ISSN: 2455-1848
INTERNATIONAL JOURNAL OF INNOVATIVE RESEARCH & GROWTH
VOLUME-I ISSUE-III JANUARY-2016
WWW.IJIRG.COM

ANTI MICROBIAL STUDIES OF METAL CHELATES IN MULTI DRUG RESISTANCE TUBERCULOSIS

[1]Sandeep gangrade & [2]Naveen kumar singh
[1, 2] Chemical Laboratory, Malwa Institute of Technology and Management Gwalior, M.P., (India)
E-mail: gangrade.sandeep@rediffmail.com

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

Keywords: *Pyrazinamide (Pyz), Isoniazid (Inz), Rifampicin (Rfn), Multi Drug Resistance Tuberculosis (MTDR-TB), Tuberculosis (TB).*

Introduction

Tuberculosis (TB) is still a challenging worldwide health problem, and mycobacterium tuberculosis remains one of the single most deadly human pathogens. The resurgence of TB over the last 15 years, even in industrialized countries where it was almost eradicated, has been favored by the pathogenic synergy with human immunodeficiency virus infection. In fact, TB and other atypical mycobacterioses are not disease frequently associated with AIDS; human immunodeficiency virus infection significantly increases the risk that new or latent TB infections will progress to active diseases. The emergence of TB has also been accomplished by the appearance of single-drug-resistant (SDR) and multi drug resistant strains of mycobacterium tuberculosis which are insensitive to one or more of the first line anti TB drugs Pyrazinamide (Pyz), Isoniazid (Inz), Rifampicin (Rfn). Indeed, a great amount of work has been done in order to acquire useful knowledge about the mechanism of action of and resistance of available anti tubercular agents. *M.* tuberculosis often becomes drug resistant as a consequence of spontaneous genetic mutations involving the molecular targets of drugs. The primary mechanism of multi drug, resistance in TB is the accumulation of mutations in individual drug target genes. However, such knowledge is not sufficient to rationally overcome drug resistance in mycobacteria. In fact, currently, combinations of two or more anti TB drugs are used to prevent the development of resistant mycobacteria sometimes it is also necessary to resort to second line drugs (ciprofloxacin, ethionamide, kanamycin and amino salicylic acid, etc.) consequently, the present anti-TB regime is rather complex and lengthy. In immuno-suppressed patients, it is also unsatisfactory. All of these serious concerns require particular attention and stimulate the continuing search for new anti mycobacterial agents and therapeutic regimes.

The term Multi Drug Resistance Tuberculosis (MTDR-TB) is used to describe strains that are resistant to two or more of the five first-line anti-TB drugs Isoniazid (Inz) Rifampicin (Rfn), Pyrazinamide (Pyz), Ethambutol (Etb), and Streptomycin (Stm). 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 (Pyz), Isoniazid (Inz), Rifampicin (Rfn), on Multi Drug Resistance Tuberculosis (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 and electron distribution. Taking in consideration the above in present study metal complexes of Pyrazinamide (Pyz), Isoniazid (Inz), Rifampicin (Rfn), anti-tubercular drugs (synthesized in the

study) have been screened for their activity towards mycobacteria, E-Coli and Streptocoli. The metal ions V(II), Zr(IV), Cd(II), La(III), Tl(I). 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 acts primarily in enzyme system.

Experimental

All chemicals used are of high purity; pure samples of Pyrazinamide (Pyz), Isoniazid (Inz), and Rifampicin (Rfn) were obtained from HOD Laboratories Ratlam India. Material used are plastic Petri plates 90 mm., synthesized metal complexes of Pyrazinamide, Isoniazid and Rifampicin, Middle brook 7H11, Agar Base, Autoclave, Incubator. Metal Chelates of Pyrazinamide (Pyz), Isoniazid (Inz), Rifampicin (Rfn) were prepared by mixing of Pyrazinamide (Pyz), Isoniazid (Inz), Rifampicin (Rfn) and the metal salt in 1:1 molar ratio and refluxing the mixture for 5 hour over water bath. The solution on refluxing gave insoluble complex, which was filtered washed and dried after re-crystallization in vacuume. The complexes were stored in airtight bottles. Serial dilutions were prepared so as 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^{o}C. After autoclaving the media was kept at 65^{o}C 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, 0.01µg and 0.001µg and then plates were spread with the help of a spreader.

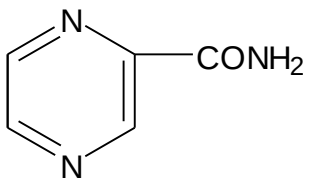


Figure-1 Pyarazinamide

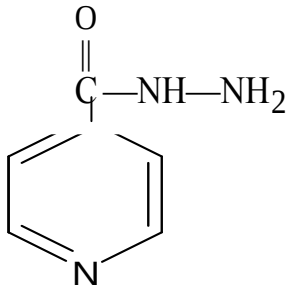


Figure-2 Isoniazid

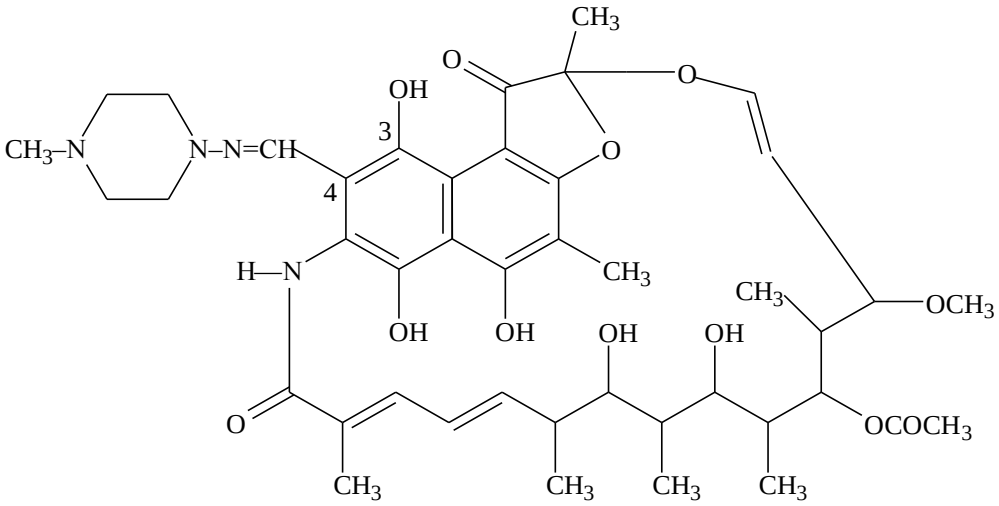


Figure-3 Rifampicin

The composition of metal complexes was ascertained by conducting conductometric methods; potentiometric methods, Melting points were recorded on a Kofler hot-state apparatus and are uncorrected. Spectra of isolate complex for NMR Spectra were recorded on a Varian 300 MHz spectrometer; chemical shifts are given in δ units relative to the internal standard

tetramethylsilane and refer to chloroform-d ($CDCl_3$) or dimethyl-d, sulfoxide solutions. Infrared spectra were recorded as Nujol mulls in the range of 4,000 to 300^{cm-1} on a Perkin-Elmer 683 spectrophotometer.

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 microorganism under the condition which exist in vivo. This anti-tuberculosis activity was carried out in Microbiology Research Center, Lupin Laboratories, Mandideep, Raisen (M.P.) 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 used was the disc colonies test

Results and Discussion

Pyrazinamide (Pyz), Isoniazid (Inz) and Rifampicin (Rfn) anti-tuberculosis agent possess chelating site and forms, stable chelate with many biologically interacting metallic ions anti-tubercular activity of Pyrazinamide (Pyz), Isoniazid (Inz) and Rifampicin (Rfn) metal complex is caused by their metal binding properties. The study revealed an effective increase in potency of Pyrazinamide (Pyz), Isoniazid (Inz) and Rifampicin (Rfn) when chelated with some metal ions. At an effective concentration 0.01µg/ml of all compounds as well as pure Pyrazinamide (Pyz), Isoniazid (Inz) and Rifampicin (Rfn) states that there was a pronounced decrease in the number of colonies as compared to pure Pyrazinamide (Pyz), Isoniazid (Inz) and Rifampicin (Rfn).

V (II), Zr(IV), Tl(I) complexes of Pyrazinamide (Pyz), Isoniazid (Inz) and Rifampicin (Rfn) inhibited the mycobacterium tuberculosis more effectively where as Cd(II), La(III) complex show moderate effect on mycobacterium tuberculosis.

Table-1 Observation after inoculation

S.No	Complex	Colour	M.P.	C.F.U. obtained
1.	Pure Pyz	White	180 ^oC	26 Colonies
2.	Pure Inz	White	170 ^oC	25 Colonies
3.	Pure Rfn	Dark Brown	190 ^oC	22 Colonies
4.	Pure V(II)	Light Green	170 ^oC	13 Colonies
5.	Pure Zr(IV)	White	250 ^oC	14 Colonies
6.	Pure Cd(II)	White	210 ^oC	17 Colonies
7.	Pure La(III)	White	200 ^oC	19 Colonies
8.	Pure Tl(I)	White	230 ^oC	16 Colonies
9.	V(II) + Pyz + Inz	White	250 ^oC	10 Colonies
10.	V(II)+ Pyz + Rfn	Light Yellow	260 ^oC	11 Colonies
11.	V(II) + Inz + Rfn	Dark Brown	280 ^oC	07 Colonies
12.	Zr(IV)+ Pyz + Inz	White	180 ^oC	11 Colonies
13.	Zr(IV)+ Pyz + Rfn	Light Brown	200 ^oC	09 Colonies
14.	Zr(IV)+ Inz + Rfn	Light Brown	230 ^oC	08 Colonies
15.	Cd(II)+ Pyz + Inz	White	190 ^oC	14 Colonies
16.	Cd(II)+ Pyz + Rfn	Dark Brown	180 ^oC	11 Colonies
17.	Cd(II)+ Inz + Rfn	Light Brown	160 ^oC	09 Colonies
18.	La(III)+ Pyz + Inz	White	190 ^oC	14 Colonies
19.	La(III)+ Pyz + Rfn	Light Brown	180 ^oC	15 Colonies
20.	La(III)+ Inz + Rfn	Light Brown	210 ^oC	13 Colonies
21.	Tl(I) + Pyz + Inz	White	130 ^oC	11 Colonies
22.	Tl(I) + Pyz + Rfn	Yellow Brown	150 ^oC	06 Colonies
23.	Tl(I) + Inz + Rfn	Light Brown	160 ^oC	07 Colonies

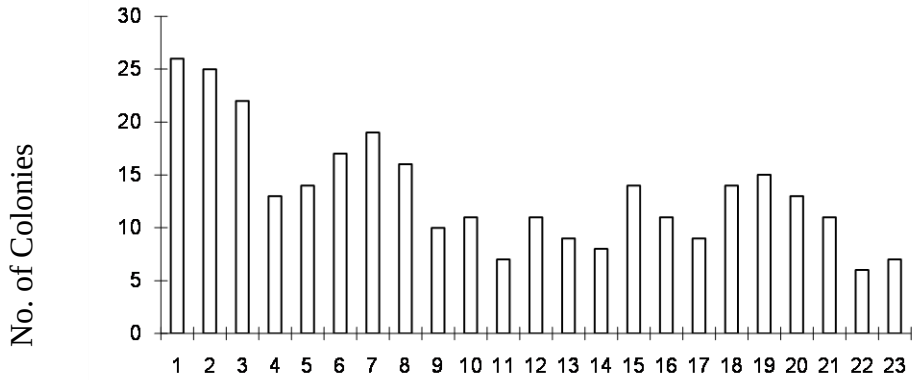
Abbreviations V(II) Vanadium, Zr(IV) Zirconium, Cd(II) Cadmium, La(III) Lanthanum, Tl(I) Thallium, Pyz- Pyrazinamide, Inz - Isoniazid, Rfn - Rifampicin CFU=Colony Formation unit)

Temp -27^o Time=21 days, Concentration= 0.0.1 µg. MIC of Pyz, Inz, Rfn= 0.1 µg

Table-2 Sensitivity Test

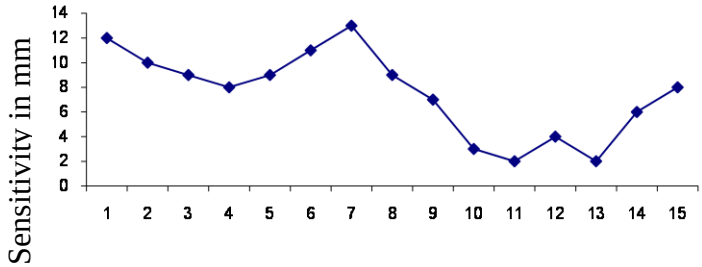
S.No	Mixed Ligand Complex	Standard Stain Staphylococci	Strain Isolated From Patients
1.	V(II) + Pyz + Inz	0 mm	12 mm
2.	V(II) + Pyz + Rfn	0 mm	10 mm
3.	V(II) + Inz + Rfn	0 mm	09 mm
4.	Zr(IV) + Pyz + Inz	0 mm	08 mm
5.	Zr(IV) + Pyz + Rfn	0 mm	09 mm
6.	Zr(IV) + Inz + Rfn	0 mm	11 mm
7.	Cd(II) + Pyz + Inz	0 mm	13 mm
8.	Cd(II) + Pyz + Rfn	0 mm	09 mm
9.	Cd(II) + Inz + Rfn	0 mm	07 mm
10.	La(III) + Pyz + Inz	0 mm	03 mm
11.	La(III) + Pyz + Rfn	0 mm	02 mm
12.	La(III) + Inz + Rfn	0 mm	04 mm
13.	Tl(I) + Pyz + Inz	0 mm	02 mm
14.	Tl(I)+ Pyz + Rfn	0 mm	06 mm
15.	Tl(I) + Inz + Rfn	0 mm	08 mm

Abbreviations V(II) Vanadium, Zr(IV) Zirconium, Cd(II)Cadmium, La(III) Lanthanum, Tl(I) Thallium, Pyz- Pyrazinamide, Inz - Isoniazid, Rfn - Rifampicin



Metal Legend Complex

Figure-4 Anti-tuberculosis Activity of Complex



Metal Legend Complex

Figure-5 Sensitivity test of mixed legend complexes

Higher Anti-tuberculosis activity of certain metal complexes than the original drug is 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 legend 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 possess 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 legend bond) on being coordinated 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 legend 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 metalo-enzyme 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 legend bond resulting in a sufficient high covalent nature of chelate molecules and hence lipid solubility. The activity of a drug depends on its bioavailability, 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 bioavailability. It is evident to note that the complexes having micro particle size leads to higher solubility and activity.

The mechanism of action suggests that Pyrazinamide (Pyz), Isoniazid (Inz) and Rifampicin (Rfn) are active as a prodrug. Susceptible organism produce deaminidase, and V(II), Zr(IV), Tl(I) complexes of Pyrazinamide (Pyz), Isoniazid (Inz) and Rifampicin (Rfn) inhibited the mycobacterium tuberculosis more effectively whereas Cd(II), La(III) complex show moderate effect on mycobacterium tuberculosis. The study clearly reveals that the mixed legend metal chelates are more active to suppress the growth of mycobacterium, and also being very helpful to suppress resistance in multi drug resistance tuberculosis.

Acknowledgement

Authors thanks to Chemistry Department for providing all laboratory facilities. Authors are extremely grateful to HOD Laboratories, Ratlam (M.P.) for providing pure samples of drug and to Lupin Laboratory Mandideep, Dist. Raisen (M.P.) for performing the work of biocidal activity.

References

1. Cohn DL, Flavia B, Raviglione MC. Clin Infect Dis., **24,** 1997, S21-30.
2. Mitchison DA. International Union Against Tuberculosis **65.** 1985, 307.
3. H. Sigel. "Metal Ions in Biological system". Marcel Dekker, New York, Vol. 1,2, 1973.
4. A. Santos. M. Gil. S. Marques. L. Gano and S. Chaves, John Libbey Eurotext, Vol. 7. 2002, 665.
5. J.J. Bodwin, A.D. Gutland, and R. Malkani, Coor. Chem. Rev. **216,** 2001, 489.
6. Iseman MD, Madsen LA. Drug-resistant tuberculosis. Clin Chest Med **153,** 1998, 341.

7 Fox, W. Int. Union of Tuberculosis. **60,** 1985, 40–49.

8. Hall, S. A., and P. E. Spoerri. J. Am. Chem. Soc. **62,** 1940, 664-665.
9. Heifets, L. B., M. A. Flory, and P. J. Lindholm-Levy. Antimicrob. Agents Chemother. **33,** 1989,1252–1254.

10 Florence, A.T. & Atthood Physieo *"Chemical Principles of pharmacy"* Mc. Millan. London **40,** 1986, 280.

11 Heifets, L.B. et al Antimicrobial agent chemotherapy, **33,** 1989,1252,

12. Mukherjee. S.L. Nahi, J., Rahymahasya, S., Lashker, S.L. and Gupta R.P. (1955) J. Pharma. Pharmacol, **7,** 1955, 36.
13. Weinberg, E.D. Bacterial Revs. **21,** 1996, 46.
14. H. Ogino. K. Tsukahara and N. Tanka, Inorg. Chem., **16,** 1977, 1215.
15. Smith E.L., Proc. Natr Acad. Sci. U.S., **35,** 1949, 80.
16. G.L. Eichhorn, H. Sigel and A. Sigel, Marcel Dekkar, *"Metal Ions in Biological System's"* New York, **25**, 1989, 81-90.
17. Goodman and Gilman *"The Pharmacological Basis of Therapeutics"* Mc. Millan N.Y. **79(5)**, 466, 1985

18. P.B. Chakrawrti, J. Indian Chem. Soc. **78,** 2001, 273.
19. A.E. Martell and M. Calvin, *"Chemistry of The Metal Chelate Compounds"*, (Chapter-(II) and III) Prentice Hall, 1959.
20. A. Jacques, *"Complex Ion in Aqueous Solutions,"* Longmans, Green and Co. Ltd., London, 1960.
21. J. Lewis and R.G. Nilkins, *"Modern Co-ordination Chemistry"*, Inter Science Publishers Inc. N.Y. 1960.
22. J.C. Bailar, "The Chemistry of The Co-ordination Compounds", Reinhold Publishing Corporation N.Y. 1956.
23. C. Lamanna and M.F. Mallette, *"Basic Bacteriology Its Biological and Chemical Background"*, William and Wilkins, Baltimore, Maryland, 1959, 853.
24. A.T. Florence and D. Atwood, *"Physical Principle of Pharmacy"*, Mac. Millan London, 1981.
25. R.J. Dubos, *"The Effect of Lipid and Serum Albumin on Bacterial Growth"*, J, Exotl. Mad., **85,** 1947, 9.
26 H. Ogino, K. Tsukahara and N. Tanka, Inorg. Chem.m **16.** 1977, 1215.
27. H. Gampp. M. Meader, C. Meyer and A.D. Zuberbuhler, Talanta, **32.** 1982, 1133.