EFFECT OF A NITRO-IMIDAZOLE ON PRIMARY EXPERIMENTAL SYPHILIS IN RABBITS*

BY

ANNE ROOF YOBBS, JOHN W. CLARK, JR., AND ARNOLD L. SCHROETER

WITH THE TECHNICAL ASSISTANCE OF

WILLIAM E. POST, JR., AND FRANCES W. FIELD

Venereal Disease Research Laboratory, Venereal Disease Branch, Communicable Disease Center, Public Health Service, US Department of Health, Education, and Welfare, Atlanta, Georgia 30333

The discovery of 2-nitro-imidazole was announced in 1955 by Nakamura, and the following year first reports of its trichomonacidal properties appeared (Horie, 1956); 4 years later Cosar and Julou (1959) reported studies on the experimental effects of 1-beta-hydroxyethyl-2-methyl-5-nitro-imidazole† *in vitro* and *in vivo* in mice. They found it to be very effective against *Trichomonas vaginalis*, and less toxic in mice, rats, and dogs than previously-tested imidazoles. Clinical trials reported by Durel, Roiron, Siboulet, and Borel (1959), by Sylvestre and Gallai (1960), by Nicol, Barrow, and Redmond (1960), and by Willcox (1960) have proven this nitro-imidazole to be a satisfactory trichomonacidal agent in both males and females when given orally, and in females in combined oral and topical therapy.

Trichomoniasis, syphilis, gonorrhoea, and the so-called "minor" venereal diseases are usually transmitted through intimate sexual contact with an infected individual. One or more of these diseases may be contracted from a single exposure, or a person previously infected with one of these diseases may later become infected with another. Since the interval required for the development of signs and symptoms varies with each disease, treatment of a symptomatic infection could conceivably affect the course of other concomitant although as yet unrecognized or inapparent disease. This effect may range from cure to minimal alteration of signs and/or symptoms. The inadvertent, inadequate treatment of infection is especially important in syphilis, a disease in which relatively short symptomatic

intervals alternate with longer periods of asymptomatic infection during which accurate diagnosis is difficult.

Although it has been stated that "Flagyl" has no effect on bacteria, and both *Candida albicans* and the Döderlein's bacillus have been found both before and after treatment (Gray, Kotcher, and Giesel, 1961), there has been no report of the use of this drug in the presence of other venereal diseases. Therefore, study of the effect of Flagyl on primary syphilis in rabbits was undertaken to determine if treatment effective against trichomoniasis in man could affect syphilis.

Method

Generally, the method set forth by Clark and Yobbs (1963) was followed. Sero-negative adult male rabbits were shaved and inoculated intracutaneously at six sites on the back with 0.2 ml. of a suspension containing 4.8×10^8 *Treponema pallidum*, Nichols strain, per cubic centimetre. These animals were followed clinically and with daily dark-field examinations. All sites on all animals had become dark-field positive before treatment was begun one week after inoculation.

Three groups, each of six rabbits, were treated with Flagyl on the following schedules:

- (1) 2 g./kg./day by mouth in two equal daily doses for 10 days;
- (2) 14 mg./kg./day, also by mouth in two equal daily doses for 10 days;
- (3) 250 mg./kg./day intramuscularly in six equal 4-hourly doses per day for 7 days.

These dosages were selected on the following bases:

- (1) 14 mg./kg./day orally—The dose range recommended by the manufacturer for use in humans (250 mg. by mouth three times a day for 10 days) (G. D. Searle and Co., 1963);

- (2) 2 g./kg./day by mouth—Mouse toxicity studies showed the LD-50 for these animals to be greater than

Note: Trade names are for identification only and do not represent an endorsement by the Public Health Service or the U.S. Department of Health, Education, and Welfare.

* Received for publication September 28, 1965.

† The substance 1-beta-hydroxyethyl-2-methyl-5-nitro-imidazole is now marketed under the name "Flagyl" (G. D. Searle and Company).

3,250 mg./kg. bodyweight when given orally (G. D. Searle and Co., 1963);

(3) 250 mg./kg./day intramuscularly—An arbitrary level within the limits of safety selected for use in intramuscular treatment.

Since the doses used were well below toxic levels reported in other animals and humans, it was not thought necessary to include a treated uninfected group as a toxicity control. Controls were a group of six untreated infected rabbits. Oral medication was given by gastric intubation. The drug used was purchased on the open market; labels stated that each tablet contained 250 mg. nitro-imidazole. Suspensions for intubation and injection were prepared by suspending crushed tablets in sterile distilled water to make concentrations of 125 mg./ml. and 2 mg./ml. The bulk of the tablet powder was insoluble in distilled water, resulting in a particulate suspension.

During treatment, back lesions were examined clinically and by dark-field microscopy each day. After the completion of treatment, clinical and dark-field examinations were performed as outlined in the published method. VDRL testing was performed after 3, 6 and, 12 weeks.

Results

Seven animals died before the completion of therapy, five animals in the group receiving the higher oral dose (2 g./kg./day) and one in each of the other two treatment groups. In addition, one animal in each oral treatment group died as a result of technical error during intubation. Thus, nine treated animals completed the planned course of treatment. No drug effect against *Treponema pallidum* was shown either in the animals which died early or in those completing treatment. All survivors and controls became serologically reactive by the VDRL test after 2 weeks of infection with syphilis.

Inoculation sites continued to be dark-field positive long after completion of treatment in all surviving animals. Over half developed dark-field positive secondary lesions 1 to 2½ months after treatment. One animal in the group receiving 14 mg./kg./day orally and one in the intramuscular group was *still* dark-field positive at an original inoculation site 5 months after treatment. Serological response to the disease was not altered by the treatment. Therapeutic data are detailed in the Table.

Pathology

There was a high incidence of sudden death in our experimental animals during treatment—six of twelve in the oral groups and one of six in the intramuscular group. Deaths followed sudden collapse; there were no observed neurological, gastrointestinal, or respiratory signs. Those animals which died during treatment were autopsied whenever possible. 5 months after therapy, when failure to affect the

TABLE
EFFECT OF A NITRO-IMIDAZOLE ON EXPERIMENTAL SYPHILIS

Dosage Schedule	Rabbit Number	No. of Doses	Total Drug (g.)	Survival After Beginning of Treatment (hrs)	Cause of Death
2 g./kg./day for 10 days Orally	1	3	15.3	36	*
	2	1	3.19	12	Technical error†
	3	6	17.76	54	††
	4	5	21.2	50	††
	5	5	18.2	50	††
	6	4	14.04	48	*
14 mg./kg./day for 10 days Orally	1	20	.47	5 mths	Killed†
	2	9	.22	103	Technical error†
	3	17	.52	200	*
	4	20	.44	4 mths	<i>Pasteurella</i> infection
	5	20	.46	5 mths	Killed†
	6	20	.43	5 mths	Killed†
250 mg./kg./day for 7 days, 4 hrly Intra-muscularly	1	42	6.88	5 mths	Killed†
	2	42	6.28	5 mths	Killed†
	3	42	5.58	2 mths	Killed (Humane)
	4	24	3.66	103	††
	5	42	5.34	5 mths	Killed†
	6	42	5.04	5 mths	Killed†

* Cause of death attributed by authors to species intolerance for drug. See text.

† Postmortem examination performed.

course of the disease was obvious in all treated animals, the survivors were killed and autopsied.

In those which died during treatment, the most striking findings at autopsy related to the liver. In the group receiving 2 g./kg./day orally, the liver was markedly enlarged; its cut surface was pale yellow with preservation of the normal gross architectural pattern. There were no apparent changes in the group receiving the lower oral dosage on macroscopic examination. The liver of the one animal which died in the group treated intramuscularly showed only slight congestion. There were no other pertinent gross abnormalities in any of the autopsied animals. The hearts and lungs were normal, as were the intestinal tracts, which contained a normal amount of formed faecal material. Residual drug suspension was found in the stomach of those on oral schedules which died during treatment. This was assumed to be pill filler, since the nitro-imidazole itself is soluble in water and readily absorbed from the intestinal tract.

The most striking microscopic changes were noted in livers from the group receiving the higher oral dosage in which there was nuclear necrosis in the centrilobular hepatic cells and less severe mid-lobular damage. The hepatic cells were swollen and sometimes ruptured; their cytoplasm was vacuolated. The periportal areas were more normal; the hepatocellular cytoplasm was coarsely vacuolated. These

changes were generalized throughout the liver. In the lower dosage groups hepatic damage was less severe. In the group receiving 14 mg./kg./day, cellular swelling and cloudy cytoplasm were seen predominantly in the centrilobular areas; severe congestion was generalized. In the group treated intramuscularly, similar mild cytoplasmic changes were seen only in the mid-lobular areas, and congestion was less marked.

No animal showed macroscopic or microscopic evidence of haemorrhage or overwhelming infection. Autopsies of animals surviving 5 months after therapy showed no significant macroscopic abnormalities. Livers were essentially normal on macroscopic and microscopic examinations; hepatic cells and architecture were normal. There was mild congestion and slightly increased perivascular fibrosis.

Discussion

It is well known that some chemotherapeutic and antimicrobial agents may affect the course of syphilis without curing the disease. Early accurate diagnosis and adequate treatment is important in the control of infectious syphilis and the prevention of late manifestations of the disease. However, early diagnosis is difficult when presenting signs and symptoms are atypical.

The incidence of trichomoniasis in females and in their male sex partners indicates a potentially high usage of safe and effective, trichomonocidal drugs. It is important that anti-treponemal effects of drugs be determined in order that physicians may be aware of possible alteration of presenting signs and symptoms of syphilis. Our studies showed no demonstrable effect on *Treponema pallidum* nor on the course of the disease in rabbits at the dosages used. We would not therefore expect that treatment of trichomoniasis with this nitro-imidazole would alter the presenting signs or symptoms of concomitant or incubating syphilis.

We attribute the high mortality in our study to intolerance to this drug at the 2,000 mg./kg./day level of the species used. Previous studies in mice, rats, and dogs have shown these species to have a higher tolerance level to this compound. Clinical use (Durel and others, 1959; Sylvestre and Gallai, 1960; Nicol, Barrow, and Redmond, 1960; Willcox, 1960) has been associated with the occasional development of only the mildest untoward reactions; there have been no reports of serious complications.

Summary

1-beta-hydroxyethyl-2-methyl-5-nitro-imidazole has been found to have no effect on experimental

syphilis in rabbits at the currently recommended dose nor at almost 150 times that amount. The high incidence of sudden death and liver damage in the high dosage group is attributed by the authors to species intolerance.

ADDENDUM

In vitro testing of 1-beta-hydroxyethyl-2-methyl-5-nitro-imidazole (Flagyl) to determine the susceptibility of *Neisseria gonorrhoeae* was performed using the method of Thayer, Field, Perry, Martin, and Garson (1960). Four strains of *N. gonorrhoeae* recently isolated from three females and one male patient were tested. Two of these strains were sensitive to 0.01 u./ml. and two to 0.05 u./ml. penicillin. No bactericidal action on the *N. gonorrhoeae* was shown at concentrations up to 0.1 mg./ml. of Flagyl. However, one strain from a female showed slight bacteriostatic effect at concentrations of 0.1 and 0.05 mg./ml. Flagyl.

REFERENCES

- Clark, J. W., Jr., and Yobs, A. R. (1963). *Brit. J. vener. Dis.* **39**, 184.
 Cosar, C., and Julou, L. (1959). *Ann. Inst. Pasteur*, **96**, 238.
 Durel, P., Roiron, V., Siboulet, A., and Borel, L. J. (1959). "Clinical Trial of a Trichomonocidal Derivative of Imidazole", presented before the French Gynaecological Society on January 19, 1959.
 Gray, L. A., Kotcher, E., and Giesel, L. O., Jr. (1961). *J. Kentucky med. Ass.*, **59**, 672.
 Horie, H. (1956). *J. Antibiot. (Tokyo)*, **9**, 168.
 Nakamura, S. (1955). *Pharm. Bull. (Tokyo)*, **3**, 379.
 Nicol, C. S., Barrow, J., and Redmond, A. (1960). *Brit. J. vener. Dis.*, **36**, 152.
 Physician's Product Brochure No. 69 (1963). G. D. Searle and Company (Chicago).
 Sylvestre, L., and Gallai, Z. (1960). *Un. méd. Can.*, **89**, 735.
 Thayer, J. D., Field, F. W., Perry, M. I., Martin, J. E., and Garson, W. (1961). "Proc. Conf. Antimicrobial Agents, Washington, 1960", ed. P. Gray, B. Tabenkin, and S. G. Bradley, pp. 352-356. Plenum Press, New York.
 Willcox, R. R. (1960) *Brit. J. vener. Dis.*, **36**, 175.

L'effet d'un nitro-imidazole dans la syphilis expérimentale primaire chez le lapin

RÉSUMÉ

On a trouvé que la drogue 1-beta-hydroxyethyl-2-méthyle-5-nitro-imidazole n'agit pas dans la syphilis expérimentale primaire chez le lapin à la dose couramment recommandée ni à une dose 150 fois plus forte. L'incidence élevée de mort subite et des lésions hépatiques dans le groupe recevant un fort dosage a été attribuée par les auteurs à une intolérance spécifique du lapin.