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ANTITUBERCULOUS EFFECT OF SOME IMIDAZOLE DERIVATIVES

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Abdel Kader and co-workers[1)2)4)5)] showed that tryptophan and some of its metabolites had tuberculostatic effect *in vitro*, *in vivo* and in human patients as well. Abdel Kader et al. also showed that histidine possessed bacteriostatic effect on Mycobacterium tuberculosis both *in vitro*[1)] and *in vivo*[6)]. This investigation was, therefore, conducted to study the effect of some imidazole derivatives on Mycobacterium tuberculosis both *in vitro* and *in vivo* in guinea pig.

METHOD OF EXPERIMENTS

Two types of investigations were carried out using Mycobacterium tuberculosis $H_{37}Rv$.

A. *In vitro* studies: studies were made to test the antituberculous effect of some imidazole derivatives on Mycobacterium tuberculosis. The compounds tested were Imidazole, Imidazole acetic hydrochloride, Imidazole lactic, Histidine methyl hydrochloride, histidinol hydrochloride, histamine, carnosine and urocanic acid.

A new modification of Proskauer-Beck and Vorwards medium[12)] was established in this work. This modification included variation of pH of medium from 7.8 to 7.0 (a series of pH variations were tried for culturing M. tuberculosis; 6.8, 7, 7.2, 7.4, 7.6, & 7.8), and the addition of 1.8 g agar in order to solidify the medium (different agar concentrations namely 1.6 g, 1.8 g, 2 g and 2.2 g were tested).

Both fluid and solid media were separately used to study the effect of Imidazole derivatives at a concentration level of 0.02 molar on the growth of Mycobacterium tuberculosis $H_{37}Rv$.

After sterilization, as described by original Proskauer-Beck medium, the inoculation with the Mycobacterium was made and the growth was recorded weekly for 6 weeks.

The results of the *in vitro* studies were further extended to *in vivo* investigations.

B. *In vivo* experiments: Therapeutic effect of Imidazole given 17.5 mg daily was examined in guinea pigs innoculated with Mycobacterium tuberculosis. This concentration was equivalent, on molar basis, to the 50 mg of histidine used previously[6)]. Four groups of animals, 8 male guinea pigs each, were selected from the laboratory stock, and their weight ranged from 250 to 300 g. They were kept on a stock diet composed of rye and green clover during the experiment period. The animals were housed separately. All animals were infected with 0.001 mg of Mycobacterium tuberculosis $H_{37}Rv$ by intramuscular injection in the right thigh. The first group (I) infected and untreated was used as the control, the second group (II) was simultaneously treated with intramuscular injection of streptomycin, 3 times a week, and 5mg isonicotinyl hydroxide daily. Treatment was continued for 15 days.

The third group (III) was given a daily subcutaneous dose of 17.5 mg imidazole as 1 m*l* sterile aqueous solution. The fourth group (IV) had to be treated with the same dose of imidazole(17.5 mg/day) 15 days after tuberculous innoculation. The duration of imidazole therapy in both groups III & IV was planned to be continued for 15 days. Yet the animals of group III died during three days of therapy. The experiment was, therefore, continued for group IV using smaller doses of imidazole, namely 3 mg. Then

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another experiment was planned using 1mg imidazole/animal/day.

All animals were left without any medication for the remaining period which lasted 8 weeks. All animals were then sacrificed and examined. The technique of Feldman and Hinshaw was exactly followed for all groups of animals studied.

All guinea pigs were examined at autopsy and sections of lymph nodes, spleen and liver were stained with Ziehl Neelsen's method and microscopically examined.

RESULTS

The imidazole compounds tested proved to have different tuberculostatic effects. The highly potent compounds were imidazole, histamine, carnosine, histidinol and histidine methyl hydrochloride. Imidazole acetic hydrochloride, imidazole lactic, however, showed slight potency, while urocanic acid had lowest effect.

In the *in vivo* experiments, on post mortem examination, the control animals (group I) showed evidence of infection manifested by enlargement and caseation with generalization specially in spleen and liver. The lungs showed discrete tubercles, discrete alveolar obstruction and increased vascularity. Ziehl-Neelsen's staining revealed the presence of T.B. organisms.

Table 1. *In Vitro* Effect of Imidazole Compounds (0.02 M) on the Growth of Mycobacterium Tuberculosis $H_{37}Rv$ in Both Fluid and Solid Media

Compound tested	Growth during 6 weeks											
	1		2		3		4		5		6	
	fl.	sol.	fl.	sol.	fl.	sol.	fl.	sol.	fl.	sol.	fl.	sol.
Control	2+	3+	3+	3+	3+	3+	4+	4+	4+	4+	4+	4+
Imidazole	+	+	+	+	+	+	+	+	+	+	+	+
Histidine methyl hydrochloride	+	+	+	+	+	+	+	+	+	+	+	+
Histamine	+	+	+	+	+	+	+	+	+	+	+	+
Carnosine	+	+	+	+	+	+	+	+	+	+	+	+
Histidinol HCl	+	+	+	+	+	+	+	+	+	+	+	+
Imidazole lactic	2+	2+	2+	2+	2+	2+	2+	2+	3+	3+	3+	3+
Imidazole acetic acid	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+	3+	3+
Urocanic acid	2+	2+	2+	2+	3+	3+	3+	3+	3+	3+	3+	3+

Table 2. Pathological Lesions in Different Organs of Different Groups of Guinea Pigs

Group examined	Lymph nodes enlargement	Spleen	Liver	Lung	Total degree of T.B. activity
Infected untreated (Control)	###	###	###	##	(###)
Streptomycin·INH treated group	1/2 −+	−	−	##	(##)
Imidazole group treated immediately after infection (17.5 mg/animal)	±	−	+	+	No activity, but severe toxicity (−)
Imidazole group treated 15 days after infection (3 mg/animal)	+	−	−	−	Moderate liver atrophy (−)
Imidazole group treated immediately after infection (1 mg/animal)	±	−	−	−	(−)
Imidazole group treated 15 days after infection (1 mg/animal)	±	−	−	1/2+	1/2+

The animals of group II (streptomycin+INH) did not show signs of generalization, and the regional lymph nodes were slightly enlarged, fibrosed and showed no caseation. Microscopic examination revealed normal spleen and liver but the lungs showed multiple tubercles with no giant cells, and consolidation of 2/3 of the lung tissue. There was also increased vascularity in the lung tissue.

The imidazole treated group III receiving a dose of 17.5 mg daily/animal, all died after three injections or 3 days. On post-mortem examination there were multiple signs of general toxicity; consolidation of whole lung tissue and infiltration of whole blood in between the alveoli. The liver showed, also increased vascularity with infiltration of blood cells in intralobular ductule and patches of fatty degeneration and necrosis with infiltration of lymphocytes. The spleen showed increase in the cells of white bulb and marked congestion.

Post-mortem examination of the group receiving imidazole 3 mg daily/animal, 15 days after infection, however, revealed normal spleen, but the liver showed 2 layers of subcapsular necrosis and degeneration as well as discrete scattered patches of degeneration of variable degree (Fig. 1). The lungs showed massive consolidation in the middle and increased vascularity of the rest of lung parenchyma, but no apparent tubercles.

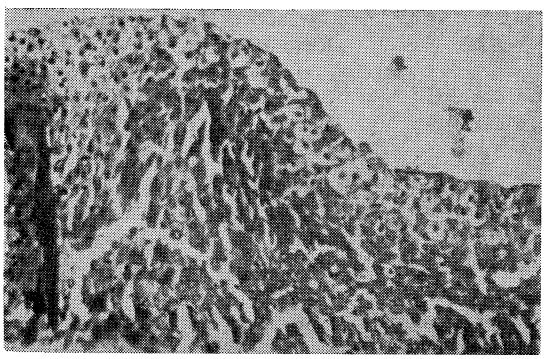

Fig. 1

Animals receiving imidazole 1 mg daily dose/animal immediately after tuberculous innoculation, showed normal spleen, liver, and lung except for some congestion of mild degree of the last two organs. However animals receiving the same 1 mg imidazole dose, 15 days after innoculation, showed normal spleen and liver, but in the lungs there could be seen some remnants of lymphocytes arranged in group-like follicles.

DISCUSSION

In the *in vitro* experiment, imidazole, the parent compound of histidine, seemed to be the most potent tuberculostatic agent. The presence of an aminated side chain in the imidazole derivatives did not lower the potency of such compounds. The deaminated side chains, however, lowered the potency of these derivatives, as shown by imidazole lactic, imidazole acetic acid and urocanic acid.

In the *in vivo* experiment, the infected untreated animals showed the typical picture of tuberculosis. The animals treated with streptomycin+INH showed normal spleen and liver, yet there was exacerbation of the tuberculous process in the lung tissue manifested by the presence of multiple tubercles and consolidation of 2/3 of lung tissue. This exacerbation in the lung was seen, six weeks after stopping the treatment and, this was not found in the untreated tuberculous animals (group I), and the facts indicate the diminished tissue resistance. This exacerbation might probably be similar to the remission which occurs in human tuberculosis whenever the antituberculous treatment by primary drugs is not continued for at least one year. In this group of animals, the traditional antituberculous drugs were able to protect both spleen and liver from the disease without any remission after stopping them, but this was not the case with the lung, which did not gain prolonged immunity and flaring up of pulmonary lesions probably happened during the

6 weeks following the short therapeutic period. This exacerbation had been explained in a previous work to be similar to the remission which occurred in human tuberculosis whenever the antituberculous therapy was not continued for at least one year.

Comparing the action of imidazole, it was found that the highest dose (17.5 mg) was highly toxic and fatal. The microscopic picture of toxicity was clearly evident in all organs examined.

The administration of 3 mg imidazole daily/animal was effectively tuberculostatic, yet it still produced serious toxic lesions in the liver, manifested as degenerative changes in two subcapsular layers of the liver.

When imidazole was given, in 1 mg daily dose/animal, it proved to be safe, since it did not present any toxic manifestations. Its antituberculous activity was also highly potent when given immediately after infection; it did not show any macroscopic or microscopic signs of tuberculosis. When administered after 15 days of infection, the same dose of imidazole, however, was not so effective as when given immediately after infection, yet it was still more effective than the streptomycin+INH treated group. This was clear from the histopathological examination of lung tissue which only presented remnants of lymphocytes trying to represent follicle formation.

The antituberculous effect of comparatively very small doses of imidazole is rather encouraging to apply this compound for human tuberculosis. This will be the aim of future work.

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