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EVALUATION OF GAMMA-STERILIZATION (^{60} CO) BY RT-PCR BY DHFR EXPRESSION DETECTION

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ABSTRACT

The improvement of techniques to detect pathogen agents in blood had reduced significantly the contamination mechanisms by hemocomponents in blood transfusion procedures. Ionizing radiation is a method that has presented several applications on medicine and in currently days has been showing special attention on blood banks which has been applied to avoid TA-GVHD development. DHFR is an enzyme constitutive in *Plasmodium* protozoa and has an important role in folate metabolism on these parasites. Detecting the expression of RNAm coder for this enzyme is possible to evaluate the viability of this parasite in blood samples. *Plasmodium chabaudi* AJ is a parasite that induces lethal malaria in rodents similar to human malaria In this work, the objective was to detect the presence of plasmodium protozoa in irradiated blood samples, infected experimentally, through the application of a RT-PCR using primers for the coder sequence of DHFR´s mRNA. We studied doses of ionizing radiation between 0 and 75 Gy. The irradiation procedures were accomplished in Center of Radiation Technology of IPEN-CNEN in a ^{60}Co panoramic source. Our results had demonstrated that RT-PCR is a sensible method to evaluate the viability of plasmodium in blood samples because the technique could detect low parasite burden in all tested samples.

1. INTRODUCTION

Blood and hemocomponent transfusion envolve procedures that have several applications on medicine due to its prescription in different pathological conditions as clotting disturbs, anemia treatment and blood volume reposition in individual cases caused by accident [1]. There is the possibility of developing immunological reactions, like the lethal form of the transfusion associated graft versus host disease [2]. Considering the possibility that several pathogens can be vehiculated by celular components in blood, can be concluded that transmission of diseases is the main risk of blood transfusion procedures [3].

The improvement of diagnosis methods for different pathogens and the application of these techniques for trial in donation process had reduced significantly disease transmission by blood transfusion. Malaria is a disease induced by *Plasmodium spp*, a protozoa that can be vehiculated by erythrocytes. There are several cases that had been demonstrated in literature about the contamination of malaria by blood transfusion. Even so this disease presents major quantity of cases in tropical regions, it can occur in the regions where there are possible of presence of human imigration from endemic areas.

Available sorologic tests frequently have low sensibility in detection of malaric infections, due this fact there are few tests avaiable for its detection what becomes Giemsa stained blood film analysis, the standard test to diagnosis malaria. Indirect immunoflorescence (IFI) is the gold standard in *Plamosdium* detection in blood, but the implementation of the required protocol can be very expensive, specially at the wild environments in whre malaria is endemic, mainly at tropical rain-forest zones.

DHFR (Dihidrofolate-reductase) is a *Plasmodium* constitutive enzyme [4] that has an important activity in folate metabolism. The detection of its expression could be a viable tool to evaluate the viability of this parasite in blood samples.

2. MATERIALS AND METHODS

2.1. Mice and Parasites

All procedures were performed according to principles of animal welfare elsewhere described [5]. Male six-week old C57Bl/6j mice obtained from Centro de Bioterismo da Fac.Medicina da Universidade de São Paulo – USP and maintained at our own facilities. Cryoperserved (N_2) erythrocitic forms of *Plasmodium chabaudi* AJ were offered from Dr. David Walliker (Univ. of California). Test groups of mice were inoculated with 10^6 parasitized red blood cells.

Parasitaemia of infected mice were determined by counting of intraerythrocitic parasites in Giemsa-stained thin films, until reaching 4% of total red blood cells. Mice were sacrificed by respiratory anaesthesia (CO_2) and blood was treated with anticoagulant (Adenin-Citrate-Dextrose, ACD) and mantained in conical tubes in ice until radiation exposure.

2.2.Irradiation procedures

Blood samples were irradiated at 0, 25, 50 and 75Gy doses without attenuators in a panoramic source using ^{60}Co as isotope (Yoshizawa Kiko Co.) at the Centro de Tecnologia das Radiações (IPEN/CNEN – SP), using dose-rate of 11,24 kGy/h. Tubes were placed in ice during the procedure. Aliquotes of samples were immediately inoculated in groups of 10 mice to perform bioassays.

2.3. Parasitaemia assessment and RT-PCR

Parasitaemia of irradiated and sham-irradiated mice was assessed daily from 3rd to 10th days post-irradiation. On same days, one mice from each group were sacrificed as described and blood was collected by cardiac puncture and conserved in TriZOL® at -80°C until extraction of total RNA. Extracted mRNA was reverse-transcribed using M-MuLV reverse transcriptase and Oligo $d(T)_{12-18}$ (Invitrogen). The cDNA libraries obtained by this method were used as templates in PCR reactions using specific primers to amplify the DHFR expressed region. β-actin amplification was used as control. Amplified products were resolved in silver-stained 6% poliacrylamide gels. Sensivity was assessed to be 0,13 pg of total cDNA (~ 0,03 parasites/μL of extracted total RNA) (data not shown).

3. RESULTS

Parasitized red-blood cells (RBC) counts were shown in Figures 1, 2 and 3.

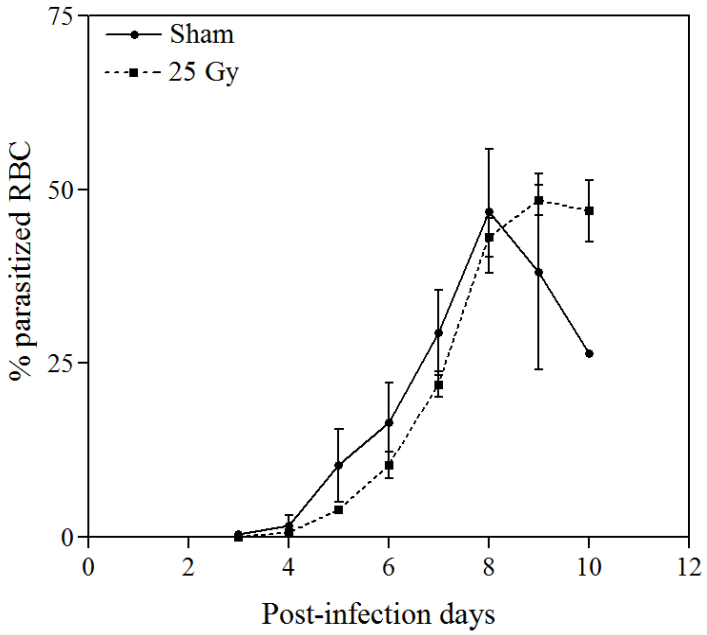

Figure 1. Parasitized red blood cell in peripheral blood of mice inoculated with blood irradiated at 25Gy, comparing with sham-group.

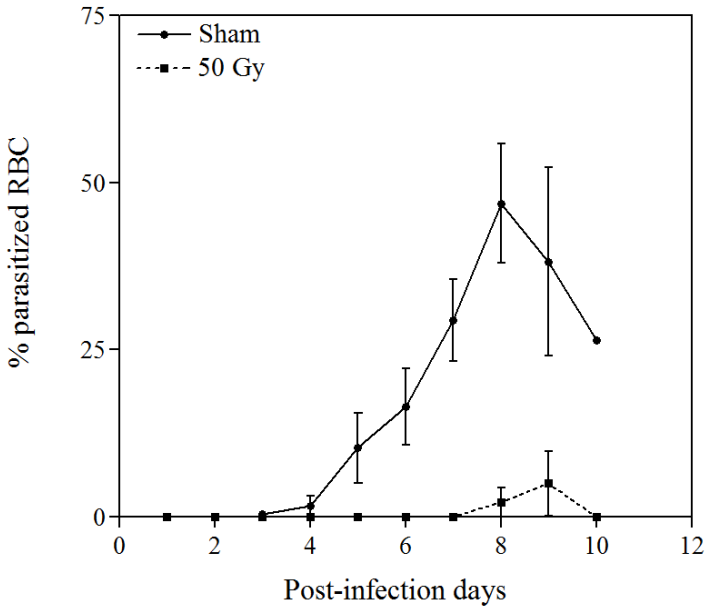

Figure 2. Parasitized red blood cell in peripheral blood of mice inoculated with blood irradiated at 50Gy, comparing with sham-group.

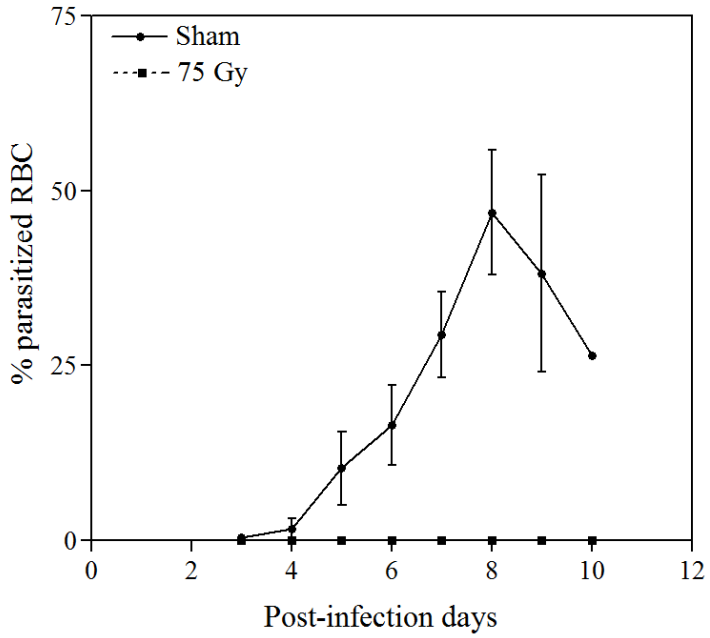

Figure 3. Parasitized red blood cell in peripheral blood of mice inoculated with blood irradiated at 75Gy, comparing with sham-group.

Electrophoresis of RT-PCR amplified products of irradiated blood samples were shown in Figure 4.

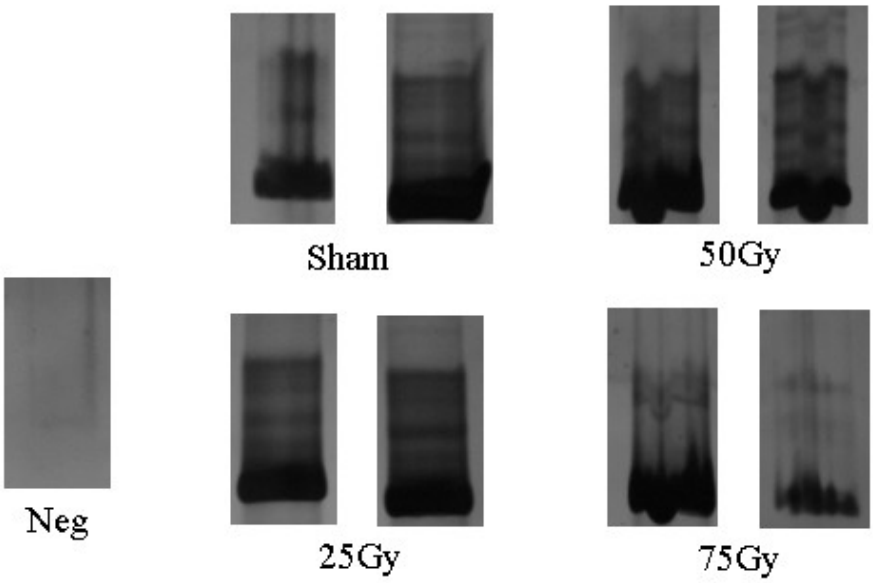

Figure 4. RT-PCR of blood samples irradiated at test doses. Neg shows non-infected sample.

Blood samples irradiated at 25Gy developed parasitaemia curve similar to infected-sham-irradiated mice. At 50Gy, parasites were observed only after 7th post-infection, and at 75 Gy no parasites were detected in analysed slides. Altough these data, all samples showed DHFR amplification.

4. CONCLUSIONS

RT-PCR of DHFR has proved to be an efficient method to detect parasite activity in blood samples. In spite of the cost (higher than blood films), it may be an important aid to prevent transfusion of malaria-infected blood. At the studied doses, ionizing radiation treatment was not effective, what may lead to test higher doses until the 200Gy limit [6], when integrity of RBC is impaired.

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