

RESEARCH NOTE

Inactivation of *Plasmodium falciparum* Parasites Using γ -irradiation

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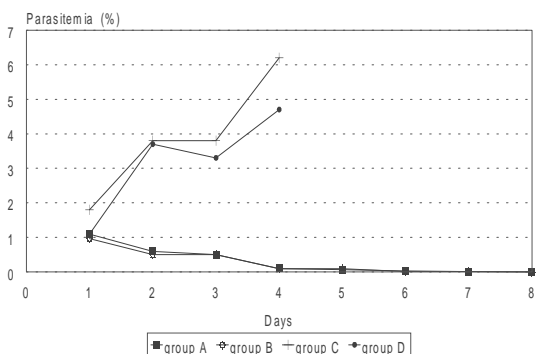
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The high and relatively stable number of malaria cases in endemic areas shows us continuously how difficult is to control malaria. In addition, besides the mosquito bites, the transmission can also occur through transfusion of infected blood, fact that concerns particularly, but not exclusively, blood Banks in malaria endemic areas. In these centers, to the worry of preventing transfusional malaria cases, is added the preoccupation of avoiding the lose of a high number of donors during the screening procedures. These facts denote the need of alternative methodologies - for the selection of blood to be used in transfusion - that could offer fast, cheap and safe results. Irradiation of blood stage *Plasmodium falciparum* parasite had mainly been performed in order to attenuate *Plasmodia* for preparing malaria vaccines but no attention has been given regarding its use with prophylactic purposes (S Waki et al. 1983 *Exper Parasitol* 56: 339-345; S Waki et al. 1985 *Z Parasitenkd* 71: 213-218). Therefore, to determine the dose of γ -irradiation which would produce attenuated parasites without affecting the erythrocyte stability was the ultimate goal of this study.

For this purpose red blood cells (RBC) parasitized with the Palo Alto *P. falciparum* strain were asynchronously cultivated in RPMI 1640 medium enriched with 10% of normal human serum at 37°C in relatively anaerobic conditions (W Trager & JB Jensen 1976 *Science* 193: 673-675). This strain of *P. falciparum* was chosen in view of its adaptability to *Saimiri* sp. monkeys in our breeding conditions. For each experiment four Petri plaques (100x15 mm) containing 1 ml of RBC and 11 ml of RPMI medium - in order to obtain a 8% hematocrit - were utilized. From these, two contained 3-5% of parasitized RBC, and two were filled with nonparasitized RBC as controls. The contents of each plate was divided in two tubes, being one of them submitted to γ -ray irradiation while nonirradiated ones were maintained at exactly the same locale conditions. The γ -irradiation was performed at the National Cancer Institute (INCA) of Rio de Janeiro, Brazil using a Cobalt-60 deep therapy apparatus at a source to mid-sample distance of 40 cm. The tubes were placed in the γ -ray source and irradiated at room temperature with a constant dose rate of 2 gray/min (1 gray /Gy = 100 rad). Parasitized and nonparasitized RBC were exposed to γ -irradiation at 10000, 15000 and 20000 CGy. After irradiation the following crossings were realized before exposure to culture: A) irradiated parasitized RBC plus irradiated nonparasitized RBC; B) irradiated parasitized RBC plus nonparasitized RBC; C) parasitized RBC plus irradiated nonparasitized RBC; and D) parasitized RBC plus nonparasitized RBC. The sensitivity of *P. falciparum* to γ -irradiation was measured by daily parasitemia calculation, as previously described (MF Ferreira-da-Cruz et al. 1995 *Parasite* 2: 23-29).

Similar results were observed with both 10000 and 15000 CGy irradiations: groups A and B showed a reduction of parasitemia till days 6 (group A) or 7 (group B) when viable forms arised followed by an increase of parasitemia levels reaching 4.5% (group A) or 3.5% (group B) around day 11. As expected, in control groups (C and D) the parasitemia developed reaching around 7% at day 2 for both groups. In the 20000 CGy irradiation experiment it was possible to observe a reduction of parasitemia levels beginning at day 2 for both groups (A and B). Around day 4, the microscopic examination was negative and no parasites were detected till day 11. Control groups showed standard growth curves (Fig.). The hemoglobin profile, evaluated by electrophoresis, and the initial and complete osmotic fragility tests (Bronstein Laboratory, Rio de Janeiro, Brazil) did not reveal any alteration in irradiated RBC that presented the same profile of non irradiated ones.

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Growth parasitemia of *Plasmodium falciparum* parasites submitted (groups A and B) or not submitted (C and D) to 20000 CGy of gamma-irradiation, cultured in normal (B and D) or irradiated (A and C) red blood cells.

These experiments provide evidences that a 20000 CGy γ -irradiation is able to abolish the original replication of erythrocytic forms of the Palo Alto *P. falciparum* strain, probably by inactivating their infectivity. According to our data 10000

or 15000 Cgy irradiation doses were enable to inactivate the parasite - despite the reduction of parasitemia - suggesting the existence of heterogenous plasmodial populations as concerns susceptibility to irradiation and pointing to the possibility that the same dose could inactivate parasites from other *P. falciparum* strains. In view of the nonexistence of defense immune mechanisms in the *in vitro* culture, it is reasonable to suppose that the dose required to abolish infectivity in these conditions is higher than that expected to inactivate the parasite *in vivo*, fact that increases the safety of the proposed dose of irradiation. Nevertheless, studies of the effects of γ -irradiation on the *in vivo* infectivity of *P. falciparum* in non human primates are necessary to clarify this question. If proven to be safe, this methodology could rescue blood bags eventually contaminated with *P. falciparum* - with low costs and no risk of inducing transfusional malaria - and help solving the problem of the chronic deficit of blood in malaria endemic areas.

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