EFFECTS OF DIFFERENT WARFARIN DOSES ON IL-6 AND COX-2 LEVELS

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ABSTRACT

Background: Warfarin has been widely used for a long time as an oral anticoagulant agent. Warfarin is reported to have inflammatory role as well. The dose of Warfarin plays an important role in up and down regulation of interacting cytokines such as IL-6 and COX-2 (Cyclooxygenase).

Objective: To investigate the effects of varying Warfarin doses on IL-6 and COX-2.

Material and Methods: Serum IL-6 and COX-2 levels were determined by ELISA.

Results and Discussion: Total 107 patients who had undergone valvular heart disease and were on warfarin therapy were included in the study of which 43 (40.2%) were taking warfarin <5mg/day, 57 (53.3%) had been put on dose 5-10mg/day and 7(6.5%) were taking >10mg/day of warfarin dose. It was observed that in patients on high dose warfarin therapy increased COX-2 caused stimulation of IL-6. Low dose warfarin down regulated the production of IL-6 and thus the synthesis of COX-2. A positive correlation was observed between IL-6 and COX-2 levels.

Conclusion: It was concluded that warfarin alters the inflammatory function of cells by directly influencing the IL-6 and COX-2 levels

Keywords: COX-2, ELISA, IL-6, Inflammation, Warfarin.

INTRODUCTION

Warfarin is reported to affect the inflammatory pathway besides its anticoagulation action. It is postulated that warfarin may employ other molecular mechanisms apart from its effect on vitamin K metabolism. Depending on the dosing requirements, certain cytokines are directly interacting with the drug which are involved in inflammation and blood coagulation[1]. This activity of warfarin acting as a pro or anti-inflammatory has been passed unobserved for more than thirty years. Few studies have suggested the pleiotropic effect of warfarin. The most important interacting inflammatory cytokine is interleukin 6 (IL-6)[2]. It has a significant role during inflammation and acts both as pro- and anti-inflammatory cytokine depending upon the low and high warfarin concentrations[3]. Inflammation is interceded by prostaglandins produced by the cyclooxygenase pathway. Almost all cells have the ability to produce prostaglandins. Arachidonic acid (AA) on receiving inflammatory stimuli is separated from plasma phospholipids by phospholipase A2. Cyclooxygenase (COX) enzymes are of two forms i.e. COX-1 and COX-2, although they catalyze the same reaction but their properties are markedly dissimilar[4]. COX-2 produces prostaglandins at the site of inflammation in various cells such as in macrophages and synovial tissues[5]. COX-2 also has pro-inflammatory functions that includes leukocyte proliferation, pain, fever and inflammation[6].

There is a dynamic interaction between the processes of coagulation and inflammation. Maclean et al examined the coagulation system that can directly affect the inflammatory regulatory pathway[7]. COX-2 might help in stimulating the IL-6 because the levels of both IL-6 and prostaglandin E2 are elevated during

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inflammation[8]. Warfarin plays an important role in up and down regulation of IL-6 e.g, low dose of warfarin (10-100 µM) inhibited the IL-6 production from murine macrophages, whereas high dose of warfarin (>200 µM) stimulated IL-6 release mediated by prostaglandins[9]. Further studies are required to understand the molecular mechanism of warfarin, depending upon its interaction with IL-6[10]. Studies are not available on the correlation of COX-2 with Warfarin doses.

Table-I: Correlation between IL-6 and COX-2 levels of a patient.

Correlation coefficient	IL-6 conc of a patient	Cox-2 conc of a patient
Sig.(2-tailed)	1	1.000**
p-value		0.001
N=number of patients	107	107

Table-II: Comparison of cytokines serum levels between control and different warfarin doses.

Warfarin dose(mg/day)	Number of patients (n=107)	COX-2 (pg/mL)	IL-6 (pg/mL)
control	5	33.6	38.7
<5	43	18.19	19.7
5-10	57	28.36	33.4
>10	07	55.67	58.09

Therefore, the aim of our study was to determine the relation of warfarin therapy with IL-6 and COX-2.

MATERIAL AND METHODS

Study Location and Population

The study was conducted at the Department of Biochemistry & Molecular Biology Post-graduate Lab, Army Medical College, National University of Sciences and Technology (NUST) over a period of 2 years from Oct 2013 to Oct 2015. Total 107 patients of valvular heart diseases, had undergone surgery (mitral, aortic, tricuspid valve replacements and repair) and were on warfarin therapy for management of anticoagulation were included.

Quantification by ELISA Assay

IL-6 and COX-2 levels were determined by COX-2 ELISA kit (Invitrogen) and IL-6 EASIA kit (Invitrogen, Biosource Europe S.A) according to the manufacturer's instructions. The quantification of samples was calculated by linear regression.

Statistical Analysis

Data were analyzed using SPSS version 22.0. Student's t-test and Spearman's rho correlation coefficient test was applied. Statistical significance was considered as $p \leq 0.05$.

RESULTS

Total 107 patients were included who had been put on Warfarin therapy after heart value replacements. Descriptive statistics were calculated for low (<5mg/day), medium (5-

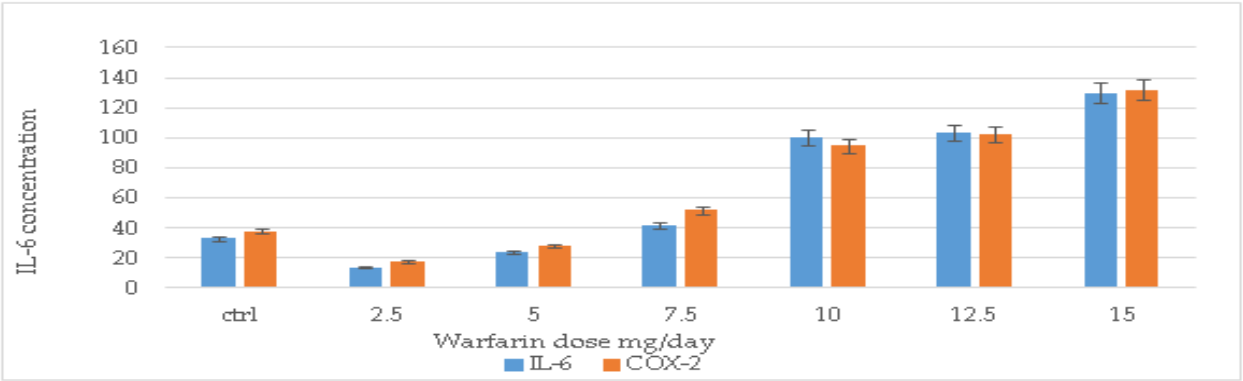

Figure-1: Comparison of IL-6 and COX-2 with different warfarin doses.

10mg/day) and high (>10mg/day) Warfarin doses. The Spearman's rho correlation analysis showed that the COX-2 and IL-6 levels were positively correlated (Correlation coefficient: 1.000, p=0.001).

IL-6 and COX-2 levels have been noted to vary in patients taking low and high-dose warfarin compared with control. The lowest concentration observed was 19.7 pg/ml of IL-6, 18.19 pg/ml of COX-2 while the highest concentration was 58.09 pg/ml of IL-6 and 55.67 pg/ml of COX-2 (table-II).

DISCUSSION

Cytokines play an important role in cell-mediated immunity. We have focused on investigating the role of specific cytokine i.e. IL-6 directly interacting with warfarin drug[11]. The mechanism of COX-2 action in cells includes stimulation of IL-6 gene transcription[12]. The effect of COX-2 on cellular IL-6 production is still under controversy that whether it has stimulatory or inhibitory affect[13]. Therefore, it was investigated on patients receiving warfarin therapy in order to explore warfarin's direct interaction with IL-6. Our results showed that COX-2 and IL-6 protein levels had significantly decreased with low dose and a markedly increased with high dose of warfarin (fig-1). Control showed normal levels of COX-2 and IL-6 while insignificant increase was noted in medium dose taking patients.

A significant positive correlation was found between IL-6 and COX-2 levels which showed that COX-2 helps in mediating the IL-6 production. This means that COX-2 gene may regulate expression of an inflammatory cytokine. It might play an important role in cell mediated immunity[14]. Therefore our results suggested that warfarin has anti-inflammatory effect on IL-6 production by decreasing the COX-2 and pro-inflammatory effect by increasing the COX-2 levels in low and high doses respectively.

CONCLUSION

The study concluded that warfarin alters the inflammatory activity of cells by influencing IL-6 and COX-2 depending on dosing regimen.

CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

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