A METHOD FOR THE PRODUCTION OF AHG RICH CRYOPRECIPITATES & THEIR USE IN THE MANAGEMENT OF BLEEDING EPISODES IN HAEMOPHILIA

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The haemostatic defect in haemophilia is a constitutionally inherited deficiency of a specific coagulation factor, antihaemophilic globulin, AHG. Management of bleeding episodes in these patients depends upon the ability to return to normal the AHG levels by the transfusion of materials containing the missing factor, such as fresh whole blood, fresh or fresh frozen plasma.

The limitation of therapy with blood or plasma is that half of the AHG activity disappears from the patient's circulation in 8-12 hours, so that repeated daily transfusions are necessary if the AHG levels are to be kept at a sufficiently high level to maintain haemostasis. In practice, it is often difficult to achieve effective AHG levels because of the large volumes required to be transfused with the consequent risk of a circulatory overload to the patient.

In recent years, several human AHG concentrates have been made available in overseas centres. This has made somewhat easier the management of bleeding episodes in these patients. However, these concentrates are not freely available in Singapore where up to recently the main therapeutic material used is fresh frozen plasma.

Management of haemophilia was transformed by the observation by Poole and Robinson, 1959, that AHG in plasma came out of solution together with fibrinogen and other proteins at low temperatures and can be separated from the rest of the plasma in this state. Subsequently, Poole and Shannon, 1965, described a method for the production of high potency concentrates of AHG using a closed bag-system. This method, with minor modifications, has been found to be very suitable for use in our Blood Transfusion Laboratories and this paper describes our initial experience over the past one year.

METHOD FOR PRODUCTION OF AHG RICH CRYOPRECIPITATES

500ml. of blood is collected from a donor into a triple ACD blood pack* containing 75ml. of anticoagulant solution according to NIH formula A. It is essential to ensure continuous mixing of the blood with anticoagulant to prevent clot formation and utilisation of AHG. After completion of the donation the donor segment tubing is sealed and cut off.

The blood pack and two satellite bags are inserted into a polythene envelope then placed in a centrifuge cup and balanced. The packs are then placed in a M.S.E. refrigerated centrifuge pre-cooled to 4°C. Centrifugation is carried out at 2,500 r.p.m. for about ten to 15 minutes after which the packs are removed from the centrifuge, and placed in a plasma extractor. The supernatant plasma is then transferred to one satellite pack leaving about one inch of plasma over the red cell layer. The connecting tubing is then sealed and cut off. The resulting red cell concentrate is then issued for the treatment of the patients with anaemia.

The plasma and transfer packs are again inserted into a polythene envelope before being immersed into an insulated container holding a mixture of ethanol and dry ice chips. The temperature of the alcohol dry ice mixture is held in the region of —50°C. to —60°C. The plasma will be frozen solid after about 15 to 20 minutes. Lately, we have found that a more rapid freezing of the plasma, within 10 minutes, could be obtained if the plasma packs were not enclosed in a polythene envelope. The frozen plasma pack is then allowed to thaw in a standard blood bank refrigerator at 4°C. ± 2°C. At the end of 18 to 24 hours the plasma will have fully thawed out leaving a cryoprecipitate—an opaque, jelly-like material which is associated

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with a high AHG activity. The thawed plasma pack is then replaced in a centrifuge cup, balanced, and then centrifuged at 2,500 r.p.m. for ten to 15 minutes. The supernatant plasma is extracted leaving the AHG rich cryoprecipitate suspended in about 10ml. of plasma in one transfer pack and the AHG poor supernatant plasma in the other. The cryoprecipitate is then stored in a freezer at —20°C. to —30°C. until required for use. The AHG poor supernatant is issued as blood volume expanders or used in the treatment of burns cases.

PREPARATION OF AHG RICH CRYOPRECIPITATES FOR TRANSFUSION

The required number of units of cryoprecipitate (1 unit = the cryoprecipitate obtained from the plasma of a 500ml. donation of blood) are removed from the freezer. The frozen packs are first placed under running water until the superficial plasma thaws out, after which they are placed in a water bath at 37°C. to accelerate the thawing which is usually complete within 5 minutes. The cryoprecipitate will go into solution giving a straw coloured liquid.

The individual units of cryoprecipitate are then pooled into an ordinary 500ml. glass bottle for transfusion into the patient using a disposable plastic blood administration set. The cryoprecipitate pool once prepared should be used for transfusion as soon as possible, preferably within an hour. Transfusion should be as rapid as possible. On the average, 10 units of cryoprecipitate equivalent to approximately 100 to 120 ml. of plasma can be easily transfused in ten to 15 minutes.

ASSAY OF AHG ACTIVITY OF CRYOPRECIPITATE

The results of the assay of AHG activity on 12 representative samples of donor plasma, the cryoprecipitate and AHG poor supernatant obtained is shown in Table I.

Assay of the AHG activity was by the method of Pitney, 1956. The cryoprecipitate was diluted with veronal buffer to obtain a final AHG concentration near to normal values for the assay.

The donor plasma taken from the plasma pack prior to freezing had an AHG activity ranging from 79% to 170% of normal with a mean value of 124%. The cryoprecipitate diluted in approximately 10-15ml. of supernatant plasma had an AHG activity ranging from 380% to 2000% with a mean of 1136%. There was a mean concentration of approximately ten times the AHG activity of normal plasma. The residual AHG poor supernatant had an AHG activity of between 6-21% with a mean of 13%.

Assay of the undiluted cryoprecipitate gel showed an AHG activity ranging between 1500% to 8000% with a mean of approximately 4900%.

Analysis of the total protein, albumin and globulin content showed slight differences in their concentration in the donor plasma, cryoprecipitate suspension and supernatant, Table II. There was a sixfold increase in the concentration of fibrinogen in the cryoprecipitate suspension compared to the plasma from which it was prepared.

CLINICAL STUDIES

Cryoprecipitate transfusions have been used over the past one year on a number of haemophilia patients with haemarthrosis, muscular haematoma, bleeding gums, and tooth extractions. In all instances the response to the cryoprecipitate transfusions has been most dramatic with almost immediate arrest of the haemorrhage and relief of symptoms.

The effect of the cryoprecipitate transfusions on the circulating plasma AHG level in seven haemophilia patients with various bleeding episodes is demonstrated in Table III.

Apart from one patient, HYC who is a mild haemophiliac, all the remaining patients had a severe clinical disability with AHG levels of less than 1%. The number of units of cryoprecipitate transfused ranged from five to 30 units. The AHG activity of the cryoprecipitate pool ranged from 325% to 895% with a mean of 586%. The post transfusion AHG level taken approximately ten minutes after completion of transfusion ranged from 21% to 65%. In six of the seven patients there was still some residual AHG activity in the plasma 24 hours after transfusion ranging from 2½% to 37%, in three of whom the levels were over 15%.

DISCUSSION

The main advantage of cryoprecipitate transfusions is that more AHG activity can be administered to the patient without risk of a circulatory overload. Infusion time for the patient is also considerably reduced thereby lessening the risk of thrombophlebitis.

TABLE I

SHOWING AHG ACTIVITY EXPRESSED AS PERCENTAGES OF NORMAL IN 12 REPRESENTATIVE DONOR PLASMA, CRYOPRECIPITATE SUSPENSION AND SUPERNATANT

Donor Plasma No.	1	2	3	4	5	6	7	8	9	10	11	12
Donor Plasma	170	144	122	167	136	79	109	108	71	137	115	130
Cppt. Suspension	1100	715	1460	2000	2000	2000	1000	380	440	840	1000	700
Supernatant Plasma	18	12	16	21	19	6	7	8½	7	13	19	9

TABLE II

SHOWING TOTAL PROTEIN, ALBUMIN, GLOBULIN AND FIBRINOGEN CONCENTRATION EXPRESSED IN GM. PERCENT IN THE DONOR PLASMA, CRYOPRECIPITATE SUSPENSION AND SUPERNATANT

	Total Protein		Albumin		Globulin		Fibrinogen	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Donor Plasma	5·5 - 6·3	5·7	2·1 - 3·2	2·8	2·2 - 3·1	2·7	0·17 - 0·30	0·22
Cppt. Suspension	5·7 - 7·3	6·4	2·2 - 3·1	2·7	2·1 - 3·6	2·9	0·62 - 2·44	1·20
Supernatant Plasma	4·4 - 5·8	5·4	1·8 - 3·2	2·7	1·9 - 3·1	7·6	0·16 - 0·24	0·19

TABLE III

SHOWING AHG ACTIVITY IN PATIENTS' PLASMA BEFORE, IMMEDIATELY AFTER AND 24HRS. AFTER CRYOPRECIPITATE TRANSFUSIONS.

Patient	Age Yrs.	Bleeding Episode	Cryoprecipitate Trans.			Plasma AHG Activity		
			Units	Vol. ml.	AHG% *	Pre Trans.	Immed. Post Trans.	24 hrs. Post Trans.
YKC	23	Muscular haematoma & haemarthroses	22	320	760	<1	60	18
GEK	9	Haemarthroses	11	200	710	<1	65	37
TTH	27	Haemarthroses	30	460	325	0	50	15
HYC	38	Muscular haematoma	20	385	375	4	45	19
WTS	53	Muscular Haematoma	20	325	625	2	55	5
GEY	7	Bleeding gums & extn. teeth	10	150	895	0	35	2½
SYS	7	Bleeding gums & extn. teeth	5	100	410	0	21	<1

* The AHG activity of the cryoprecipitate refers to that of the cryoprecipitate pool.

Our initial clinical studies have shown that cryoprecipitate transfusions are more effective than equal volumes of fresh or fresh frozen plasma in controlling haemorrhagic episodes in haemophiliacs. Adequate plasma AHG levels can be relatively easily achieved and maintained by cryoprecipitate transfusions compared to the use of fresh frozen plasma. Thus, fresh plasma given in maximum volumes of 15-25ml. per kilogram bodyweight could only raise the AHG levels in severe haemophiliacs to between 15-20% of normal, whereas much higher levels could be achieved with smaller volumes of cryoprecipitate suspensions in the same patients.

There are a number of practical implications of treatment with AHG rich cryoprecipitates. Firstly, patients must be persuaded to present themselves as soon as bleeding occurs and not to delay seeking treatment in the hope that the bleeding will subside spontaneously. Relief of symptoms, especially pain, restoration of joint mobility, and arrest of further bleeding can be expected within minutes of the prompt administration of cryoprecipitates. Apart from the very mild haemorrhage, treatment will have to be repeated daily for a few days and with experience we have found it possible to treat patients on an outpatient basis. This results in a saving in the use of hospital bed space and cutting down on the frequency of patients being kept away from school or work.

The finding of the rather low AHG activity of the cryoprecipitate pool compared to the individual units was rather surprising. Whilst AHG activity of over 1000% of normal is not uncommon in individual units, the highest level obtained in the cryoprecipitate pool was 895%. There was no apparent reason for the discrepency apart from the possibility that the individual cryoprecipitate suspensions were diluted with a larger volume of supernatant plasma. Another possibility is that there has been some loss of AHG activity in the process of preparation of the cryoprecipitate pool.

The finding of significantly high AHG levels 24 hours after transfusion suggests a longer survival of AHG rich cryoprecipitates compared to fresh frozen plasma. This would suggest that with repeated daily transfusion one can expect a cumulative effect with progressive increase in the post transfusion AHG levels. This effect has been demonstrated in three patients undergoing major surgery under cover of daily cryoprecipitate transfusions, Kwa, 1968.

In the selection of donors for collection of blood for cryoprecipitate production, no special criteria were applied apart from the standard criteria applicable to all donors, Kwa et al 1966. Although the practice in some centres is to select group AB donors only, no such selection was applied in our laboratory because of the relatively small number of group AB donors available. Donors of all groups were accepted provided their plasma did not contain any atypical blood group antibody. However, care was taken to ensure that when pooling the individual cryoprecipitate units, there was a representative mixture of plasma of all groups. With this practice we have so far not encountered any examples of intravascular haemolysis occurring with cryoprecipitate transfusions.

Because of the observation that cryoprecipitate produced from plasma with high AHG levels generally had a higher AHG activity, some Centres make it a point to only collect blood from donors with high AHG titres. For practical reasons, we have not found it possible to routinely determine the AHG levels of all donors beforehand because of the great difficulty we already have in recruiting donors and making them wait or come up on a second occasion for their donations would make the situation worse.

The ideal would be to select individuals with high AHG titres and submit them to a programme of plasmapheresis thereby ensuring sufficient supplies of high titre plasma from a small number of donors, Simson et al, 1966.

The implications of cryoprecipitate production are also considerable for a blood transfusion laboratory. Although we have at present only some 50 odd haemophilia patients under our care, many hundreds of units of blood will have to be processed weekly to supply enough cryoprecipitate for their use. At present, cryoprecipitate production is barely sufficient to meet the requirements for treatment of minor bleeding episodes and an occasional dental extraction. Reserves have not reached the stage where they are considered adequate to cover emergencies.

Although it might be said that the single unit method for the production of AHG rich cryoprecipitate is far from efficient, in a situation like ours where other forms of concentrates are not available, the development of a simple method such as this has definite advantages.

Firstly, it does not require expensive or specialised equipment, apart from the refrigerated centrifuge which is standard equipment for all blood banks. The greatest expense is for the triple blood packs required for the collection of

blood. Secondly, the use of the triple pack has allowed an economic use of the different blood components which are by-products of the process. Thus from one individual donation, in addition to cryoprecipitates, concentrated red cells and plasma are made available. The use of the quadruple pack will allow the collection of a fourth component, platelet concentrates, which could be utilised for the treatment of patients with thrombocytopenia.

In terms of production costs, with one technician working full time we have been able to produce 16-20 units of cryoprecipitate per day at very little extra cost and without placing a strain on the availability of blood for other patients.

SUMMARY

A simple, efficient and inexpensive method for the production of AHG rich cryoprecipitates which could be easily adopted by any routine hospital blood bank or blood transfusion service has been described.

Its effective use in the management of spontaneous haemorrhagic episodes in a number of haemophilia patients is demonstrated.

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