British Journal of Pharmaceutical Research
6(4): 278-283, 2015, Article no.BJPR.2015.069
ISSN: 2231-2919

SCIENCEDOMAIN *international*
www.sciencedomain.org

Effect of Ascorbic Acid on Co-trimoxazole Induced Hyperbilirubinemia in Rats

Nabin Wagle[1*], Hari Prasad Sapkota[1], Nim Bahadur Dangi[1], B. Sreedhar Naik[1], Y. Balakrishna[1], S. Nagarjuna[1] and Y. Padmanabha Reddy[2]

[1]Division of Pharmacology, RIPER, Saigram Krishnamreddy Palli Cross, Chiyyedu, P.O. Anantapuramu-5155721 (A.P.), India.
[2]Division of Analysis, RIPER, Saigram Krishnamreddy Palli Cross, Chiyyedu, P.O. Anantapuramu-5155721 (A.P.), India.

Authors' contributions

This work was carried out in collaboration between all authors. Preparation of manuscript and first submission was done by author NW, designed of study was done by author NBD and HPS protocol writing, literature searches, analyses of the study was performed in collaboration of all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJPR/2015/14719
Editor(s):
(1) Faiyaz Shakeel, King Saud University, Riyadh, Saudi Arabia.
(2) Vasudevan Mani, Universiti Teknologi MARA (UiTM), Selangor, Malaysia.
(3) Selwyn Hurwitz, Laboratory of Biochemical Pharmacology, Emory University, School of Medicine, Department of Pediatrics, USA.
(4) Ke-He Ruan, Director of the Center for Experimental Therapeutics and Pharmacoinformatics (CETP), Faculty of Medicinal Chemistry and Pharmacology, Department of Pharmacological and Pharmaceutical Sciences, University of Houston, USA.
Reviewers:
(1) Anonymous, India.
(2) Anonymous, Taiwan.
(3) Chung-Ming Chen, Department of Pediatrics, Taipei Medical University, Taiwan.
(4) Tarek M. Heikal, Pesticide Chemistry Department, National Research Centre, Egypt.
(5) Anonymous, USA.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=985&id=14&aid=8620

Original Research Article

Received 16th October 2014
Accepted 4th March 2015
Published 30th March 2015

ABSTRACT

Aims: To evaluate the effect of ascorbic acid on co-trimoxazole induced hyperbilirubinemia.
Place and Duration of Study: Laboratory of Raghavendra Institute of Pharmaceutical Education & Research (RIPER), between July 8, 2014 to July 23, 2014.
Methodology: Rats were divided into three groups of six each, Control group were treated with vehicle, Negative control group were treated with co-trimoxazole (36 mg/kg of body weight) and Test group were treated with ascorbic acid (500 mg/kg) and co-trimoxazole (36 mg/kg of body

**Corresponding author: Email: nabinwagle2001@gmail.com;*

weight). All the groups were treated once daily for a period of 15 days. After one hour of oral administration, blood were collected by retro-orbital method on 1st, 5th, 10th, and 15th days and bilirubin levels were estimated by using semi-auto analyser.

Results: Animals treated with co-trimoxazole have none significantly increased on bilirubin level at day 1st but bilirubin levels were significantly increased on 5th and 15th days of test (*P*<.001) when compared to normal group.

However, Animals treated with ascorbic acid + co-trimoxazole have none significantly decreased on bilirubin level on day 1st but from day 5th today 15th bilirubin levels were significantly decreased (*P*<.001), when compared to negative control group.

Conclusion: On administration of ascorbic acid to rats, the hyperbilirubinemia which was induced by Co-trimoxazole decreases. So, the combination therapy of co-trimoxazole and ascorbic acid has the beneficial effect over hyperbilirubinemia caused by co-trimoxazole.

Keywords: Ascorbic acid; co-trimoxazole; hyperbilirubinemia; albumin; hepatic mRNA.

1. INTRODUCTION

Ascorbic acid is a natural, water soluble, slightly acidic vitamin. As it was found that it is used as a vitamin C in animal, then it was renamed as ascorbic acid from L-hexuronic acid [1]. It is an essential nutrient found mainly in fruits and vegetables. It support for maintenance of bones, blood vessels and skins and promotes the healing of cuts, abrasion and wounds. It appears to lessen the risks of developing high blood pressure, heart disease, and help to regulate the cholesterol level [2]. Generally, ascorbic acid is required for collagen formation and tissue repair by acting as a cofactor in the post translational formation of 4-hydroxy proline in Xaa-Pro-Gly sequences in collagens and other proteins. Ascorbic acid is reversibly oxidised to dehydroascorbic acid in the body. These two forms of vitamins are believed to have importance on oxidation-reduction reactions [3]. Ascorbicacid also suppresses the elevation of hepatic mRNA level of hem oxigenase-1, the rate limiting enzyme of bilirubin biosynthesis [4].

Co-trimoxazole or trimethoprim (1): sulfamethoxazole (5) is an antibiotic used in the treatment of variety of bacterial, fungal, protozoal infections. Co-trimoxazole is generally considered bactericidal, although its components are individually bacteriostatic [5]. Its actions are antifolate in nature, inhibiting both De Novo folate biosynthesis and metabolism [6]. Sulfamethoxazole inhibits bacterial synthesis of dihydrofolic acid by competing with PABA (ParáAmino Benzoic Acid) and trimethoprim blocks production of tetrahydrofolic acid by inhibiting the enzyme dihydrofolate reductase. Which blocks two consecutive steps in bacterial biosynthesis of the essential nucleic acid and proteins [7]. Sulfonamide therapy in children lead to hepatic damage during treatment and a liver function test shows the alteration of plasma globulin [8]. Sulfonamide also decreases the activity of glucuronyl transferase in the liver. Thus, it increases the unconjugated bilirubin. Where unconjugated bilirubin causes the nerve cell necrosis [9].

Co-trimoxazole was claimed to be more effective than either of its components individually in treating bacterial infections, although this was later disputed. Its use has been restricted in many countries to very specific circumstances where its improved efficacy has been demonstrated [10]. The global problem of advancing antimicrobial resistance has led to a renewed interest in the use of co-trimoxazole more recently [11].

Sulfamethoxazole displaces bilirubin from binding site on serum albumin. The bilirubin is then free in systemic circulation which leads to hyperbilirubinema.

Here,

Plasma protein ⟶ Albumin
Displacement drug ⟶ Sulfamethoxazole
Displaced compound ⟶ Bilirubin
Outcome ⟶ Hyperbilirubinemia

Bilirubin is the yellow breakdown product of normal hems catabolism. Heme is found in haemoglobin, principal component of RBC. Excreted in bile as urine and elevated levels may indicate certain diseases mainly the yellow discoloration in jaundice. Bilirubin consists of an open chain of four pyrrole-like rings which are connected into a larger ring, called a prophyrin ring. Bilirubin conjugated with glucuronic acid which makes it water soluble. It has capacity to capture the light energy.

Biliverdin react with biliverdin reductase gives bilirubin on oxidation gives again biliverdine, which have the physiological role as cellular oxidation [12].

Bilirubin level in rat; Total bilirubin- 0.2-0.55 mg/dl.

2. MATERIALS AND METHODS

2.1 Drugs

Co-trimoxazole was purchased from the retail pharmacy (B.N. SEPMAX, GSK, and Batch No. N579), ascorbic acid (L-Ascorbic acid, Fisher scientific, product No.16544, Lot No.46716810-1) and distilled water were received from the Lab house of RIPER.

2.2 Animals

Above six-week old albino rats (body weight 200-250 gm) were obtained from central animal house of RIPER and maintained under standard laboratory conditions (room temperature 24ºc±2ºc, relative humidity 50-55%, 12/12 hours light/dark cycle) with free access to a commercial rodent diet and tap water.

2.3 Ethical Approval

The Institutional Animal Ethics Committee (878/ac/05/CPCSEA/014/2013) has approved the experimental protocol at post graduate department of pharmacology, Raghavendra Institute of Pharmaceutical Education & Research (RIPER), K.R.palli cross, Chiyyedu (post), Anantapuramu, Andhra Pradesh, India – 515721.

2.4 Instruments

Semi-auto analyser- ERBA, CHEM-7

2.5 Preparation of Drug Solution

a) Weigh 162.49 mg of co-trimoxazole powder and dissolve in 15 ml of distilled water, which gives 10 mg/ml concentration.
b) Weigh 1000 mg of ascorbic acid and dissolve in 10 ml of distilled water, which gives 100 mg/ml concentration.

2.6 Experimental Design

Rats were divided into three groups of six each and the groups were as follows

Group I- Control group (No treatment)
Group II- Negative control group treated with co-trimoxazole (36 mg/kg of body weight)
Group III- Test group treated with ascorbic acid (500 mg/kg) and co-trimoxazole (36 mg/kg of body weight)

Animals were marked as head, body, tail and kept in different cages each having 3 animals for their identification.

All the above groups except group I were treated once daily for the15 days.

After one hour of oral administration of drug(s) blood was collected by retro-orbital method on day 1^{st}, 5^{th}, 10^{th}, and day 15^{th} and bilirubin levels was estimated by semi-auto analyser respectively.

2.7 Statistical Analysis

The results were expressed as Mean ± SEM. The differences were compare using One Way Analysis of Variance (ANOVA) and subsequently followed by Tukey's multiple comparison test using Graph Pad Prism-6 software.

3. RESULTS

Normal group was treated with vehicle i.e. distilled water because in both drug solutions we had used distilled water as vehicle for the 15 days. For negative control groups, Co-trimoxazole was administered for same period and the level of total bilirubin was estimated on day 1^{st}, day 5^{th}, day 10^{th}, and day 15^{th}.Similarly for test group, Ascorbic acid and co-trimoxazole was administered for the period of 15 days and the level of total bilirubin was estimated on day 1^{st}, day 5^{th}, day 10^{th}, and day 15^{th}.

The average value of each groups on respective day was estimated by statistical analysis and the data regarding those groups were given in Table 1.

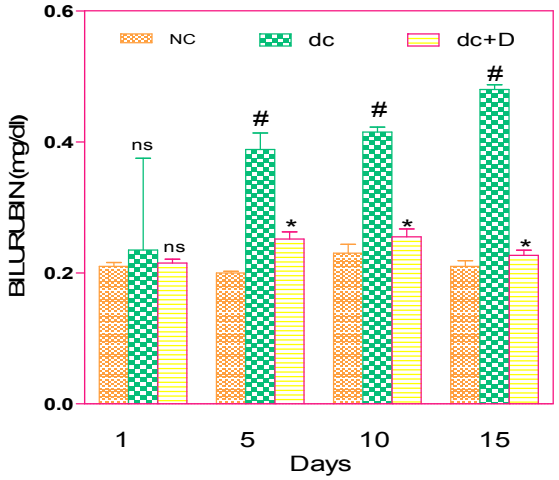
The average value of bilirubin levels found in normal, co-trimoxazole and ascorbic acid + co-trimoxazole groups on day 1^{st}, day 5^{th}, day 10^{th}, and day 15^{th} were plotted as Fig. 1.

4. DISCUSSION

Hyperbilirubinemia is a common abnormality among patients with notable liver or biliary

disease. It may also be observed in patients with systemic illnesses, such as sepsis and cardiogenic shock. The frequencies of the liver and biliary diseases that causes hyperbilirubinemia are described for each specific disease. Most of the drug are metabolised in liver. Normally on hepatic dysfunction, there is increased in bilirubin level. In case of developing countries jaundice is the most common disease mainly symptomized by hyperbilirubinemia as well as on using various drugs, there is a chance of increase in bilirubin level as side effect.

Thousands of biologically active phytochemicals have been identified in plant foods, e.g., grains, nuts, legumes, vegetables, and fruit. Of these plant food groups, vegetables and fruit are the most botanically diverse [13]. The removal of F&V from individual's habitual diets and when they started the basal diet period may have contributed to the bilirubin increase. Li et al. [14] reported that incubation of rat hepatocytes with bilirubin (within the physiological range of serum bilirubin levels in rats) increased UGT1A1 mRNA expression, suggesting that bilirubin modulates its own metabolism. This may explain in part the reversion to baseline bilirubin concentrations from d 8 to d 15 of the basal diet [14].



NC- normal control
dc- disease control
dc+D- disease control+drug (ascorbic acid)

Fig. 1. Bilirubin profile of normal, co-trimoxazole and ascorbic acid + co-trimoxazole groups
ns– Non significant; # - P < .001 when compared to normal group; - P< .001 when compared to co-trimoxazole treated group*

Table 1. Comparison of bilirubin levels in normal, negative control and test groups

S. no	Group treated	Bilirubin level (mg/dl)			
		Day 1	Day 5	Day 10	Day 15
1	Normal group	0.2100±0.0057	0.2000±0.0025	0.2300±0.0135	0.2100±0.0085
2	Co-trimoxazole (36 mg/kg-PO)	0.2350±0.014^{ns}	0.3883±0.0254$^{\#}$	0.4150±0.0076$^{\#}$	0.4800±0.0073$^{\#}$
3	Ascorbic acid (500 mg/kg-PO) + Co-trimoxazole (36 mg/kg-PO)	0.2150±0.0067^{ns}	0.2517±0.0110*	0.2550±0.0120*	0.2267±0.0080*

All values are expressed as Mean ± SEM
ns - Non significant; # - P <.001 when compared to normal group; - P<.001 when compared to co-trimoxazole treated group*
Animals treated with co-trimoxazole has none significantly increased on bilirubin level at day 1st but from day 5th today 15th bilirubin levels were significantly increased, when compared to normal group.
Animals treated with ascorbic acid + co-trimoxazole has none significantly decreased on bilirubin level on day 1st but from day 5thtoday 15th bilirubin levels was significantly decreased, when compared to negative control group

Ascorbic acid is a common diet, which have the positive role against hyperbilurubin. On administration of ascorbic acid we can minimise the side effect i.e. hyperbilirubinemia of many drugs by reducing the level of bilirubin. On metabolism of some drug leads to the elevation of hepatic mRNA level of heme oxygenase-1, the rate-limiting enzyme of bilirubin biosynthesis. Due to excessive elimination of the heme oxygenase-1 the bilirubin level increased beyond the limit. Where, ascorbic acid supresses the elevation of hepatic mRNA level of heme oxygenase-1, due to which there is inhibition of excessive production of the bilirubin [3,4].

Co-trimoxazole is a broad spectrum antibiotic with a side effect as hyperbilirubinemia on albino rats, which was decreased by given ascorbic acid. It was studied for 15 days on which we found increased bilirubin level on negative control group gradually from day 1 (0.2350±0.0140) today 15 (0.4800±0.0073) when compared to normal group. Whereas in test group the bilirubin level was significantly decreased from day 1 (0.2150±0.0067) today 15 (0.2267±0.0080) when compared to negative controlled group. So on co-administration of the ascorbic acid and co-trimoxazole have the beneficial effect over hyperbilirubinemia caused by co-trimoxazole.

Recent work supports the concept that bilirubin functions as an antioxidant at concentrations within the serum reference interval and may provide protection against atherosclerosis, coronary artery disease and inflammation [15,16]. Increased serum levels of β-carotene, retinol, bilirubin and total antioxidant status are associated with reductions in breast cancer risk [17].

Emerging work demonstrates that serum bilirubin is a novel biomarker implicated in cardiovascular and metabolic diseases. However, Paul et al. [18] have a limited understanding of the influence of flavonoid-rich fruit and vegetable consumption on bilirubin levels, their findings suggest an association between flavonoid-rich fruit and vegetable consumption and bilirubin levels. It confirmed by prospective and experimental studies, that regular consumption of flavonoid-rich fruits and vegetables should be promoted to increase levels of bilirubin [18], which can be included as the limitation of our study.

5. CONCLUSION

From the above results it was concluded that administration of ascorbic acid to rats decreases the hyperbilirubinemia which was induced by Co-trimoxazole.

CONSENT

It is not applicable.

ACKNOWLEDGEMENTS

We consider it as a great honour to express our deep sense of gratitude and indebtedness to Mr K. Somasekhar Reddy M. Pharm, (PhD), and Mr A. Sudheer M. Pharm, for their valuable encouragement and support fortheir excellent guidance, constant encouragement, every scientific and personal concern throughout the course of investigation and successful completion of our work.

We are grateful to express our special thanks to Mr P. Nagendra Kumar, Lab. Technician for his continuous assistance throughout our work. We also extend our thankfulness to librarian, all technical and non-teaching staff for their timely support.

It gives us immense pleasure to thank our parents for their magnificent and timely support with which we came to this position.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Lachapelle MY, Drouin G. Inactivation dates of the human and guinea pig vitamin C genes. Genetica. 2010;139(2):199–207.
2. Available:http://www.medterms.com/script/main/art.asp.articlekey=12536
3. Available:http://www.drugbank.ca/drugs/DB00126#pharmacology
4. Yamaguchi T, Horio F, Hashizume T, Tanaka M, Ikeda S, Kakinuma A, Nakajima H. Biochem Biophys Res Commun. 1995; 214(1):11-19.
5. Martindale: The complete drug reference. London, UK: Pharmaceutical Press; 2011.
6. Bactrim, Bactrim DS. Trimethoprim / sulfamethoxazole dosing, indications,

interactions, adverse effects, and more. Medscape Reference. WebMD; 2014.
7. Available:http://www.drugs.com/ppa/trimethoprim-sulfamethoxazole-co-trimoxazole
8. Marie A. Andersch. Ann Intern Med. 1943; 19(4):622-628.
9. American academy of paediatrics. Available:http://www.aap.org
10. Available:http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=54670067
11. Goodman & Gillman's, the pharmacological basis of therapeutics, 10th edition, the vitamins page no. 1768-1769.
12. Available:http://en.wikipedia.org/wiki/Bilirubin
13. Lampe JW. Health effects of vegetables and fruit: assessing mechanisms of action in human experimental studies. Am J Clin Nutr. 1999;70:475–490.
14. Li YQ, Prentice DA, Howard ML, Mashford ML, Desmond PV. Bilirubin and bile acids may modulate their own metabolism via regulating uridine diphosphate-glucuronosyltransferase expression in the rat. J Gastroenterol Hepatol. 2000; 15:865–870.
15. Mayer M. Association of serum bilirubin concentration with risk of coronary artery disease. Clin. Chem. 2000; 46:1723-1727.
16. Hunt SC, Kronenberg F, Eckfeldt JH, et al. Association of plasma bilirubin with coronary heart disease and segregation of bilirubin as a major gene trait: The NHLBI family heart study. Atherosclerosis. 2001;154:747-754.
17. Ching S, Ingram D, Hahnel D, Beilby J, Rossi E. Serum levels of micronutrients, antioxidants and total antioxidant status predict risk of breast cancer in a case control study. J. Nutr. 2002;132(2):303-306.
18. Loprinzi PD, Mahoney SE. Association between Flavonoid-Rich fruit and vegetable consumption and total serum bilirubin. Angiology. 2014;66(3):286-290.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=985&id=14&aid=8620