Available online at http://www.ijcrls.com

International Journal of Current Research in Life Sciences
Vol. 07, No. 04, pp.1885-1887, April, 2018

ISSN: 2319-9490

RESEARCH ARTICLE

IN VITRO EFFECTS OF ROSUVASTATIN ON MYCOBACTERIUM TUBERCULOSIS

[1,2]Pratik Kumar, [1,3]Maneesh Kumar, [1]Harish Kumar and [1,*]Jainendra Kumar

[1]Department of Biotechnology, College of Commerce, Arts & Science, (Magadh University, Bodh Gaya), Patna, India
[2]Intermediate Reference Laboratory, TBDC building, Patna, India
[3]Department of Virology, Rajendra Memorial Research Institute of Medical Sciences (ICMR), Agamkuan, Patna-800007, Bihar, India

Received 28th February, 2018; Accepted 02nd March, 2018; Published 30th April, 2018

ABSTRACT

Discovery of new anti TB drugs is the necessity of prime importance to control the global epidemic of Tuberculosis (TB). To ensure the sensitivity of the drug over a range of different strains of *Mycobacterium tuberculosis* (M.TB), new targets are required. Rosuvastatin has been studied to inhibit the key MEP pathway enzyme during *in silico* investigations. We have treated M.TB culture with different concentrations of rosuvastatin *in vitro.* We observed delay in growth of cultures with 100 µg/ml and 200 µg/ml concentration of rosuvastatin as compared with controls and cultures with lower rosuvastatin concentrations. Based on these results, we can assume that rosuvastatin has some growth retarding effects on M.TB and treatment with higher concentrations may restrict the M.TB growth.

Key words: Tuberculosis, MEP pathway, Rosuvastatin.

Citation: Pratik Kumar, Maneesh Kumar, Harish Kumar and Jainendra Kumar, 2018. "In vitro effects of rosuvastatin on mycobacterium tuberculosis " *International Journal of Current Research in Life Sciences*, 7, (04), 1885-1887.

INTRODUCTION

A group of mycobacterial species called as Mycobacterium Tuberculosis Complex (MTBC) is the agent of disease Tuberculosis (TB) (Huard *et al.,* 2006). Drug resistant forms of TB are the biggest challenge for the TB control programme (Prasad, 2010). Streptomycin was the first antibiotic discovered to have anti TB effects. After that, other anti TB drugs were discovered and to deal with the slow growing bacilli with a thick mycolic acid covering, a combination therapy with four key anti TB drugs was designed (Zumla *et al.,* 2013; Kumar *et al*., 2017). The combination therapy with four drugs (rifampicin, isoniazid, pyrazinamide and ethambutol) was implemented in India under DOTS (Directly Observed Treatment short course) strategy (*Treatment of tuberculosis: guidelines,* WHO 2010). Inappropriate utilization of anti TB drugs, incomplete treatment course and usage of poor quality drugs was observed to be responsible for the failure of DOTS strategy and ultimately emergence of drug resistant TB (Kumar 2017). We need more effective drugs like rifampicin and isoniazid. At present, we are facing huge burden of Multi drug resistant (MDR) TB cases in India (Global Tuberculosis Report, 2017). After MDR and XDR (Extensively drug resistant) cases, progression of TDR (Totally drug resistant) TB has been observed in India (Udwadia, 2012).

***Corresponding author:* Jainendra Kumar**
[1]Department of Biotechnology, College of Commerce, Arts & Science, (Magadh University, Bodh Gaya), Patna, India.

At present, many of the antibiotics share a common target and thus resistant in one drug confers different degrees of cross resistance with others. For example, levofloxacin and ofloxacin share a high degree of cross resistance as both drugs target the bacterial DNA gyrase. So, priority is being given to search new targets to avoid any risk of cross resistance or pre existing mutation. Isoprenoid biosynthesis pathway is essential for survival of bacteria (Singh *et al.,* 2007) and enzymes of this pathway do not share homology with human. So, this pathway can be considered as a new target. Isoprenoids show high structural diversity (Sacchettini and Poulter, 1997) and they are basically polymer of isoprene units. The precursors are isopentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP). M.TB synthesizes these precursors through Methylerythritol phosphate (MEP) pathway which is different from the Mevalonate pathway of humans. Several studies have been done to find out suitable targets to inhibit MEP pathway in M.TB. Based on *in silico* investigations, Kumar *et al*., (2017) proposed that rosuvastatin would bind and inhibit the binding of substrate (CTP) to the key enzyme of MEP pathway. Rosuvastatin competitively inhibits the HMG CoA reductase, a critical enzyme of Mevalonate pathway for isoprenoids production (White, 2002).
In the present study, we are performing the *in vitro* effects of rosuvastatin on M.TB growth.

MATERIALS AND METHODS

All the laboratory work was carried out at Intermediate Reference Laboratory, Patna under Biosafety level-3 facility.

Fresh culture of H37Rv strain of M.TB was taken for the study. Susceptibility testing was done on liquid media (modified middlebrook 7H9 broth) containing MGIT (Mycobacteria Growth Indicator Tube) and positive culture not older than 5 days was taken for testing (Siddiqi, 2006). Rosuvastatin was purchased from Sigma and Dimetyhyl sulphoxide (DMSO) was used as solvent. Concentrations were prepared by using the formula mentioned below.

$$A = \frac{V \times C \times D}{P}$$

Where, "A" is the amount to be taken in mg,
"V" is the volume to be prepared in ml,
"C" is the desired concentration of compound in µg/ml,
"D" is the dilution factor and
"P" is the potency of compound in µg/mg.

10 mg of rosuvastatin in 1 ml of DMSO taking 50 as dilution factor and 1000 µg/mg potency. Further concentrations were prepared by serial dilution. Finally, concentrations of 100 µg/ml, 50 µg/ml, 20 µg/ml, 10 µg/ml, 5 µg/ml and 2 µg/ml along with the mother concentration (200 µg/ml) were prepared. Control tubes were taken; one was direct and second with 1:100 dilution to study the growth in terms of proportionate method. Finally, both batches were loaded inside the MGIT 960 instrument and daily inventory were taken for growth analysis.

RESULTS AND DISCUSSION

Table 1 represents growth units observed in daily inventory for different MGIT tubes. We observed initial growths in control tubes and lower growth units in tubes with higher rosuvastatin concentration.

Table 1

S.No	Lab No.	Type	Day 1	Day 2	Day 3	Day 4	Final ZN result	BHI result
1	4616	GC	0	0	433	1505	Positive	Sterile
2	4616	GC 1:100	0	0	0	280	Positive	Sterile
3	4616	200 µg/ml	0	0	0	480	Positive	Sterile
4	4616	100 µg/ml	0	0	0	510	Positive	Sterile
5	4616	50 µg/ml	0	0	230	1299	Positive	Sterile
6	4616	20 µg/ml	0	0	281	1458	Positive	Sterile
7	4616	10 µg/ml	0	0	380	1510	Positive	Sterile
8	4616	5 µg/ml	0	0	305	1499	Positive	Sterile
9	4616	2 µg/ml	0	0	280	1015	Positive	Sterile
10	4996	GC	0	0	338	1016	Positive	Sterile
11	4996	GC 1:100	0	0	0	301	Positive	Sterile
12	4996	200 µg/ml	0	0	0	255	Positive	Sterile
13	4996	100 µg/ml	0	0	388	1285	Positive	Sterile
14	4996	50 µg/ml	0	0	505	1505	Positive	Sterile
15	4996	20 µg/ml	0	0	400	1588	Positive	Sterile
16	4996	10 µg/ml	0	0	510	1800	Positive	Sterile
17	4996	5 µg/ml	0	0	550	1936	Positive	Sterile
18	4996	2 µg/ml	0	0	530	1988	Positive	Sterile

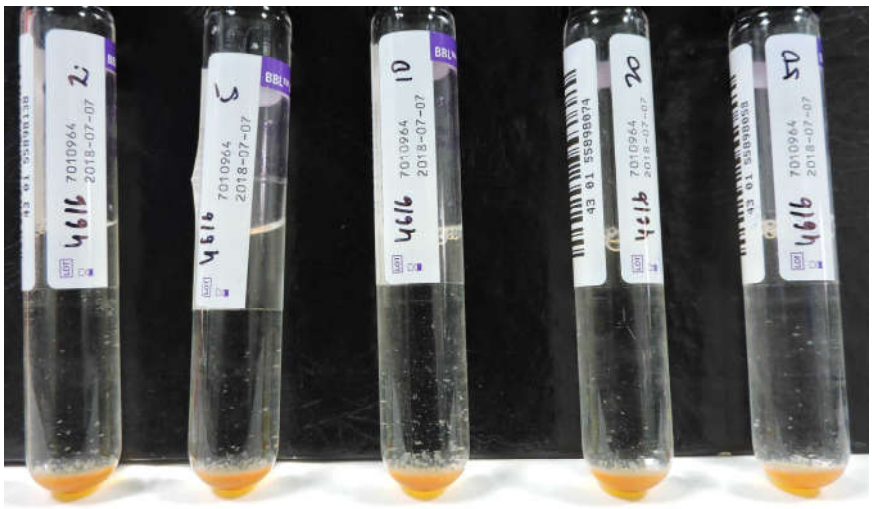

Figure 1

Two batches of culture were tested for compound susceptibility. For susceptibility testing, 4 ml of liquid media was taken in MGIT and 500 µl of MGIT OADC supplement was added into the media. Further, 100 µl of dissolved compound added and finally 400 µl of fresh culture was inoculated into each tube. Each batch contains different tubes with different concentrations along with control tubes. Two We prepared maximum concentration of 200 µg/ml by adding

Figure 1-3 represents growth in different tubes. Visual inspection supports the pattern of growth units and growth in control and lower concentrations was relatively rapid and more than that of tubes with higher concentration. The growth in 1:100 diluted control tubes was more or less similar with the growth in tubes with higher concentration of rosuvastatin. Growth unit started on 4th day for both 1:100 diluted controls and tubes with 100 & 200 µg/ml concentrations.

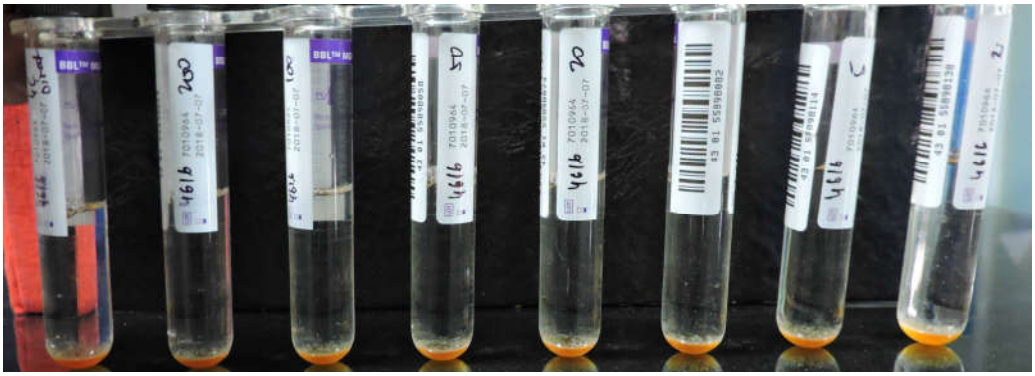

Figure 2

Figure 3

This clearly represents that rosuvastatin has some growth retarding effects on M.TB growth. India has committed to end TB by 2025 (Kumar, 2017). The country needs more stringent strategies and more focus on research is needed (Pai *et al*., 2017). We are in urgent need of new anti TB drugs to treat the existing drug resistant cases of TB. We observed inhibitory effects of rosuvastatin over growth of M.TB in liquid media. However, more studies with higher concentrations and with other statin compounds can add to our observations.

Acknowledgement

We are thankful to The Director, IRL, Patna for his support. We also thank the Laboratory team of IRL, Patna for their help and support.

REFERENCES

Huard, RC., Fabre, M., deHaas, P., Oliveira Lazzarini, LC., van Soolingen, D. and Caosin, D. 2006. Novel genetic polymorphisms that further delineate the phylogeny of the *Mycobacterium tuberculosis* complex. *J Bacteriol.,* 188: 4271-87.

Kumar, M., Kumar, P., Kumari, P. and Kumar, J. 2017. Inhibition of ISPD enzyme to control the growth of *Mycobacterium tuberculosis*, *International Journal of Advanced Researc*h, 5(5), 1309-1313.

Kumar, P. and Dewanjee, S. 2017. Evolution of Rapid TB Diagnostics technique and its potential impact on Tuberculosis epidemics in India, *Journal of Scientific and Engineering Research,* 4(2):35-40.

Kumar, S. 2017. Winners and losers in India's science budget. *Science*, 3.

Kumar, P., Goswami P., Kumari, R., Kumar, M. and Kumar, S. 2017. Comparative Study of LED Fluorescent Microscopy with the Conventional ZN based Microscopy for diagnosis of Microbiologically confirmed TB cases. *International Journal of Advanced Research,* 5 (6): 1083-1086.

MGIT Procedure Manual by Salman H. Siddiqi and Sabine Rüsch-Gerdes, 2006.

Pai, M., Bhaumik, S. and Bhuyan, S.S. 2017. India's plan to eliminate tuberculosis by 2025: converting rhetoric into reality.

Prasad, R. 2010. Multidrug and extensively drug-resistant TB (M/XDR-TB): problems and solutions. *The Indian journal of tuberculosis*, 2010. 57(4): p. 180-191.

Sacchettini, J.C. and Poulter, C.D. 1997. Creating isoprenoid diversity. *Science,* 277 (5333), 1788-9.

Singh, N., Chevé, G., Avery, M.A. and McCurdy, C.R., 2007. Targeting the methyl erythritol phosphate (MEP) pathway for novel antimalarial, antibacterial and herbicidal drug discovery: inhibition of 1-deoxy-D-xylulose-5-phosphate reductoisomerase (DXR) enzyme. *Current pharmaceutical design*, 13(11): 1161-1177.

Udwadia, Z.F. 2012. MDR, XDR, TDR tuberculosis: ominous progression.

White, C.M. A review of the pharmacologic and pharmacokinetic aspects of rosuvastatin. *The Journal of Clinical Pharmacology*, 200, 42(9):963-970.

World Health Organization and Stop TB Initiative (World Health Organization), *Treatment of tuberculosis: guidelines*. World Health Organization 2010.

World Health Organization, Global Tuberculosis Report, 2017.

Zumla, A., Nahid, P. and Cole, S. T. 2013. Advances in the development of new tuberculosis drugs and treatment regimens. *Nat. Rev. Drug Discov.*, 12, 388–404.

****