Effects Of Vitamin A On The Immunologic Deficiencies After Thermal Injury

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VİTAMİN A'NIN YANIKTA İMMÜN SİSTEMDE MEYDANA GELEN BOZUKLUKLAR ÜZERİNE ETKİSİ

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SUMMARY

Effects of vitamin A on the immune system were investigated in thermal injury. The study was performed on 40 male Swiss-Albino rats. A third degree burn of 30-33% body surface was done by immersing the back of the anasthetized animal in 99° C water.

The cellular immunity was studied in 20 rats by rejection of a full thickness skin graft. The humoral immunity was also studied in 20 rats by injecting sheep erythrocytes (SRBC) intraperitoneally and measuring the hemaglutination antibody titers. Ten of the rats in each group received vitamin A while the other ten animals were given saline, intraperitoneally. At the end of the experiment, the liver, spleen, thymus were removed and weighted.

The skin graft was rejected in 18.5 + 3.20 days in the controls and 12.37 + 1.76 days in the vitamin A treated group (p < 0.05). In this group of animals, only the wet weight of thymus in vitamin A group was significantly higher than in the control group (p < 0.05). The hemaglutination antibody titers were between 1/8 - 1/32 in the control and 1/8-1/256 in the vitamin A group on the 8th post injection day (p + 0.05). The same titers were between 1/8 - 1/16 in the control and 1/8 - 1/128 in the vitamin A group on the 14th post injection day (p < 0.05). In this group of animals the wet weights of thymus and spleen in the vitamin A treated group were significantly higher than in the control group (p<0.05).

This study suggests that vitamin A stimulates the cellular and humoral immunity in thermal injury. This is apparent with the earlier rejection of the skin grafts and higher antibody titers against the sheep erythrocytes in the vitamin A treated group.

Key Words: Thermal injury, Immune system, Cellular immunity, Humoral immunity, Vitamin A.

ÖZET

Deneysel yanıkta vitamin A 'nın immiin sistem üzerine olan iyileştirici etkisi araştırıldı. Deney için 40 tane Swiss-Albino erkek sıçan kullanıldı. Hayvanların sırtları 99°C 'de suya batırılarak vücut yüzeylerinin % 30-35'ini kaplayan, 3. dereceden yanık meydana getirildi.

Hücresel immunité 20 sıçanda greft rejeksiyonuyla, humoral immunité de kalan 20 sıçanda koyun eritrositlerine karşı oluşan hemoglutinasyon antikor titrajlarıyla değerlendirildi. Her gruptan 10 sıçana vitamin A, kontrol grubundaki 10 sıçana da serum fizyolojik intraperitoneal olarak verildi. Deney sonunda hayvanların karaciğer, dalak ve timusları çıkarılıp tartıldı.

Ortalama greft rejeksiyon günü kontrol grubunda 18.5 + 3.20 gün, vitamin A verilen grupta 12.37 + 1.76 gün (p < 0.05) olarak tesbit edildi. Bu grupta sadece Umusun ağırlığı vitamin A verilenlerde önemli derecede artmış olarak bulundu. Koyun eritrositlerine karşı oluşan hemaglutinasyon antikor titrajı 8. günde kontrol grubunda 1/8 - 1/32, vitamin A verilen grupta 1/8 - 1/256, 14. günde ise sırasıyla 1/8 - 1/16 ve 1/8 - 1/128 olarak ölçüldü (p < 0.05). Bu grupta ise, timus ve dalağın ağırlıkları vitamin A verilenlerde önemli ölçüde artmış olarak bulundu (p < 0.05).

Bu çalışma, yanıkta vitamin A 'nın hücresel immüniteyi greft rejeksiyonunu hızlandırarak, hümoral immüniteyi verilen koyun eritrositlerine karşı hemaglutinasyon antikor titrajım artırarak stimule ettiğini göstermektedir.

Anahtar Kelimeler: Yanık, İmmiin sistem, Hücresel immunité, Hümoral Immunité, Vitamin A.

In most burn centers 80 to 85% of the mortality folk wing severe thermal injury is due to sepsis (36). One should have adequate concomitant immune responses in order to be successfully treated with antibiotics for septic complications (51, 52). Al-though appropriately selected systemic antibiotic to which the organism is highly sensitive is adminis-tered to an experimental animal with infected burns, death may not be prevented (50). Therefore, the most important problem is to control infection in thermal injury.

Clinical and experimental studies have shown that there is close connection between the altered host defenses in burn and sepsis. First, of all the skin, the primary mechanical barter to bacterial in-vasion is damaged by thermal injury. At the burned tissues, local agglutination of endothelial and red blood cells, microvascular thrombosis cause severe microcireulatory failure. Thus inflamatory cells cannot reach the burned area and contact with bacteria (35, 38). Alexander has stated that chemo-taxis and intracellular killing of neutrophils de-crease in burn trauma (1, 2. 3). Thermal injury also affects macrophages by decreasing their migration, phagocytosis and intracellular killing (15. 25, 43). The fact that the reticuloendothelial system func-tions were depressed in severe burn, has been noted (6, 41, 49). In addition, a number of reports have provided that, all immunoglobulins and complement components significantly decrease (26, 27. 39, 44), allograft survival period prolongs (6, 24, 40, 42, 45) and delayed hypersensitivity reactions alter (14, 21, 22, 35, 48) in burn injury.

A variety of pharmacologic agents have been used in order to improve altered immune responses, such as BCG, thymosin, levamisole, cyclophos-phamide, cimelidine, indomethaein (20, 22, 25, 36, 53). The present experimental study examines ef-fects of vitamin A on altered cellular and humoral immunity in the burn trauma.

MATERIAL AND METHODS

This study was done at the Department of Sur-gical Research in Hacettepe University. 40 adult male Swiss-Albino rats, weighing approximately 200-350 gm. were used.

The animals were anesthetized with Na-Pento-barbilal administered intraperitoneally (20-25 mg/kg body wt), shared of hair over the back and placed in a device to limit and control burn size. A third degree burns covering 30-35% of the body surface area were produced by immersion of the bacj« and flanks in water at 99 C for 10 seconds (13'). Im-mediately after burn, 10 ml saline per 100 gm of body weight were given intraperitoneally to diminish mortality from acut hypovolemia.

Twenty of 40 rats used in this experimental study, received 200 IU/gm/day vitamin A (Retinil Palmitat Aquasol-A) intraperitoneally for 15 days starting on the 5th day before the burning. The twenty rats of control group received an equal volume of saline intraperitoneally for 15 days.

Each group was divided into two subgroups. The cellular and humoral immunity were studied in one each subgroup of vitamin A and saline treated groups separately. Cellular immunity was evaluated by rejection of the skin graft. At the day of burning, the full thickness skin grafts which were 2 cm in diameter and received from sacrificed donor rat were sutured on a unburned place on the back of rats by technique described in detail else-where (37). Signs of necrosis were observed daily. The day of necrosis of 100% of the graft was regarded as the time of rejection.

Twenty rats in which the humoral immunity was studied, were injected intraperitoneally with 0.5 ml/25 gm body wt. of a 10% suspension of washed sheep red blood cells (SRBC) at the time of burning. 0.5 ml samples of sera were obtained from the blood drawn by cardiac puncture on 8th and 14th days after injection. Hemaglutination titers were determined using microtitration technique (17).

At the end of the experimental procedures, all rats were sacrificed. Their livers, spleens and thy-muses were removed and their wet weights were estimated.

The data were analyzed with the Mann-Whitney "u" test statistical significance was assumed at $p<0.05$.

RESULTS

1 Allograft Rejection

2 rats in the control group and 2 rats in the vita-min A group died during burning or two days after burning and they were discarded from the series. The remaining 8 rats of each group were followed accord-ing the experimental protocol (Table - I). In the control group, the earliest rejection time was 13 days and the latest one was 22 days. The mean rejection time for controls was 18.5 ± 3.29 days. In the vitamin A treated group the erliest and the latest rejection times were 10 and 15 days respectively. The mean rejection time for vitamin A group was 12.37 ± 1.76 days. The difference was statistically significant ($p<0.05$).

2. Hemaglutination Antibody Titers

The humoral responses to sheep red blood cells in the control and vitamin A treated groups were measured as hemaglutination antibody titer. Some of the animals died during the experiments. Therefore

7 blood samples on 14th day in the control group, 8 blood samples on the 8th day and 7 blood samples on the 14th day in the vitamin A treated group could be measured (Table II).

The hemaglutination antibodytiters were between 1/8 - 1/32 in the controls and 1/8 - 1/256 in the **vitamin** A group on the 8th post injection day. In the vitamin A treated group, the antibody response to SRBC increased in a great measure ($p < 0.05$). The same titers were between 1/8 - 1/16 **in** the controls and 1/8 - 1/128 in the vitamin A treated group on the 14th post injection day. This **increase** was not significant statistictieafly ($p < 0.05$).

Table 1

Rejection Days of Skin Allografts

Rats	vitamin **A Group**	Control **Group**
1	**14.**gün	**20.** nün
2	**12.** gün	**20.** gün
3	**15.** gün	1 8. gün
4	**10.** gün	**13.** gün
5	13. gün	**22.** gün
6	1 2, gün	14. gün
7	13. gün	**20.** gün
8	**10.** gün	**21.** gün
Mean rejection day	**S 2.3 75 *i* 1.767**	18.**5 i 3**.2i).5
P		$p < 0.05$

Table - II

The Hemaglutination Antibody Titers

	8th **day**		**14th** day	
Rais	**Control**	Vit. **A**	Control	Vit. **A**
1	**1/8**	**1/16**	**1/16**	**1/32**
2	**1/8**	**1/128**	**1/16**	**1/8**
3	**1**/3 **2**	**1/128**	**1**/8	**1/64**
4	**1/32**	**1**/8	l/S	**1/3 2**
5	**1/8**	1/**128**	**1/8**	**1**/8
6	**1/8**	**1/128**	**1/8**	**1/8**
7	**1/16**	**1/256**	**1**/8	**1/8**
8	**1**/3 2	**1/128**		
9	**1**/32			
.10	**1/8**			
P	**p<0.05**		p > **0.Ü5**	

3. The Weights of the Reticuloendothelial System Organs

In the experiment of cellular immunity, the mean weights of the liver, spleen and thymus were 12.76 + 1.76 gin, 1.01 + 0.19 gm 0.372 + 0.068 gm in the control group and 11.44 + 1.85 gm, 1.226 + 0.63 gm, 0.649 + 0.24 gm in the vitamin A treated group respectively (Table-Ill). The increase of the thymus weight was statistically significant in the vitamin A treated group ($p < 0.05$). In the experiment of humoral immunity, the mean weights of the same organs were 12.33 + 2.13 gm, 0.978 + 0.15 gm, 0.146 + 0.063 gm in the control group and 12.50 + 4.16 gm, 1.44 *i* 0.27 gm 0.474 + 0.041 gm in the vitamin A treated group respectively (Table-IV). The increases in the weights of the spleen and thymus were statistically significant- ($p < 0.05$T

DISCUSSION

Formerly deaths following severe thermal injury took place rather early in the postburn period because of the hypovolemia. Modern concepts of fluid and electrolite replacement managed to survive most of the burned patients reported in the literature. Today the ones who can survive in the initial shock phase, have been dying due to infectious complications. It has been demonstrated in experimental animals that thermal trauma results in an increased susceptibility to bacterial infections and this is due to the defects in host defenses developing after burn (19, 30, 34, 40). Markley and Smallrnan studied the number and function of T and R cells from the spleens of burned and normal mice. The results showed a significant decrease in the number and spontaneous mitotic activity of both T and B ceils postburn period. (32).

ft has been shown that immunological responses remain normal in burns smaller than 20‘?. body surface. As the burn size increases, host defense mechanisms become progressively abolished (5, 6, 22, 28). Because of this, 30-35% third degree burn was produced in this experimental study.

Skin graft rejection is commonly agreed to represent a good measure of cellular immunity (18). We also evaluated the cell-mediated immunity with skin graft rejection in this study. After burning, the mean rejection time was 1.8.5 + 3.295 days in the control and 12.375 + 1.767 days in the vitamin A treated group. It has been stated that the mean rejection time of the skin allograft in the unburned animals is 10-12 days (18, 40). The prolongation of the skin graft survival time in the control group given saline injections, indicated that cellular immunity was depressed in the burned animals. The shortening of the graft survival time in the vitamin A treated group showed that the altered cellular immunity was stimulated by vitamin A.

Table - III

The Wet Weights of RES Organs in the Cellular Immunity Studied Group

	Thymus		Liver		Spleen	
Rats	**Vit.** A	**Control**	**Vit.** A	**Control**	**Vit.** A	**Control**
1	1.025	0.247	13.185	11.680	1.195	0.720
2	0.640	0.395	12.170	15.495	0.880	1.043
3	0.970	0.440	10.900	11.335	1.240	1.115
4	0.485	0.420	11.600	10.555	0.740	1.225
5	0.510	0.355	13.100	13.740	0.555	1.305
6	0.470	0365	10.6 80	12.315	1.270	0.980
7	0.355	0.445	12.450	14.615	1.300	0.932
8	0.740	0.3 20	7.460	12.410	2.630	0.870
Mean Weights	0.649 T 0.24	0.3 72 * 0.068	11.443 T 1.855	12. 768 + 701	1.226 * 0.63	1.017 + 197
P	p < 0.05		p > 0.05		p < 0.05	

Table - IV

The Wet Weights of RES Organs in the Humoral Immunity Studied Group

	Thymus		Liver		Spleen	
Rats	**Vit. A**	**Control**	**Vit. A**	**Control**	**Vit.** A	**Control**
i	0.546	0.140	11.730	10.802	1.630	1.080
2	0.4 70	0.247	15.100	11.330	1.623	1.075
3	0.440	0.147	18.035	10.910	1.682	1.073
4	0.451	0,117	10.3 20	11.985	1.145	1.177
≥	0.467	0.205	7.355	16.705	1.157	0.798
		0.114		13.478		0.860
		0.055		11.100		0.788
Mean Weights	0.4 7 t					
Mean Weights	0.4 74 т 0.041	0.146 + 0.063	12.5 + 0.063	12.33 + 2.13	1.447 + 0.27	0.978 + 0.15
P	p < 0.05		p > 0.05		p < 0.05	

The effects of vitamin A on immune system were investigated in various studies (8-12, 16). It has been shown that the lymphocyte transformation response decreases in the animals given synthetic diet devoid of vitamin A. After vitamin A is supplemented their diet for three days, it returns to former levels (31). Benjamin et al. demonstrated that the survival rates of mice injected with Pseudomonas aeruginosa and Candida albicans, are enhanced with vitamin A treatment in pharmacological doses. Vitamin A has no direct toxic effect on the microorganisms. This appears to be due to the fact that vitamin A increases the host resistance by stimulating reticuloendothelial (RES) phagocytic cells and en-

haneing the intracellular killing of ingested microorganisms (9),

In our study, the increase of the weight of thymus in the vitamin A treated group was statistically significant. This increase suggests that it is related to the hypertrophy of the thymus regulating the cell-mediated immunity. In the same group, the graft rejection was also accelerated suggesting that the proliferation and function of T lyphocytes were enhanced. Production of hemaglutination antibodies to SRBC importantly increased on the 8th day in the vitamin A group. This significant increase in the antibody titers on the 8th day might indicate that the IgM type primary antibody response in the vitamin A group, was much more enhanced than that in the controls. These figures returned to normal levels in both the control and vitamin A treated groups on the 14th day.

Artурson et al. (7) found a depression in immunoglobulin G. M, A, D and E concentrations two days after burn in 15 adults. Among these, the immunoglobulin M concentration was most severely-depressed. The concentrations of immunoglobulins returned to normal levels by the end of the second week. McClung (46) examined serum immunoglobulins A, M and G in 100 children after severe burns. The concentration of these decreased significantly following burns.

Krishnan et al. (47) stated that there was a marked atrophy of thymus and spleen in rats rendered vitamin A deficient. In the thymus the cortex was depleted of lymphocytes. The number of germinal centers were reduced in the spleen. The titers of hemaglutinations in response to the sheep erythrocytes were lower than the pair-fed controls.

Results reported in present study show that, vitamin A can stimulate both cellular and humoral immunological responses observed after thermal injury. Although the mechanisms of vitamin A stimulation of irnmunoregulatory system are not clear and require further study, it is therefore hypothesized that vitamin A plays a role on the immune system as an adjuvant and steroid antagonist (11, 14, 23, 29).

In addition, the weights of reticuloendothelial system organs were found to be increased in both the cellular and humoral immunity studied groups in our experiments. These observations might suggest that T and B lympocytes producing cellular and humoral imrnun responses respectively, were proliferated to a great extent by vitamin A.

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