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EFFECT OF A RADIOSENSITISER,WITHAFERIN A ,ON THE FREE RADICAL METABOLISING ENZYMES OF NEUTROPHILS, IN CARCINOMA OF UTERINE CERVIX SUBJECTED TO RADIOTHERAPY

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ABSTRACT

Radiosensitisation influences the enzymes of free radical metabolism.These enzymes are also a part of the respiratory burst mechanisms. Withaferin,an active component obtained from the dried root extracts of withania somnifera(ashwagandha),showed radiosensitising effects in animals. Hence, a study of the respiratory burst enzymes namely NADPH oxidase ,Myeloperoxidase, Glutathione,Glutathione peroxidase,Glucose 6 phosphate dehydrogenase and Superoxide dismutase were undertaken in leukocytes of patients with carcinoma of uterine cervix , which would perhaps aid in the understanding of radiosensitising mechanisms of Withaferin and increase the therapeutic outcome in cancer patients. Blood samples were collected from stage IIIB carcinoma of uterine cervix patients(n=20), before starting treatment(baseline),after 15 days and after 30 days of treatment with RT and Withaferin. Age and sex matched controls(n=25)were also considered for comparison. A significant decrease in NADPH oxidase was observed in baseline samples of cancer patients when compared to controls. Except for this variation, there was no change in the activities of other enzymes, when cancer patients were compared to controls or when baseline values were compared with that of follow up samples, in case of patients. This study therefore implies that radiosensitising actions of withaferin may not be mediated through free radical metabolism.

KEY WORDS

Radiotherapy, radiosensitiser, Withaferin

INTRODUCTION

Polymorphonuclear leukocytes mediate cytotoxicity towards cancer cells through superoxide anion, hydrogen peroxide, hydroxyl radical and hypohalite, all of which are generated during respiratory burst[1] Similar processes are involved in achieving tumor cell killing through radiotherapy. Radiosensitisers , by sensitizing the tumor cells to radiation are bound to make the treatment more effective. One of the underlying principles for the action of radiosensitiser being, mimicking oxygen, [2] so as to generate free radicals. Withaferin A , the active component obtained from the alcoholic extracts of the dried roots of the plant Withania somnifera(ashwagandha) showed significant antitumor and radiosensitising effects in experimental tumors induced in mice without any noticeable systemic toxicity [3.4].A similar study involving cancer patients could offer benefits to this population.

Cancer of uterine cervix is one of the leading causes of death among women worldwide[5].Withaferin as a radiosensitiser was thought to enhance tumor cure in these patients. To understand the radiosensitising effects of withaferin, prooxidants and antioxidants that are commonly associated with respiratory burst were evaluated in the present study in stageIIIB , uterine cervical carcinoma, and then compared with the values obtained after radiation and withaferin therapy.

MATERIALS AND METHODS

20 cases of carcinoma of uterine cervix (stage 111 B) were considered for the study. All patients were treated with radiation, at Kasturba Medical college, Hospital, Mangalore, India.

INCLUSION CRITERIA:

All patients selected were aged between 30 and 70 years. All cancer patients were selected based on the Karnofsky's performance scale KPS>70%[a.]. Patients had no previous history of treatment and received radiotherapy at a dose of 60 Gy in 30 fractions over 6 weeks.

a. Cares for self but unable to carry out normal activity:shows some signs or symptoms of the disease

EXCLUSION CRITERIA

All patients were subjected to thorough clinical examination and those with severe systemic illness like diabetes mellitus, coronary artery disease and tuberculosis were excluded.

All patients with carcinoma of cervix were treated with withaferin prior to radiotherapy,at a dose of 400mg/m^2 ,2 hrs prior to each sitting. The Institutional Ethical Committee had approved the drug trials.

Age and sex matched healthy non hospitalized controls(n=25) were considered for the comparative study with the patients.

CHEMICALS:

NADPH,Riboflavin,L-methionine and Glutathione standard were obtained from SRL company

limited. Glucose 6 phoshate 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.) TypeIII from Bakers yeast from Sigma chemicals,U.S.A.

Heparinised vacuotainers were purchased from Babul Biomedicals Pvt.Ltd,Ahmedabad.

5 ml of venous blood was collected from patients in three stages

a) 0 days of radiation (Baseline sample)
b) 15 days of radiation (I follow up sample)
c) 30 days of radiation (II follow up sample)

Leukocyte suspension was prepared according to the reported protocol [6]. NADPH oxidase was determined by monitoring the reduction of cytochrome C by the superoxide generated by the enzyme in the presence and absence of superoxide dismutase [7] .

Myeloperoxidase was estimated by the method of Matheson[8].Glucose6 phosphate dehydrogenase was estimated by recording the rate of change of absorbance at 340nm due to the production of NADPH by this enzyme [9] .The method for estimating glutathione was based on the method of Beutler[10] . GSH-PX activity was determined by recording the decrease in absorbance due to depletion of NADPH at 340nm for 5 min [11].SOD was determined according to the method of Beauchamp and Fridovich[12], based on inhibition of nitroblue tetrazolium reduction. Protein content in the leukocyte suspension was determined by Lowry's method[13]. All enzyme activities were expressed as Units /mg protein in the leukocyte suspension.

STATISTICAL ANALYSIS

Kruskal Waalis test was used for comparingn between independent groups. Wilcoxons rank sign test for as used for comparing the follow up cases. P value> 0.05 was considered considered to be significant.

RESULTS

A significant decrease in NADPH oxidase was observed in cancer patients prior to any treatment(baseline samples).This parameter did not show significant improvement following combination treatment with radiation and withaferin. There were no significant changes in other parameters studied, namely, MPO,G6PD,GSH,GSH-PX and SOD in baseline values when compared to controls or when baseline values were compared with follow up samples.

DISCUSSIONS

When activated by a variety of stimuli, human neutrophils become capable of lysing tumor cells which is an inducible event associated with the generation of reactive oxygen intermediates and perhaps, other toxic mediators, resulting from a contact of monocytes with tumor cells[14]. This would mean an increase in the activity of NADPH oxidase and MPO that generate ROS.On the contrary, our findings show a decreased activity of NADPH oxidase in cancer patients ,with no significant changes in MPO and other antioxidant enzymes namely SOD,GSH-PX, the cellular
reductant GSH and the NADPH generating enzyme G6PD.This can be attributed to a decreased immunity in cancer patients, due to a suppression in the production of superoxide as indicated by the decreased activity of NADPH oxidase. Some of the earlier reports are in agreement with this finding[15,16] In order to improve the efficacy of treatment ,radiotherapy was combined with withaferin, a proven radiosensitiser in animal studies [3,4] . Withaferin being a lactone, behaves as an antiproliferative agent to tumor cells in invitro studies, and also senisitises the tissues to oxidative damage when exposed to oxidative stress[17], perhaps due to its prooxidant nature. Certain studies have reported withaferin to be an antioxidant [18,19].These effects could well be reflected in the blood cells in general and leukocytes in particular where respiratory burst mechanisms occur. A delicate balance between the two mechanisms is essential to achieve promising results as a radiosensitiser.

TABLE-1

Leukocyte parameters in controls and patients treated with radiation and withaferin (Values are Mean ± SD)

Parameters	Controls n=25	Patients Baseline n=20	Patients I followup n=20	Patients II follow up n=20
NADPH oxidase nmoles of O_2^- produced/ mg protein	35.10±29.60	12.95 ± 16.19*	19.30±26.03	19.00±23.74
Myeloperoxidase U/mg protein	3.22±2.03	4.74 ± 3.40	4.89±2.98	6.06±3.85
Glutathione nmol/mg protein	348.31±499.24	313.43± 390.84	258.66±318.4	431.45±368.18
Glutathione peroxidase nmol of NADPH oxidised /min/mg protein	98.32± 100.36	87.61 ±61.10	106.13±100.5	197.77±419.15
Glucose 6 phosphate dehydrogenase nmol of NADP reduced/min/ mg protein	39.54±19.96	51.03 ±27.98	47.89±24.60	57.18±20.20
Superoxide dismutase U/mg protein oxidised/min/ mg protein	14.66±11.87	15.30 ±14.06	22.84±18.05	20.70±19.81

=P<0.05 comparison between controls and cancers

CONCLUSION

The results of the present study are contradictory to the suggested hypothesis, due to nonsignificant results when patients were compared with controls and follow up cases. Therefore assigning the role of free radical generator as the mode of action to withaferin in human cancers subjected to RT ,is uncertain. More relevant studies need to be undertaken before arriving at a conclusion.

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