Brief Original Article

Modulation of apoptosis against *P. falciparum* by low dose radiation in human PBMCs

Sompal Singh[1,2], Ramesh C. Dhiman[2] and Aditya P. Dash[2]

[1]*Radiation Biosciences, Institute of Nuclear Medicine & Allied Sciences (DRDO), Delhi, India*
[2]*National Institute of Malaria Research (ICMR), New Delhi, India*

Abstract

Background: Implications of low dose radiation (LDR) have been well reported in cancer therapy but data is scanty on the therapeutic application of LDR in infectious diseases.
Methodology: Human peripheral blood mononuclear cells (PBMCs) were cultured and exposed to 0.07 Gy. *P. falciparum* infected RBCs were mixed with the PBMCs after five hours of irradiation. Thereafter, PBMCs were monitored for micronuclei and apoptosis.
Results: The low dose pre-irradiated PBMCs which were subsequently challenged with parasite, showed a reduction in micronuclei frequency and apoptosis as compared to controls.
Conclusion: LDR inhibited apoptosis against *P. falciparum* in human PBMCs.

Key words: Low dose radiation, micronuclei, apoptosis, *P. Falciparum*

J Infect Dev Ctries 2009; 3(11):869-872.

(Received 23 June 2009 – Accepted 28 October 2009)

Introduction

Infection with malaria parasite occurs with a disturbance of immune homeostasis. It is associated with severe immune mediated pathology due to excessive inflammatory responses [1]. Malaria specific antibodies mediate inhibition of parasite cytoadherence, inhibition of erythrocyte invasion, and antibody dependent cytotoxicity [2]. Cell-mediated immune effector mechanisms include macrophage activation by IFN-γ to kill parasitized erythrocytes and inhibit parasite growth [3]. However, Malaria parasites have evolved to acquire diverse immune evasion mechanisms which are critical for the ability of the parasite to evade the host immune response. Depletion of lymphocyte population is one of the important factors of immuno-suppression which may be due to apoptosis. Parasite exotoxins and soluble S-antigen are released when infected RBCs rupture. These toxins may be responsible for the reduction in the number of lymphocytes during the malaria infection. Excessive damage in the DNA results in the cell death by apoptosis. Micronucleus is the DNA segment which becomes separated from the genome and acts as a biomarker of DNA damage. A direct relationship has been found between the occurrence of micronuclei and apoptotic cells [4].

The most lethal form of malaria caused by *Plasmodium falciparum* poses a major problem in treatment because of increasing resistance to antimalarial drugs. Of various alternatives available for the control of parasitic/bacterial diseases, LDR has been found to be beneficial. Some studies on the therapeutic application of LDR in non cancerous diseases such as diabetes, arthritis and infectious diseases are also available [5-7]. Furthermore, it is also known that LDR exposure reduces apoptosis in human PBMCs. Circulating PBMCs primarily include T cells and monocytes, which play defensive roles against plasmodium infection. Therefore, we planned to test the hypothesis whether LDR plays any role in the modulation of apoptosis in healthy human PBMCs against *P. falciparum*.

Materials and methods

Human PBMCs (1×10^6 cells/ml) from healthy volunteers (fellow scientific workers who consented to provide 5 ml peripheral blood, aged 20-30 yrs, non-smokers and no medical history within the last one month) were cultured in RPMI-1640 with 25 mM HEPES supplemented with 10%

Table 1. Micronuclei frequency in human PBMCs challenged with *P. falciparum* after low dose ^{60}Co-γ-irradiation

Donor	uRBCs		iRBCs		% decrease in micronuclei frequency
	(0 Gy)	(0.07Gy)	(0 Gy)	(0.07Gy)	
1	20	13	38	28	*26.3%
2	22	15	48	30	*37.5%
3	12	13	21	22	-
4	12	12	27	27	-
5	14	15	29	20	*31.0%
6	12	15	40	33	*17.5%
7	18	19	48	36	*25.0%
8	15	16	36	27	*25.0%
9	8	10	29	22	*24.1%
10	16	16	37	28	*24.3%

% decrease in micronuclei frequency in PBMCs challenged with *P. falciparum* (iRBCs) = (MN_{0Gy} - $MN_{0.07Gy}$) × 100/MN_{0Gy}. PBMCs were stained with Hoechst 33342 and total 1,000 cells were scored per sample at 400X magnification under fluorescent microscope. * indicates significant ($p < 0.05$) decrease in the number of micronuclei per 1,000 cells in low-dose irradiated cells with respect to their respective controls. (Gy- Gray; uRBC-uninfected red blood cell; iRBC-infected red blood cell)

human AB^+ heat inactivated serum, 1 mg/ml glucose, 40 μg/ml of gentamycin sulfate and 2 mg/ml sodium bicarbonate. These cells were stimulated with 10 μg/ml phytohemagglutinin. Cells were irradiated with a low dose (0.07 Gy) of ^{60}Co-γ-rays (45 rad/min) after 22 hours of culture initiation. After five hours of irradiation, routine culture of *P. falciparum* strain MRC2 (NIMR, India) containing trophoziotes and schizonts [1×10^6 parasite (iRBCs)/ml] was added to PBMCs culture and fresh human RBCs were also added accordingly [8]. The cultures were maintained in flat-bottom 96-well culture plates and incubated in 5% CO_2 at 37°C. Student's t-test was used for statistical analysis of the data.

Results

Cultured PBMCs were monitored for the micronuclei frequency and apoptosis. Micronuclei frequency was significantly ($p < 0.05$) enhanced in PBMCs challenged with *P. falciparum* without pre-irradiation with LDR in all donors (Table 1). On the contrary, the level of micronuclei frequency was reduced (17.5-37.5%) in the LDR pre-exposed PBMCs cultured with *P. falciparum* (except in donors 3 and 4) as compared to their controls (Table 1). In a similar fashion, low dose pre-irradiation drastically reduced (25.6-68.2%) apoptosis in PBMCs of eight donors subsequently challenged with *P. falciparum* as compared to non pre-irradiated PBMCs (Table 2). The parasite induced the apoptosis more than two-fold in PBMCs which were not pre-exposed with a low dose of radiation. No effect of LDR was observed in the PBMCs of donors 3 and 4.

Discussion

Reduction in micronuclei frequency is the marker of enhanced DNA repair in the cells. In our earlier findings, induction in DNA repair was reported in 0.07 Gy exposed human PBMCs subsequently treated with 5.0 Gy of radiation [9]. Here also, low dose pre-irradiation reduced micronuclei in PBMCs challenged with *P. falciparum* as compared to the controls. Therefore, low dose pre-irradiation might be responsible for enhanced DNA repair in the PBMCs cultured with a parasite. The correlation between micronuclei frequency and apoptosis has been reported as directly proportional; *i.e.,* under conditions of reduced apoptosis, micronucleus formation was also reduced [4]. Induction of apoptosis has been observed during *P. falciparum* infection in human lymphocytes, endothelial cells, hepatocytes and neurons [10-12]. It may also be the cause of lymphopaenia which occurs in the course of malaria because acute malaria infection causes a depletion of lymphocyte populations in the peripheral blood [13,4]. However, Riccio *et al.* suggested that there is no correlation of apoptosis with lymphopaenia in the progression of malaria [14]. Interestingly, low dose pre-irradiation reduced apoptosis in the PBMCs challenged with parasite as compared to non-irradiated PBMCs as shown in Table 2. This reduction in apoptosis by LDR against parasite suggests a positive effect of LDR in the therapy of malaria. In the absence of LDR, induction in micronuclei and apoptosis appeared in PBMCs cultured with parasite (Tables 1 and 2). Two donors (3 and 4) showed no effect of LDR on micronuclei frequency and apoptotic cells in the presence of *P. falciparum*. This variation among the donors may be due to the variability of LDR response in the different individuals because it is a genetically governed phenomenon [15].

LDR activates T cells, NK cells, and B cells, resulting in activation of the immune system and reduction in apoptotic cells induced by high dose radiation in healthy individuals [16-18]. At the molecular level, expression of key proteins involved in the process of apoptosis with LDR exposed human PBMCs is also being studied in our laboratory. Reduced expression of pro-apoptotic proteins and induction of pro-survival proteins was observed in low dose radio-adapted human PBMCs (data not

Table 2. Modulation of apoptosis in LDR pre-exposed human PBMCs *P. falciparum*

Donor	uRBCs		iRBCs		% decrease in apoptosis
	(0 Gy)	(0.07Gy)	(0 Gy)	(0.07Gy)	
1	18	15	42	23	*45.2%
2	16	12	54	24	*55.5%
3	11	11	23	24	
4	10	12	29	30	
5	9	8	32	22	*31.2%
6	6	6	26	16	*38.4%
7	14	13	35	21	*40.0%
8	12	12	34	25	*26.4%
9	11	10	39	29	*25.6%
10	15	13	41	28	*68.2%

% decrease in apoptosis in PBMCs challenged with *P. falciparum* (iRBCs) = ($apoptosis_{0Gy}$ - $apoptosis_{0.07Gy}$) × 100/$apoptosis_{0Gy}$. PBMCs were stained with Hoechst 33342 and total 1000 cells were scored per sample at 400X magnification under fluorescent microscope. *indicates significant ($p<0.05$) decrease in the number of micronuclei per 1000 cells in low dose irradiated cells with respect to their respective controls.

shown). In another study, low dose UVA-induced suppression of cutaneous lesions has been reported in mice infected with *Leishmania* [19,7]. These studies also reported that suppression of cutaneous development was due to the systemic and upregulation of IFNγ and TNFα. Thus, the present study provides a clue for the therapeutic efficiency of LDR against malaria infection.

Conclusion

LDR-induced suppression of apoptosis in human PBMCs cultured with *P. falciparum* has relevance in the restoration of immune function in malaria patients. Further experimental studies are needed on various model systems infected with parasites to ascertain the usefulness of LDR.

Acknowledgements

The authors wish to acknowledge the Director, Dr. R. P. Tripathi, (INMAS, Delhi) for providing the radiation facility. This work was financially supported by the UGC through a fellowship grant to the first author. The support provided by Dr. C. R. Pillai, Emeritus Scientist, ICMR and Dr. Vinita Singh, NIMR is also acknowledged.

References

1. Schofield and Grau GE (2005) Immunological processes in malaria pathogenesis. Nat Rev Immunol 5: 722-735.
2. Artavanis-Tsakonas K, Tongren JE, Riley EM (2003) The war between the malaria parasite and the immune system: immunity, immunoregulation and immunopathology. Clin Exp Immunol 133: 145-152.
3. Tsuji M, Zavala F (2003) T cells as mediators of protective immunity against liver stages of *Plasmodium*. Trends Parasitol 19: 88-93.
4. Vukicevic V, Kampfinger K, Stopper H (2004) Influence of altered apoptosis in human lymphoblastoid cell lines on micronucleus frequency. Toxicol Lett 147: 187-95.
5. Wang GJ, Li XK, Sakai K, Cai L (2008) Low-dose radiation and its clinical implications: diabetes. Hum Exp Toxicol 27: 135-142.
6. Nakatsukasa H, Tsukimoto M, Ohshima Y, Tago F, Masada A, Kojima S 2008) Suppressing effect of low-dose gamma-ray irradiation on collagen-induced arthritis. J Radiat Res 49: 381-389.
7. Khaskhely NM, Maruno M, Uezato H, Takamiyagi A, Ramzi ST, Al-Kasem KM, Kariya K, Toda T, Hashiguchi Y, Gomez Landires EA, Nonaka S (2002) Low-dose UVB contributes to host resistance against *Leishmania amazonensis* infection in mice through induction of gamma interferon and tumor necrosis factor alpha cytokines. Clin Diagn Lab Immunol 9: 677-686.
8. Artavanis-Tsakonas K, Riley E M (2002) Innate immune response to malaria: rapid induction of IFN-γ from human NK cells by live *Plasmodium falciparum*-infected erythrocytes. J Immunol 169: 2956-2963.
9. Singh S, Bala M, Kumar R, Kumar A, Dhiman SC (2009) Modification in the expression of Mre11/Rad50/Nbs1 complex in low dose irradiated human lymphocytes. Dose Response 7: 193-207.
10. Kassa D, Petros B, Mesele T, Hailu E, Wolday D (2006) Characterization of peripheral blood lymphocyte subsets in patients with acute *Plasmodium falciparum* and *P. vivax* malaria infections at Wonji Sugar Estate, Ethiopia. Clin Vaccine Immunol 13: 376-379.
11. Mukherjee P, Devi YS, Chauhan VS (2008) Blood stage malaria antigens induce different activation-induced cell death programs in splenic CD4 (+) T cells. Parasite Immunol 30: 497-514.
12. Toure FS, Ouwe-Missi-Oukem-Boyer O, Bisvigou U, Moussa O, Rogier C, Pino P, Mazier D, Bisser S (2008) Apoptosis: a potential triggering mechanism of neurological manifestation in *Plasmodium falciparum* malaria. Parasite Immunol 30: 47-51.
13. Lisse IM, Aaby P, Whittle H, Knudsen K (1994) A community study of T lymphocyte subsets and malaria parasitaemia. Trans R Soc Trop Med Hyg 88: 709-710.
14. Riccio EK, Junior IN, Riccio LR, Corte-Real S, Morgado M, Daniel-Ribeiro CT, de Fatima Ferreira-da-Cruz M (2003) Malaria associated apoptosis is not significantly correlated with either parasitemia or the number of previous malaria attacks. Parasite Immunol 90: 9-18.
15. Correa CR, Cheung VG (2004) Genetic variation in radiation-induced expression phenotypes. Am J Hum Genet 75: 885-890.
16. Pandey R, Shankar BS, Sharma D, Sainis KB (2005) Low dose radiation unduced immunomodulation: effect on macrophages and CD8+ T cells. Int J Radiat Biol 81: 801-812.
17. Kojima S, Ishida H, Takahashi M, Yamaoka K (2002) Elevation of glutathione induced by low-dose gamma rays and its involvement in increased natural killer activity. Radiat Res 157: 275-280.
18. Shigematsu A, Adachi Y, Koike-Kiriyama N, Suzuki Y, Iwasaki M, Koike Y Nakano K, Mukaide H, Imamura M, Ikehara S (2007) Effects of low-dose irradiation on enhancement of immunity by dendritic cells. J Radiat Res 48: 51-55.

19. Khaskhely NM, Maruno M, Takamiyagi A, Uezato H, Kasem KM, Hosokawa A, Kariya K, Hashiguchi Y, Landires EA, Nonaka S (2001) Pre-exposure with low-dose UVA suppresses lesion development and enhances Th1 response in BALB/c mice infected with *Leishmania* (Leishmania) *amazonensis*. J Dermatol Sci 26: 217-232.

Corresponding Author
Sompal Singh, MSc
National Institute of Malaria Research (ICMR)
Sector-8, Dwarka, New Delhi-100 077 (India)
Phone: +91-11-25364701
Fax: +91-11-27604279
E-mail: somuatar@gmail.com

Conflict of Interest: No conflict of interest is declared.