COMPARATIVE EVALUATION OF DEXAMETHASONE WITH CYCLOPHOSPHAMIDE, CHLORAMPHENICOL AND CYPROHEPTADINE HYDROCHLORIDE AS IMMUNOSUPPRESSIVE AGENTS ON SKIN HOMOGRAFTS IN RATS AND RABBITS*

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Summary: An experimental study on immunosuppressive action of cyclophosphamide, chloramphenicol and cyproheptadine hydrochloride in comparison with the control and dexamethasone was undertaken on skin homografts in rabbits and rats. The survival period of skin homografts in cyclophosphamide treated group was significantly increased ($P<0.01$) in rabbits, but there was insignificant increase in rats ($P>0.01$). With chloramphenicol there was significant increase in survival period in both the species ($P<0.01$). Cyproheptadine did not show significant result in any of the species. The probable mechanisms of immunosuppressive action have been discussed.

Key words: immunosuppression

INTRODUCTION

A considerable amount of work has been devoted in recent years to the suppression by chemical agents of immunological reaction following homotransplantation, (2,8,10).

The aim of this research has been to study in laboratory animals the homograft reaction and how it may be modified by various drugs to prolong the survival period of the grafts. Dexamethasone was selected as the standard drug because of known immunosuppressive effect of corticosteroids in various animal species and human beings (2,3,7,9,13,16, 18). Cyclophosphamide was chosen because of its inhibitory effect on antibody formation. so far no body has reported immunosuppressive effect of chloramphenicol on skin homografts in albino rats, although in rabbits it has been studied by Weisberger *et al.* (21,22). As homograft reaction has been associated with actively acquired immunity conforming to the pattern of antigen antibody reaction, cyproheptadine hydrochloride (antihistaminic and antiserotonin) was selected to see whether it has some role to play in preventing homograft reaction.

MATERIALS AND METHODS

This study was conducted on 88 rabbits of either sex, weighing between 1 to 1.5 *kg* and 112 albino rats of either sex, weighing between 150-200 *gms*. The animals were fed *ad lib* on stock diet. The animals were divided in to different groups as shown in Table I & II. The dosage schedule of various drugs is also given in the same table.

Full thickness skin homografts were transplanted under ether anaesthesia according to the method of Medawar (15). The grafts were cut in the manner of pinch grafts from the clean skin of the dorsal aspect of the trunk on either side of the spine.

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The skin of the prepared area was lifted up in a conical elevation with fine dog toothed forceps and sliced off with rounded margins by firm horizontal stroke with No. 12 scalpel. The subcutaneous tissue was sliced off with a sharp knife. The grafts were transplanted and fixed with a number of interrupted fine thread sutures at the periphery. Then a ball of impregnated vaseline gauze was fixed on the surface of the graft and dressing was done.

The drugs were started on the day of homografting and continued till there was graft rejection. Biopsy specimens were removed on 6, 9, 12 and 15 days after operation and then as often as felt necessary. For taking biopsies animals were anaesthetized with ether and a small rectangular piece was removed and sent for processing. Total and differential leucocytic count of the animals was done before the grafting, on 6th day post-operatively and at the end of the experiment.

RESULTS AND DISCUSSION

Control Group : Macroscopically, all the homografts showed primary healing and "took" well to the graft bed. The changes these grafts then underwent are briefly as follows:

Grafts became swollen, colour changed from pink to dirty yellow, green or brown. The surface of the graft became dry, hard and epidermis could be peeled off to reveal the dermis. Microscopically the grafts showed signs of primary healing on 6th day of grafting and were well vascularized. In the later biopsies massive round cell infiltration and destruction of the epidermis was seen.

Drug treated Groups: The macroscopic and microscopic appearances and sequence of events were the same as in the control group but the speed of their progress was delayed under the influence of the drug resulting in longer periods of survival of the grafts as seen in the Table I & II . Total and differential leucocytic count in the drug-treated groups also showed some differences as compared to the control and dexamethasone-treated groups.

In the present study, the macro and microscopic findings in the control group which finally led to graft rejection were in record with the findings reported by several workers (1,2,10, 15,16). There was systemic lymphocytosis in control group. This observation is in conformity with that of Billingham *et al.* (2). In the dexamethasone-treated animals there was a decrease in number of total lymphocytes.

The mean survival period in dexamethasone-treated group in both species was significantly prolonged ($P<0.01$) as compared to the control group. This prolongation could be ascribed to inhibitory effect of corticosteroids on local inflammatory and other cellular activities that accompany the breakdown of foreign epithelium, as evidenced by fine or scanty granulation tissue, absence of epithelial hyperplasia and cellular proliferation

TABLE I: Effect of various drugs on survival period of skin homografts in rabbits.

S. No.	*Group*		*Dose mg/kg*	*Percentage solution*	*Frequency of administration*	*Route of administration*	*Lethality percentage*	*Mean survival of graft (days) ±S.D.*	*P Value with control control group*	*P Value with standard (Dexamethasone)*
(1)	(2)		(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
1.	Control		Saline	—	Daily	Subcutaneous	—	10.30±1.25		
2.	Dexamethasone treated	R_1	2	0.1%	Daily	Subcutaneous	10	12.00±2.29	<0.01	
		R_2	4	—	Daily	Subcutaneous	20	14.00±1.22	<0.01	
3.	Cyclophosphamide treated	R_1	25	10%	Alternate days	Intraperitoneal	—	17.10±1.79	<0.01	<0.01
		R_2	50	—	Alternate days	Intraperitoneal	40	18.83±2.22	<0.01	
4.	Chloramphenicol treated	R_1	50	2%	Daily	Intraperitoneal	—	19.50±4.49	<0.01	<0.01
		R_2	100	—	Daily	Intraperitoneal	20	19.12±2.48	<0.01	
5.	Cyproheptadine hydrochloride treated		10	0.3%	Daily	Intramuscular	10	9.66±1.11	>0.05	>0.05

TABLE II : Effect of various drugs on survival period of skin homografts in albino rats.

S.No.	*Group*		*Dose mg/kg*	*Percentage solution*	*Frequency of administration*	*Route of administration*	*Lethality percentage*	*Mean survival of graft (days) ±S.D.*	*P Value with control group*	*P Value standasd (Dexamethasone)*
(1)	(2)		(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
1.	Control		Saline	—	Daily	Subcutaneous	—	8.58±1.176	—	—
2.	Dexamethasone treated	R_1	10	0.1%	Daily	Subcutaneous	8.33	13.00±1.612	<0.01	—
		R_2	20		Daily	Subcutaneous	41.66	15.14±1.112	<0.01	—
3.	Cyclophosphamide treated	R_1	50	10%	Alternate days	Intraperitoneal		13.58±1.378	<0.01	>0.05
		R_2	100	—	Alternate days	Intraperitoneal	25	13.22±2.199	<0.01	
4.	Chloramphenicol succinate treated	R_1	100	2%	Daily	Intraperitoneal	—	15.00±2.00	<0.01	>0.05
		R_2	200	—	Daily	Intraperitoneal	33.33	17.12±1.357	<0.01	
5.	Cyproheptadine hydrochloride treated		20	0.3%	Daily	Intramuscular	12.5	8.28±1.213	>0.5	>0.5

and delayed vascularization (2,16). The other possibility could be due to lympho-cytolytic activity of the drug as observed by minimal or no lymphocytic infiltration of the graft as compared to control group and leucopenia and lymphopenia (6,24).

The mean survival period in cyclophosphamide-treated group in rabbits was significantly prolonged ($P < 0.01$) as compared to control and the standard group. In rats, the survival period was significantly prolonged as compared to the control group ($P < 0.01$). When compared with standard drug, cyclophosphamide did not compare favourably. The same results have been reported by other workers (8,19). The prolonged survival of the skin homografts with cyclophosphamide could possibly be due to antimitotic effect, (4) or inhibitory effect on antibody formation.

The mean survival period in chloramphenicol treated group in both the species was significantly increased ($P < 0.01$) as compared to control and standard group. The antibiotic chloramphenicol inhibits the binding of mRNA to ribosomes and thus inhibit protein synthesis (20,21, 22,23). This drug has also been shown to inhibit antibody formation in tissue-cultures (1). This could explain the underlying mechanism for the immunosupperssive action of chloramphenicol.

There was no effect of cyproheptadine hydrochloride on skin homograft survival period as compared to control and dexamethasone group, in both the sepecies.

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