International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.3, No.1, pp 580-584, Jan-Mar 2011

Evaluation of Protective effect of reduced Glutathione on 5-Fluorouracil-induced changes in Cholesterol Profile

Sarbani Dey Ray*

Dr. B C Roy College of Pharmacy & Allied Health Sciences, Bidhannagar, Durgapur, 713 206, India

***Corres. author: sarbanidey_in@yahoo.co.in**
Tel: +919434443946, Fax: +91343-2532679

Abstract: The study was designed with an aim to evaluate the protective effects of reduced glutathione on 5-fluorouracil (5-FU)-induced Cholesterol profile *in vitro*. Goat blood was used as lipid source for the model. In the cholesterol profile total cholesterol (TC) and high density lipoprotein (HDL) cholesterol content of goat blood was determined. 5-FU and reduced glutathione were added at a concentration of 0.012, 0.05 mg / g of goat blood respectively. The study reveals that 5-FU has the induction capacity to produce changes in cholesterol profile (12.164 and 13.328% for total cholesterol as well as -5.502 and -16.594 for HDL cholesterol at 1 and 2 hrs of incubation with respect to corresponding controls). But after addition of reduced glutathione with 5-FU total cholesterol level was decreased (7.724 and 8.448%) and HDL cholesterol level was increased (29.312 and 12.426%) in comparison to 5-FU treated group. Incubation of blood samples only with reduced glutathione also shows a tendency of decrease in total cholesterol (-16.888 and -16.852%), but HDL-cholesterol contents (27.016 and 4.914%) were increased in comparison to control or 5-FU-treated group respectively. These observed data indicate that reduced glutathione has protective effect on 5-FU-induced changes in cholesterol content. Interpretation of the results is supported by analysis of variance and also by statistical multiple comparison analysis using least significant different procedure. For total cholesterol content reduced glutathione treated group is statistically significantly different from 5-FU treated as well as 5-FU and reduced glutathione-treated group. In case of HDL-cholesterol content 5FU treated group is statistically significantly different from 5-FU and reduced glutathione-treated group as well as only reduced glutathione-treated group.
Key words: 5-fluorouracil, reduced glutathione, Total cholesterol, HDL.

Introduction

Lipid peroxidation is a degenerative process that affects unsaturated membrane lipids under conditions of oxidative stress[1]. This complex process is believed to contribute to human aging and disease by disrupting the structural conformation, the packing of lipid components and ultimately the function of biological membranes. Oxidative stress may cause lipid peroxidation by damaging the DNA-sugar and oxidizing protein by introducing carbonyl group into the side chains of protein molecules[2, 3]. Reactive oxygen species and other pro-oxidants cause the decomposition of ω3 and ω6 polyunsaturated fatty acids of membrane phospholipids leading to the formation of aldehydic end products including malondialdehyde (MDA), 4-hydroxy-2-nonenals and 4-hydroxy-2-alkenals (HAKs) of different chain length. These aldehydic molecules have been considered as ultimate mediators of toxic effects elicited by oxidative stress occurring in biological

membrane[4]. Oxidative stress in cells can be initiated by the addition of Fe2+ in the presence of dioxygen. This stress will result in lipid peroxidation and subsequent formation of lipid radicals[5, 6].

Free radical mediated oxidative stress results usually from deficient natural antioxidant defenses and act as a main factor in the pathophysiology of various diseases and ageing[7]. Various antioxidants and free radical scavengers have been suggested to be general cytoprotective agents of therapeutic benefit[8]. 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[9].

5-Fluorouracil (5-FU), an anticancer drug, has several toxic side effects besides its antitumor property. It has been reported that 5-FU produces nephrotoxicity on Wistar rats[10]. It has also been observed that the drug induces cardiotoxicity[11] because 5-FU treatment causes impairment in the myocardial antioxidant defense system and leads to cardiac peroxidation and it has been postulated that antioxidant therapy might have therapeutic advantage[12]. Lipid peroxidation induction capacity of drugs may be related to their toxic potential as exemplified by adriamycin-induced cardiotoxicity, which occurs through free radical mediated process [13].

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[14]. In view of the above findings and the ongoing search of the present authors for antioxidant that may reduce drug induced lipid peroxidation[15-18], the present work has been carried out *in vitro* to evaluate the antiperoxidative potential of reduced glutathione on cyclophosphamide-induced changes in cholesterol content in goat blood sample.

Experimental

5-Fluorouracil was kindly gifted from Dabur Research Foundation, Ghaziabad, India, reduced glutathione were from Sigma Chemicals Co. St. Louis, MO, USA, and 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 Durgapur 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.

Estimation of total cholesterol and HDL-cholesterol from goat blood

Determination of cholesterol concentration was performed in one step method[19] with the help of cholesterol test kit. The determinations were done at 2 and 24 hrs of incubation and it was repeated for five times. In each case there were three samples. After the specified hours of incubation, 2 ml of blood was withdrawn from the ear vein of rabbits. The 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 rabbit 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 HDLCholesterol 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[20, 21].

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

Type of model	Time of incubation (hrs)	Analysis of variance and multiple comparison
In vitro	1	F1=119.008 [df=(2, 8)], F2=2.79 [df=(4, 8)], Pooled variance (S^2)*=10.289, Critical difference (p=0.05)# LSD=6.04 , Ranked means** (D, DA) (A)
	2	F1=143.57 [df=(2, 8)], F2=5.66 [df=(4, 8)], Pooled variance (S^2)*=9.14, Critical difference (p=0.05)# LSD=5.692, Ranked means** (D, DA) (A)

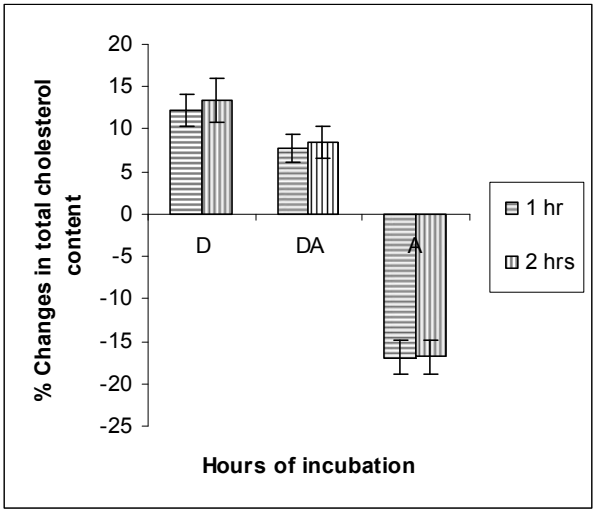
Theoretical values of F: p=0.05 level F1=4.46 [df=(2,8)], F2=3.84 [df=(4,8)] P=0.01 level F1=8.65 [df=(2,8)], F2=7.01 [df=(4,8)], F1 and F2 corresponding to variance ratio between groups and within groups respectively; * Error mean square, # Critical difference according to least significant procedure (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 content

Type of model	Time of incubation (hrs)	Analysis of variance and multiple comparison
In vitro	1	F1=237.42 [df=(2, 8)], F2=1.41 [df=(4, 8)], Pooled variance (S^2)*=7.984, Critical difference (p=0.05)# LSD=5.32, Ranked means** (D) (DA, A)
	2	F1=65.84 [df=(2, 8)], F2=0.64 [df=(4, 8)], Pooled variance (S^2)*=17.23, Critical difference (p=0.05)# LSD=7.81, Ranked means** (D) (DA, A)

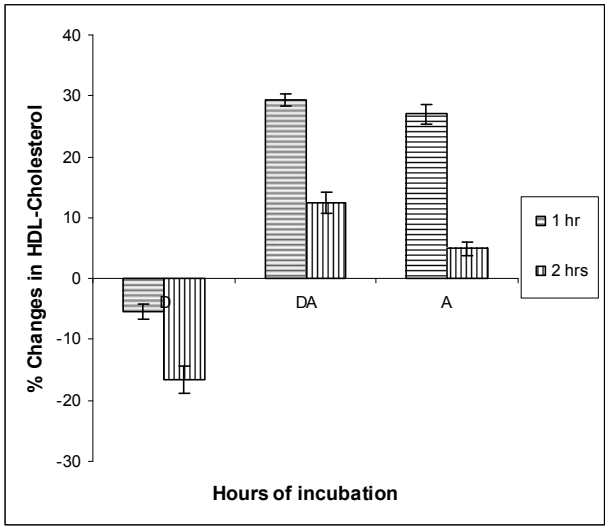
Theoretical values of F: p=0.05 level F1=4.46 [df=(2,8)], F2=3.84 [df=(4,8)] P=0.01 level F1=8.65 [df=(2,8)], F2=7.01 [df=(4,8)], F1 and F2 corresponding to variance ratio between groups and within groups respectively; * Error mean square, # Critical difference according to least significant procedure (LSD) **Two means not included within same parenthesis are statistically significantly different at p=0.05 level.

Figure 1: Effects of reduced glutathione on 5-FU-induced changes in total cholesterol



Changes in total cholesterol profile (n=5); D, DA & A indicate only 5-FU -treated, 5-FU & reduced glutathione -treated and only reduced glutathione –treated samples.

Figure 2: Effects of reduced glutathione on 5-FU-induced changes in HDL cholesterol



Changes in HDL-cholesterol profile (n=5); D, DA & A indicate only 5-FU -treated, 5-FU & reduced glutathione -treated and only reduced glutathione –treated samples.

Results and Discussion

It was observed from Figure 1-2 that goat blood treated with 5-FU caused an increase in total cholesterol content (12.16 and 13.33 %) with respect to corresponding control. But the HDL cholesterol level (-5.50 and -16.59%) was reduced in comparison to control group. These observations suggest that 5-FU can change the cholesterol profile. It was further found that incubation of blood sample with 5-FU and reduced glutathione produce a decrease in total cholesterol (7.72 and 8.45%), but the HDL-cholesterol contents (29.31 and 12.43%) were increased in comparison to 5-FU -treated group respectively. Incubation of blood samples only with reduced glutathione also shows a tendency of decrease in total cholesterol (-16.89 and -16.85%), but HDL-cholesterol contents (27.01 and 4.91%) were increased in comparison to control or 5-FU-treated group respectively. These results suggest that reduced glutathione could inhibit 5-FU-induced changes in cholesterol profile. 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 5-FU-treated, 5-FU and reduced glutathione-treated and only reduced glutathione-treated. But within a particular group, differences (F2) are insignificant which shows that there is no statistical difference in animals in a particular group.

Conclusion

These findings indicate that 5-fluorouracil has the ability to change the cholesterol profile by inducing lipid peroxidation which may be related to its toxic potential. The results also suggest the antiperoxidative effects of reduced glutathione and demonstrate its potential to reduce 5-fluorouracil induced lipid peroxidation and thus to increase therapeutic index of the drug by way of reducing toxicity that may be mediated through free radical mechanisms.

References

1. Girotti AW, Lipid hydroperoxide generation, turnover and effector action in biological systems. J Lipid Res 1998; 39: 1529–1542.
2. Colburn NH, Moses HL, Stanbridge EJ, (Eds.), Growth Factors, Tumor Promoters and Cancer Genes. New York, Alan R Liss Inc, 1998, 239-247.

3. Goidstein BD, Witz G, Amomso M, Troll W, Protease inhibitors antagonize the activation of polymorphonuclear leukocyte oxygen consumption. Biochem Biophys Res Commun 1979; 88: 854-860.
4. Parola M, Belloma G, Robino G, Barrera G, Dianzani, MU, 4-Hydroxynonenal as a biological signal: molecular basis and pathophysiological implications. Antioxid Redox Signal 1999; 1: 255-284.
5. Halliwell B, Gutteridge JMC, Free radicals in Biology and Medicine, Oxford, Oxford University Press, 1989; 195-205.
6. Wagner BA, Buerrner GR, Burns CP, Increased Generation of Lipid-derived and Ascorbate Free Radicals by L1210 Cells Exposed to the Ether Lipid Edelfosine. Cancer Res 1993; 53: 711-713.
7. Kampkotter A, Nkwonkam CG, Zurawski RF, Timpel C, Chovolou Y, Watjen W, Kahl R, Investigations of protective effects of the flavonoids quercetin and rutin on stress resistance in the model organism Caenorhabditis elegans. Toxicology 2007;234: 113-123.
8. Ratty AK, Sunamoto J, Das NP, Interaction of flavonoids with 1, 1-diphenyl-2-picrylhydrazyl free radical, liposomal membranes and soybean lipoxygenase-1. Biochem Pharmacol 1988; 37: 989-995.
9. Halliwell B, Drug antioxidant effects-A basis for drug selection. Drugs 1991; 42: 569-605.
10. Skretkowicz J, Sekulska M, Danilewicz M, Wagrowska-Danilewicz M, Polakowski P.Biol. Signals 1996; 5, 51.
11. Simbre C, Duffy A, Dadlani H, Miller L, Lipshultz E. Pediatr. Drugs 2005; 7, 187.
12. Durak L, Karaayvaz M, Kavutcu M, Burak Cimen M. Y, Kacmaz M, Buyukkocak S, Serdar Ozturk H. J. Toxicol. Environ. Health 2000; 59, 585.
13. Luo X, Evrovsky Y, Cole D, Trines J, Benson L. N, Lehotay D. C. Biochim. Biophys. Acta 1997; 45, 1360.
14. Eliot RS, Stress and the heart, New York, Futura Publishing Company, 1974; 41-63.
15. Ray S, Roy K, Sengupta C, Cisplatin-induced lipid peroxidation and its inhibition with ascorbic acid. Indian J Pharm Sci 2006; 68:199-204.
16. Ray S, Roy K, Sengupta C, Evaluation of protective effects of water extract of Spirulina platensis (blue green algae) on Cisplatininduced lipid peroxidation. Indian J Pharm Sci 2007; 69: 378-383.
17. Ray S, Sengupta C, Roy K, In vitro Evaluation of protective effects of ascorbic acid and water extract of Spirulina platensis (blue green algae) on 5-fluorouracil-induced lipid peroxidation. Acta Pol Pharm-Drug Res 2007;64: 335-344.
18. Ray S, Roy K, Sengupta C, Exploring the protective effect of ascorbic acid and water extract of Spirulina platensis (blue green algae) on methotrexate-induced lipid peroxidation. Iranian J Pharm Sci 2007; 3:217-228.
19. Wybenga DR, Pileqqi VJ, Dirstine PH, Di Giorquio J, Direct manual determination of serum total cholesterol with a single stable reagent. Clin Chem 1970; 16: 980-984.
20. Snedecor GW, Cochran WG, *Statistical Methods*, *New Delhi, Oxford & IBH Publishing Co Pvt Ltd, 1967; 375-385*.
21. Bolton S, Statistics, in, Gennaro AR, (Ed.), *Remington: The Science and Practice of Pharmacy*, *Philadelphia, Lippincott Williams & Wilkins, 2001, 124-158*.

*****