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STUDY AND ANALYSIS OF COMBINED DRUG THERAPY TO FIGHT AGAINST MULTI DRUG RESISTANCE TUBERCULOSIS (MTDR-Tb)

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Abstract

On Mycobacterium bacteriological activity has been conducted with metal chelates (complexes) of Pyrazinamide, Isoniazid and Rifampicin the results obtained were compared with that of the parent drug. The study reveals that few metal chelates (complexes) show a remarkable resistance as compared with the parent drug.

***Key Words**: Pyrazinamide, Isoniazid, Rifampicin, Multi Drug Resistance tuberculosis.*

Introduction

It is evident that metal complexes play an important role in biological activity of drugs. Present study was carried out to see the effect of metal complexes of Pyrazinamide, Isoniazid and Rifampicin on MTDR-Tb. It is being shown that in many cases metal complexes were more potent than as compared with the pure drug. The increase in potency is due to binding of a drug with metal ions dressed it up with some special physico-chemical properties helpful in its biological activity such as low redox potential and low dissociation constant, lipid solubility's and electron distribution . Taking in consideration the above in present study metal complexes of Pyrazinamide, Isoniazid and Rifampicin, anti-tubercular drugs (synthesized in the study) have been screened for their activity towards mycobacteria, E-coli and Streptocoli. The metal ions used for the present study and for the preparation of chelates include the essential elements required by the body, they are involved in wide variety of biochemical functions in the body but most act primarily in enzyme system viz. V (II), Zr (IV), Cd(II), La(III), Tl(I).

Experiment

All the chemicals were used of high purity. A.R. grade pure sample of Pyz, Inz, & Rfn were obtained from HOD Laboratories Ratlam India. Pyz, Inz, & Rfn metal complexes were prepared by mixing of Pyz, Inz, & Rfn and the metal salt in 1:1 molar ratio and refluxing the mixture for 41/2 hour over water bath. The solution on concentration gave insoluble complex, which was

filtered washed and dried. (after recrystallisation) in vaccume. The complexes were stored in air tight bottles.

The composition of metal complexes was ascertained by conducting conductometric methods; potentiometric methods and I.R., N.M.R. E.S.R. spectrum of isolated complexes were recorded. Melting point electronic spectra was obtained.

The anti microbial agents are used for therapy of disease depend upon for effectiveness upon their capacity to inhibit the multiplication of or to kill the invading micro-organism under the condition which exist in vivo. This anti-tuberculosis activity was carried out in Microbiology Research Center, Lupin Laboratories, Mandideep, and Bhopal using the standard methods according to approved Laboratory Techniques. The anti-tuberculosis drug and its complexes were screened in vivo for the sensitivity test. The method use was the disc colonies test due to Bauer et al[(8)] Material used for this study are as follows :- Plastic Petri plates

90 mm. Synthesized metal complexes of Pyrazinamide., Middle brook 7H11 Agar Base, Autoclave, Incubator.

Results and Discussion

Pyz, Inz and Rfn anti-tuberculosis agent possess chelating site and forms, stable chelate with many biologically interacting metallic ions Anti-tuberculosis activity of Pyz, Inz and Rfn metal complex is caused by their metal binding properties .The study revealed an effective increase in potency of Pyz, Inz and Rfn when chelated with some metal ions. At an effective concentration 0.01µg/ml of all compounds as well as pure Pyz, Inz and Rfn states that there was a pronounced decreased in the number of colonies as compared to pure Pyz, Inz and Rfn V (II), Zr(IV), Tl(I) Complexes of Pyz, Inz and Rfn inhibited the M-tuberculosis more effectively Whereas Cd(II), La(III) Complex show moderate effect on mycobacterium tuberculosis.

Serial dilution was prepared so to obtain the desired concentration of 0.1 ug/ml, 0.01 ug/ml, and 0.001 ug/ml in autoclaved distilled water. 10.5 grams 7H11 media was dissolved in 200ml of distilled water and stir properly and 2.5ml glycerol was added. The volume was making up to 450ml and media was autoclaved for 15 minutes at 121'C. After autoclaving the media was kept at 65^{o} for two hours and 20ml was poured into each plate under sterile conditions. Bacteria H37RV was added to serially diluted compounds so that the final concentration become 0.1µg, 0.01 µg and 0.001 µg and then plates were spread with the help of a spreader. The observation was obtained after twenty one days. This is tabulated in table-1

Table-1

Physical Properties and Antituberculosis Activity of Pyrazinamide Complex

Temp $= 27^{o}$ Time = 27 days

Concentration = 0.0.1 µg. MIC of Pyz, Inz, Rfn = 0.1 µg

S.No	Complex	Colour	C.F.U. obtained
1.	Pure Pyz	White	26 Colonies
2.	Pure Inz	White	25 Colonies
3.	Pure Rfn	Dark Brown	22 Colonies
4.	Pure V(II)	Light Green	13 Colonies
5.	Pure Zr(IV)	White	14 Colonies
6.	Pure Cd(II)	White	17 Colonies
7.	Pure La(III)	White	19 Colonies
8.	Pure Tl(I)	White	16 Colonies

9.	V(II) + Pyz + Inz	White	10 Colonies
10.	V(II) + Pyz + Rfn	Light Yellow	11 Colonies
11.	V(II) + Inz + Rfn	Dark Brown	07 Colonies
12.	Zr(IV) + Pyz + Inz	White	11 Colonies
13.	Zr(IV) + Pyz + Rfn	Light Brown	09 Colonies
14.	Zr(IV) + Inz + Rfn	Light Brown	08 Colonies
15.	Cd(II) + Pyz + Inz	White	14 Colonies
16.	Cd(II) + Pyz + Rfn	Dark Brown	11 Colonies
17.	Cd(II) + Inz + Rfn	Light Brown	09 Colonies
18.	La(III) + Pyz + Inz	White	14 Colonies
19.	La(III) + Pyz + Rfn	Light Brown	15 Colonies
20.	La(III) + Inz + Rfn	Light Brown	13 Colonies
21.	Tl(I) + Pyz + Inz	White	11 Colonies
22.	Tl(I)+ Pyz + Rfn	Yellow Brown	06 Colonies
23.	Tl(I) + Inz + Rfn	Light Brown	07 Colonies

(Pyz- Pyrazinamide, Inz - Isoniazid, Rfn - Rifampicin CFU=Colony Formation unit)

Higher Antituberculosis activity of certain metal complexes than the original drug may be due to the fact that complexation with metal imparts some important characteristics to the drug, which are helpful in its biological activity e.g. low dissociation constant (strong metal ligand bond) special redox potential, electron distribution and solubility's. It also helps in the natural process of bond formation and bond cleavage and the group transfer reactions.

As a result, the metal complex has increased duration of action and possesses enhanced blood concentration, which may probably be due to a comparatively faster diffusion of the metal chelate and through the organisms due to its more liposoluble (more covalent metal to ligand bond) on being co-ordinated with the metal ion forming stable chelates. The higher biocidal activity of the metal chelate may also due to the combined bioactive effect of the metal and the ligand and the higher concentration of the ligand in the chelate (1:2 M.L)

The anti growth (inhibition) of the bacteria species may be due to the exchange of trace metal of the metaloenzyme with the metal ions of the chelate under test and/or due to steric control of the encumbered and bulky chelate molecule. The results of present study clearly indicate formation of (M: L) 1:2 chelates with involvement of N-atom in metal to ligand - bond resulting in a sufficient high covalent - nature of chelate molecules and hence lipid - solubility. The activity of a drug depends on its bio-availability, which in turn depends, apart from other factors, upon its particle size. It has been shown that reduction in particle size increases activity, it increases the solubility of the drug and hence its bio-availability. In most of the case the complexes having high activity have micro - particle size, which helps their higher solubility.

The mechanism of action suggests that Pyz, Inz and Rfn may be active as a prodrug. Suseptable Organism produce deaminidase, and V^{+2}, Zr^{+4}, Tl^{+1} Complexes of Pyz, Inz and Rfn inhibited the M tuberculosis more effectively Whereas Cd^{+2}, La^{+3} Complex show moderate effect on mycobacterium tuberculosis.

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