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SUBJECT: The Therapeutic Effect of Total Body Irradiation Followed by Infusion of Stored Autologous Marrow in Humans

Purpose of Study:

The goal of this study is to test the hypothesis that total body irradiation followed by infusion of stored autologous marrow is effective palliative therapy for metastic malignancy in human beings. Total body irradiation has been used for palliation by others (1,4,5,6) but the dose used has been limited to less than 200 R because of the bone marrow depression which follows large doses of radiation.

A combination of total body radiation and infusion of autologous bone marrow may make it possible to use larger doses of total body radiation. Effective total body radiation therapy without hematologic morbidity would be the most innocuous and least disturbing form of therapy available for the palliation of patients who have extensive neoplasm.

According to Pegg (1) "Autologous marrow aspiration and storage may be considered a wise precaution when new drugs and treatment schedules are being explored; it may then be possible

to treat the occasional unexpectedly severe hemopoietic depression more effectively." Mathe (2) has demonstrated in one patient that after 800 rad total body irradiation, preceded by 4 days administration of methyl-nitro-imidazolylmercaptopurine, a successful take of homologous marrow occurred.

It has already been proved that intravenous administration of bone marrow cells will repopulate the marrow space in mice (19), guinea pigs (19), rabbits (20), and dogs (21). In mice (22), rabbits (23) and dogs (21) intravenous administration of stored marrow cells prevented death after an otherwise lethal dose of radiation. It is not possible to apply this information directly to human beings because of the variable response of different species. We, and others (24) have established in vitro viability of bone marrow cells stored at -80 degrees C. for as long as one year. However, almost all in vivo studies in human beings where marrow transplantation has been attempted did not involve total body radiation or adequate doses of radiomimetic chemotherapy. Therefore, it is necessary to test in humans the effectiveness of marrow in repopulating the marrow space following total body radiation and to proceed with developmental techniques and refinements of marrow infusion. In the management of patients with malignancy who are to receive myelotoxic agents or of patients who incur marrow injury in other ways (e.g., warfare, nuclear accident of exposure in space) the availability of stored autologous marrow may well be life saving.

Proposed Procedures:

Patients will receive 200 rad total body irradiation as treatment for metastatic malignancy. (A control group includes patients who have already been irradiated at the 200 rad dose level and did not receive bone marrow infusion.) A second group of patients will be treated with 200 rad total body irradiation, followed by infusion through filters* of their previously stored marrow immediately after irradiation.

Patients selected will be those who are to receive total body irradiation in the treatment of metastatic malignancy from various primary sites, excluding neoplasms which are treated by internal radiation, e.g., carcinoma of the thyroid. They will have stable hematologic values and may have received local

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* The marrow will be infused intravenously with filtration through successive #50, #100 and #200 mesh filters (corresponding to openings of 297, 149 and 74 microns). Filters and tubing will be sterile and pyrogen free. Initially, all marrow will be infused through the filters. If the filtered marrow does not repopulate the marrow space, then unfiltered marrow will be infused to determine whether filtration was the reason the infusion was not effective.

radiation previously. Patients will be hospitalized at the Cincinnati General Hospital and will be treated with 200 rads (midline absorbed tissue dose) of total body irradiation at an exposure dose rate of 4-6R/min. from a Cobalt-60 source. This dose of radiation approximates the therapeutic and hematologic effects of 0.4 - 0.6 milligrams of nitrogen mustard per kilogram body weight (1,4,5,6).

Complete blood counts including hemoglobin, hematocrit, erythrocyte count, leukocyte count, platelet count, reticulocyte count, differential smears and cell indices will be obtained three to five times in the twenty-one days pre-irradiation period and 1, 2, 3, 6, 9, 12, etc. days after irradiation according to the standard test days of Thoma and Wald (7). Pre- and post-irradiation immunologic capabilities will be evaluated as well. Additional counts will be obtained as clinically indicated. Granulocyte reserves will be tested with etiocholanolone so as to ascertain the safety of giving 200 rads to a patient (see enclosed protocol).

Bone marrow specimens for cytologic and pathologic examination will be obtained from the sterum and posterior ilium prior to irradiation.

Bone marrow from the posterior ilium may be stored during the week prior to irradiation by the method of Kurnick (8, 9) or may be kept at 4 degrees C. under sterile conditions for the brief period of radiation and infused immediately afterward. The

bone marrow will be cultured for sterility prior to freezing. In vitro viability of the marrow will be estimated by trypan blue exclusion initially (8), but tests utilizing tissue culture (10), DNA synthesis (11), and acridine orange (12) are being developed.

Peripheral blood data will be compared to those which we have already obtained in patient who have been irradiated at the same dose level but have not received marrow infusion.

There will be a continuous review of the results as they are being obtained. If it becomes obvious that the results are statistically significant, the study will be terminated.

The data from these two groups of patients (with and without autologous marrow transfusion) will be compared with data obtained from patients with similar neoplasms who have been untreated and from patients who have received chemotherapy (25), according to age, sex, race, type, duration and extent of neoplasm. As a result of this comparison, it will be possible to determine the palliative value of total body irradiation at this dose level with and without bone marrow infusion.

Potential Hazards:

With any irradiation therapy there is the danger of radiation injury. We have observed consistent depression of hematologic values in the control group. Two hundred rads of total body radiation has therapeutic effectiveness equal to 0.4 - 0.6 mgm/kilo of nitrogen mustard. At this dose not only would

an observable therapeutic effect be anticipated but also at 200 rads the effect of marrow reinfusion will be detectable.

In this study the prodromal morbidity immediately after irradiation (anorexia, nausea, vomiting) would approximate 80% and responds well to phenothiazines. As to mortality figures at the dosages to be evaluated, in human beings the LD50 for bone marrow aplasia occurs at about 350 R and "intestinal death" occurs above 1000 R. With hospital care it is felt that a human LD50 may approximate 500 R or higher. The doses proposed are below these levels.

When the mean survival time of patients treated with less than 100 rad, 150 rad, and 200 rad are compared there is no progressive decrease related to dose (Tables I, II). If the patients with survival times of 9 and 10 days are excluded, and the patients still living at the present time are included, there is still no significant difference in survival time which can be associated with the irradiation as determined by several statistical tests. In fact the data suggest that a therapeutic effect from the radiation therapy has been obtained.

The use of autologous and isogeneic marrow in humans has been investigated by others (8, 9, 13, 14). When marrow has been infused too rapidly, cerebral hemorrhage, hemoglobinemia and hemoglobinuria have been observed. These hazards have not been encountered when the rate of infusion has been maintained below 60 drops/min.

There is disagreement as to the need for filtration. Kurnick does not feel it is necessary at the time of infusion; Thomas, Ferrebee, and Pillow (13,14,15) recommend its use to minimize the incidence of pulmonary embolism. A series of filters will be used with the now operational constant rate infusion pump.

To date, we have infused unfiltered marrow intravenously in five patients. One patient had hemoglobinuria. Autopsy material from three of these patients has been studied by Drs. E. A. Gall and B. Yamaguchi. No evidence of pulmonary emboli, pulmonary infarction or other disease as a result of marrow infusion was found on microscopic examination.

All patients will be followed carefully clinically. Hematological data are accumulated at frequent intervals and all measures to control infection will be used. They may include plastic barrier "Life Islands", reverse isolation, and laminar air flow units if needed. Prophylactic antibiotic therapy will not be prescribed.

Previous Work Done in This Area:

During the past seven years an ongoing study of the metabolic effects of total body irradiation has been in progress. (See enclosed Protocols A and B). To date, over 70 patients have been treated with 25 to 200 rad total body irradiation. The clinical courses of these patients has paralleled that of comparable patients treated with other agents. Bone marrow from

23 patients has been stored; it has been infused intravenously in 5.

Marked hematologic depression occurred in all patients who received more than 125 rad total body radiation. There was a total leukocyte count depression below 2000 WBC/cubic mm. and platelet count depression 25-40 days after irradiation. The mean minimum leukocyte count of previously untreated patients who received 200 rad total body radiation only was 1264 ± 1140, and was 1140 ± 816 when there had been previous therapy. The nadir of the leukocyte counts of patients who received 200 rad was 983± 369.

A comparison of the hematologic changes in patients receiving partial body irradiation with those who received total body irradiation revealed a paucity of change in the total WBC, platelets, and hematocrit even when the patient received more than 200 rad partial body radiation. In 2 of the 10 patients who were given partial body irradiation, lymphopenia did not occur until 48 or 72 hours after exposure.

Infusion of stored autologous bone marrow has been completed in 5 patients. The marrow was infused intravenously without filtration (13). It now appears that 9 billion nucleated cells/cubic mm. will be necessary for successful autologous grafting.

Chromosome studies have been performed in these patients. In 4 patients who were studied in detail, endoreduplication has

been seen with increased incidence in the peripheral blood chromosome preparations (16). This observation may be associated with malignancy but was increased following total body irradiation. As an outgrowth of the experience with endoreduplication, the question as to whether the malignant cell was the one which might be undergoing endoreduplication was considered. Therefore, a method of cell separation utilizing a Ficoll gradient and ultracentrifugation was undertaken. Friedman, et al., have reported adequate separation of lymphocytes from granulocytes. Chromosome culture techniques following this cell separation have revealed metaphases (17).

Studies of a serum factor in irradiated patients which breaks chromosomes of normal cells are underway also.

Another facet of considerable interest in patients receiving large doses of total body irradiation has been the question of immunologic response. In cooperation with A. J. Luzzio, studies of serum obtained from patients before and after total body irradiation have been performed. These data suggest an alteration in the antigenicity of human serum gamma globulin as a result of <u>in vivo</u> irradiation (18). Immediate and delayed hypersensitivity reactions before and after radiation, using the patient as his own control, are also under study. (See attached protocol C).

<u>Method To Be Used in Procuring Consent of Subjects Above</u>:

Consent forms, as attached, have been used since May 1, 1965. These forms are signed only after the patient has been advised of the research and study aspect of the procedures to be used. The patients are told this is a form of therapy but that benefits cannot be guaranteed. They are advised of the possible complications, including the possibility of pulmonary infarct following marrow infusion. Since total body radiation is another mode of treatment for patients with an ultimately fatal disease, it is only correct to indicate the possibility of therapeutic advantages. All patients are informed that a risk exists, but that all precautions to prevent untoward results will be taken.

General Remarks:

Many studies have been carried out in animals regarding the protective effect of bone marrow following radiation. They are well summarized in these paragraphs from Pegg's book. (1)

"The possibility of protecting mice and guinea pigs with allogenic haemopoietic cells was first demonstrated by Lorenz and Congdon (Lorenz, et al., 1952; Congdon, et al., 1952). Similar results have been obtained in rabbits (Hilfinger, et al., 1953; Porter, 1957, Porter and Murray, 1958; Hupka, et al., 1961); rats (Gorizontov, et al., 1963) and, after some initial failures (Weston, 1958; Rothberg and Akeroyd, 1958), successful protection has also been obtained in the monkey (Crouch and Overman, 1957; Overman, 1958). Similarly, there have been many failures in irradiated dogs (Porter and Couch, 1959; Stecher and Sullivan,

1959; Alpen and Baum, 1958; Puza, et al., 1961; Jordan, et al., 1961) but the Cooperstown group using more closely related dogs have reported more successful allogenic protection (Ferrebee, et al., 1958; Thomas, et al., 1958; Thomas et al., 1959). Others have since confirmed these findings (Trumbull, et al., 1963; Winchell, et al., 1963)."

"The survival of irradiated mice infused with guinea pig marrow was reported by Congdon, et al., 1952, but others have been unable to confirm this (Barnes and Loutit, 1954). There is, however, wide agreement that rat bone marrow will protect the irradiated mouse (Vos, et al., 1956; Nowell, et al., 1956; and Gengozian and Makinodan, 1957.)"

It is most important that methods of protecting against the undesirable effects of therapeutic efforts--radiation or other cytotoxic agents--be developed. Since hematologic depression is the limiting biologic response to radiotherapy under 1000 rads, it is the first for which protective methods must be developed. Based on animal work, it would seem that autologous marrow is the safest protective technique to be used initially.

<u>This study is proposed as a continuation of the work cited for the following purposes:</u>

1. To evaluate the role of total body radiation and autologous bone marrow in the treatment of extensive neoplasms;

2. To evaluate autologous bone marrow transfusion in the treatment of bone marrow depression from radiotherapy;

3. To determine whether stored autologous bone marrow therapy may play a role in treatment of bone marrow depression following acute radiation exposure in warfare or occupationally induced accidents.

This work is considered to be of vital importance not only for improving the survival of patients with far advanced cancer but for the survival of the citizens of this nation in the event of nuclear warfare or a major peacetime radiation accident.

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UNIVERSITY OF CINCINNATI MEDICAL CENTER
FACULTY COMMITTEE ON RESEARCH
VOLUNTARY CONSENT STATEMENT

*I_____ of _____
(Patient) (normal subject)

being of the age of majority and of sound mind and body, voluntarily and without force or duress, consent to participate in a scientific investigation which is not directed specifically to my own benefit, but in consideration for the expected advancement of medical knowledge, which may result for the benefit of mankind.

I have been informed of and understand the nature, duration, and purpose of the study the method and means by which it is to be conducted, the inconvenience and hazards to be expected, and the effects upon my health and person which may possibly come from participation in the experiment, as follows:

Purpose: To kill tumor cells and at the same time study the effects of radiation on blood and urination of the whole body.

Procedure: Radiation of the whole body.

Risks: The chance of infection or mild bleeding to be treated with marrow transplant, drugs, or transfusion as needed.

I understand that I may, at any time during the course of the experiment, revoke my consent, in writing, and withdraw from the experiment.

I acknowledge that no guarantee or assurance has been made to me as to the results that may be obtained, and I hereby waive any and all claims for liability, except for negligence, on the part of the medical personnel involved, the University of Cincinnati, its Hospital and its Medical School, which otherwise might have inured to me or my heirs, as a result of this medical procedure.

I certify that I have read and am competent to fully understand this consent and that the explanations listed above were, in fact, made.

Volunteer_____ Date_____

Investigator_____ Date_____

Witness (1)_____ Date_____

*In case of subject under age, the parent or guardian should be the responsible party and should sign on his behalf.

NOTE: Copy to Patient/normal subject, Research File and Patient's Chart.