) through a no. 16 bone-marrow needle. Catheterization of the external carotid artery was performed either directly, through a purse-string suture, or by cannulating the superior thyroid branch, by the techniques of Sullivan and Duff and their colleagues (12, 13).¹ The position of the catheter tip was confirmed by radiopaque angiography, by tissue distribution of injected fluorescent dye (Fluorescite, C. F. Kirk Co., N. Y.) under ultraviolet light, and by determination of the circulation time from catheter to mouth with sodium dehydrocholate.

¹ The surgical procedures were performed by Drs. R. Houlihan, P. Smith, J. Mark and T. Goentch.

Laboratory Studies. Total and differential leukocyte counts were performed by standard techniques. Aliquots of bone-marrow aspirates were evaluated for cellularity by centrifugation in calibrated small-bore tubes and by microscopic examination of Wright-Giemsa-stained preparations.

Radioactive iron (^{59}Fe) was given intravenously, 50 μc , in the form of ferrous citrate (Ferrutope, E. R. Squibb and Sons, N. J.). A sodium iodide crystal scintillation probe, shielded by a lead collimator with a 3-cm aperture, placed 5 cm above the skin surface, was used for external monitoring.²

RESULTS

Stomatitis, alopecia, and leukopenia were observed in all patients who received IUDR at the dosage indicated, without concomitant protective infusions of thymidine (TDR) into the external carotid artery (Table I). Painful bullous or ulcerated lesions on the lips or buccal mucosa usually appeared shortly after completion of the 5-day course and persisted for periods up to 3 weeks. Loss of hair was extensive after the third week but, after a period of nearly total baldness, normal regrowth of hair occurred if administra-

² This procedure was performed in the radioisotope unit in cooperation with Dr. C. H. Chang.

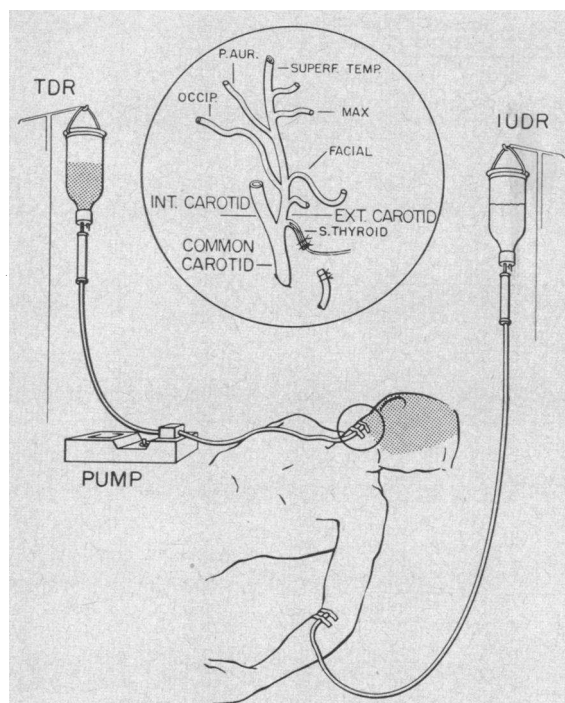


FIG. 3. ANATOMICAL DIAGRAM OF THE INFUSION PLAN DEMONSTRATING INTRA-ARTERIAL ADMINISTRATION OF TDR INTO THE EXTERNAL CAROTID ARTERY THROUGH A SIGMAMOTOR PUMP WHILE IUDR WAS INFUSED INTRAVENOUSLY INTO THE ANTECUBITAL FOSSA. Insert shows the catheter in position and the circulatory distribution of the external carotid artery.

tion of the drug was discontinued. In every instance the leukocyte count fell below 4,000 cells per mm^3 and usually remained below 3,000 cells per mm^3 for several days.

All four patients who received protective infusions of TDR into the external carotid artery (Table I) developed neither bilateral stomatitis nor a comparable degree of hair loss on the protected side (Figure 5, a). The activity of IUDR, in areas distant from the arterially infused site, was not compromised by recirculation of the dos-

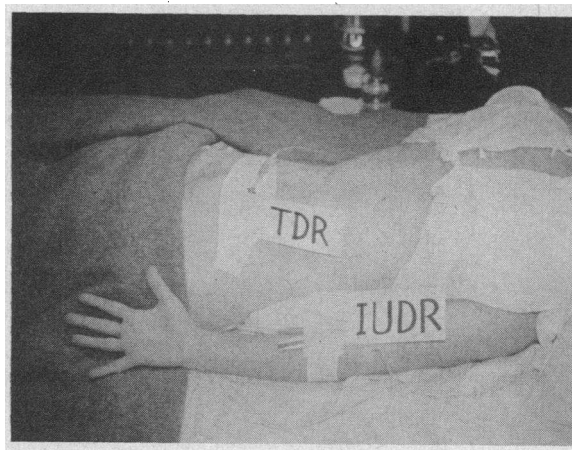


FIG. 4. PATIENT E.W. RECEIVING INFUSION OF TDR THROUGH A NO. 16 NEEDLE INSERTED INTO THE MARROW CAVITY OF THE RIGHT POSTERIOR ILIAC CREST WHILE IUDR WAS ADMINISTERED INTRAVENOUSLY INTO THE RIGHT FORE-ARM.

age of TDR, as evidenced by the contralateral alopecia and by the levels of leukopenia, which were comparable with or more prolonged than those observed with identical courses of IUDR alone (Figure 6). During this study injections of fluorescent dye or of sodium dehydrocholate were found to be convenient and rapid bedside methods of confirming the desired position of the catheter. In the case of sodium dehydrocholate, an immediate bitter taste was reported if the catheter was correctly placed, while the delay of a full circulation time of approximately 14 seconds signified that the tip of the catheter had been misdirected.

Three of the above four patients received subsequent courses of IUDR with concomitant infusions of TDR directly into the marrow cavity of the posterior iliac crest (Table I). In every instance, stomatitis and nearly total alopecia (Figure 5, b) were observed, but no protection of the peripheral leukocyte count (Figure 7) and no

TABLE I
Regional protection with thymidine during therapy with 5-iodo-2'-deoxyuridine

TDR infusion	No. of cases	Leukopenia	Stomatitis	Alopecia
None	12	12	12	12
Ext. carotid	4	4	0	0 protected side 4 other side
Iliac marrow	3	3	3	3

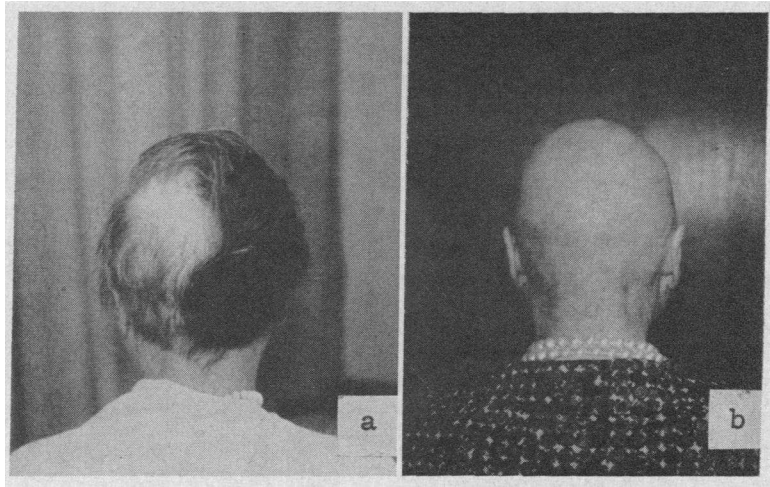


FIG. 5. PATIENT R.B. DEMONSTRATING MARKED REDUCTION OF HAIR LOSS (a) AFTER SYSTEMIC COURSE OF IUDR THERAPY WITH CONCOMITANT RIGHT EXTERNAL CAROTID ARTERY INFUSION OF TDR. TOTAL ALOPECIA IN THE SAME PATIENT (b) AFTER AN IDENTICAL COURSE OF THERAPY WITH IUDR WITHOUT TDR PROTECTION TO THE HEAD.

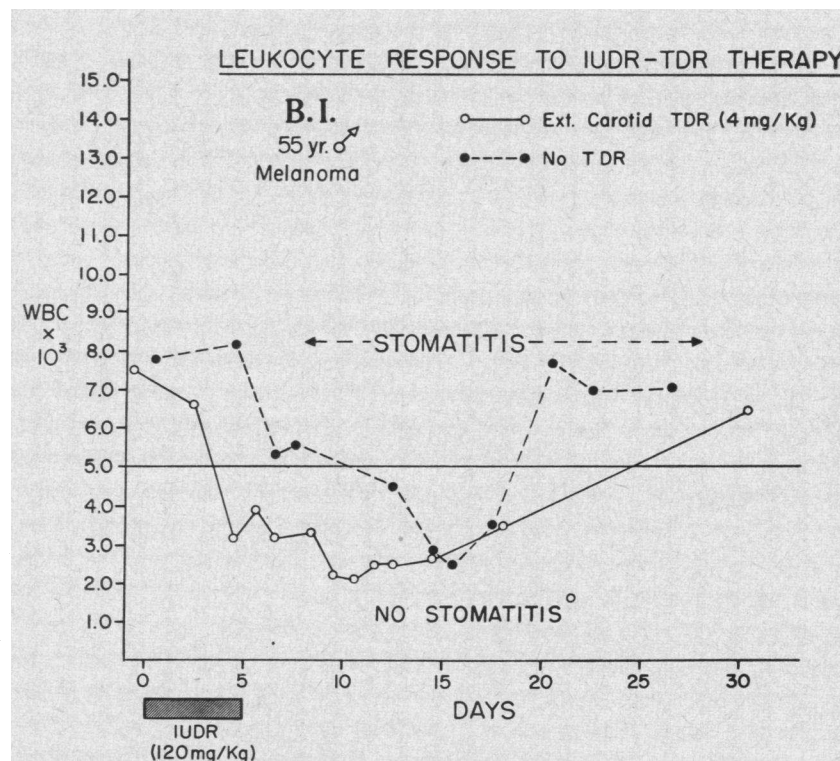


FIG. 6. LEUKOCYTE RESPONSE IN TWO SEPARATE BUT IDENTICAL COURSES OF IUDR THERAPY IN PATIENT B.I., ONE WITH (○—○) AND ONE WITHOUT (●---●) THE INFUSION OF TDR INTO THE EXTERNAL CAROTID ARTERY, ILLUSTRATING NO INHIBITION OF SYSTEMIC IUDR EFFECT BY RECIRCULATION OF THE DOSE OF TDR USED.

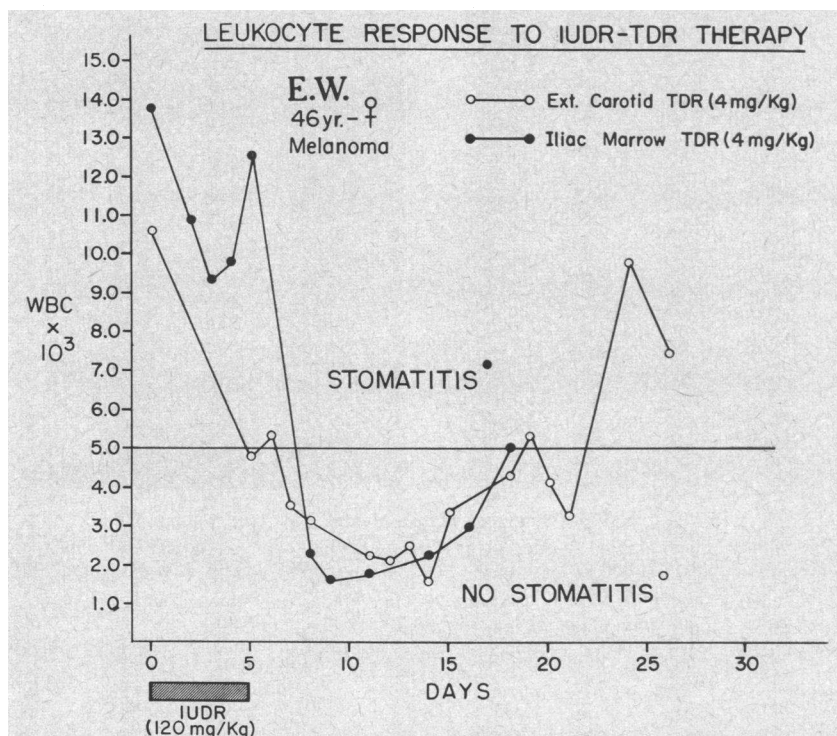


FIG. 7. COMPARABLE LEUKOCYTE RESPONSE IN TWO SEPARATE BUT IDENTICAL COURSES OF IUDR THERAPY AND TDR IN PATIENT E.W. When TDR was infused into the external carotid artery (○—○), protection against bilateral stomatitis and hair loss on the infused side was observed, but when TDR was administered into the marrow cavity of the posterior iliac crest (●—●), no protection of the peripheral leukocyte count could be demonstrated.

significant difference in the cellularity of bilateral marrow aspirates could be demonstrated. Patient R.B. also received 50 μ c of Fe^{59} on the tenth day after initiation of therapy (Figure 8), at the nadir of the induced leukopenia. An increase in radioactivity up to fivefold at 6 hours was obtained by external monitoring over the infused marrow site, as compared with that of the opposite posterior iliac crest.

DISCUSSION

Several clinical techniques have been devised in recent years in order to obtain enhanced antineoplastic effects with the greatest possible amount of protection to normal tissues of the host. These have included extracorporeal storing of autologous bone marrow (14, 15), isolation-perfusion of various regions of the body (16), administration of drugs into the arterial supply of tumors (17), and occlusion of the circulation to bone marrow areas

by balloon catheters (18) or pneumatic tourniquets (19).

By combining the intermittent intramuscular administration of folinic acid (citrovorum factor) with continuous intra-arterial infusions of methotrexate (amethopterin), Sullivan and his co-workers were able to deliver this antimetabolite to patients with advanced head and neck (12) or pelvic neoplasms (20) at unusually high doses (in the range otherwise expected to produce fatal systemic toxicity). Of particular interest was the suggestion that, in some of the cases, administration of methotrexate into one external carotid artery resulted in greater local toxicity on the infused side (12). This implied that the local effects achieved by high concentrations of a compound were not reversed by concomitant systemic administration of a physiologically utilized antagonist. Accordingly, it was conceivable that the opposite effect could be obtained: protection of a

vulnerable region of the body by maintaining high local concentrations of a normal metabolite while the corresponding antimetabolite was circulating systemically. The system described was designed to test this hypothesis.

In the present series the total dose of IUDR was not increased above the usual limit of systemic administration, but at these levels, which must be reached to achieve tumor inhibition, toxicity has been consistent and reproducible. Previous studies (1, 7) have shown that, after a 2-hour infusion of IUDR- I^{131} in man, significant blood levels of the intact compound, as reflected by the rate of urinary excretion, persisted for 2 hours after completion of the infusion. Hence, the protective solution of thymidine was infused during the entire 4 hours and was started 15 minutes before the IUDR (Figure 2), in an attempt to saturate the enzymes responsible for the formation of the corresponding 5'-phosphate derivatives (Figure 1). Although 60 times more IUDR than TDR was given during the first 2 hours, the antimetabolite was diluted in the entire blood volume,

whereas TDR was diluted, initially, only in the blood flow of the external carotid artery. The actual concentrations of TDR and IUDR achieved in the arterially infused area are not known, but it is likely that they were similar in order of magnitude and, on the basis of previous animal experiments (5), such levels presumably should afford a 30-fold preferential utilization of TDR. The schedules used, however, were chosen arbitrarily and do not necessarily constitute the best possible combination of the two agents. Determination of the systemic pharmacological response to IUDR was based on the peripheral leukocyte count, rather than on tumor inhibition. The former criterion was preferred, since it is a more reliable and quantitative index of drug activity which can be reproduced in the same patient or compared in different individuals. The results of this study indicate not only that protection was obtained, but also that the systemic action of IUDR was not compromised by the recirculation of TDR at this dosage level. It is evident that any claim for regional protection, as opposed to

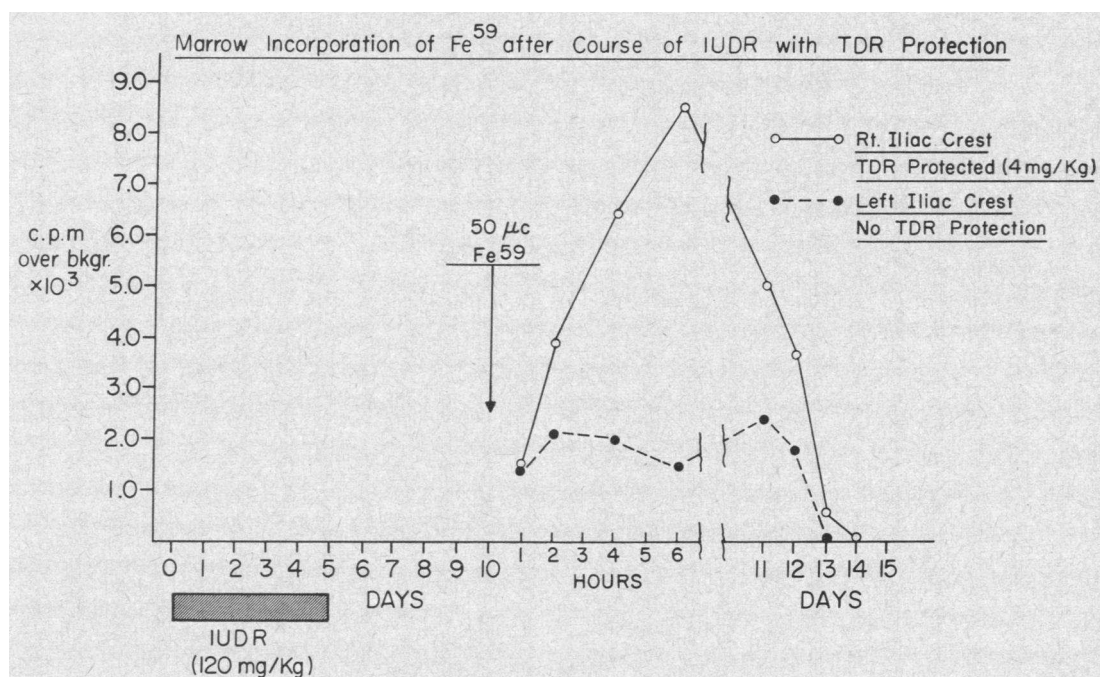


FIG. 8. RADIOACTIVE IRON (Fe^{59}) INCORPORATION STUDY IN PATIENT R.B. ON TENTH DAY AFTER INITIATION OF 5-DAY COURSE OF INTRAVENOUS THERAPY WITH IUDR, WITH SIMULTANEOUS PROTECTION OF THE BONE MARROW OF THE RIGHT ILIAC CREST WITH TDR. External monitoring over TDR-infused marrow site (o—o) demonstrated increased radioactivity as compared with the unprotected opposite side (●---●).

the total neutralization of drug action, can be made only in the presence of both findings.

The localized nature of this protection, as demonstrated by the relative difference in hair loss on the protected and unprotected side (Figure 5, a), was not observed with respect to stomatitis. A possible explanation for this was provided by examination of the scalp and oral cavity with an ultraviolet light source after the injection of fluorescent dye into the catheter. The pattern of fluorescence in the scalp indicated a relatively unilateral distribution, whereas varying amounts of fluorescent dye quickly reached the contralateral oral mucosa, a finding that suggested a rich collateral circulation.

Although stomatitis can be extremely uncomfortable and alopecia is cosmetically important, bone marrow depression is, at present, the toxic factor that limits the dose of IUDR. Accordingly, an attempt was made to achieve regional protection of a bone marrow area by the easiest and most direct approach: infusion of thymidine directly into the marrow cavity of the posterior iliac crest (Figure 4). Although it was realized that this approach might not permit a significant quantity of marrow to be reached, such a trial was desirable because of its technical simplicity. The absence of adequate hematopoietic protection was reflected by the inability to prevent leukopenia (Figure 7), but the occurrence of stomatitis and nearly total alopecia further confirmed the finding that the protection of the oral mucosa and of the scalp, previously observed, was not attributable to the administration of thymidine per se, but rather to the high local concentrations obtained by arterial infusion.

That some protection to a localized area of marrow may have been achieved is suggested by a study of the incorporation of Fe^{59} in one patient (Figure 8), and the possibility of protecting larger areas of hematopoietic tissue *in situ* seems to be worth pursuit, since it could afford a large number of shielded, viable cells already in their normal habitat. However, a better route of administration must be sought in order to insure more diffuse distribution of the protective agent to the marrow. Preliminary studies in dogs indicate that the internal iliac artery, a major source of blood supply to the iliac crest, can be used for this purpose (21). Similar efforts to achieve

bone marrow protection by this route are now in progress in patients with advanced neoplasms (22).

Finally, it should be noted that the IUDR-TDR combination merely represents a model system and that a number of other drug-antidote combinations or even regional hypothermia could be tried. While it is too early to predict the ultimate practical value of this method, it may offer another approach to achieving higher doses of antineoplastic drugs with greater protection to the normal tissues of the patient.

SUMMARY

Fourteen patients with metastatic neoplasms were treated daily for 5 days with intravenous doses (110 to 120 mg per kg) of 5-iodo-2'-deoxyuridine (IUDR), an antagonist of thymidine (TDR); in each of these individuals stomatitis, alopecia, and leukopenia were demonstrated.

Four of these patients received identical courses of IUDR with concomitant infusions of 4 mg of TDR per kg into the external carotid artery. Regional protection was demonstrated by the total absence of stomatitis and by marked reduction of hair loss on the protected side. The contralateral alopecia and comparable levels of leukopenia observed with each course of therapy indicated that the activity of IUDR, in areas distant from the arterially infused site, was not compromised by the recirculation of the dose of TDR that was used.

Attempts to achieve regional protection of the bone marrow during IUDR therapy by direct infusions of TDR into the posterior iliac crest of three patients failed to prevent leukopenia, but increased incorporation of Fe^{59} over this area in one patient suggested localized protection.

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