Preliminary Communications

Combined Immunosuppressive Action of Phytohaemagglutinin and Azathioprine (Imuran) on Dogs with Renal Homotransplants

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Phytohaemagglutinin (P.H.A., an extract of *Phaseolus vulgaris* seeds) causes small human lymphocytes to enlarge and undergo mitotic division (Nowell, 1960). The mechanism of this lymphocyte transformation is not known, although it has been ascribed to the antigenic properties of the material. Humble (1964) has used P.H.A. systemically in human cases of aplastic anaemia and found evidence of stimulation of bone-marrow function in all six patients treated. He noted the appearance in the peripheral blood of lymphocytes of increasing size and of increasing cytoplasmic basophilia which continued for many weeks after cessation of treatment. Holm *et al.* (1964) showed that P.H.A. rendered normal lymphocytes in tissue culture cytotoxic for target cells of homologous origin.

We felt it would be of interest to investigate the effect of systemically administered *Phaseolus vulgaris* extracts on the progress of animals receiving renal transplants.

Materials and Methods

P.H.A. was prepared from *Phaseolus vulgaris* seeds by the method of Hurn and Wood (1965). Material of two different degrees of purity was used: (1) "Crude P.H.A."—the neutralized acid extract of the ground beans; this material is highly antigenic in rabbits; (2) "Purified P.H.A."—obtained by dialysis, gel-filtration chromatography, and iso-electric precipitation of the crude P.H.A. This material had 10 to 20 times the mitogenic activity of the crude P.H.A. on a weight basis, while it was far less antigenic in rabbits than the crude extract.

Adult unrelated dogs (of either sex) were divided into five experimental groups which received the treatment shown in Table I.

Renal transplantation was performed with vascular sutures of the renal vessels to the iliac vessels and implantation of the ureter into the bladder. The animal's own two kidneys were removed at the same time. Total white blood cell count, haematocrit, and blood urea were estimated twice weekly and smears of the peripheral blood were examined. The sera of some of the dogs in group II were examined for the presence of antibodies to P.H.A. by the Ouchterlony gel-diffusion technique.

Animals which died after transplantation were examined and the histological appearances of lungs, liver, spleen, bone-marrow, lymph nodes, intestine, and the transplanted kidney were noted. Evidence of rejection in the transplant was graded as follows:

+ : Minimal cellular infiltrate.
+ + : Moderate cellular infiltrate and/or moderate vascular changes, more than 50% viable cortical tissue remaining.
+ + + : Severe cellular infiltrate and/or vascular changes, less than 50% viable cortical tissue remaining.

TABLE I

Group	P.H.A.	Azathioprine	Renal Transplantation
I	Crude P.H.A. 50 mg. i.v. daily from day 1 to 5	Standard dosage from day of transplant	Day 3
II	Crude P.H.A. immunizing course Day 1, 200 mg. with Freund's adjuvant i.m. Day 14, 200 mg. with Freund's adjuvant i.m. Day 21, 200 mg. aqueous i.m. „ 23, 100 mg. „ i.v. „ 27, 200 mg. „ i.v.	Standard dosage from day 32	Day 32
III	Purified P.H.A., i.v. Day 1 and 2, 30 mg. „ 3, 4, and 5, 15 mg.	Standard dosage from day 3	Day 5
IV	As group III	Nil	As group III
Va	Nil	As group III, starting 2 days before renal transplantation	
Vb	Nil	As group I, starting day of renal transplantation	

Standard azathioprine dosage: 10 mg./kg. orally for two days; 5 mg./kg. thereafter unless the white-cell count fell below 2,000 when the dose was lowered.

Results

The results of each group are summarized in Table II. In the animals treated with P.H.A. alone there was no evidence of marrow or lymph-follicle depression, and peripheral blood

TABLE II

Dog	Survival in Days	Homograft Reaction	Cause of Death
		Group I	
1	33	+	Sepsis
2	28	+ +	Uraemia. Pneumonia
3	13	+ +	Uraemia
4	4	—	Infarcted kidney
5	37	+	Marrow depression
6	7	+ +	Pneumonia. Rejection
7	11	±	Ascites. Pneumonia
8	17	+ ±	Sepsis
9	8	0	Marrow depression. Pneumonia
10	11	±	Pneumonia
11	19	0	Distemper
12	17	0	Distemper
		Group II	
1	4	—	Arterial thrombosis. Uraemia
2	4	—	Uraemia. Arterial thrombosis
3	31	+ +	Uraemia
4	8	+ + +	Abdominal haemorrhage
		Group III	
1	47	+	Anaemia. Sepsis
2	33	0	Pneumonia
3	21	0	Pneumonia
4	120	—	Last blood urea 120 ± mg./100 ml. Alive
5	5	±	Ascites
6	8	0	Pneumonia
7	16	0	Pneumonia
8	6	0	Haemorrhage
9	9	0	Peritonitis
10	6	0	Pneumonia
11	7	0	Ascites. Last blood urea 67 mg./100 ml.
12	16	0	Pneumonia
13	5	0	Pneumonia
14	15	Infarcted	Pneumonia. Transplant infarcted
15	41	0	Pneumonia
		Group IV	
1	12	+ + +	Rejection
2	27	0 to ±	Last blood urea 43 mg./100 ml. Pneumonia.
3	16	+ + +	Rejection
4	16	+ + +	Rejection.
		Group Va	
1	19	+	Lung slight consolidation
2	9	+	Intussusception
3	12	0	Pneumonia
4	9	+ + +	Rejection
5	20	+ + +	Rejection. Pneumonia.
		Group Vb	
1	32	+ + +	Rejection
2	28	+ + +	Rejection
3	18	+ + +	Rejection

smears only occasionally revealed blast cells as noted by Humble (1964). In the animals given azathioprine in addition to P.H.A., marrow, lymph-follicles, and peripheral blood appearances were similar to those in those dogs given azathioprine alone. There was no apparent difference in the dogs given azathioprine two days prior to renal transplantation compared with those given azathioprine on the day of transplantation. In those dogs given combined purified P.H.A. and azathioprine (Group III) 5 out of 6 showed no homograft reaction after 14 days or more, and the 7th dog was still alive at 120 days with a blood urea of approximately 120 mg./100 ml. One dog dying at five days had minimal rejection (±). In contrast, of the dogs treated with azathioprine alone, all those living beyond 14 days had a homograft reaction between + and +++. In a previous study (Calne *et al.*, 1962) two of the 14 dogs treated with azathioprine alone surviving beyond 14 days had no homograft reaction.

Discussion

The animals in group I were the first to be studied, and at the time it was thought that the results in this group indicated a possible potentiating effect of P.H.A. on the action of azathioprine in delaying rejection of the transplanted kidney. It was felt that P.H.A. might be acting in one of two ways: either (1) as a potent antigen acting in competition with the foreign kidney, (2) as a stimulus to some or all of the immunologically competent cells to undergo mitotic division, thus rendering them more specifically susceptible to the action of azathioprine.

The choice of P.H.A. preparation and dosage was designed to separate these two hypothetical modes of action in groups II and III respectively. Groups IV and V were controls.

The animals in group II suffered considerable morbidity from the multiple injections and were particularly liable to develop abscesses in relation to the injection of Freund's adjuvant. The incidence of thrombosis of the renal vessels after apparent satisfactory anastomoses was very high and the animals appeared to be particularly prone to haemorrhage. Not all the animals developed detectable antibodies to P.H.A. One dog survived 31 days, but insufficient animals could be brought to successful operation for this group to throw any light on the mode of action of P.H.A.

In group III, where a short course of purified P.H.A. was given intravenously in large dosage before transplantation, the immunosuppressive action of azathioprine seems to have been reinforced (cf. group V), the most pronounced effect being seen in the degree of graft rejection detected at necropsy. Experience in rabbits given purified P.H.A. intravenously has shown that it is not very antigenic under these circumstances, and it might therefore be assumed that its immunosuppressive effect was unlikely to be due to antigenic competition. The findings in the animals of group IV, which received P.H.A. but no azathioprine, suggest that P.H.A. had immunosuppressive activity on its own. Transplant function in untreated animals usually ceases in five to eight days with ++ or +++ rejection; Dempster (1953) and Simonsen *et al.* (1953) agreeing with results from our laboratories. The benefit of P.H.A. alone was far less than that of P.H.A. + azathioprine, since three out of four animals showed marked rejection at the time of death, which was itself delayed by only a few days. However, the fourth dog died after 27 days from pneumonia with a blood urea of 43 mg./100 ml. and no homograft reaction.

The above experiments do not explain the mode of immunosuppressive action of P.H.A., though they would suggest that it may be related to blast transformation and mitogenesis of lymphoid cells.

Summary

Phytohaemagglutinin was administered systemically in dogs who received renal homotransplants. The purified fraction, administered intravenously prior to renal transplantation, demonstrated some immunosuppressive activity when given alone and potentiated the immunosuppressive action of azathioprine (Imuran) when the two substances were given together. The P.H.A. produced no observed toxic effect of its own on the marrow.

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R. Y. Calne, M.S., F.R.C.S.,
Honorary Consultant and Senior Lecturer in Surgery, Surgical Unit, Westminster Hospital, London.

J. R. Wheeler, M.D.,
Research Fellow, Surgical Unit, Westminster Hospital, London.

B. A. L. Hurn, M.D., M.C.PATH.,
Clinical Pathologist, Wellcome Research Laboratories, Beckenham, Kent.

References

Calne, R. Y., Alexandre, G. P. J., and Murray, J. E. (1962). *Ann. N.Y. Acad. Sci.*, **99**, 743.
Dempster, W. J. (1953). *Brit. J. Surg.*, **40**, 447.
Holm, G., Perlmann, P., and Werner, B. (1964). *Nature (Lond.)*, **203**, 841.
Humble, J. G. (1964). *Lancet*, **1**, 1345.
Hurn, B. A. L., and Wood, M. (1965). In preparation.
Nowell, P. C. (1960). *Cancer Res.*, **20**, 462.
Simonsen, M., Buemann, J., Gammeltoft, A., Jensen, F., and Jørgensen, K. (1953). *Acta path. microbiol. scand.*, **32**, 1.