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A CLOSED METHOD FOR FILTRATION OF HUMAN BONE MARROW

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ENCLOSURE 18

These studies were performed in conformation with the "recommendations guiding doctors in clinical research" as stated in the Declaration of Helsinki of the World Medical Association (1964).

ABSTRACT

Since bone marrow depression was the most life threatening radiation effect in patients given 150-200 rad total body radiation, we began storing bone marrow for autologous infusion. Though we have used unfiltered stored marrow in the past, we have developed a closed method of filtration for use in man.

With the infusion filtration system there has been no difference in the means of the cell counts of marrow before or after filtration in nineteen experiments. In vitro viability studies were not changed by filtration in seventeen studies. Viability was not influenced by storage at -80° Centigrade from five and one-half to twenty-six months.

This study supports the concept that this method of filtration will not interfere with the effectiveness of autologous bone marrow in controlling the severe hematologic depression associated with chemotherapy or radiation injury.

INTRODUCTION

Marked hematologic depression occurred in eighteen consecutive patients who received 150-200 rad total body radiation. The total leucocyte count fell below 2000 WBC/mm^3, 25 to 40 days after irradiation. The mean minimum leucocyte count of previously untreated patients who received 150 rad total body radiation alone was 1264 $\pm$ 1140, and was 1140 $\pm$ 816 when there had been previous therapy. The nadir of the leucocyte counts of patients who received 200 rad was 983 $\pm$ 366 WBC/mm^3.

Since bone marrow depression was the most life threatening radiation effect at the doses used, we began storing bone marrow for autologous infusion after irradiation. We have infused autologous stored human bone marrow intravenously <u>without filtration</u> into six patients. One patient had hemoglobinuria for twelve hours after infusion at 100 drops per minute. Autopsies were performed in three patients five days, eight days, and nine days respectively after infusion. No evidence of pulmonary emboli, pulmonary infarction or other disease as a result of marrow infusion was found on microscopic examination.

There is disagreement as to the need for filtration. Kurnick (1,2) does not think it is necessary prior to infusion. Thomas, Ferrebee and Pillow (8,1,6) recommend filtration to minimize the incidence of pulmonary embolization. Though we have not encountered embolization, we have developed a simple closed method of filtration for use in man.

METHODS

The bone marrow was stored by a modification of the method previously described by Kurnick (2). Approximately 100 ml. of material

was aspirated from each posterior iliac crest. The marrow suspension was diluted with an equal amount of 30% glycerol - Osgood media. This mixture, in quantities of 10 ml. per culture tube, was cooled at 1° Centigrade per minute to -40° Centigrade. The tubes were then placed in polyethylene bags and stored at -80° Centigrade.

On the day of infusion, the frozen marrow-glycerol-Osgood media was thawed at 4° Centigrade. Two parts of it were diluted with one part 33 1/3% dextrose in water and placed in a Fenwal plastic transfer pack. The complete infusion filtration system consisted of a Fenwall transfer pack, Sigmamotor administration set, filter assembly, and Sigmamotor pump (Figure 1). The pump had a variable speed control, but for the experiments to be reported, was set to deliver 4 ml/min. Within the filter assembly* (Figure 2), there were successive #50, #100, #200 mesh filters corresponding to openings of 297, 149, and 74 microns. The filter assembly was constructed of stainless steel with Teflon washers so that it could be disassembled for cleaning. It was then reassembled, sterilized and made pyrogen free.

We investigated the total cell count of a volume of marrow suspension and the viability of stored marrow cells before and after filtration through the infusion filtration system described. Cell counts are reported as cells per ml. (2). Viability studies, as determined by dye exclusion, utilized trypan blue (1).

*Supplied by Buckeye Supply Company, 7775 Montgomery Road, Cincinnati, Ohio 45236.

RESULTS

We studied bone marrow from fourteen patients. The marrow had been stored from five and one-half to twenty-six months prior to study. Though the storage temperature had usually been -80° Centigrade, at times, due to transient equipment failure, the temperature rose to levels as high as -40° Centigrade for periods up to twenty-four hours.

Cell counts were performed on nineteen specimens of bone marrow from fourteen patients before and after filtration (Table 1). The mean of the cell counts prior to filtration was 3.8 ± 2.0 cells x 10^6/ml. and 3.0 ± 1.6 x 10^6/ ml. after filtration. When these data were subjected to a non-parametric median test, there was no significant difference in the two groups at the 0.05 level though counts after filtration were usually lower. The sensitivity of the cells to injury by filtration was not influenced by the duration of storage at low temperatures.

Trypan blue exclusion studies were performed on seventeen specimens of bone marrow obtained from twelve patients (Table 2). The mean of the % viability before filtration was 55.3 ± 8.9% and 55.5 ± 8.1% after filtration. There was no significant difference between these two groups. Percent viability as determined by this dye exclusion technique was not influenced by the length of storage up to twenty-six months or by filtration through the system described.

If the bone marrow was not filtered, macroscopic clumps of material, as large as 3 mm. in length, were seen passing through the tubing. On microscopic preparations from unfiltered marrow, clumping was visualized (Figure 3). Following filtration, no macroscopic particles were seen and usually individual separated cells were observed on microscopic examination (Figure 4).

DISCUSSION

One of the first tenets in the handling and preparation of human tissue is to minimize external manipulation whenever possible. This thought led us to the use of unfiltered marrow in the past. However, there was little question that filtered marrow would have advantages over unfiltered marrow if the ability to repopulate the marrow space was not altered by filtration. With filtration, a needle as small as 22 gauge can be used for intravenous infusion. The closed system described minimizes the possibility of contamination and eliminates extra manipulation, so that autologous marrow transfusion can be performed easily outside of an operating pavilion. Therefore this modified technique removes another stumbling block to further investigation of marrow repopulation in man.

Since there has been no significant alteration in the cell count or in the viability of the cells when studied in vitro, there does not appear to be a deleterious effect from filtration. Only the trypan blue dye exclusion test of in vitro viability is reported. Further in vitro investigation of the cellular viability with tritium labeling of cells, acridine orange studies, and other tissue culture techniques (6) will be performed.

The ultimate answer as to whether filtration alters the ability of stored autologous human marrow to repopulate the marrow space can come only from an adequately controlled investigation in man. This study supports the view that filtered autologous marrow should be as effective as unfiltered marrow in the management of myelosuppression due to radiation therapy, chemotherapy or accidental radiation exposure in industry, in warfare, or in space.

SUMMARY

An easy-to-use closed infusion filtration system for autologous stored human bone marrow has been developed. There has been no difference in the means of the cell counts of marrow before or after filtration in nineteen experiments. In vitro viability studies were not changed by filtration in seventeen studies. There was no change in viability after storage from five and one-half to twenty-six months.

This study supports the concept that filtration through this closed system will not interfere with the effectiveness of autologous human bone marrow in controlling the severe hematologic depression associated with chemotherapy or radiation injury.

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Table 1

Case Number	Storage Time (months)	Cell Count ($\times 10^6$/ml) Before Filtration	After Filtration
068	5.5	5.6	4.1
065	5.5	1.6	0.8
067	6	4.2	3.3
065	7	1.4	0.6
066	10	5.3	4.4
064	11.5	4.8	4.1
064	12	3.6	3.1
063	13.5	9.8	8.2
056	17	3.5	2.9
050	21	6.2	2.6
050	21.5	2.2	2.4
052	22	3.2	3.5
052	22.5	3.2	3.5
047	23	4.4	3.2
049	24	4.3	3.6
048	25	1.8	1.4
x1	26	3.0	2.5
x2	26	3.0	2.2
047	26	1.3	1.2
Mean ± S.D.		3.8 ± 2.0	3.0 ± 1.6

Table 2

Case Number	Storage Time (months)	% Viable Cells Before Filtration	% Viable Cells After Filtration
068	5.5	69	70
065	5.5	--	--
067	6	58	46
065	7	--	--
066	10	51	56
064	11.5	53	54
064	12	64	60
063	13.5	63	57
056	17	63	56
050	21	38	36
050	21.5	49	46
052	22	52	69
052	22.5	47	55
047	23	44	58
049	24	69	52
048	25	63	64
x1	26	60	52
x2	26	52	60
047	26	45	53
Mean ± S.D.		55.3 ± 8.9	55.5 ± 8.1

FIGURES

1. Infusion-filtration system.

2. Filter assembly.

3. Clumping of unfiltered marrow. (X970).

4. Filtered marrow. (X970).

Figure 1

INFUSION-FILTRATION SYSTEM

Figure 2

FILTER ASSEMBLY

Figure 3

CLUMPING OF UNFILTERED MARROW (X970)

Figure 4

FILTERED MARROW (X970)