

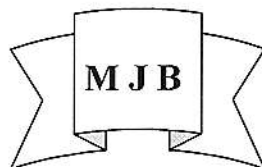
## Vitamin D<sub>2</sub> As Humoral Immunosuppressant in Rabbit

Ibrahim, M.S.Shnawa

Samah, A.Kadum\*

Department of Biology, College of Science, Babylon University , Hilla , P.O. Box 4. IRAQ.

\* The Institute of local Ecology, Babylon University , Hilla , P.O. Box 4. IRAQ.



### Abstract

Serial concentrations of vitamin D<sub>2</sub> that ranges from 1.7-34 IU were made in 2% egg albumin aqueous solution and used for rabbit priming through multisite and oral protocols. Meantime, same vitamin D<sub>2</sub> concentration incorporated in the diluting buffer of the hyperimmune and immunesera of anti EA specificity and titred against egg albumin coated erythrocytes.

The immunomodulation effect was scored as either titre enhancement or titer reduction .vit. D<sub>2</sub> was found as being humoral immunosuppressant and of dose dependent type . The dose response relationship was of simple linear type . Such suppression effect was evident both invivo during the immune response time curve and invitro on ready made anti EA antibody titers .The humoral immunotoxicity parameters as NOL threshold and activity range were determined vit. D<sub>2</sub> can be of help in maintenance and treatment of autoimmune disease and graft incompatibility conditions.

### الخلاصة

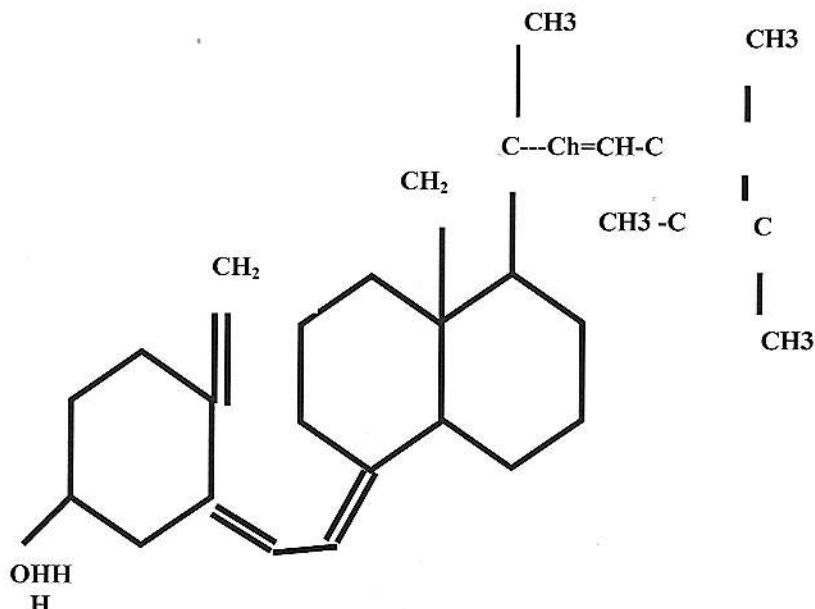
حضرت تراكيز متدرجة من فيتامين D<sub>2</sub> بمدى 1.7 الى 34 وحدة دولية في المحلول المائي لأح البيض بتركيز 2% . وقد استخدم هذا التركيز مع أح البيض في تحفيز الارانب بمنوال تمنيع متعدد الحقن ومنوال عبر الفم . وجرى في نفس الوقت تحضير هذه التخافيف بالدارى المستخدم في الاختبار المصلي لامصال مفرطة التمنيع واخرى منيعة متخصصة أح البيض لدراسة اثر هذا الفيتامين على الضد الموجود في المصل وفي الزجاج . تم الحكم بحصول التحوير المناعي اما بزيادة عيار الضد المتخصص عن مجموعة السيطرة او بنقصانه عن هذه المجموعة .

ووجد بأن فيتامين D<sub>2</sub> مثبط للمناعة الخلطية وهذا التثبيط معتمد على الجرعة والعلاقة بين التركيز والتثبيط علاقة خطية بسيطة سلبية في طريقتي التمنيع لحيوان المختبر وفي حالة الضد الجاهز في المصل بالزجاج . هذه النتيجة قد تساعد في علاج مرض المناعة الذاتي او عدم التوافق في الغرس النسيجي او غرس الاعضاء .

### Introduction

The fat soluble vitamin (FSV) are a group of vitamins that are soluble in fat and alcohol . They are isoprenoid derivative including vit . A,K,D and E . vit D. however, is a term that specific a

group of eleven sterol compounds that have vit D activity . Calciferol or vit . D<sub>2</sub> can be obtained from exposure of an ergosterol molecule or the provitamin D<sub>2</sub> (Fig-1) to ultraviolet or sun light (1 , 2)



**Figure 1** The structural formula of vit D<sub>2</sub>

several studies have been published about the immunomodulating effects of vit A,C,E and D<sub>3</sub> (3 , 4) . The objective of the present work was to report on the invivo and invitro effect of vitamin D<sub>2</sub> on immune response of rabbit to (EA) egg albumin antigen, in combination with graded doses of that vitamin .

**Material and Methods**

**1- Animals :** Thirty three male rabbits *O. caniculus* were elected from a group of rabbits after screening for ecto , endo and blood parasites as well as the naturally occuring anti EA antibody titres Such rabbits were kept ad libitum . They were assorted in the eleven groups, each of there .

**2- Antigen :** A solution of 2% egg albumin BDH in sterile distilled water was made as an antigen for the test and control groups .

**3- Adjuvant :** Turkish made fluid sunflower oil was used as adjuvant .

**4- Vitamins :** Vitamin D<sub>2</sub> in ergocalciferol from Roussel / Diamant Houchest was dissolved in ethyl alcohol and completed to the required dose by sterile saline . This helps as test vitamin . Vitamin A from kahira pharmoceutical and chemical industry co. Cairo , Egypt . Stocks of the vitamin was solved in 2cc ethyl alcohol then completed to 100cc , from which the required concentration was made .

**5- Immunization Protocol :** Multisite injection (5) and oral dosing protocols (6) were followed as :

Multisite		Oral	
EA	3R	EA	3R
EA + SFO	3R	EA + Vit. A	3R
EA + Vit. A	3R	EA + Vit. D <sub>2</sub> 3.4 IU	3R
EA + Vit. D <sub>2</sub> 0.17 IU	3R	EA + Vit. D <sub>2</sub> 6.8 IU	3R
EA + Vit. D <sub>2</sub> 3.4 IU	3R	EA + Vit. D <sub>2</sub> 34 IU	3R
EA + Vit. D <sub>2</sub> 34 IU	3R		

**6- Serology :** Blood was collected by cardiac puncture and sera obtained (7) Mucosal immunoglobulin was separated as in Hassan, 2002 . Immunomodulation was scored as enhancement or decrease of titre than EA control animals . Vitamin D<sub>2</sub> graded concentrations were incorporated in the diluting buffer for hyperimmune and immune anti EA antisera invitro (8) .

**7- Statistics :** Means , rates and regression analysis were done as in (9) .

### Results

The anti EA humoral immune responses were of higher titers in multiste injection protocol than in oral dosing protocols

Systemic humoral responses as indicated by titers were higher than mucosal immune responses .

Vitamin D<sub>2</sub> serial concentration 1.7-34 IU were inducing proportional reduction in the anti EA antibody titers as the concentration of vit D<sub>2</sub> increased , the anti EA antibody titers decreased . Such decrease was dose dependent . The dose response curve was of simple linear negative type . Both at systemic and mucosal levels . Likewise , both in multisite and oral protocols .

Vit D<sub>2</sub> dose effect was more evident in oral mucosal responses than on others (Tables 1&2) .

The invitro effect of vit D<sub>2</sub> graded concentration on the ready made antibody in hyperimmune and immunesera was as that of invivo .

The immunotoxicity parameters of vit. D<sub>2</sub> were determined as NEL threshold and effectivity range (Tables 2&1)

### Discussion

Vitamin D<sub>3</sub> exerts multiple effects on cytokines and major histocompatibility complex class II

expression in monocyte and lymphocytes (10) It can suppress the cellular and humoral immune responses (11).

Vitamin D<sub>2</sub> suppress anti EA antibody activity both invivo and invitro whatsoever , that immunization rout. Invivo it may interferes with antigen processing , antigen presentation and antigen recognition by the B lymphocyte system as well as it may inhibited the proliferation and expansion of B lymphocyte and /or antibody secretion release (12 , 13). On the invitro level it may interferes with antibody affinity , avidity and /or the forces involved in epitope- paratope reactions (14) therefore , vit.D<sub>2</sub> is immunomodulant , immunosuppressant , antigen independent and B cell suppression (Tables 1&2) (15)

Hence , beside it's uses for avitaminosis D<sub>2</sub> , it can be of use as immunosuppressant in graft incompatibility and autoimmune diseases (11).

### References

- 1- Champe. , P.C. & Harvey , R .A. (1994) .Biochemistry . 2<sup>nd</sup> ed. Lippincott- Williams and Wilkins , London , 337.
- 2- Stryer, L . 1995 Biochemistry . 4<sup>th</sup> ed. Freeman Company , New York .
- 3- Ortuno, J.;Cuosta A ; Esteban , A.E. & Mesegner , J. . . Vet. Immunol . Immuno pathol. ,2001 79 ,167.
- 4- Griffin,M.D.; L. W.J. Phan , V.A. ;Bachman , L.A. Mekan , O.J. & Kamar, R. . PNAS., 2001, 98,12, 6500 .
- 5- Al Shahery , M.A.N. & Shnawa , I .M. S. . Vet. Med . J.cairo . ,1989 ,37 , 2 , 291 .
- 6- Hassan, A.J. ,2002,. Some Aspects of the Dose and Rabbit immune System, Ph.D. Thesis . Babylon University.
- 7- Garvey , J.S. ; Cremer , N.E. and Sussdorf D.H. ,1977, Methods in

Immunology . Benjamin Press , Readings .  
 8- Crucishanck, R; Duguid , J.P ; Marmoin B.R & Swain , R.H.A .1975. Medical Microbiology.Vol 2 , 12<sup>th</sup> ed . Churohiu – Living ston . London , 459.  
 9- Dawod , K. & Al-Yas , A..1991. Statistical Methods for Agricultural Experimental . Rescarchs Mosul Univ.Press . Iraq  
 10- Kreft, B. ;Brzoska, S ; Doehn .C, Daha , MR.; Van - Der- Woude , F.J. & sacks , K ., J.1996, 155 ,4, , 1448.  
 11- Bouillon , R.; Verstuyf , A. ; Branisteanan , D.; Waer , M. & Mthieu , C.,Verh-K-Acad- Genesk- Belg .,1995 ,57,5, 371.  
 12- Brandtzaeg , P.; Farstad , I.N . ; Johansen ,F.E . ; Worderhang , I.N. & Yamanaka , T . . Immunol Rev.,1999, 171 , 45 .  
 13- Kacmar ,P. ; Pistl, J ; & Mikula, I . . Acta .Vet . Brno.,1999, 68,57.  
 14- Roitt , I . ; Brustoff , J. and Male , D. ,2001, Immunology. 6<sup>th</sup> ed .Mosby , London .  
 15- Hennessy , L.R.& Baker , J.J.R. ,1994, Immunomodulation . In : Stites, D.P.Terr, A.I.& Parslow, T.G. (eds) . Basic and Clinical Immunology . 8<sup>th</sup> ed ., Printice-Hall Inter. Inc. 781.

**Table 1** The immunomodulating Effect of Vit D<sub>2</sub> in Rabbit

Vit D <sub>2</sub> IU/ ml / kg (x)	Systemic		Mucosal		ST / MT		Immunotoxicity			Classification
	PHA (y <sub>1</sub> )	Pt (y <sub>2</sub> )	PAH (y <sub>3</sub> )	Pt (y <sub>4</sub> )	PHA	PT	NEL	Threshold	Activity Range	
Multisite										*Antigen independent dose dependent *Bioorganic immuno- suppressent
0	3840	3840	192	256	20/1	15/1				
1.7	320	1920	40	128	8/1	15/1	<1.7	1.7	1.7-34	
3.4	90	960	9	48	10/1	16.8/ 1				
34	40	160	4	24	10/1	6.6/1				
$\hat{Y}_1 = 6105.6 - 514.9x$ ; $r_1 = 0.44$ $\hat{Y}_2 = 2041.6 - 31.8x$ ; $r_2 = 0.79$					$\hat{Y}_3 = 87.1 - 2.64x$ ; $r_3 = 0.524$ $\hat{Y}_4 = 124.8 - 1.1x$ ; $r_4 = 0.504$					
Oral (x)	(y <sub>5</sub> )	(y <sub>6</sub> )	(y <sub>7</sub> )	(y <sub>8</sub> )						As in above
0	128 0	640	32	16	40/1	40/1				
3.4	800	160	12	8	8.7/1	20/1	<3.4	3.4	3.4- 34	
6.8	480	160	16	10	30/1	16/1				
34.0	20	60	13	4	6.7/1	15/1				
$\hat{Y}_5 = 977.71 - 30.11x$ ; $r_5 = 0.88$ $\hat{Y}_6 = 370.69 - 10.47x$ ; $r_6 = 0.62$					$\hat{Y}_7 = 22.49 - 0.61x$ ; $r_7 = 0.78$ $\hat{Y}_8 = 12.37 - 0.26x$ ; $r_8 = 0.51$					

**Table 2** The in vitro Vit D<sub>2</sub> effect on hyperimmunesera and immunesera Specific to EA , Invitro

Vit D <sub>2</sub> IU (x)	Hyperimmunesera		Immune	Immunotoxicity			Classification
	SFO-EA (y <sub>1</sub> )	Vit A-EA (y <sub>2</sub> )	Sera-EA (y <sub>3</sub> )	NEL	Threshold	Range	
Systemic							*Antigen independent *Bioorganic immuno-suppressent
0	10240	5120	5120				
1.7	7680	3840	5120	<1.7	1.7	1.7	
3.4	2720	1280	120			↓	
34	320	480	40			34	
$\hat{Y}_1 = 5935.1 - 66.55x$ $\hat{Y}_3 = 3730.81 - 115.684x$ $\hat{Y}_2 = 3644.32 - 98.4x$ $r_1 = 0.21$ $r_2 = 0.42$ $r_3 = 0.644$							
Mucosal	y <sub>4</sub>	Y <sub>5</sub>					As in above ↓
0	384	256	256				
1.7	256	256	256	<1.7	1.7	1.7	
3.4	128	128	*				
34	16	64	*			34	
$\hat{Y}_4 = 284.9 - 8.4x$ $r_4 = 0.51$ $\hat{Y} = 222.37 - 4.86x$ $r_5 = 0.35$							