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PROSPECTIVE TREATMENT FOR *MYCOBACTERIUM ULCERANS* INFECTION (BURULI ULCER) IN RAT AND MICE THROUGH COMBINED THERAPY WITH RIFAMPICIN AND SEPTRAN

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'BURULI ULCER'/Bairnsdale ulcer of man was described in 1948[1], but the effective treatment against this infection even today, is far from perfection. Some success has been obtained when the treatment is started early, otherwise the mainstay of treatment is surgery—excision or curettage with limited success[2]. The organism is reported to be resistant to many of the antituberculars[3]. We report here a combination therapy with rifampicin and septran which has arrested the pathogenicity of the organism in both rats and mice.

Random bred animals were used in the study. Duckrey rats weighing between 40–60 g were divided into 8 groups (table 1). The left hind footpad of each animal was inoculated with 1 mg (approx. 2.3×10^8 cfu) of *Mycobacterium ulcerans* in 0.03 ml of diluent (M/15 disodium hydrogen phosphate pH 8.7). Treatment was started after 54th day of infection and 6 injections per week were given. In all, 53 injections were given rifampicin dose was kept constant at 3 mg/kg but the dose of septran was kept varying at 10, 20 and 30 mg/kg alone and the same dosage in combination with rifampicin (table 1). The drugs were emulsified in 1 drop of tween 80 in saline and were given intraperitoneally. The criterion of effectiveness of the drug dose was reduction in foot pad inflammation after treatment since *M. ulcerans* produces severe inflammation of the foot pad. The thickness of both normal and the infected foot pads was measured weekly by means of Vernier Calipers and the difference in thickness between infected and normal foot pad actually represented the degree of inflammation.

In a second experiment the combination of septran and rifampicin was tested in mice. Park strain of mice bred randomly weighing 20 g ± 2 from CDRI animal house were taken for the study.

They were divided into ten groups (table 2). The dose of septran was kept constant at 30 mg/kg level and the dosages of rifampicin were varied at 2.5, 5, 10 and 15 mg/kg. The route of administration of the drugs was subcutaneous in this case. Mice were infected intravenously with 1 mg of *M. ulcerans* (approx. 2.3×10^8 cfu). Treatment with the drugs started from the day of infection and 6 injections per

Table 1 *Combined effect of rifampicin and septran against Mycobacterium ulcerans infection in rat foot pad model.*

Treatment	Dose mg/kg	Footpad inflammation in cm			Percent decrease in footpad inflammation
		9th week	11th week	14th week	
Untreated control	Nil	0.17 ± 0.01	0.13 ± 0.01	0.14 ± 0.02	18
Septran	10	0.15 ± 0.03	0.11 ± 0.05	0.09 ± 0.01	40
Septran	20	0.18 ± 0.06	0.15 ± 0.04	0.08 ± 0.01	56
Septran	30	0.18 ± 0.03	0.12 ± 0.03	0.08 ± 0.01	56
Rifampicin	3	0.17 ± 0.04	0.12 ± 0.02	0.06 ± 0.01	62
Septran + Rifampicin	10 + 3	0.31 ± 0.09	0.11 ± 0.04	0.14 ± 0.05	53
Septran + Rifampicin	20 + 3	0.32 ± 0.11	0.12 ± 0.03	0.06 ± 0.02	81
Septran + Rifampicin	30 + 3	0.31 ± 0.10	0.13 ± 0.03	0.06 ± 0.02	80

Table 2 *Combined effect of rifampicin and septran against M. ulcerans infection in mouse I.V. model*

Treatment	Dose mg/kg	No. of mice	Survivors on day 30 of challenge	Percentage survivors on day 30 of challenge	Mean survival time + standard error
Untreated control		20	0	0	7.0 ± 0.6
Septran	30	12	0	0	12.0 ± 0.35
Rifampicin	2.5	11	0	0	8.3 ± 0.8
Rifampicin + Septran	2.5 + 30	14	0	0	10.0 ± 1.0
Rifampicin	5.0	12	0	0	16.2 ± 1.6
Rifampicin + Septran	5.0 + 30	11	11	100	82.9 ± 7.1
Rifampicin	10	11	10	91	85.0 ± 9.3
Rifampicin + Septran	10 + 30	12	12	100	113.2 ± 9.5
Rifampicin	15	10	10	100	87.5 ± 5.5
Rifampicin + Septran	15 + 30	15	15	100	144.0 ± 5.8

week were given upto a total of ten. The mean survival time of each group was calculated and other parameters of study were followed.

In the rat foot pad model the natural decrease in inflammatory response percentage of untreated control group was only 18 upto 14th week of infection. The value of the corresponding period with rifampicin alone was 62 and that of septran alone was 40, 56 and 56 in increasing doses. The corresponding values with combination was much higher and reached up to 81 when 20 mg of septran and 3 mg of rifampicin were used. This clearly indicated that there was additive action of the drugs when used in combination.

In the second experiment with mice, the results presented in table 2 clearly show that the combination therapy, even when used the other way round (*i.e.* septran dose was kept constant and rifampicin dose was varied) and through a different route, has definite effect in arresting the pathological action of the pathogen and the MST of the infected animals was considerably increased upto 144 days when 15 mg of rifampicin and 30 mg of septran were used. Corresponding values of untreated control were only 7 days. The pathological score of the visceral organ also indicated the effectiveness of the treatment since the lesions were comparatively far less than in the controls. This also indicated the effectiveness of the drug combination in mice.

This combination of drug therapy with rifampicin and septran succeeded with many other drug combinations and may help in the treatment of this infection, along with excisional surgery.

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A BACTERIAL LEAF SPOT OF *BAEL* (AEGLE MARMELOS CORREA) IN RAJASTHAN AND A REVIVED NAME OF THE BACTERIUM

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BROWN spots were found on leaves of *Bael* trees in villages Baparawal and Barapal, 26 and 29 km respectively from Udaipur city during the month of August and September 1980–82. A bacterium was isolated from the infected leaves and this was purified by dilution plate method.

In nature brown spots were formed on leaves, twigs and fruits and under humid conditions yellow bacterial ooze was seen. The spots often coalesce and shot holes were observed in the infected leaves. By artificial inoculation, using carborandum abrasion technique, symptoms could be reproduced.

The bacterium is rod-shaped, gram negative, motile with single polar flagellum, capsulated, asparagine not utilised as sole source of carbon and nitrogen, pro-