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Refadin *as* Humoral Immunomodulant in a lapin Models

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Abstrect:

Serial subtheraputic concentrations of Refadin® that ranged from 0-6 mg/rn/kg were made in 2v. egg albumin antigen. Two protocols for immunopriming rabbits were attempted, as oral and multiinjection. Same concentrations were incorporated in vitro in the diluting buffur of immune and hyper-immunesera of anti EA Specificity. In each case immunomodulation was scored as enhancement of anti EA

titres or reduction in anti EA titres using microtitrehaermagglutination assay. It has been found that; the priming rout, the nature of adjuvant used, the nature of antibody as serum or mucosal and the drug concentration as well as the approach nature as in vitro or in vivo, were the factors affecting the humoral immune modulation. This immuilomodulation was in form of immunosuppressive type, antigen independent, dose dependent in which the antigen was T independent, B cell mitogen. The dose — response relation was of simple linear negative type. Based upon this, results drug induced immunosuppression in tuberculus patients is quitepossible. ‘. .

Introduction:.

Refadin® is an antibacterial antibiotic produced by *Streptomyces rneditrranei* in three forms. In which the usuall theraputic form is the Refadin B®.(EgOfOtr, 1985) This antibiotic is helpful in treatment of chronic bacterial infections like tuberculosis.(Ba, 1976; Lorian, 1986; Grang, 1988 & Swerlick *et.al.,1998)* It is genotoxic effect has been *investigated.(shnawa, OO3) Its immunological influences* have rarely been documented.(shnawa, 2001) The aim of the present work was at the investigation of the possible

immunomodulating effect of Refadin® in a lapin experimental model.

Materials and Methods:

Animals: Thirty three rabbits (0. caniculi) were brought from local breed. Check for ecto and endoparasites as well as blood parasites were done. Test bled to check for anti EA natural antibodies. The antibody and parasite free rabbits were the test animals. Otherwise, replenishing of anti EA and parasite bear rabbit was done. These animals were acclimatried to housing conditions and kept ad libitum and grouped into 11 group each of three. One as control and the rest were test groups.

Antigen: Egg albumin 2% aqious solution were made from egg albumin flakes of BDH Inc in sterile saline. Drugs: Refadin® 0.3, 0.6, 1.2. and 6 mg/mi were prepared from Refadin® 150 mg Lillely Co. Chloramphenicol® in 10 mg/mi was made in sterile saline from Chloramphenciol 250 mg Samara Drug Industry Iraq. Vitamin A is theraputic Co. was made in sterial saline from.

1. Adjuvant: Sunflower oil of Turkish origin was used in

ratio of volume to volume with EA antigen as adjuvant.(AL Shahary& Shnawa, 1989)

2. Immunizatian Protocol: Muitisite injection protocol(AL Shahary & Shnawa, 1989) and oral dosage protocol (Hassan, 2002) were followed.

3. Serology: Blood samples were collected from test and control groups of rabbits by cardic puncture and sera were separated.(AL Shahary & Shnawa, 1989) Gut mucosal immunoglobulins were separated from duodenum.(sITha.va & Thwa,ni 2000) Haemagglutination and interfacial precipitation were ersuded as fl.(Garvey et. aL, 1977) For in vitro studies the diluting saline serial refadin concentration.

Results:

The multisite injection protcol produced higher anti EA antibodies than that of the oral dosing protocol. The systemic immune responses were higher than mucosal immune responses Passive haemagglutinin is rather more sensitive than the interfacial tube precipitation test. (Table 1).

Refadin® was found to be immunotoxic as it reduced the specific anti

EA antibody titres in rabbits. This reduction was more makked in oral Than multisite protocol. The dose response relation was of simple linear negative type and the effect is dose dependent. The ratio of systemic to mucosal responses was reduced as the concentration was increased (Tables1&2).

The no effect levels were ranged from < 0.3 to 0.6 mg/mI. The threshold concentration were ranged from 0.3 to 0.6 mg/mi The range of effect concentration were. from 0.6 to 6 mg/ml (Table 2).

Discussion:

Refedin® can be classified as humoral immunomodulant and is being of immunosuppressive type (Tables .1 & 2). Such immunosuppressive action is of dose dependent type as reduced by the reduction of anti EA antibody titres as the concentrations were increased.(Kacrner et. at.. 1999; Herniessy & Baker, 1994)

The Refadin® in vivo effect may explained on the bases of the interference, with EA

1esentation and recognition by B lymphocytes (Hennessy & 'Baker, 1994; Kaemer L at., 1999) While its in vitro, influences, can be attributed to its effect on affinity, avidity of antibody and I or the forces involved in epitope-paratope reaction as well as to the disturbance in reaction dynamicity.(p10et. at., 2001)

Such findings are being of clinical bearings since it constitute an alrm to clinician treating tuberculus patients. Drug dilution during prolonged therapy rejmins is predictable.Hence immunosuppression is possible. The immunosuppressive effect of other antibiotic has been reported by other workers.(Muneer *et. al.*. 1988 & Chakrabarty *ci, at,,* .1981)

Table (1): The in vitro Refadin ® effect on hyperimmune sera induced by SF0, Vit. A together with regression analysis

Refadin Concentration	SF0 anti EA	Vit A anti EA	GA anti EA
	Systemic		
0	10240	5120	3840
0.3 0.6	1280	1920	320
320		960	240
6.0	20	80	20
X	Y1	Y3	Y5
= 41704.871 – 861.206x, r1 0.74 yA = 21515.625 —430.997 x, r3 = 0.499 ‘rY51621.1298.3x ,*r6=0.5* Mucosal			
0		192	192
	Y2	.8	Y
= 139.926 – 35.016 x, r2 0.583 yA 129.807 – 22.432 x, r4 = 0.647 yA21 , r60.47			

Table 2: Humoral Immunotoxicity Parameters in Lapin Model

Priming	Parameter	Effecting Threshold Concentration range	
Method	NEL		
In vivo Systemic and mucosal multisite	0.3	0.6	0.6 – 6
Oral	0.3	0.6	0.6—6
In vitro Systemic and mucosal multisite	0 6	1 2	1 2 - 6

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