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Effect of gamma irradiation on cytokines released by platelets during storage

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ABSTRACT

Most published gamma irradiation studies are on the cytokines secreted by leucocytes than platelet cytokines. If cytokines secretion is suppressed by irradiation then it will be an important application of irradiation. To know this we planned to measure cytokines like Regulated upon activation normal T cells expressed and secreted (RANTES), platelet factor 4 (PF-4), Transforming growth factor βeta1 (TGF-β) and βeta Thromboglobulin (β TG) during the storage of irradiated (IR) and non-irradiated (NI) platelets. Ten platelet concentrate (PCs) were prepared using platelet rich plasma and buffy coat methods each and 10 by apheresis on Trima Cell separator. Each PC was transferred in a transfer bag and IR at about 25 Gy and stored at 22 °C in platelet agitator. Samples were taken from NI and IR bag each on 0 day, 3rd day and 5th day from supernatant for cytokine analysis. The samples were stored below −50 °C for cytokines assays using commercial ELISA kits. RANTES levels were in the range of 12–400 ng/ml on 0 day and increased to 108–800 ng/ml on 5th day in NI platelets and 104–800 ng/ml in IR platelet. PF4 increased from 0 day to 5th day showing levels 300–1500 ng/ml in both types of PC. β-TG range was 748–5258 ng/ml in NI and 878 –4638 ng/ml in IR platelets on 5th day. TGF-β1 increased up to 780–38431 pg/ml in NI and 461–50,000 pg/ml in IR PC. The study showed that comparison of cytokine levels during the storage in NI and IR platelets was not significant by Mann–Whitney U- test ($p > 0.05$). Significant increase was observed in these cytokine levels from 0 day to 3rd and 5th day in NI as well as IR samples by Wilcoxon Signed- Rank test ($p < 0.05$).

1. Introduction

Gamma irradiation of 25 Gy to cellular components of human blood is essential to prevent transfusion associated graft versus host disease (GVHD) (Pelsynski, Moroff, Luban, Taylor, & Quinonoes, 1994). Platelets do not contain nucleus and therefore host no DNA, platelets do inherit a genome in the form of messenger RNA. They contain numerous specialized organelles including α and dense granules, lysosomes,

Abbreviations: RANTES, Regulated upon activation normal T cells expressed and secreted; PF-4, platelet factor 4; TGF-β, Transforming growth factor βeta; β TG, βeta Thromboglobulin; PC, platelet concentrate; IR, irradiated; NI, non-irradiated; GVHD, graft versus host disease; PRP, platelet rich plasma; BC, Buffy coat; DAE, Department of atomic energy; BRNS, Board of research in nuclear sciences; BARC, Bhabha Atomic research centre; SDP, Single donor platelet.

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microperoxisomes and mitochondria (Mc Redmond et al., 2004). In a recent study of 5 Gy and 7 Gy irradiation damage mechanism on ultrastructures of platelets in rat cells there was profound destruction at the cell membrane because of the free radicals formed by ionizing irradiation. There was cytoplasmic outflow and organelle damage where the platelet granules were reduced markedly (Ji, Dong, & Chung, 2012). It is possible that if the granules are reduced the release of cytokines may also be suppressed by irradiation. If so then it will be an important application of irradiation. Therefore this study was undertaken and it will be interesting and important to know if there is any suppression of cytokine secretion by platelets after irradiation by measuring levels of cytokines like RANTES, PF4, TGF-β and β-TG in NI and IR platelets.

2. Material and methods

2.1. Sample collection

The study was approved by the institutional ethics committee. It was funded by the BRNS, (DAE). Gamma irradiator, BI 2000 was purchased from Board of radiation and isotope technology, (DAE), Mumbai and regulatory permissions were obtained from atomic energy regulatory board.

After selection of blood donor as per Drugs and cosmetics rules 1945, the blood was collected in triple or quadruple bags containing Citrate–Phosphate–Dextrose-A1 from donors attending blood donation camps and donating in-house (Saran, 2003). PCs were prepared within six hours of blood collection.

2.2. Preparation of PC

Two types of PC were prepared namely PRP-PC and BC-PC using two different principles for separation. Ten PRP-PC were prepared from triple bags by centrifuging the bags at 20–22 °C at light spin and then heavy spin. PC was left undisturbed for one hour, and then the platelets in plasma were resuspended by gently mixing. Platelets were stored at 20–22 °C in platelet incubator with agitator (Benson et al., 1996). BC method was employed for preparation of 10 PCs using 'top and bottom' bags on Optipress II component extractor of M/S Fenwal (Bharucha, Chiewsilp, & Bhasin, 2002). Ten SDP were prepared using cell separator Trima (Gambro Bct) and stored at 20–22 °C.

2.3. Irradiation

All PCs were divided into two parts. NI part was named as "A" and IR as "B". Part B was put in the sample chamber of blood irradiator BI-2000 in inverted position, so no tubing was protruded outside the sample chamber and irradiated at about 25 Gy, dose rate 6.9 Gy/min (irradiated for 3 min 37 s).Co-60 is the source of radiation in BI2000. Five ml PC sample of A and B parts was collected on 0, 3 and 5th day aseptically and after centrifugation, one ml aliquots were prepared and stored below −50 °C for measurement of cytokines. Before assay the samples were thawed at room temperature and centrifuged at 3000 rpm for 5 min. Cytokine analysis was done using ELISA kits for RANTES (Human CCL5-R & D Systems), PF-4 (Abcam), β-TG (USCAN Life Science Inc.) and TGF-β1 (eBioscence Inc.) on Fully automated ELISA system 'Freedom evo' (TECAN)

2.4. Statistical analysis

In this study Median, minimum, maximum and Non-parametric tests like Mann–Whitney and Wilcoxon signed rank test were used because the data obtained were not normally distributed (Mann & Whitney, 1947; Fay & Proschan, 2010).

3. Results

Total 30 units including 10 each of PRP-PC, BC-PC and SDP were taken in this study. RANTES, PF-4, β-TG, and TGF-β1 were measured from NI and IR PCs at 0, 3 and 5th days interval. The data of three types of PC were pooled for presentation of results.

Table 1 shows median levels of RANTES on different days of storage. The level on 0 day was below 70 ng/ml in half of the samples in both groups and five samples showed the values above 200 ng/ml in NI and seven in IR. The levels gradually increased from 0 day to fifth day and no sample was below 100 ng/ml and 50% of the samples were above 350 ng/ml reaching maximum to 800 ng/ml.

Table 2 shows median PF4 on different days of storage. On 0 day it was below 400 ng/ml in half of the samples in both groups and three samples showed the values above 700 ng/ml. The levels gradually increased from 0 day to fifth day and no sample was below 300 pg/ml and > than 50% of the samples were above 1300 ng/ml reaching maximum to 1500 ng/ml.

Table 3 shows median β-TG on different days of storage. β-TG level on 0 day was below 650 ng/ml in half of the samples in NI and ten in IR. Three samples showed the values around 2000 ng/ml in NI and around 1700 ng/ml in IR. The levels gradually increased from 0 day to fifth day and no sample was <700 ng/ml and >50% of the samples were above 2000 ng/ml reaching maximum to 5000 ng/ml.

Table 4 shows median TGF-β1 on different days of storage TGF-β1 level on 0 day in more than half of the samples in NI group were above 2000 pg/ml reaching maximum to 11,500 pg/ml. In IR group 20 samples were below 2000 pg/ml and maximum was 5400 pg/ml. The levels gradually increased from 0 day to fifth day and no sample was below 780 ng/ml and > than 50% of the samples were above 5000 pg/ml reaching maximum to 38,000 pg/ml in NI group and up to

Table 1 – Median RANTES levels in NI and IR PC on different days of storage.

Platelet concentrate	RANTES (ng/ml)		
	0 Day	3rd Day	5th Day
Non irradiated n = 30	67 (12–400)	319 (88–800)	382 (108–800)
Irradiated n = 30	88 (13–400)	296 (86–800)	383 (114–800)

Figures in the parenthesis show min–max values.