Research Article

PROTECTIVE ROLE OF ALPHA-TOCOPHEROL ON DOCETAXEL -INDUCED CHANGES IN CHOLESTEROL PROFILE

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ABSTRACT
This *in vitro* study was designed to evaluate the protective effects of alpha-tocopherol on docetaxel-induced changes in cholesterol profile. Goat blood was used as lipid source for the model. Total cholesterol (TC) and high density lipoprotein (HDL) cholesterol content of goat blood was determined. The results showed that docetaxel had the capacity to produce changes in cholesterol profile. Alpha-tocopherol had the capacity to inhibit docetaxel-induced changes in cholesterol profile. Interpretation of the results is supported by analysis of variance and also by statistical multiple comparison analysis using least significant different procedure.

Keywords: Docetaxel, alpha-tocopherol, Total cholesterol, HDL-cholesterol.

INTRODUCTION

Docetaxel is a semi synthetic derivative of paclitaxel which is primarily used for the treatment of breast, ovarian and non-small cell lung cancer. Docetaxel produces several toxic side effects due to damage of normal cell like hair follicles, bone marrow and other germ cells[1]. It was reported that docetaxel has the capability of inducing lipid oxidization and membrane damage in human hepatoma cells[2]. Free radicals are constantly produced inside our body and also removed by endogenous antioxidant defence system. Reactive oxygen species and other pro-oxidants cause the decomposition of ω_3 and ω_6 polyunsaturated fatty acids of membrane phospholipids leading to the formation of aldehydic end products including malondialdehyde (MDA), 4-hydroxy-2-nonenals (4-HNE) and 4-hydroxy-2-alkenals (HAKs) of different chain length[3]. In case of reduced or impaired defense mechanism and excess generation of free radicals that are not counter balanced by endogenous antioxidant defense exogenously administered antioxidants have been proven useful to overcome oxidative damage.

Cholesterol is a fatty lipid produced by the liver and is crucial for normal body functioning. It exists in the outer layer of every cell in our body and is transported in the blood plasma of all animals. It is the main sterol synthesized by animals and small amounts are also synthesized in plants and fungi. Several factors like nutrition, diet, weight, physical activity, age, gender, heredity, alcohol etc affect the cholesterol level in blood[4-5] Serum cholesterol or its fractions like low density lipoproteins (LDL), high density lipoproteins (HDL) content have been found responsible for many diseases. Cholesterol and lipoprotein levels correlate well with the risk of cardiovascular diseases[6].

In view of the above findings and the ongoing search of the present authors for antioxidant that may reduce drug induced lipid peroxidation[7-10] the present work has been carried out *in vitro* to evaluate the antiperoxidative potential of alpha-tocopherol on docetaxel-induced changes in cholesterol content in goat blood sample.

MATERIALS AND METHODS

Pure sample of docetaxel used in present study was provided by Fresenius Kabi, Kalyani, India. Alpha tocopherol was from CDH Pvt. Ltd., New Delhi. Cholesterol test kit was from Span Diagnostic Ltd., Surat, India. All other reagents were of analytical grade.

Collection and preservation of goat blood

The goat (*Capra capra*) blood was collected from Silchar Municipal Corporation approved outlet. Appropriate quantity of blood as per the requirement for determination of a specific parameter was collected in a sterile vessel containing sodium citrate. Then the whole blood was divided equally. The first portion was kept as control (C), while the second portion was treated with docetaxel (D) at a concentration of 0.143 µM/g blood. The third portion was treated both with docetaxel at a concentration of 0.143 µM/g blood and antioxidant (alpha-tocopherol) at a concentration of 0.189 µM/g blood (DA). The fourth one was treated only with the above mentioned antioxidant alone at a concentration of 0.189 µM/g blood (A). After treatment with docetaxel and / or antioxidant, the different portions of blood samples were initially shaken for 5 hours at ambient temperature and total cholesterol and HDL-cholesterol content of different proportions were estimated. Then the samples were stored at 10-12 ^{0}C for 24 hours for next determinations.

Estimation of total cholesterol and HDL-cholesterol from goat blood

Determination of cholesterol concentration was performed in one step method[11] with the help of cholesterol test kit. The determinations were done at 5 and 24 hrs of incubation and it was repeated for three times. In each case there were three samples. After the specified hours of incubation, 2 mL of blood samples were centrifuged at 2000 rpm for 15 minutes and the supernatant (plasma) was separated out. After that total cholesterol and high density lipoprotein cholesterol of the goat blood were determined.

Total cholesterol

The Total Cholesterol (TC) was calculated by using the following formula

Total Cholesterol (mg / dL) = (O.D. of Test / O.D. of Standard) x 200

HDL cholesterol
Step-I

HDL- cholesterol separation: 0.2 mL of the supernatant was transferred into a centrifuge tube and to it 0.2 mL of reagent 3 from test kit was added. Then it was shaken well to mix and the tubes were kept at room temperature for 10 minutes. It was centrifuged at 2000 rpm for 15 minutes to obtain a clear supernatant.

Step-II

HDL-cholesterol determination: The test sample was prepared by mixing 3 mL of reagent 1 from test kit with 0.12 mL of the supernatant obtained from the step-I. The centrifuge tubes were shaken well and the tubes were kept in the boiling water bath exactly for 90 sec. The tubes were cooled immediately at room temperature under running tap water. The O.D. of Standard (S) & Test (T) were measured at 560 nm against reagent 1 as blank. The content of HDL Cholesterol was calculated by using the following formula:

HDL-Cholesterol (mg / dL) = (O.D. of Test / O.D. of Standard) x 50

Statistical analysis

For *in vitro model* of experiment, interpretation of the result is supported by analysis of variance (ANOVA) and multiple comparison analysis using least significant different procedure[12-13].

RESULT AND DISCUSSION

It was observed from Figure 1 that goat blood treated with docetaxel caused an increase in total cholesterol content (11.19 and 5.77 %) with respect to corresponding control. But the HDL cholesterol level (-8.35 and -3.40%) was reduced in comparison to control group (Figure 2) at 5 and 24 hours of incubation. These observations suggest that docetaxel can change the cholesterol profile. It was further found that incubation of blood sample with docetaxel and alpha-tocopherol produce a decrease in total cholesterol (-12.7 and -3.54%), but the HDL-cholesterol contents (5.45 and 2.7%) were increased in comparison to both control and docetaxel-treated group respectively. Incubation of blood samples only with alpha-tocopherol also shows a tendency of decrease in total cholesterol (-8.06 and -1.77%), but HDL-cholesterol contents (2.52 and 1.75%) were increased in comparison to control or docetaxel-treated group respectively.
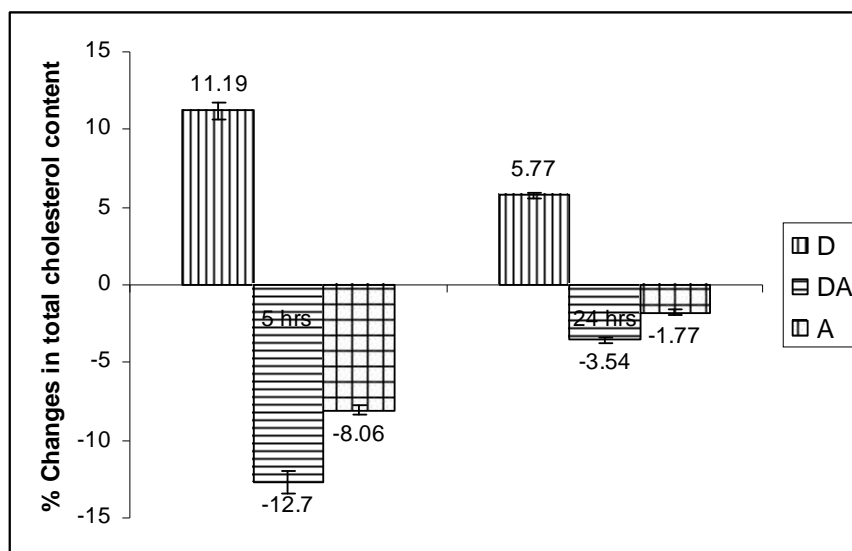
**Fig. 1: Effects of alpha-tocopherol on docetaxel-induced changes in
total cholesterol profile (n=3); D, DA & A indicate only docetaxel-treated,
docetaxel & alpha-tocopherol -treated and only alpha-tocopherol –treated samples**
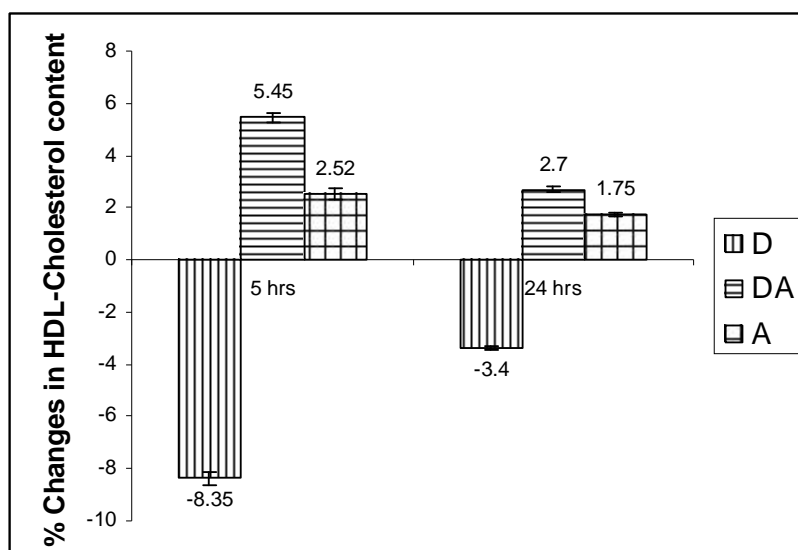

**Fig. 2: Effects of alpha-tocopherol on docetaxel-induced changes in
HDL-cholesterol profile (n=3); docetaxel-treated, docetaxel &
alpha-tocopherol -treated and only alpha-tocopherol –treated samples**

To compare means of more than two samples, multiple comparison analysis along with analysis of variance was performed on the percent changes data of various groups (Table 1-2). It is seen that there is significant differences among various groups (F1) such as docetaxel-treated, docetaxel and alpha-tocopherol-treated and only alpha-tocopherol-treated group. But within a particular group, differences (F2) are insignificant which shows

that there is no statistical difference in animals in a particular group. If F-test is significant and more than two treatments are incorporated into the experiment it may not be obvious immediately which treatments are different. To solve the problem multiple comparison analysis is suggested. Least significant different procedure [12-13] is applied on the percent changes data of various groups such as docetaxel-treated (D), docetaxel and alpha-

tocopherol (DA) and only alpha-tocopherol - treated (A) with respect to control group of corresponding time. It was observed that the level of total cholesterol and HDL-cholesterol content (Table 1 &2) in docetaxel-treated, docetaxel and alpha-tocopherol-treated as well as only alpha-tocopherol-treated groups are significantly different from each other.

Table 1: ANOVA & Multiple comparison for changes of total cholesterol

Name of the antioxidant	Time of incubation (hrs)	Analysis of variance and multiple comparison
Alpha-tocopherol	5	F_1=454.3[df=(2,4)], F_2=0.69[df=(2,4)], Pooled variance $(S^2)^*$=1.06, Critical difference $(p=0.05)^\#$ LSD=1.94 Ranked means** (D) (DA) (A)
	24	F_1=572.89 [df=(2,4)], F_2=0.19[df=(2,4)], Pooled variance $(S^2)^*$=0.128, Critical difference $(p=0.05)^\#$ LSD=0.67 Ranked means** (D) (DA) (A)

Theoretical values of F: p=0.05 level F_1=6.94 [df=(2,4)], F_2=6.94 [df=(2, 4)] F1 and F2 corresponding to variance ratio between groups and within groups respectively; D, DA & A indicate only docetaxel-treated, docetaxel & alpha-tocopherol-treated and only alpha-tocopherol–treated samples * Error mean square, # Critical difference according to least significant procedure (LSD) **Two means not included within same parenthesis are statistically significantly different at p=0.05 level.

Table 2: ANOVA & Multiple comparison for changes of HDL-cholesterol

Name of the antioxidant	Time of incubation (hrs)	Analysis of variance and multiple comparison
Alpha-tocopherol	5	F_1=1513.26[df=(2,4)], F_2=2.25[df=(2,4)], Pooled variance $(S^2)^*$=0.104, Critical difference $(p=0.05)^\#$ LSD=0.61 Ranked means** (D) (DA) (A)
	24	F_1=1004.2[df=(2,4)], F_2=0.12[df=(2,4)], Pooled variance $(S^2)^*$=0.032, Critical difference $(p=0.05)^\#$ LSD=0.34 Ranked means** (D) (DA) (A)

Theoretical values of F: p=0.05 level F_1=6.94 [df=(2,4)], F_2=6.94 [df=(2, 4)] F1 and F2 corresponding to variance ratio between groups and within groups respectively; D, DA & A indicate only docetaxel-treated, docetaxel & alpha-tocopherol-treated and only alpha-tocopherol–treated samples * Error mean square, # Critical difference according to least significant procedure (LSD) **Two means not included within same parenthesis are statistically significantly different at p=0.05 level.

CONCLUSIONS
The results from the above study indicate docetaxel's induction property to change cholesterol profile. The results also showed the protective effects of alpha-tocopherol and demonstrate its potential to reduce docetaxel-induced changes in cholesterol profile and thus to increase therapeutic index of the drug by way of reducing toxicity that may be mediated through free radical mechanisms. However a detailed study has to be carried out.

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