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Overcoming the antimicrobial fasting property of the dormant *Brucella* by combining bacterial reactivators and antiBrucella drugs in mice

Hussein AbdulKadhim A.
Pharmacology and Therapeutics
Ali. J. Fakhriddeen (Medical Microbiology)

Abstract:-

Brucella is one of the microorganism that is characterized by its ability to persist although an effective immunity and a course of antimicrobial are provided. *Brucella* dormancy is the major contributor for this persistence.For this reason brucellosis gains its clinical significance as recurrent disease.In a trial of overcoming this dormancy state immunity of the tested mice(induced with intraarticular brucellosis) has been modulated with short course of dexamethasone to enhance *Brucella* suscebtabilty to antimicrobials. Sensitive thermometer was used to evaluate the average topical left knee joint hotness in response to treatment. Evaluation of knee joint aspirate every 3 days for the average count of PMN and MN inflammatory cells in addition to the number of the stained Brucella bacilli per high power field light microscope in addition to autopsy histopathological sectioning of the leftkneejointdirectlyafterthe10 days.Mice joints temperature have been measured and showed an obvious and statistically significant increased in temperature in untreated mice(40.1 degrees at the end of monitoring course) whereas a less sharp raise noticed in dexamethasone plus doxycycline plus SMS-TM (slfamethoxazole – trimethoprim) treated group which was lesser even than antimicrobial only treated group. There was a statistically significant decline in the number of knee joint inflammatory cells in group C as compared with the standard treated and untreated groups at $P<0.05$. A *Brucella* count in untreated mice was the largest 3400 at the end of the course in comparison with negligible count noticed in group C at the end of treatment.

Dexamethasone has decreased the percentage of inflammation to 20% at the end of treatment in comparism with untreated group (100% affected joints) and SMS-TM plus doxycycline only treated group(40%).

From the overall results, adding a short course of immunomodulator like dexamethasone is in need for further clinical evaluation since it may improve the health state during treatment in addition to enhancing antimicrobial effects.

Key words: Brucella , dexamethasone , dormant

Introduction:-

Bacterial dormancy is one of the major causes of latent infections.It is attributed to the down-regulation of the target bacterial proteins for antimicrobial agents(1). This makes those bacteria to be antimicrobial fast, a phenomenon commonly encountered with latent and endemic bacterial infections like brucellosis, salmonellosis and tuberculosis(2).In proliferation & in other cases the germ can remain dormant or latent in us for many years or even a lifetime while we remain healthy (3). Dormancy, although by itself is an inactive microbial maneuver, it stands for the more dangerous virulence factor giving rise to drugs insusceptibilty by *Brucella*, and therapeutic obstacles including the need for more prolonged antibacterial courses and an increase in the emergence rate of drug resisting *Brucella* strains (4). Trials of overcoming dormancy are become repeatedly conducted to treat these infectious diseases with more shorter and effective antimicrobial regimens. An in vivo mode of bacterial inactivation starts with changing the pH and the constituents of the tissue involved with *Brucella* through increasing of catabolic rate of the patient with precipitation of metabolic acidosis, in addition to the action of the innate cytokines, opsinins, vasoactive amines, cell membrane derived cytokines and the subsequent immune elements like immunoglobulins, and phagocytic lysosomal enzymes(5). On the other hand, bacterial growth factors pass to exhaustion. All those factors induce *Brucella* gene directed inactivation with capsule and spore synthesis and downregulation of growth factor receptors and enzymes. Cell wall and protein synthesizing enzymes like transpeptidase will no longer be susceptible for penicillins and aminoglycosides. Partial activation of these enzymes with amino acid and transient immune modulation is theoretically an approach for overcoming latent chronic
infections like malta fever due to its dormancy.

Materials and Methodes:-

Three groups of mice each contained 5 laboratory mice of mixed sexes with an average body weight of 20-25 g and aged between 6-8 wks were given standard oxoid diet ad libitum and breed with standard plastic cages.

All mice were induced with Brucella arthritis of *Brucella abortus* spp by inoculating *B. abortus* intra-articularly into the left knee joint of the mice in a dose of 20 colony per joint. The groups are :

1- Group a: (n=5) induced and untreated mice apart of giving 2ml of distilled water orally daily throughout 10 days.

2- Group b: (n=5) induced with Brucella arthritis and treated with 20 mg /kg of doxycycline with 20 mg/kg sulfamethoxazole and 5 mg/kg trimethoprim in 2 ml of distilled water daily for 10 days

3- Group c: similar to group b with additon of 40 mg/kg dexamethsone orally once daily for only the first 2 days then continuation of the rest of treatment throughout the 10 days.

Isolation and identification of *Brucella abortus*

The microorganism had been provided from bacteriology lab.of the hospital after being isolated from the blood of infected patient and further cultivated on the chocolate agar. Serological method was used to identify the strain.

Monitoring

Clinical, histopathological and microbiological parameters were used to follow up the response. These parameters were repeated every 3 days.

A) Clinically:the local signs of arthritis were assessed as the knee joint hotness, redness, tenderness, dysfunction and swelling. Sensitive thermometer was used to evaluate the average topical left knee joint hotness in response to treatment.

B) Bacteriologically: a septic evaluation of knee fine needle aspirate every 3 days for the average count of PMN and MN inflammatory cells in addition to the number of the stained *Brucella* bacilli per high power field light microscope.

C) Histopathologically :an autopsy histopathological sectioning of the left knee joint directly after the 10 days of the treatment being completed for assessing the relative grades of arthritis.

Results:-

1- Mice knee joint temperature findings

Table (1) shows the effect of antibrucella regimens on joint temperature in comparison with the untreated mice which was 40.1c°during six days, while this effect in mice treated with antibiotics was 38.8c° during one and six days, whereas this effect in mice treated with antibiotics plus dexamethasone was 37.9c° during three days.

Groups	Time of treatment and mice knee joint temperature in C					Test of significance At P<0.05
	Day 0	Day 1	Day 3	Day 6	Day 9	
Untreated	37.5 +/- 3	39.22+/- 4	38.34+/- 2	40.12+/- 3	38.23+/-4	Not sig.
Doxycycline plus SMS-TM	37.4+/- 2	38.78+/- 3	37.8+/-4	38.81+/- 4	37.55+/- 3	significant
Doxycycline plus SMS-TM plus dexamethasone	37.5+/- 4	37.6+/-3	37.92+/- 5	37.65+/- 2	37.45+/- 3	significant

From these results above, joint temp. increased in untreated mice whereas it was lesser in mice treated with antibiotics and it is also lesser in mice treated with such antibiotics plus dexasmethasone

2- findings of mice knee joint polymorph nuclear cells(PMN) and mononuclear cell(MN)

Table (2) appears the effect of different antibrucella regimens on knee joint aspiration PMN and MN cells count in mice. The WBCs count in knee joint in untreated mice was the largest 19 during six days, while the WBCs count in the treated mice with antibiotics was the largest 15 during one day only , whereas the WBCs count in the treated mice with antibiotics plus dexamethasone was the largest 11 also during one day.

Groups	Time of treatment and PMN and MN cells count (x 1000 per cubic mm)					Test of significance At P<0.05
	Day 0	Day 1	Day 3	Day 6	Day 9	
Untreated	0	15.64+/-1	18.54+/-2	19.12+/-2	18.6+/-1	Not sig.
Doxycycline plus SMS-TM	0	14.75+/-2	13.76+/-1	12.45+/-2	12.33+/-1	significant
Doxycycline plus SMS-TM plus dexamethasone	0	11.12+/-2	10.28+/-1	8.78+/-1	6.77+/-1	significant

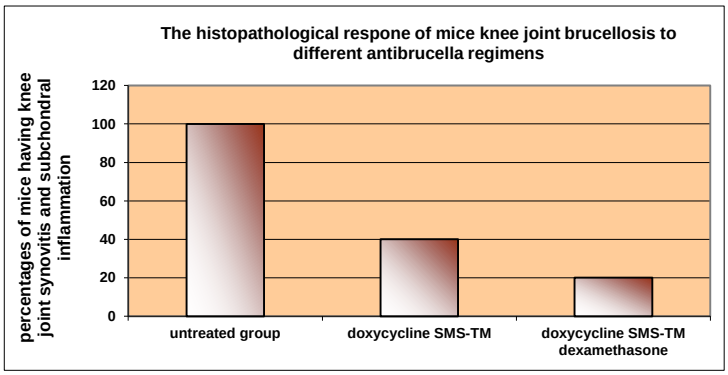
From the above results, WBCs count in knee joint increased in untreated mice , but it is the less in the mice treated with antibuotics , but it is the lesser in the mice treated with both antibiotics and dexamethasone in short time (1 day) . Thus the dexamethasone drug is consider as a fast track agent to minimize WBCs count.

3- Mice knee joint *Brucella* count findings

Table (3) reveals effect of different antibrucella regimens on *Brucella* count in mice knee joint. A *Brucella* count in untreated mice was the largest 3400 during nine days, while a *Brucella* count in mice treated with antibiotics plus dexamethasone was the largest 1400 during three days , whereas a *Brucell* count in mice treated with antibiotics only was the largest 500 during three days.

Groups	Time of treatment and *Brucella* count					Test of significance At P<0.05
	Day 0	Day 1	Day 3	Day 6	Day 9	
Untreated	20+/- 3	35+/- 4	2345+/- 22	3208+/- 31	3420+/- 32	Not sig.
Doxycycline plus SMS-TM	20+/- 2	234+/- 9	536+/- 11	345+/- 7	123+/- 5	significant
Doxycycline plus SMS-TM plus dexamethasone	20+/- 2	211+/- 10	1422+/- 21	204+/- 12	12+/- 2	significant

The current study refers to increase of no. of *Brucella* count in untreated mice, will reach to the largest no. , versus others. A *Brucella* count was more affected in the mice treated with antibiotics plus dexamethasone than these mice treated with antibiotics only.



4- Findings of Mice knee joint histopathology

Figure(1) shows that untreated group from mice was the largest group %100 having knee joint synovitis and subchondral inflammation , while after this group come mice treated with antibiotics %40 then mice group treated with antibiotics plus dexamethasone was the lowest group %20 having knee joint synovitis and subchondral inflammation.

Discussion:-

Persistence of microbial viability is one of the most dangerous property in regard to virulence. Brucellosis presents after an incubation period normally ranging from 1- 4 weeks, but may be as short as 1 week or as long as a year, without adequate and prompt antibiotics treatment, some patients develop a chronic brucellosis syndrome with many features of chronic fatigue syndrome.

Reasons of *Brucella* persistence could be attributed to its resistance to nutritional stress, since the vegetative bacteria of this genus can stay in a dormant state conferring on them a high resistance to chemical and physical agents prolonging its survival in the environment.

Some studies attribute *Brucella* persistence to genes , those required for initiating infection and those required only for long – term persistence , suggests that *B. abortus* uses distinct sets of virulence determinants to establish and maintain chronic infection in mice (6). The greater number of virulence genes required for chronic infection versus acute disease may reflect the requirement for additional adaptations to ensure long – term persistence, such as those which prevent clearance of *B. abortus* by the host immune system. Many factor have been found to reactivate a dormant *Brucella* including vaccination, immunosupprasants , immunocompromization .The idea behind the current work is to try a transient suppression of immunity to enhance reactivation of *Brucella* so that in become vulnerable for the given antiBrucella drugs(7).

Mice joints temperature have been measured and showed an obvious and statistically significant increased in temperature in untreated mice(40.1 degrees at the end of monitoring course) whereas a less sharp raise noticed in dexamethasone plus doxycycline plus SMS-TM treated group which was lesser even than antimicrobial only treated group. This low febrile state in group C could add another beneficial therapeutic value behind combining a short dexamethasone course . Thus a dexamethasone drug regards important factor to minimize joint temperature in mice.

In regard to WBCs count in knee joint. There was a statistically significant decline in the number of knee joint inflammatory cells in group C as compared with the standard treated and untreated groups at $P<0.05$ (t test),the mean joint WBCs at the end of treatment in group A,B and C were 19000, 1300,7000 /cubic mm respectively.

From these results mentioned above , WBCs count in knee joint was significantly decreased in in SMS-TM plus doxycycline plus dexamethasone in comparison with other groups .Thus the dexamethasone drug is consider as a fast track agent to minimize joint infiltration with inflammatory cells.

A *Brucella* count (as a total bacteria per high power field of the microscope) in untreated mice was the largest 3400 at the end of the course, whereas a spike of 1400 *Brucella* in the antimicrobial plus dexamethasone treated group during three days although a negligible count notice at the end of treatment

which was statistically significant decrease in *Brucella* count at P<0.05. group B showed minimal differences from group C in this parameter.

Knee joint synovitis and subchondral sclerosis were the most important histopathological parameters for assessment of antibrucella treatment. Dexamethasone has decreased the percentage of inflammation to 20% at the end of treatment in comparison with untreated group (100% affected joints) and SMS-TM plus doxycycline only treated group(40%). Clinical study of *Brucella* arthritis in human showed nearby findings (8). This improvement in histopathological outcome in group C could be attributed to the anti-inflammatory effect of dexamethasone in addition to the expected antidormant activity that caused a partial activation of *Brucella* and a sufficient exposure for the antimicrobial regimen.

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صفة تغلب التجويع للمضاد الميكروبي للبروسيلا الساكنة عن طريق اعادة التنشيط البكتيري ومضاد البروسيلا في الفئران

علي جابر فخر الدين حسين عبد الكاظم
جامعة الكوفة / كلية الصيدلة

الخلاصة:-

تعد البروســـللا من الاحياء المجهرية دات القدرة على البقاء بالرغم من وجود جهاز المناعة وتقديم المضـــادات الجرثومية وقد يعزى دلك لخاصية السبات ولهدا السبب اكتسبت البروسلا اهميتها السريرية كمرض قابل للانتكاس. وكمحاولة للتغلب على ظاهرة السبات لدى البروسللا فقد تم تحوير مناعة الفئران التي حقنت بالبروسللا داخل الركبة حيث تم اعطاؤها الدكساميثازون لمدة قصيرة.

تم بعد دلك مراقبة حرارة مفصل الفئران بالمحرار الحساس وكدلك تقييم السائل المسحوب من الركبة مجهريا بواسطة حساب عدد الكريات البيض وعدد بكتريا البروسللا في حقل المجهر العالي بالاضافة الى التقييم النسيجي لمفصل الركبة بعد انتهاء عشرة ايام من مدة العلاج.

اظهرت النتائج ان حرارة مفصـــل الركبة بلغت 40.1 درجة مئوية في المجموعة الغير معالجة بالمقارنة مع الحرارة الطبيعية في المجاميع المعالجة كما لوحظ انخفاظا معتدا في عدد كريات الدم البيض في مفاصل المجموعة المعالجة بالدكساميثازون. بينما كان عدد البروسللا في مفاصل الفئران الغير معالجة 3500 في حقل المجهل العالي فقد اصبح عددها مهملا في مفاصل الفئران للمجموعة سي في نهاية فترة العلاج. اما بالنسبة للفحص النسيجي لمفصل الركبة فقد انخفضت نسبة التهاب بطانة المفصل وتليف الغضروف المفصـــلي الى 20% في الفئران المعالجة بالدكســـاميثازون مع المضـــادات الجرثومية و40% في مجموعة العلاج بالمضـــادات الجرثومية فقط و100% بالمجموعة الغير معالجة. ومن مجموع النتائج فان اضافة المحورات المناعية لفترة قصيرة كالدكساميثازون الى المضادات الجرثومية بحاجة الى تقييم

سريري اوسع لاحتمال ان يحسن من اثار البروسللا ويزيد فاعلية المضادات الجرثومية.