# دراسة على التريكينيلا سبيرالس
# أستعمال مضادات حامض الفوليك فى علاج عدوى التريكينيلا التجريبية

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## الملخص

يعتبر الروبيدوميس عقار مضاد للانقسام الاختزالى . ويستعمل للمرة الأولى فى علاج عدوى التريكينيلا التجريبية فى الفئران البيضاء . ويستعمل فى جرعة مساوية ل ٢ ملى جرام لكل فأر فى اليوم . ويختزل العقار شدة غزو الطور المحوصل وغير المحوصل للأمعاء بنسب ٧٧٪ ، ٩٠٪ ، ٩٦٪ ، ٩٨٪ مع تأثير سام بسيط وخصوصا فى طور العدوى المبكر .

وعند استعمال الثيابندازول مع عقار الروبيدوميسن أعطى نتائج باهرة . فقد اختزل شدة العدوى فى الطور الأخير لمرض التريكينيلا بنسبة ١٠٠٪ .

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# STUDIES ON TRICHINELLA SPIRALIS FOLIC ACID ANTAGONISTS IN THE TREATMENT OF EXPERIMENTAL TRICHINOSIS

(With 2 Tables and 3 Figures)

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## SUMMARY

Rubidomycin is an antimitotic drug and used for the first time in the treatment of experimenal trichinosis in white rats. It is used in a dose of 2mg/rat/day. It gives 77%, 90%, 96% and 98% reduction in the intensity of invasion in intestinal migrating non encapsulated and encapsulated phases of the disease respectively with little toxic effects especially in the early trichinosis.

Combined with thiabendazole it gives excellent results, almost 100% reduction in the intensity of infection during late trichinosis.

## INTRODUCTION

It has been shown by many workers that thiabendezole has a good effect in the treatment of trichinosis. The mechanism of action of this durg has been proved to be on the embryonal tissue of TRICHINELLA SPIRALIS (CAMPBELL and CUCKLER, 1966; and OZERTSKOVOSKAYA, 1967).

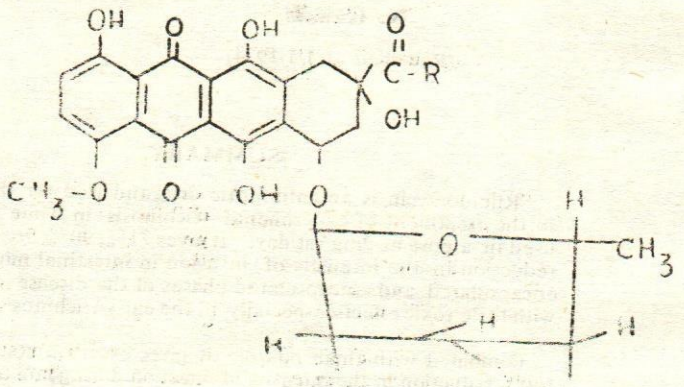
The group of drugs known in medical work and have this mechanism of action are the folic acid antagonists e.g. Methotrexate, indoxan, thiapta and rubidomycin. Methotrexate, was tested by the worker in white rats infected with *Trichinella spiralis* and has been shown to give 83% and 92% reduction in the intensity of muscle larvae when given to the rats during the stages of non encapsulated and encapsulated muscle larvae (GAWISH, 1970).

Due to the toxic effect of the antimitotic drugs the worker has reduced the dose of methotrexate and combined it with thiabendezole. The combination gives excellent results especially in the treatment of late trichinosis.

In the present work, the worker tests for the trichinicidal effect of other antimitotic agent namely Rubidomycin and also the results of its combination with thiabendezole.

## MATERIAL AND METHODS

1 Rubidomycin is an antimitotic drug available in solution. It is folic acid antagonist and potent inhibitor of epithelial and connective tissue proliferation. Its main toxic effect is on the bone marrow resulting in anaemia, leukopenia and thrombocytopenia. It is used in a dose of 2 mg/rat per day. The drug is of the anthracyclines with the following formula.




*Mechanism of action*

Physiochemical studies of the interaction between anthracycline antibiotics and DNA suggest that the ring portion intercalates between adjoining nucleotide pairs. Additionally, with the stabilizing effect of the amino sugar, the drug binds tenaciously to DNA preventing DNA, directed RNA or DNA transcription.

*Absorption, Metabolism and Excretion*

The initial half-life of Rubidomycin in plasma is about 45 minutes. It goes into kidney, spleen, liver and lung. It is mainly excreted in bile, and about one sixth is excreted in urine. The plasma protein bound drug has a half-life of over 40 hours, delaying clearance of part of each drug dose.

*Side effects*

— Bone marrow depression which result in haemorrhagic manifestion and ulceration in the mouth.

— Cardiac toxicity in the form of arrhythmia and cardiorespiratory symptoms.

*Clinical uses*

It is as antimitotic agent in leukaemia, sarcomas, lymphomas and bladder carcinoma.

2. Female white rats weighing 130 mg each are used as experimental animals. They are parasite free.

*Experiment A*

Testing for the trichinicidal effect of Rubidomycin :

44 white rats were used in this experiment. They were divided into 4 groups, 8 rats each and 12 control. Rats were infected per os, by 50 living trichinella larvae per animal.

The larvae were obtained from the muscles of infected white rats by acid-pepsin digestion.

Rubidomycin was given in a dose of 2 mg/rat per day intragastric by special needle according to the following scheme :

*Group I.* - The drug was given for 3 days starting from the second day post infection.

*Group II.* - The drug was given for 7 days starting from the fourth day post-infection.

*Group III.* - It was given for 7 days starting from the 14th day post-infection.

*Group IV.* - Received the drug for 7 days starting from the 21st day of infection.

These periods correspond to :

I. Young intestinal forms.

II. Fully developed intestinal forms and migrating larvae.

III. Non encapsulated muscle stage.

IV. Encapsulated muscle stage.

Rats were examined daily for haemorrhagic manifestations and buccal ulceration. Blood films were done every other day. They were killed 3 months post infection and the number of larvae per gm diaphragm were calculated. The ability of the obtained larvae to infect other animals was also tested by giving them to white mice whose muscles examined for larvae after one month.

*Experiment 'B'*

Combination of Rubidomycin and Thiabendezole in the treatment of muscle phase of trichinosis.

Since many drugs have been found to give excellent results in the treatment of early trichinosis e.g. thiabendazole, this combination was tried only in late trichinosis during which many patients present themselves with chronic myositis.

*Technique* : 36 white rats weighting 150 gm each were used. They were females and parasite free. They were divided into 3 groups, 12 rats for each group. Rats were infected by living *Trichinella* larvae. Hundred larvae per animal were given by mouth. Two groups were given thiabendezole in a dose of 50 mg/kg/day by mouth and Rubidomycin in a dose of 2 mg/animal/day by mouth also. The third group remained as a control.

*Group I.* — Received the drugs from the 14th to the 20th day post infection.

*Group II.* — Received the drugs from the 21st to the 26th day post infection.

These periods corresponded to the nonencapsulated and encapsulated muscle phase respectively.

Rats were killed three months after infection and the following steps were performed:

*a*) The number of larvae per gram muscle diaphargm were calculated.

*b*) Changes occuring inside the larvae itself were noticed and its ability to infect other rats was tested for.

## RESULTS

The results of the two experiments were tabulated as follows :

This table shows the following :

TABLE 1 : Number of Trichinella larvae per gram muscle diaphragm of white rat received Rubidomycin in a dose of 2 mg/rat/day in various stages of infection

| Group No | Time of adminis tration | Number of rats | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | Average |
| I | 2.4 day | 4220 | 5220 | | | | | | | | | | | 4720 |
| II | 4.10 day | 1804 | 2344 | 1624 | 2030 | | | | | | | | | 1950 |
| III | 14.20 day | 875 | 780 | 666 | 956 | 814 | 590 | 711 | 610 | | | | | 750 |
| IV | 21.27 day | 401 | 339 | 380 | 415 | 298 | 200 | 308 | 319 | | | | | 332 |
| Cntrol | group | 21785 | 19243 | 20112 | 18999 | 21008 | 19881 | 20341 | 19898 | 20978 | 21144 | 18071 | 20999 | 20205 |

1. The average number of larvae per gram muscle diaphragm in group I is 15485 less than the control group. This means 77% reduction in the intensity of infection.

2. The average number of larvae per gram muscle diaphragm in group II is 18255 less than the control group. This means 90% reduction in the intensity of infection.

3. The average number of larvae per gram muscle diaphragm in group III is 19455 less than the control group. This means 96% reduction in the intensity of infection.

4. The average number of larvae per gram muscle diaphragm in group IV is 19873 less than the control group. This means 98% reduction in the intensity of infection ($P > 3.15$).

5. Six rats died from group I and four died from group II. The dead rats show haemorrhages in the intestine and internal organs and also ulcerations in the mouth probably from the toxic effect of the drug during the acute stage.

6. Blood films show slight reduction in the white cells during the course of treatment.

Table 2 shows 100% reduction in the intensity of infection caused by *Trichinella spiralis* in the experimental animals used in this work. Under the microscope the larvae are immotile and vaculated. When given to white mice or rats, these animals showed no larvae in their muscles 45 days after their infection.

## DISCUSSION

Since the discovery of Trichinella spiralis worm and the description of the disease caused by it namely trichinosis, many drugs have been tried in the treatment of this disease, Sulphanilamide Phenothiazine, Tetrachloroethylene, Heterazan, Cadmium oxide Dithiazanine iodide Neguvon, Sulphanil Halaxon, Corticosteroids and recently Thiabendezole. These drugs gave partial effect on the intestinal forms of the parasite. Thiabendezole has an excellent effect on the intestinal forms and satisfactory effect on the muscle stage of trichinosis (CAMPBELL and CUCKLER, 1966; FROTEEVA, OZERTCKOVOSKAYA and TAREEV 1968; GAWISH, 1970).

TABLE 2 : Number of Trichinella larvae per gram muscle diaphragm of white rats received Rubidomycin in a dose of 2 mg/rat per day + Thiabendezole in a dose of 50 mg/kg/day

| Group No | Time of administration | Number of rats | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | Average |
| I | 14.20 day | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 4 | 1 | 2 | 5 | 0 | 1 |
| II | 21.26 day | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 1 | 0 | 0 |
| III | Control | 40611 | 41213 | 39789 | 39099 | 40001 | 38989 | 40351 | 40111 | 39999 | 40522 | 36891 | 39891 | 39769 |

The folic acid antagonist was introduced for the first time by the worker in 1970 (unpublished work), when he used methotrexate during the non encapsulated and encapsulated stages of the parasite. The reduction of the intensity of muscle invasion was 83% and 92% for the two stages respectively. Combination of thiabendezole and methotrexate has been tried also by the worker in 1970 and the results were successful with little toxic manifestions (89% for non encapsulated phase and 90% for the encapsulated stage).

Few months later in 1970, GRETILLATE published that he obtained good results when used methotrexate against muscle phase of trichinosis in guinea pigs and rabbits. Based on this finding, the worker tried other anti-mitotic drugs in the treatment of trichinosis. Rubidomycin used in the present work gives better effect than methotrexate especially against muscle phase, 96% and 98% for non encapsulated and encapsulated stages, $P > 3.15$ which is statistically significant. The drug has also good effect against intestinal and migrating forms of the parasite but with toxic effect which resulted in the death of some rats. It seems that the animals cannot tolarate the action of the drug during acute trichinosis. So the worker limits the use of Rubidomycin to the treatment of chronic trichinosis *i.e.* non encapsulated and encapsulated muscle phase (Fig. 1 & Fig. 2). In the second experiment when combination of Rubidomycin and Thiabendezole were tried in the treatment of chronic trichinosis very astonishing results were obtained. The combination has eradicated the infection 100% during the muscle phase of the disease with nearly no apparent toxic manifestations in the dose of 50 mg/kg per day Thiabendezole and 2 mg/rat/day Rubidomycin.

The mechanism of action of Rubidomycin is prevention of proliferation of embryonal tissue so it kills the larvae or prevents their development and growth. Also it prevents proliferation of fibroblasts, so the capsule is not formed or ill-formed and this gives free passage of both drugs to reach larvae in the muscles and exert their action on them.

Now we can say that, to treat trichinosis, the following scheme can be applied :

1. Early trichinosis is treated by thiabendezole in a dose of 50-100 mg/kg/day for one week.

2. Late trichinosis is treated by the combination of thiabendezole and Rubidomycin in a dose of 50 mg/kg/day and 2 mg/rat/day for the two drugs respectively for one week.

This work to be of value, it must be tried on the trichinised human being.

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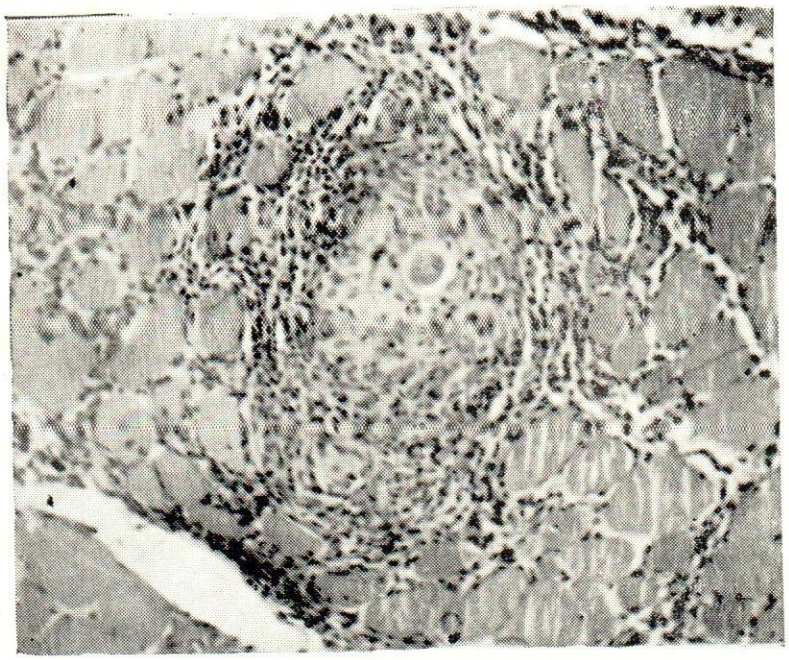

Fig. 1.—Section in the muscles of the thigh of white rat showing non encapsulated larva of *T. spiralis* surrounded by cell infilterate. H. and E. (× 120).

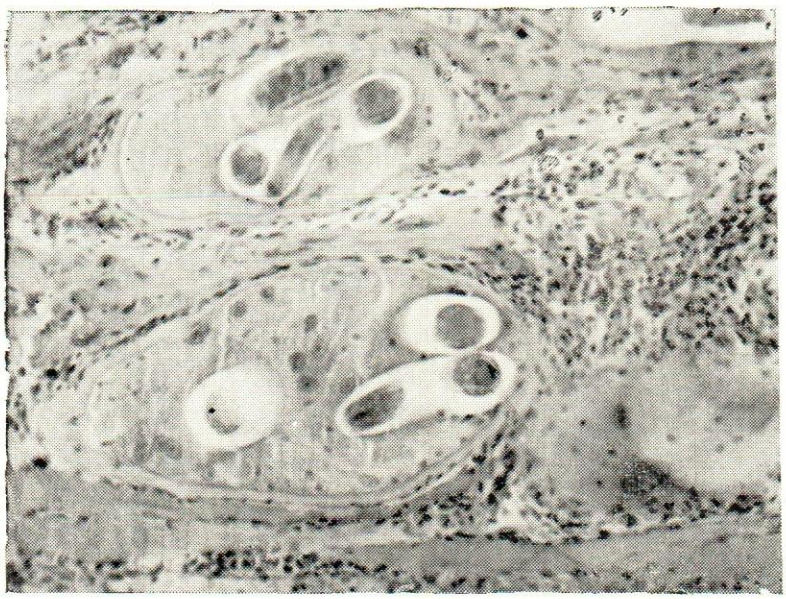

Fig. 2—The diaphragm of the white rat showing encapsulated *T. spiralis* larvae. H and E. (X 310)

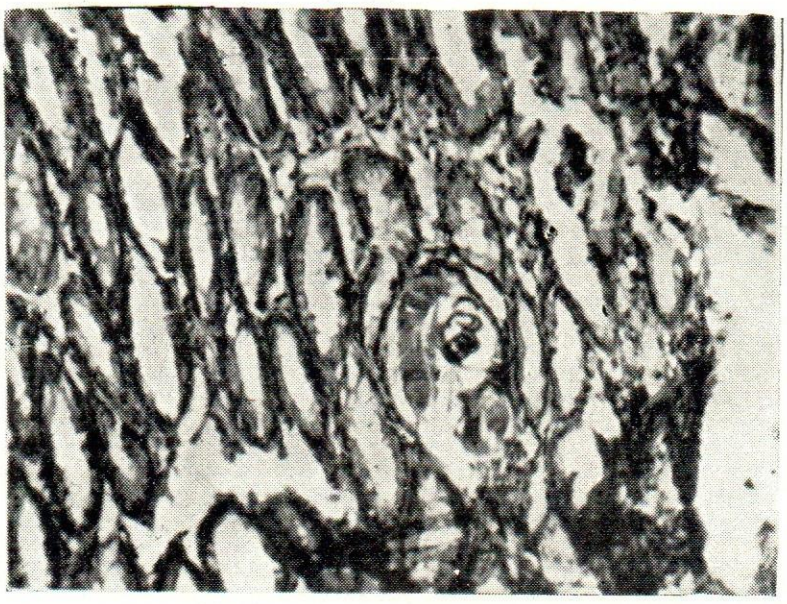

**Fig. 3.—Section in the intestine of white rat, showing female *T. spiralis* laying its larvae in lymphatic space. H. and E. (× 310).**