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The Role of Tacrolimus and Sirolimus in Modulation Humeral Immunity in Male Albino Rats

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Abstract
The object of this study ware to modulated the effect of Tacrolimus and Sirolimus in Humeral immunity epically on titration of antibody and interlukin-2 in albino male rats. Fiftieth adult albino male rats were housed and arbitrarily divided into five equal groups (ten rats/ group)and administered as follows for 90 days :control group received distilled water ,groups T1 received sirolimus therapeutics dose 0.02mg/ kg B.W and T2:received sirolimus two fold dose 0.04mg/ kg BW and T3: received tacrolimus therapeutic dose 0.05mg/ kg BW and T4:received tacrolimus two fold dose 0.1mg/ mg B W.blood sample ware collect from each group in 30,60,90 days of experimental .Passive hemagglutination test used to assessment titrations of antibody, direct Enzyme Linked Immune Sorbent Assay used to assessment titrations of interlukin-2.The results at 30 days the titration of interleukin-2 ware decreased in all treated groups as a camper with control group and the decreasing depended on the dose and the time of exposure. The more significant in T2 and T4 in .30,60,90 days of experimental and the Tacrolimus showed more significant in reduction of interlukin-2 titrations than Sirolimus while the titration of antibody also show decreased in all treated groups T1,T2,T3,and T4 showed significant ($p<0.05$) in 30,60,90 days in comparison with the control one. Also the all treated group showed more decreased in 60,90 day and that more clear than in 30 day of experimental .The sirolimus more significant on the titrations of antibody than tacrolimus.
Keywords:Tacrolimus, Sirolimus, interlukin-2, Antibody.

Introduction

Tacrolimus (also FK-506 or fujimycin, trade names Prograf , Advagraf Protopic is an immunosuppressive drug that is used mainly after allogeneic organ transplant to reduce the activity of the patient's immune system and so lower the risk of organ rejection it is a 23-membered macrolide lactone discovered in 1984 from the fermentation broth of a Japanese soil sample that contained the bacteria Streptomyces tsukubaensis. It reduces interleukin-2 (IL-2) production by T-cells.with **(1).** Tacrolimus is a calcineuron inhibitor. It is also used in a topical preparation in the treatment of atopic dermatitis (eczema) with a dose of 0.5,1,3 and even 5 mg**(2).**sever refractory uveitis after bone marrow transplants with dose 5mg its primary use in veterinary medicine is for the treatment of Keratoconjunctivitis Sicca in dogs. Tacrolimus can also be used to treat dermatologic problems such as Superficial Keratitis, Miliary Dermatitis and Atopic Dermatitis (Veterinary customer survey results ,2013). Sirolimus known as rapamycin, is a macrolide (one of a group of drugs containing a macrolide ring) produced by the bacteria Streptomyces hygroscopicus. It has immunosuppressant functions in humans and is used to prevent rejection in organ as treatmant with a dose of 2mg and profelalses transplantation; it is especially useful in kidney transplants. It prevents activation of T cells and B cells by inhibiting their response tointerleukin-2 (IL-2)**(3).** (. Sirolimus is also used as a coronary stent coating. Sirolimus works, in part, by eliminating old and abnormal white blood cells **(4).** Sirolimus is effective in mice with autoimmunity and in children with a rare condition called autoimmune lymphoproliferative syndrome (ALPS) **(5).**

Materials and methods

Fifty albino male rats, aged 12-16 weeks with weight ranged (250-300g), supplied from the animal house of the College of veterinary medicine Baghdad university were used for the administration of tacrolimus and sirolimus .They were housed and maintained in a conventional animal facility, with controlled conditions of temperature (20 ± 5ºC). The animals were fed on special formula feed pellets and given water add libitum, throughout the experiment, each group of rats were housed in a plastic cage containing hard-wood chip as bedding. The bedding was changed every three days to ensure a clean environment**.** The rats that ware subjected to study ware divided in five equal groups each one consist of 10 rats and treated orally through gastric lavage with tacrolimus and serolimus for 90 days as fallowing group one(T1) Administered 0.02 mg/kg b.w Sirolimus as therapeutic dose.Group tow (T2) Administered 0.04 mg/kg b.w Sirolimus as two fold dose.Group three(T3) Administered 0.05mg/kg b.w Tacrolimus as therapeutic dose .Group four(T4) Administered 0.1 mg/kg b.w Tacrolimus as two fold dose.Group fivecontrol administered distilled water. The blood sample ware collect at 30,60,90,days of experimental in tube continuing anticoagulation. After the blood drown from heart puncture from anesthetized rats (intramuscular injections of ketamine (90mg/ kg B.W.)and xylazine (40mg/ kg B.W.) and then separate the serum which storeg in (-20 C) the used to measured the passive hemagglutination test by (Alton et al., 1975) and the interlukin-2 titration by the Elisa protocol titration of commercial komobiotic company.

Results

The result of interlukin-2 titration

There are significant p<0.05 in the IL-2 titration in all treated group incomparison with IL2- titration of control group especially the decrease was proration the dose and time of exposure in desendigly manner 30,60,90 days the prominent reduction in IL-2 titration observed in both treated group T2 and T4 which were received double dose of sirolimus and tacrolimus respectively .IL-2 especially after 90 day of treatment in comparison with there titration after 30 day of treatment (table1)

Table 1. titration of interlukin-2(IL-2 (pg/ml))in serum of male rat exposed orally to tacrolimus&sirolimus with therapeutic &two fold dose for 30,60,90 day

Groups	Day			LSD value
	30	60	90	
Control	298.41 ± 2.09 A a	295.36 ± 2.09 A a	297.32 ± 3.87 A a	8.674 NS
T1	127.22 ± 13.89 B a	93.31 ± 1.78 B b	24.38 ± 61.49 B c	29.441 *
T2	53.24 ± 2.09 CD a	33.99 ± 1.98 D b	15.94 ± 0.72 B c	5.290 *
T3	83.25 ± 8.06 C a	45.49 ± 1.07 C b	30.49 ± 4.61 B b	18.034 *
T4	41.92 ± 2.25 DE a	28.67 ± 1.25 E b	17.02 ± 0.95 B c	4.896 *
LSD value	22.019 *	4.987 *	42.073 *	---
* (P<0.05).				

Different capital letters mean significant differences' (P<0.05) between the group
Different small latter mean significant diffrences (P<0.05) within the group
periods.
-T1= therapeutic dose of sirolimus (20µg/kg.B.W) dosing orally for 90 days.
-T2=two fold dose of sirolimus (40 µg/kg.B.W) dosing orally for 90 days.
-C=control group given distilled water orally.
T3= therapeutic dose fo tacrolimus(50 µg/kg.B.W) dosing orally for 90 days
T4= two fold dose of tacrolimus (100 µg/kg.B.W) dosing orally for 90 days-n=10 (number of animal).

Result of passive hemaglutination test

The All treated group T1,T2,T3,and T4 showed significant (p<0.05) in titration decrease of antibodies at 30,60,90 days in comparison with the control one.Olso the All treated group (T1,T2,T3,T4) showed significant(p<0.05)decrease in antibodies titer descending with the period of exposure at 60and 90 day in comparison with there value at 30 day of treatment(table 2)

Table 2Passive hemaglotination test (PHT) titration of antibody in serum of male rat that exposed to tacrolimus &sirolimus with therapeutic& tow fold dose after 30&60and 90 day

Groups	Day			LSD value
	30	60	90	
Control	921.60 ± 298.54 A a	563.20±125.41 A b	422.40 ± 166.40 A bc	172.45 *
T1	358.40 ± 94.06 B a	41.60 ± 9.60 B b	18.40 ± 5.87 B b	109.66 *
T2	83.20 ± 19.20 B a	27.20 ± 9.99 B b	3.00 ± 1.41 B b	31.85 *
T3	628.80 ± 182.34 A a	41.60 ± 9.60 B b	6.80 ± 2.49 B b	128.64 *
T4	236.80 ± 112.50 B a	36.80 ± 11.7 B b	2.80 ± 0.48 B b	98.47 *
LSD value	501.07 *	167.66 *	219.70 *	---
* (P<0.05).				

Different capital letters mean significant (P<0.05) between the group
Different small latter mean significant (P<0.05) within the group
-T1= therapeutic dose of sirolimus (20µg/kg.B.W) dosing orally for 90 days.
-T2=two fold dose of sirolimus (40 µg/kg.B.W) dosing orally for 90 days.
T3= therapeutic dose fo tacrolimus(50 µg/kg.B.W) dosing orally for 90 days
T4= two fold dose of tacrolimus (100 µg/kg.B.W) dosing orally for 90 days
-C=control group given distilled water orally.

Discussion

Discussion of interlukin-2

Interleukin is a type of cytokine signaling molecule in the immune system. It is a protein that regulates the activities of white blood cells (leukocytes, often lymphocytes) that are responsible for immunity. IL-2 is part of the body's natural response to microbial infection, and in discriminating between foreign ("non-self") and "self". IL-2 mediates its effects by binding to IL-2 receptors, which are expressed by lymphocytes. Also IL-2 has key roles in key functions of the immune system, tolerance and immunity, primarily via its direct effects on T cells. In the thymus, where T cells mature, it prevents autoimmune diseases by promoting the differentiation of certain immature T cells into regulatory T cells, which suppress other T cells that are otherwise primed to attack normal healthy cells in the body. IL-2 also promotes the differentiation of T cells into effector T cells and into memory T cells when the initial T cell is also stimulated by an antigen, thus helping the body fight off infections**(6).** Its expression and secretion is tightly regulated and functions as part of both transient positive and negative feedback loops in mounting immune responses and tamping them down. Through its role in the development of T cell immunologic memory, which depends upon the expansion of the number and function of antigen-selected T cell clones**(7).**dendritic cell(DCs) have previously been identified as a potential source of IL-2 cytokine. The most effective stimuli to trigger IL-2 production in DCs by activating the calcineurin/NFAT signaling pathway.as for as (DCs), which have developed the capacity to respond to ligation of specific pathogen recognition receptors (PRRs) in order to trigger adaptive immune responses. many different responses induced by PRR ligation in DCs, the have found that specific agonists can stimulate translocation of the transcription factor “nuclear factor of activated T-cells” (NFAT) to the DC nucleus . resulting in the activation and regulation of gene expression **(8),(9).** Interestingly, one of the gene products regulated by NFAT in DCs is the interleukin (IL)-2 **(8).**

Tacrolimus is a calcenurin inhibitor leading to de-phosphorylated of nuclear factor activated T cell(NFAT) while the sirolimus block mammillae target of rapamycin leading to inhabit activation and proliferation of T cell,B cell and antigen presenting cell (APC) as for as Rapamycin inhibits IL-2-driven proliferation by down regulating the expression of genes required for key processes required for cell cycle progression**(10).**so it effect direct and indirect but the tacrolimus effective directly on NFAT by inhibition of calcenurin**(8).** this effectiveness clearly appearing in the rats interlukin-2 level after 30 day of treatment as compere with sirolimus that show less effective in level of interlukin-2 .but after 60 of treatment in the same period of treatment showed there were inhabited of IL-2 level still in the same manner as after 30 day of treatment representing by the more potential in the group that treated by tacrolimus as a compared with the group treated with sirolimus.After 90 day of exposure also we observed the sever inhibition of interlukin-2 level of all treated group ,but the story here is deferent since the treated sirolimus group showed more potent inhibition after on the IL-2 level incomparesion to the group treated by tacrolimus ,we though this is may be have accumulative effect of sirolimus after long standing treatment which not found in the calcenurin inhibitor(tacrolimus)

(11). as for as there are another way in case of sirolimus treatment there are more likely to event of the hepatotoxicity which leading to increasing serum sirolimus concentration**(12).**

Disccution of passive hemaaglutination test

Passive hemaaglutenation test is a tools to detect humeral immunity that represent by antigen antibody reaction that show an a agglutinations mass.The titrations of agglutination reduced significantly in the groups that dosed sirolimus as a compere THE GROUPS group that dosed tacrolimus because the tacrolimus not directly effecting antibody production it inhibited T cell mitogen activated B cell immunoglobulin (IgG) productions **(13).** While the sirolimus directly affect the antibody production by inhibit the important cytokines to the proliferation,activation and remain survival of B cell which called PI3K which when activation leading to mobilization of calcium and B cell become inhibited B cell activation and increase chance of apoptosis and down stream so leading to decreasing IgG producing **(14).**

Generally present study demonstrated that sirolimus elicited lower value of Abs titer as compare with tacrolimu.This may be associated with the potentiality of the sirolimus which consider more potent than tacrolimus for reduce of CD4 Tcell and CD8 Tcell which depended on antigen exposure on APCs and density of peptide MHC –complex on APCs**(15).**more than tacrolimus

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