

EFFECT OF OCIMUM FLAVONOIDS AS A RADIOPROTECTOR ON THE ERYTHROCYTE ANTIOXIDANTS IN ORAL CANCER

Reshma K*, Ashalatha V Rao[#], Dinesh M** and Vasudevan DM***.

*Department of Biochemistry, Centre for Basic Sciences, Kasturba Medical College, Mangalore-575 004

[#]Department of Biochemistry, Father Muller's Medical College, Mangalore

**Department of Oncology, Amrita Institute of Medical Sciences, Elamakkara, Cochin

***Amritha Institute Of Medical Sciences, Elamakkara, Cochin

ABSTRACT

Flavonoids extracted from the leaves of Indian holy basil, *Ocimum sanctum* showed promising results as radioprotector in rodents. Hence it was thought pertinent to analyze the antioxidants of erythrocytes in oral cancer patients who were concurrently treated with radiation and ocimum flavonoids. Oral cancer patients consisted of 2 groups. Group A (n=17) received radiation alone while Group B (n=17) received radiation and ocimum flavonoids (OF). Samples of heparinised blood were collected prior to treatment, 15 and 30 days respectively after treatment. Blood from normal healthy volunteers were taken as controls (n=25). Erythrocytes were analyzed for the antioxidants viz. glutathione, glucose 6 phosphate dehydrogenase, glutathione peroxidase and superoxide dismutase by the standard methods. Results of analysis indicated that erythrocytes from cancer patients (Group A and B) had significantly high glutathione levels before as well as after treatment compared to controls. It was observed that Group B which received OF showed a significant reduction in glutathione levels in comparison with Group A. All the other parameters showed no statistical significant changes. Results of the study suggest that erythrocytes from cancer patients responded to oxidative stress by elevating glutathione levels, while a decrease in glutathione levels observed in Group B, could be due to the free radical scavenging effect of OF, sparing the glutathione. However OF did not seem to exert its effect on other antioxidants of erythrocytes.

KEYWORDS

Radiotherapy, Radioprotector, Ocimum flavonoids, antioxidants

INTRODUCTION

DNA damage by oxygen derived free radicals (ODFR) are important contributors to cancer development (1). Earlier works state that antioxidant defense system function inefficiently in tumor cells leading to accumulation of reactive oxygen metabolites (ROM) which further enhance the hazardous effects (2,3). Further more Radiotherapy (RT) involves free radical generation in order to kill the tumor cells. Increase in lipid peroxidation in the blood of oral cancer patients undergoing RT with a concomitant decrease in antioxidant enzymes have been reported (4). Radioprotector (RP), that protect the normal tissues

from the deleterious effects of RT, are currently in use for increasing the efficacy of RT. Many of them function by detoxifying the free radicals (5). Leaves of ocimum sanctum contain flavonoids which when administered to mice treated with radiation resulted in increasing the activities of antioxidant enzymes and decreasing lipid peroxidation (6). Ocimum flavonoids act by scavenging the hydroxy radicals (7). The present study has been undertaken to study the influence of ocimum flavonoids (OF) on the erythrocyte antioxidant defense potential in oral and oropharyngeal cancer patients who were concurrently treated with RT and OF. Erythrocytes were chosen because they act as 'sinks' for H_2O_2 and O_2^- produced in the tissues and plasma.

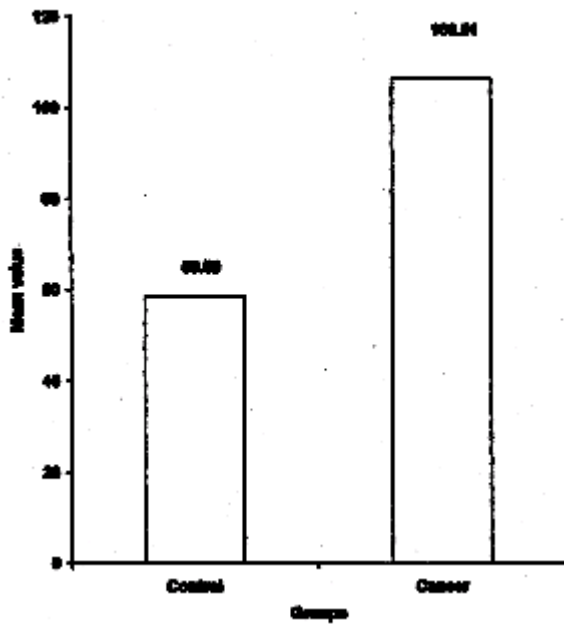
Author for correspondence

Dr. Reshma Kumarchandra
Department of Biochemistry
Centre for Basic Sciences
Kasturba Medical College
Mangalore-575 004

MATERIALS AND METHODS

The study group consisted of 34 (26M;8F) hospitalized patients having squamous cell carcinoma of oral cavity and oropharynx aged between 30 and 70 years and 25 age and sex matched controls. Stage III (T3) and stage

ERYTHROCYTE GLUTATHIONE (GSH) IN CANCER OF HEAD AND NECK
(nmoles/gHb)



IV (T4) of cancers were selected with a *PS-KP< 70%(8) and #Ecos scale<2 (9). These patients had no previous history of treatment. Those with severe systemic illness like Diabetes mellitus, coronary artery disease and tuberculosis were excluded in this study. The selected patients were randomly divided into 2 groups.

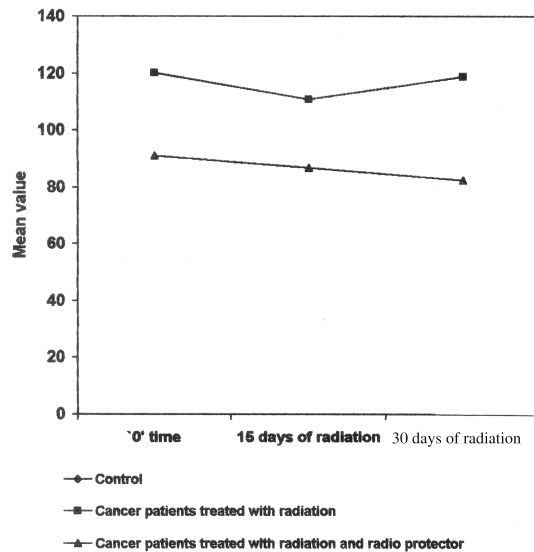
Group A : Patients (n=17) received radiotherapy at a dose of 46 Gy in 23 sittings over whole neck, 5 times a week for 4 weeks; followed by a booster dose of 1-20 Gy in 7-10 sittings (5 times/week) thereafter to the primary L₁ and L₂ lymphnodes.

Group B : Patients (n=17) received RT at the same dosage and an additional drug comprising of aqueous extract of ocimum flavonoids in the form of capsules. These capsules were prepared as per reported protocol(10). Each capsule consisting of 60 mg(1.32 mg/kg body wt) of the drug,was given to the patients orally, half an hour prior to radiation, during each sitting.

NADP⁺, NADPH, Riboflavin, L-methionine and Glutathione standard were obtained from SRL company Ltd. Glucose 6 phosphate was purchased from Loba chem. Cyanomethemoglobin standard was bought from Ranbaxy. DTNB was obtained from SISCO, NBT, from S.D.fine chem.. Ltd. Cumene hydroperoxide from Fluka, Ag L Buchio, Switzerland and glutathione reductase (E.C. 1.6.4.2.) Type III from Bakers yeast from Sigma Chemicals, U.S.A. Heparinised vacuotainers were purchased from Babul

Fig. 2

ERYTHROCYTE GLUTATHIONE (GSH) IN CANCER DURING TREATMENT
(nmoles/gHb)



Bio Medicals Pvt. Ltd. Ahmedabad.

5 ml of blood was collected by venepuncture from the patients, in heparinised vacutainers in 3 stages.

- 0 days of radiation {Baseline sample}.
- 15 days of radiation (I follow up sample)
- 30 days of radiation (II follow up sample) - all patients were not available for the study.

Likewise 5 ml of blood was collected from controls. Blood samples were kept in an upright position at room temperature for an hour. Once the plasma separated erythrocytes were collected and the hemolysate was prepared by the reported method(11). This hemolysate was used for estimation of different parameters. Glutathione(GSH) was assayed by the method of Beutlar etal (12).Glutathione peroxidase(GSH-PX) by the method of Paglia and Valentine(13),(14).Glucose-6-phosphate dehydrogenase (G6PD) was estimated by monitoring the increase in absorbance at 340nm(15).Superoxide dismutase (SOD) was assayed by the method of Beauchamp and Fridovich (16),(17). Haemoglobin(Hb) concentration in the RBC was determined by Cyanomethaemoglobin method (18). The activities of GSH was expressed as nmol/gHb and the activities of other antioxidants were expressed in terms of units /gHb.

Table 1A. ERYTHROCYTE ANTIOXIDANT PARAMETERS IN CONTROLS AND PATIENTS TREATED WITH RADIOTHERAPY

Parameters	MEAN ± S.E.M			
	Controls	Patients		
	n=22	Baseline n=17	I Followup n=17	II Followup n=7
GSH (nmoles/gHb)	58.59 ± 6.20	*120.08 ± 17.22	*110.86 ± 11.52	*118.80 ± 14.70
GSH-PX (nmoles of NADP red/min/gHb)	14.45 ± 1.46	12.18 ± 2.87	13.73 ± 3.38	12.05 ± 4.30
G6PD (nmoles of NADPH oxi/min/gHb)	2.79 ± 0.30	3.34 ± 0.49	2.58 ± 0.40	2.50 ± 0.44
SOD (units/gHb)	6203.09 ± 386.80	7294.73 ± 1750.20	7064.53 ± 776	9144.50 ± 1523.20
*P < 0.01; significant compared to control.				

Table 1B. ERYTHROCYTE ANTIOXIDANT PARAMETERS IN CONTROLS AND PATIENTS TREATED WITH RADIOTHERAPY AND OCIMUM FLAVONOIDS

Parameters	MEAN ± S.E.M			
	Controls	Patients		
	n=25	Baseline n=17	1 Followup n=17	II Followup n=7
GSH (nmol/gHb)	58.59 ± 6.20	**90.84 ± 8.87	**86.69 ± 9.90	**82.42 ± 6.89 ^a
GSH-PX (nmol NADPH oxi/min/gHb)	14.45 ± 1.46	24.92 ± 4.90	16.94 ± 4.62	18.47 ± 6.21
G-6-P-D (nmol NADP red/min/gHb)	2.79 ± 0.30	2.45 ± 0.30	2.17 ± 0.33	2.35 ± 0.19
SOD (units/gHb)	6203.09 ± 386.80	6553.84 ± 617.30	7620.53 ± 872.70	6897.57 ± 525.70
** p<0.05 compared to controls a: p<0.05 significant between group A and group B on comparing the second follow up samples (mean difference followed by student 't' test)				

STATISTICAL ANALYSIS

Mann Whitney 'U' test was used for comparison between independent groups. Wilcoxon's rank sign test was used for comparing the follow up cases. Whenever the baseline data for certain parameters varied in 2 groups mean difference for the paired

values in baseline and follow up cases were analysed by student 't' test. The differences were considered significant when the probability was p<0.05.

RESULTS

Table 1A, displays the mean values ± S.E.M of GSH, GSH-PX, SOD and G6PD in erythrocytes from cancer patients treated with Radiotherapy (Group A).

All the parameters showed significantly different values at the baseline level (i.e. before starting any treatment for cancer patients). Therefore we selected the particular statistical analysis (i.e. mean difference). Each of the follow up samples from each group were compared with baseline by paired 't' test and the mean difference was calculated in both the groups (Group A and Group B). The mean difference obtained in the 2 groups was further compared by student 't' test and a p value < 0.05 was considered to be significant.

A significantly higher erythrocyte GSH level was found in cancer patients ($p < 0.05$). There was no significant differences in the level of other parameters studied in erythrocytes from cancer patients, compared to controls. In the follow up samples from the patients subjected to radiotherapy, GSH levels in erythrocytes remained significantly high. No significant change was observed in other parameters. In the group of patients receiving radiation and radioprotector, erythrocyte GSH levels remained high in the first and second follow up samples compared to controls. Comparison of the follow up samples of group A and Group B showed a significant decrease in GSH in the latter, that received radioprotector.

Table 1B, shows the mean values \pm S.E.M. of GSH, GSH-PX, G-6-P-D and SOD in erythrocytes from patients receiving radiotherapy and ocimum flavonoids (Group B).

DISCUSSION

Glutathione is the most prevalent intracellular thiol exerting its antioxidant properties by scavenging hydroxy radicals and singlet oxygen, thus protecting the cell against these species. Elevated level of GSH in various cancer tissues (Oral cancer, lung squamous cell carcinoma, cervical and other squamous cell carcinoma) have been reported by Wong et al (19) which has been attributed to the abnormal proliferative activities in cancer tissues. High levels of GSH observed in the present work implies a proportionate increase in erythrocytes as well. Krishnamurthy and Jaya (20) state that there is a significant increase in antioxidant enzymes of erythrocytes namely SOD, GSH-PX, GSH reductase and G6PD in oral cancer. The present work observes no corresponding increase in other antioxidant parameters, which suggests that GSH is the most potent antioxidant in combating free radical injury which is a consequence of cancer and RT. Another possibility for elevation of GSH could be attributed to the reactivation of some enzymes by GSH, that have been inhibited by exposure to large amounts of free radicals that are generated during RT which act as oxidants of the enzymes.

GSH + enzymes (inactive) S-S-G enzyme (active) SH + GSSG

In fact Bhattathri (21) is of the opinion that plasma GSH level could be used as a predictor for individual sensitivity to acute radiation mucositis in the oral cavity. A decrease in the GSH level after administration of OF perhaps indicates the role of latter as a free radical scavenger as most of the radioprotectors act by scavenging the free radicals. One of the extensively studied radioprotector amifostine also acts by scavenging the free radicals. Administration of amifostine in cyclophosphamide treated rats brought about elevations in the enzymatic antioxidants like SOD, GSH-PX, catalase and glutathione reductase as well as elevations in nonenzymatic antioxidants like GSH to control levels while lipid peroxidation was inhibited (22). The fact that ocimum flavonoid treatment has decreased GSH suggests that ocimum by itself could have spared the GSH of its effects, as both share the common property of scavenging OH^\bullet radicals (7). This could be the probable reason for the non influential role of OF on SOD and GSH-PX that are involved in scavenging O_2^- and peroxy radicals respectively.

No noticeable variation in G6PD may suggest that the source of NADPH for generating GSH is not G6PD, but preferably certain other oxidative reactions. At the same time, clinical picture of these patients revealed no significant improvements in mucositis. Therefore establishing the role of OF as a radioprotector in human subjects is rather ambiguous. A reasonable derivation for different observations in rodents and human subjects could be that the results are species/dosage dependant.

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