A STUDY OF THE PROTECTIVE PROPERTIES OF DIMETHYLSULFOXIDE ON BONE MARROW CELLS DURING RAPID FREEZING TO − 79°C

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The transplantation of viable bone marrow cells has acquired considerable importance as a technique in the treatment of radiation sickness. The simplest and most practicable method of storing bone marrow cells in order to preserve their viability is to keep them in nutritive media at temperatures of 3-5°. However, as has been demonstrated by several research workers [1, 3], this method does not allow the cells to be preserved in a biologically active condition for more than 2 weeks.

Over the last ten years many attempts have been made to keep bone marrow cells at low temperatures and to preserve their viability by using glycerine as a protective agent [5, 9, 10, 14].

Quite recently, several research workers [4, 6, 11] have shown that dimethylsulfoxide (DMSO) possesses a similar protective effect to that of glycerine on bone marrow cells during deep freezing, but that the effect of the former compound is more marked [4, 7, 13]. In addition, DMSO has been found to be less toxic than glycerine [6] which permits the direct transfusion of bone marrow cells without any preliminary washing.

In experiments, involving the preservation of bone marrow cells at low temperatures with glycerine, DMSO, etc. as protective agents, it is usual to begin the freezing process by gradually lowering the temperature 1° per min to between −15 and −25° and then to complete the process by rapid refrigeration to −79° [8, 12].

This present article is concerned with a study of the protective effect of DMSO on bone marrow cells during rapid freezing and describes how it is possible to preserve such cells in a viable condition at −79° for a lengthy period of time.

EXPERIMENTAL METHODS

The bone marrow cells were obtained from long bones of young rats belonging to the inbred August strain. Fragments of bone marrow were isolated in Parker's No. 199 medium, consisting of 10% isologous serum and 5% glucose; they were broken up by aspiration through a syringe. A 10% solution of DMSO solution was added to the cell masses obtained in this way, after which 1 ml amounts of suspension (containing $5\text{-}6.10^7$ cells) were poured into glass tubes, 6 mm in diameter and the latter were placed in a refrigerator (at 3-5°C). After various periods of contact with DMSO, the tubes with bone marrow cells were transferred to vessels containing a mixture of solid carbon dioxide and 95° alcohol and, subsequently to a container with solid carbon dioxide alone (at −79°) for prolonged storage.

The thawing out process was carried out in a water bath at 40°. Survivability of the bone marrow cells after freezing and thawing out was determined by staining in a 1% aqueous eosin solution or 0.1% solution of Trypan blue, and also on the basis of the protective influence of frozen and fresh cells transplanted into isologous animals receiving a daily dose of experimental radiation equivalent to 600 r.

TABLE 1. Results of Experiments to Determine the Survivability of Rat Bone Marrow Frozen to −79° in a Medium with Dimethylsulfoxide

Expt. No.	Initial No. of living cells (as %)	No. of living cells (as %) after freezing and thawing out for different periods of time (in days)						
		7	14	21	30	45	60	75
1	89	74	81	79	73	83		85
2	89	81	76	80	77	76		76
3	87	83	82	80	76		67	
4	87	75	73	79	77	78		78
5	85	83	78	81	82	80		
6	85	80	77	80	73		66	
7	85	85	77	81	81	86	86	
8	87	85	81	80	76		84	
9	78	87	81	85	77		79	
10	89	87	82	81	78	80		70

TABLE 2. Effect of Isotransplantation of Frozen and Fresh Bone Marrow Cells on the Survival of Rats Which Have Received a Lethal Radiation Dose

Bone marrow	No. of injected cells (in millions)	Period of preservation of cells −79° (wks)	No. of rats at beginning of expt.	No. of surviving rats after injection of cells	
				30 days	60 days
Fresh.	85	—	14	8	7
Frozen	85	2—5	12	6	6
Not injected (controls)			13	2	0

EXPERIMENTAL RESULTS

First of all, we considered it necessary to investigate the effect of different periods of time, during which bone marrow cells were in contact with DMSO prior to freezing, on their survivability during freezing, and subsequent thawing, for no definite information regarding this was available in the literature. We, therefore, carried out 5 series of experiments, in which 50 samples of bone marrow cells were kept in contact with DMSO solution for 10, 15, 30 min, 1 h and 24 h, before being frozen. All the samples were then maintained in a frozen condition at −79° for several hours.

The results of these tests indicated that over the range 10 min to 24 h the period of contact between bone marrow cells and DMSO prior to freezing of the former was not significant in relation to survivability of the cells to refrigeration: the number of cells which remained viable after freezing and thawing was almost the same as that for cells which were not subjected to these processes.

On the basis of these findings bone marrow cells were subsequently kept in contact with DMSO for 30-60 min prior to freezing in all our experiments.

The results of our survivability determinations of bone marrow, rapidly frozen to −79° in a medium containing DMSO and maintained at this same temperature for periods varying from 7 weeks to $2^1/_2$ months, are set out in Table 1.

We investigated a total of 55 samples of bone marrow cells taken from 10 rats.

It is evident from Table 1, that 3 weeks storage at −79° has practically no effect on the viability of bone marrow cells: the numbers of living cells in all the samples are essentially the same as they were before freezing.

With more prolonged periods of preservation (30-75 days), the number of cells surviving remains unchanged in many samples, but in certain cases there is a reduction of 13-20% as compared with the original value.

It is evident therefore, that DMSO exhibits a well marked, protective influence on refrigerated rat marrow cells which are subsequently thawed.

Our preliminary observations have revealed that DMSO serves as a reasonably effective protective agent for use during the freezing of intact bone marrow. In experiments involving samples of bone marrow from the sternum of human corpses, it has been established that the cells retain their viability following rapid freezing to −79° in a medium containing DMSO and subsequent thawing (as tested by staining methods).

The survivability of rat bone marrow, frozen to −79° in a medium containing DMSO and maintained at that particular temperature for 2-5 weeks has also been studied by comparing the therapeutic effect of isotransplantation of cells from fresh and frozen marrow on rats of the August strain which had received a lethal dose of radiation. The results of our preliminary observations are set out in Table 2.

It can be seen from Table 2, that of the 14 irradiated rats which were injected with 85 million cells of fresh bone marrow, 8 survived for 1 month and 7 for 2 months; of the 12 irradiated rats injected with the same number of cells from refrigerated bone marrow, 6 animals survived for a corresponding length of time; by contrast only 2 of the 13 irradiated rats in the control group survived for 1 month and both these died before the end of 2 months.

These findings suggest that the survival of animals which have been subjected to lethal irradiation and have subsequently received a transfusion of bone marrow cells frozen to a low temperature is dependent on the particular cells having retained their full functional capabilities.

In this way, our findings testify to the fact that DMSO is a useful protective agent in the rapid freezing to −79° and in the prolonged preservation of bone marrow cells. The rapid freezing method using DMSO for the preservation of bone marrow is a simple one and does not require special or complex apparatus. It may be used for storage and prolonged preservation of bone marrow.

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