Study of damage to red blood cells exposed to different doses of γ-ray irradiation

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Background. The aims of this research were to study alterations in the ultrastructure of red blood cells, the changes in concentrations of plasma electrolytes and the killing effect of lymphocytes in samples of blood exposed to different doses of γ-ray irradiation.

Materials and methods. Blood samples were treated with different doses of γ-ray irradiation and then preserved for different periods. Specimens were prepared for standard electron microscopy and transmission electron microscopy. At the same time, changes in the concentrations of Na^+, K^+ and Cl^- and pH values in the plasma as well as Fas and FasL expression of lymphocytes before and after irradiation were determined.

Results. The proportions of reversibly and irreversibly transformed cells, for example, echinocytes, sphero-echinocytes, and degenerated forms, increased with increasing doses of irradiation and storage period, while the number of discocyte shaped red blood cells decreased. The change in K^+ concentration was greater than that of Na^+ or Cl^- after irradiation and was dosage-dependent. Plasma pH was influenced by different doses of radiation and storage time. After exposure to ^{137}Cs γ-irradiation, the expression of both Fas and FasL in lymphocytes differed significantly from that in the control group: the expression was positively correlated with irradiation dose (r=0.95, 0.96), but no significant difference in the Fas/FasL ratio was observed ($P>0.05$).

Discussion. We conclude that the ultrastructure of red blood cells is not changed obviously by irradiation with some doses of γ-rays and various periods of storage. However, irradiation does have some dose-dependent and time-dependent adverse effects on the erythrocytes.

Keywords: red blood cells, γ-rays, irradiation, ultrastructure.

Introduction

As early as the 1970s, there was already research on the irradiation of blood products. γ-ray irradiation of blood is now considered the main way to prevent transfusion-associated Graft-versus-Host disease. Numerous studies on the irradiation of red blood cells have been reported, but with conflicting results[1-8]. These studies have focused on cellular immunity, lymphocyte-killing effect, red blood cell activity (ATP), function (2,3-DPG) and fragility, free haemoglobin level, concentrations of potassium ions (K^+), sodium ions (Na^+), and chloride ions (Cl^-), etc. Generally, the studies indicated that although there was some damage to the red blood cells after irradiation and that the damage was positively correlated with irradiation dose, the impact on the activity and function of red blood cells was not massive. Although γ-ray irradiation of red blood cells is commonly performed, little information is available on the effects of irradiation on cell ultrastructure and expression of Fas and FasL on lymphocytes.

Normal cell morphology is the key to the maintenance of cellular functions. Any changes of cell structure will impair or cause loss of cell function. Therefore, by observing variations in red blood cell morphology and plasma electrolyte concentrations, the extent of damage of erythrocytes irradiated by ^{137}Cs γ-irradiation can be inferred.

At present, there are no unified international standards for the radiation dose to use for blood. In the USA, the Food and Drug Administration requires a central dose of 25 Gy and a minimum dose of 15 Gy to

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other points in the container. In the United Kingdom, a minimum dose of 25 Gy is recommended[5]. It would, therefore, be useful to determine the best radiation dose and causes of damage to red blood cells, including the effects of irradiation on the activity and function of erythrocytes and the effects of storage time on the irradiated blood. We, therefore, investigated the changes in ultrastructure of red blood cells, electrolytes and pH stored for different periods after irradiation with different doses of ^{137}Cs γ-irradiation.

Materials and methods

Sample collection

The leucocyte-reduced red blood cell units were provided by Ningbo Blood Centre. These units were discarded blood units with unqualified alanine transaminase levels within a week of collection. ACD-B additive solution was used for storage. The ages of the ten male and ten female blood donors ranged from 18 to 55 years. None of blood donors had a history of diabetes, severe hypertension, or other diseases which could significantly influence the rheological properties of the donated blood. The total volume of blood was 400 mL for each test, with samples of 50 mL used for each irradiation dose. The volume of the experimental samples was 5 mL.

Sample processing

The leucocyte-reduced red blood cells were stored for 28 days under standard blood banking conditions (2-6 °C). Samples were blinded by randomising and serial numbering. The 400 mL units were divided into seven aliquots of 50 mL labelled group A, B, C, D, E, F and G and then irradiated by a blood irradiator (Gamma Cell/3000 Elan, Canada MDS Nordion, Ottawa, Canada) at the doses of 0 Gy (not irradiated), 10 Gy, 15 Gy, 25 Gy, 35 Gy, 45 Gy and 55 Gy, respectively.

The radionuclide used for irradiation was caesium-137 chloride. Total activity: set of one C-3001 sources. The radiation source intensity was the total activity of ^{137}Cs radioactive sources: 1377Ci (50.9TBq) (1 November 2006). The absorbed dose rate was calculated to be 4.9 Gy/min (28 November 2006); the overall uncertainty of the absorbed dose rate measurement was ±2.5% at the 95% confidence level.

Samples were then taken from each group (group A to G) on days 0, 7, 14, 21 and 28 after irradiation for the ultrastructural studies and measurements of ions, pH, Fas and FasL.

Preparation of samples for scanning electron microscopy

Each group of blood samples was irradiated with different doses of γ-rays, before taking 50 μL specimens from each group. These blood specimens were fixed in phosphate-buffered (pH 7.2-7.4) 3% glutaraldehyde (purchased from Sinopharm Chemical Reagent Co. Ltd., Shanghai, China) for 2 hours (at 4 °C), washed three times in phosphate buffer (4 °C, pH 7.2-7.4), fixed in 1% osmic acid (purchased from Ted Pella Inc., Redding, California, USA) for 1-2 hours, washed three times in phosphate buffer (4 °C, pH 7.2-7.4), dehydrated in a graded alcohol series (30%, 50%, 70%, 80%, 90% and 100 %), fixed in tert-butyl alcohol (3:1, purchased from Sinopharm Chemical Reagent Co. Ltd.) and frozen at −40 °C. The specimens were freeze-dried with a freeze dryer (Model ES-2030, Japan Hitachi, Tokyo, Japan) and covered with a layer of gold palladium[9] (Carbon Coating Unit, E-1010/E-0635, Japan Hitachi), before undergoing scanning electron microscopy analysis.

The scanning electron microscope (S-3400N, Japan Hitachi) had an acceleration voltage of 10 kV for observations. First, we browsed the entire sample plate in the 400x zoom, then randomly selected regions with moderate cell density and amplified these regions 1,000-4,000 times. The percentages of the different morphological forms of red blood cells were determined by counting 1,000 cells in the randomly chosen fields.

The different cell shapes were identified using Bessis's classification[10]. Red blood cells that take the form of echinocytes and stomatocytes are capable of returning to the discocyte shape under certain conditions. These red blood cell shape changes are considered potentially reversible transformations. In contrast, red blood cells assuming the shapes of sphero-echinocytes, spherostomatocytes, spherocytes, and ovalocytes and degenerated forms are irreversibly changed cells.

Preparation of samples for transmission electron microscopy

The groups of blood samples were irradiated by different doses of γ-rays. Next, a 50 μL specimen of blood was taken from each sample. The specimens were fixed in phosphate buffered (pH 7.2-7.4) 3% glutaraldehyde (purchased from Sinopharm

Chemical Reagent Co. Ltd.) for 2 hours (4 °C), washed three times in phosphate buffer (4 °C, pH 7.2-7.4), fixed in 1% osmic acid (purchased from Ted Pella Inc.) for 1-2 hours, washed three times in phosphate buffer (4 °C, pH 7.2-7.4), dehydrated in a graded alcohol series (30%, 50%, 70% and 90%), and further dehydrated in graded acetone (90% and 100%) (purchased from Sinopharm Chemical Reagent Co. Ltd.), washed three times in absolute acetone, infiltrated by Low Viscosity Embedding Media (Spurr's Kit, Catalogue #14300, purchased from Electron Microscope Science, Hatfield, Western Massachusetts, USA, consisting of ERL 4221, DER 736, NSA and DMAE) 1:1 and 2:1, left overnight, embedded in pure Spurr's mixture and polymerised at 70 °C for 8 hours. Ultrathin sections were cut and stained. The percentages of discocytes, echinocytes, stomatocytes, sphero-echinocytes, spherostomatocytes, and spherocytes were determined by counting 1,000 cells in randomly chosen fields.

Observation conditions: transmission electron microscope (H-7650, Japan Hitachi), acceleration voltage: 60 kV, magnification, 4,000x to 8,000x.

Measurements of K^+, Na^+ and Cl^- and pH

We used an electrolyte analyser (AFT-500, Hangzhou Kangli high technology Co. Ltd., Hangzhou, China) and corresponding diagnostic kits to determine the concentrations of K^+, Na^+ and Cl^-, and pH values. The procedures were performed according to the manufacturer's instructions.

Measurement of Fas and FasL expression

Samples were exposed to ^{137}Cs irradiation at doses of 0 Gy, 15 Gy, 25 Gy, 30 Gy, 35 Gy, 45Gy or 55 Gy. The irradiation was uniform (sample placed in the middle of the blood cup centre plane position). Irradiated 4.5 mL specimens from each group were treated with purified lymphocyte cell separation medium (Axis-Shield, Liverpool, UK). The lymphocytes acquired by flow cytometry for the analysis were suspended at a concentration of $3x10^6$ cells/mL and the levels of expression of Fas and FasL were measured using a BD FACSCalibur flow cytometer and fluorescein isothiocyanate (FITC)-labelled antibodies (Becton Dickinson Medical Devices Co. Ltd. New Jersey, USA).

Statistical analysis

Data were analysed using SPSS 10.0. Changes in K^+, Na^+ and Cl^- ion concentrations were assessed by two-way analysis of variance (ANOVA, F-test). The inter-group factor was irradiation storage duration (days), and the intra-group factor was irradiation doses, the effect was erythrocyte morphological changes (the proportion of different forms of red blood cells). F-test analysis was used to evaluate the intra-group factor, while multi-group χ^2 analysis was used to evaluate the inter-group factor. The differences were considered statistically significant at P values <0.05.

Results

Changes in the concentrations of K^+, Na^+, and Cl^-

The results are shown in Table I. In whole blood plasma without irradiation which was stored from 7 to 28 days, the concentration of K^+ increased from 4.78 mmol/L to 13.50 mmol/L. In samples irradiated with 10-55 Gy and stored for 7 to 28 days, the concentration of K^+ increased from 5.16 mmol/L to 28.60 mmol/L, more rapidly than in the control group. In particular, in samples irradiated at 55 Gy and stored for 28 days, the concentration of K^+ rose quickly to 28.6 mmol/L, much higher than in the control group (F-test, P<0.01). The K^+ concentration differed significantly between samples stored for different periods (F-test, P<0.01). In contrast, the concentration of Na^+ decreased from 146.88 mmol/L to 143.11 mmol/L in the control group, and from 147.18 mmol/L to 139.68 mmol/L in the group of samples irradiated with 55 Gy. The rate of decrease in the experimental group was significantly greater than that in the control group (F-test, P<0.01). Differences were statistically significant between the groups treated with different doses of radiation. The change in K^+ concentration was more significant than the changes in Na^+ and Cl^-; the changes were dose-related.

Changes in pH

As blood yields acids in the process of metabolism, its pH changes (Table I). The pH values of the control group samples ranged from 6.32 to 6.39, while those of the irradiated samples ranged from 6.39 to 7.07. Differences between the groups of samples irradiated with different doses and the groups of samples stored for different periods were statistically significant (F-test, P<0.01). It was obvious that the pH of blood was influenced by both irradiation dose and storage time.

Table I - The changes of K^+, Na^+, Cl^- and pH in red blood cell units irradiated by different doses*.

Time (day)	Index	Group (doses, Gy)						
		A (0)	B (10)	C (15)	D (25)	E (35)	F (45)	G (55)
0	K^+	4.78*	5.16	5.08	5.13	4.90	5.15	4.88
	Na^+	146.88	147.13	145.07	146.82	147.16	146.32	147.18
	Cl^-	74.06	74.80	74.39	74.28	75.25	75.28	75.05
	pH	6.39	6.82	7.07	6.87	6.78	6.85	6.67
7th	K^+	6.10	8.36	9.08	11.33	13.95	14.85	15.32
	Na^+	145.88	147.10	145.01	142.82	145.16	146.02	146.16
	Cl^-	75.09	76.86	76.59	75.28	76.85	78.39	77.25
	pH	6.37	6.80	6.96	6.77	6.74	6.85	6.67
14th	K^+	7.60	12.74	14.42	17.74	20.85	22.93	23.54
	Na^+	144.71	145.53	142.17	140.79	142.88	144.49	144.06
	Cl^-	76.25	77.91	77.91	78.61	80.11	80.79	80.33
	pH	6.35	6.79	6.76	6.70	6.72	6.64	6.53
21st	K^+	10.25	18.57	18.36	19.35	20.89	23.64	27.60
	Na^+	143.52	144.39	141.82	138.67	139.04	142.10	141.96
	Cl^-	77.43	81.14	78.41	79.89	82.14	81.67	82.92
	pH	6.34	6.65	6.41	6.52	6.55	6.53	6.52
28th	K^+	13.50	21.00	21.47	23.81	25.99	27.29	28.60
	Na^+	143.11	144.32	140.61	137.44	136.60	140.39	139.68
	Cl^-	78.14	85.03	82.91	81.89	82.67	82.40	83.98
	pH	6.32	6.46	6.39	6.50	6.51	6.53	6.51

Legend: *The values were the mean of 20 cases.
K^+, Na^+, Cl^- unit: mmol/L; K^+ ANOVA: $F_{Group}=12.28$, $F_{0.01(5,24)}=3.90$, $P<0.01$: $F_{Time}=57.78$, $F_{0.01(7,24)}=3.50$, $P<0.01$; Na^+ ANOVA: $F_{Group}=7.65$, $F0.01_{0.01(5,24)}=3.90$, $P<0.01$: FTime=32.15, $F_{0.01(7,24)}=3.50$, $P<0.01$; Cl^- ANOVA: $F_{Group}=5.88$, $F0.01_{0.01(5,24)}=3.90$, $P<0.01$: F Time=49.10, $F_{0.01(7,24)}=3.50$, $P<0.01$; pH ANOVA: $F_{Group}=8.00$, $F0.01_{0.01(5,24)}=3.90$, $P<0.01$: F Time=14.00, $F_{0.01(7,24)}=3.50$, $P<0.01$.

Electron microscope observation

The normal red blood cells in the viewing field were bi-concave disc-shaped cells with diameters ranging from 7-8 μm, a thickness of 1-2 μm, and a smooth surface. On days 0 and 21 of storage of non-irradiated blood, discocytes were the predominant type of cell (Table II), accompanied by only a few irreversibly changed red blood cells (Figures 1, 2, 3, 4, 5, 6). By the 28th day of the storage, the proportion of normally shaped discocytes had decreased significantly from 88.0% to 52.4%, and the proportions of reversibly and irreversibly changed red blood cells had significantly increased. The percentage of reversibly changed red blood cells had increased from 8.5% to 32.2% and the percentage of irreversibly changed red blood cells had increased from 3.5% to 15.4% (Table II). As the duration of storage extended from 7 days to 28 days, the number of normal erythrocytes was statistically reduced (Table II). According to F-tests, the proportion of different forms of erythrocytes was preserved. The results on the 7th and 14th days were statistically significantly different ($F=27.19$, $F_{0.01\ (3,10)}=7.56$, $P<0.01$ for the 7th day and $F=10.27$, $F_{0.01\ (3,10)}=7.56$, $P<0.01$ for the 14th day). However, the results on the 21st and 28th days were not statistically significant different ($F=2.33$, $F_{0.05\ (2,10)}=4.10$, $P>0.05$ for the 21st day and $F=0.85$, $F_{0.05\ (2,10)}=4.10$, $P>0.05$ for the 28th day. It was clear that the duration of storage strongly affected red blood cell morphology.

At the end of storage, the majority of red blood cells were echinocytes, sphero-echinocytes, stomatocytes, spherostomatocytes, spherocytes, ovalocytes, and degenerated shapes. (Figures 1, 2, 3, 4, 5, 6). The number of abnormally shaped red blood cells continued to increase as the storage period prolonged and by the 28th day of storage, the percentage of abnormally shaped red blood cells reached 100% in the group of samples irradiated with 55 Gy.

When the erythrocytes were irradiated with 35 Gy and stored for 21 days, the proportions of normal cells, reversibly changed red blood cells and irreversibly changed red blood cells were 52%, 31% and 17% respectively, which were close to those of non-irradiated erythrocytes stored for 28 days, for which the corresponding proportions were 52%, 32% and 16% respectively.

Table II - Changes of red blood cell shape, irradiated by different doses.

Irradiation Doses (Gy)	Day of storage	Proportion ratio of different red blood cell (RBC) shapes (%)			
		Discocyte	**Reversibly changed RBC**	**Irreversibly changed RBC**	**P Value**
0#	0	88.0±2.6	8.5±1.6	3.5±1.5	
	7th	86.1±2.9	11.3±1.7	2.6±1.6	F=27.65
	14th	73.3±3.1	18.4±1.9	8.3±1.8	$F_{0.01(2,8)}$=8.65
	21st	57.5±3.6	29.1±2.6	13.4±2.3	P <0.01
	28th	52.4±4.2	32.2±3.7	15.4±3.1	
10	7th	85.6±2.7	10.1±1.7	4.3±1.6	
	14th	70.5±3.0	20.2±1.8	9.3±1.8	F=11.46
	21st	60.7±3.5	29.6±2.7	9.7±2.5	$F_{0.01(2,6)}$=10.92; P <0.01
	28th	41.2±4.0	39.1±3.6	19.7±3.0	
15	7th	81.0±2.7	12.1±1.8	6.9±1.7	
	14th	62.9±3.0	25.7±2.1	11.4±2.0	F=7.02
	21st	55.3±3.7	30.4±2.8	14.3±2.6	$F_{0.05(2,6)}$=5.14; P <0.05
	28th	34.0±4.1	43.4±3.5	22.6±3.2	
25	7th	77.1±2.8	16.3±1.9	6.6±1.8	
	14th	61.9±3.1	25.5±2.0	12.6±2.1	F=6.19
	21st	52.6±3.8	31.1±3.0	16.3±2.7	$F_{0.05(2,6)}$=5.14; P <0.05
	28th	31.5±4.2	45.6±3.6	22.9±3.4	
35	7th	75.0±3.0	15.3±2.0	9.7±1.7	
	14th	60.2±3.3	24.6±2.6	15.2±2.3	F=2.94
	21st	50.8±3.8	31.4±3.1	17.8±3.0	$F_{0.05(2,6)}$=5.14; P >0.05
	28th	21.0±4.3	52.5±3.8	26.5±3.3	
45	7th	58.1±3.1	25.6±2.0	16.3±1.9	
	14th	44.3±3.5	30.2±2.8	25.5±2.5	F=0.33
	21st	40.0±3.7	26.6±3.2	33.4±3.1	$F_{0.05(2,6)}$=5.14; P >0.05
	28th	8.5±4.0	20.1±4.1	71.4±4.5	
55	7th	42.2±3.5	22.3±2.7	35.5±2.8	
	14th	30.9±3.8	23.2±3.0	45.9±3.5	F=4.16
	21st	17.0±4.0	21.6±3.5	61.4±4.0	$F_{0.05(2,6)}$=5.14; P >0.05
	28th	0	15.6±4.0	84.4±5.0	

Legend: *The values were mean ± standard deviation (SD), based on 1,000 erythocytes from 20 samples. The results were obtained from comprehensive analyses by transmission and scanning electron microscopy.
#The erythrocytes before ^{137}Cs irradiation, control group; 7th day: F=27.19, $F_{0.01(2,10)}$=7.56, P<0.01: 14th day: F=10.27, $F_{0.01(2,10)}$=7.56, P<0.01; 21st day: F=2.33, $F_{0.01(2,10)}$=4.10, P>0.05; 28th day: F=0.85, $F_{0.01(2,10)}$=4.10, P>0.05.

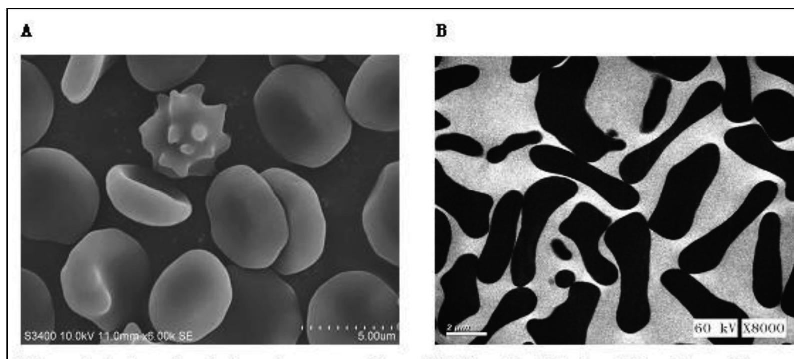

Figure 1 - A. Scanning electron microscope picture of red blood cells subjected to 15 Gy irradiation, after 7 days of storage. Original magnification 6,000x. The dominant cells are discocytes and only a few irreversibly changed red blood cells can be seen.
B. Transmission electron microscope picture of red blood cells. Original magnification 8,000x.

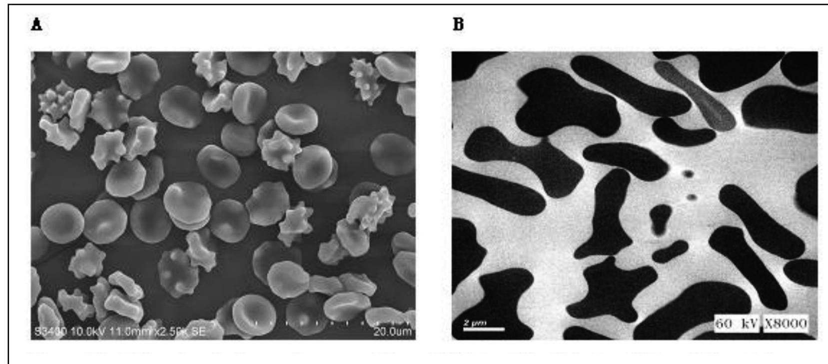

Figure 2 - A. Scanning electron microscope picture of red blood cells subjected to 35 Gy irradiation, after 21 days of storage. Original magnification 2,500x. Numerous echinocytes and sphero-echinocytes can be seen.
B. Transmission electron microscope picture of red blood cells. Original magnification 8,000x.

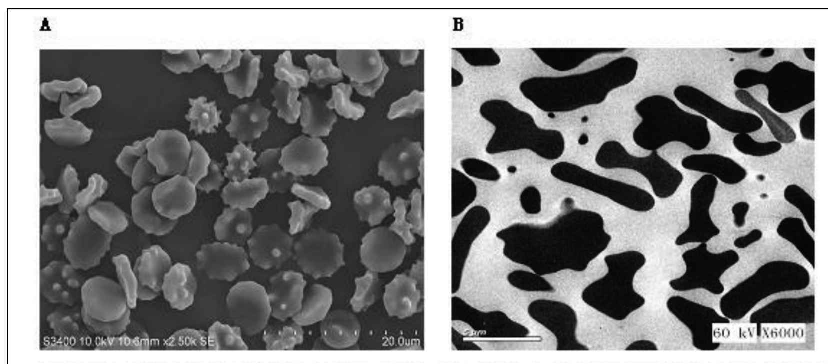

Figure 3 - A. Scanning electron microscope picture of red blood cells subjected to 45 Gy irradiation, after 21 days of storage. Original magnification 2,500x. Sphero-echinocytes and degenerated forms dominate among irreversibly changed cells.
B. Transmission electron microscope picture of red blood cells. Original magnification 6,000x.

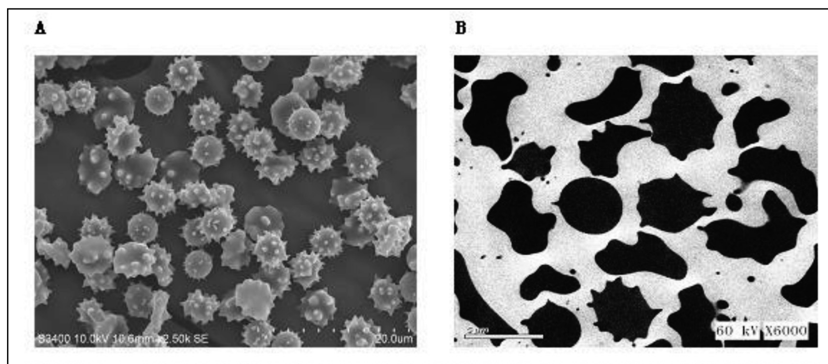

Figure 4 - A. Scanning electron microscope picture of red blood cells subjected to 55 Gy irradiation, after 28 days of storage. Original magnification 2,500x. Almost all cells are irreversibly changed cells.
B. Transmission electron microscope picture of red blood cells. Original magnification 6,000x.

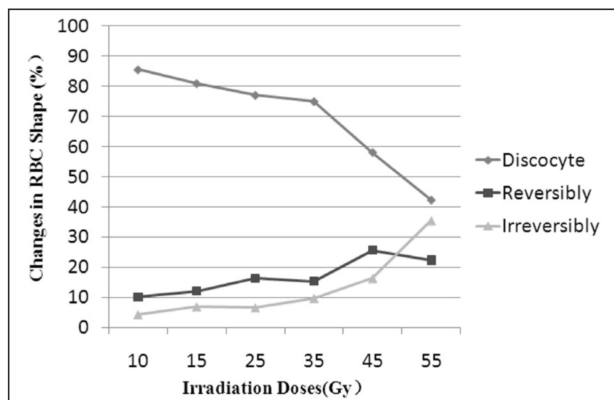

Figure 5 - Changes in red blood cell (RBC) shape after irradiation and storage for 7 days. Reversibly: reversibly changed RBC, Irreversibly: irreversibly changed RBC.

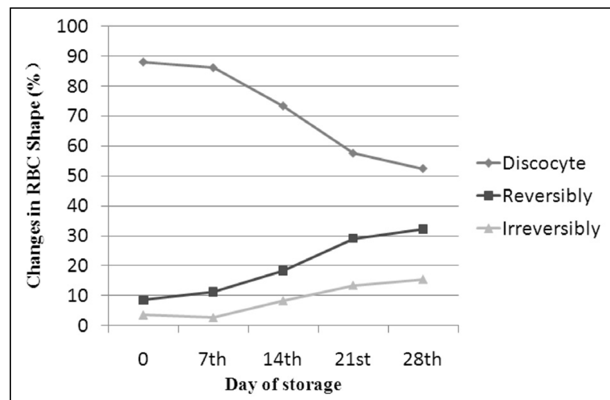

Figure 6 - Changes in red blood cell (RBC) shape without irradiation. Reversibly: reversibly changed RBC, Irreversibly: irreversibly changed RBC.

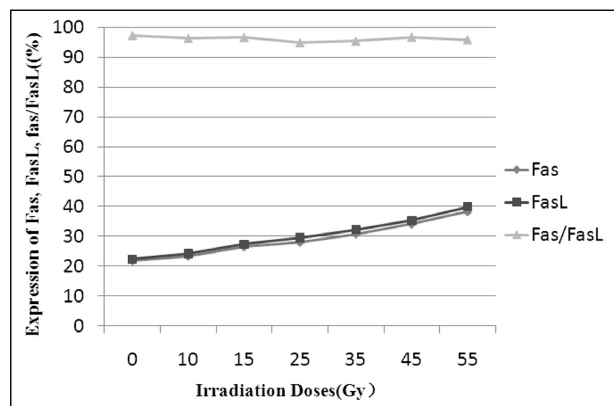

Figure 7 - Line diagram of the expression of Fas, FasL and Fas/FasL.

Table III - Fas and FasL protein expression of lymphocytes in blood irradiated by 137Cs γ-rays.

Group (Gy)	Fas (%, x±s)	FasL (%, x±s)	Fas/FasL (%, x±s)
0	21.75±2.12*	22.34±2.34	97±0.91
10	23.29±2.50	24.16±1.28	96±1.95
15	26.42±2.11	27.34±3.01	96±0.70
25	27.97±2.88	29.46±5.07	96±0.59
35	30.78±3.34	32.23±4.07	95±0.82
45	34.11±2.67	35.25±4.93	96±0.68
55	38.29±3.18	39.89±3.83	96±0.73
F test	P<0.05	P<0.05	P>0.05

*The values are mean ± standard deviation.

Fas and FasL expression

The levels of Fas and FasL expression of lymphocytes are shown in Table III and Figure 7. The levels of Fas and FasL expression of lymphocytes increased significantly after irradiation ($P<0.05$), and were positively correlated with the radiation doses ($r=0.95$, 0.96). The Fas/FasL ratio did not differ significantly between pre- and post-irradiation samples ($P>0.05$) and was not related to the dose of irradiation.

Discussion and conclusion

Red blood cells travel in the circulation for about 120 days and constantly change shape from a biconcave disc of 8 μm diameter to a cigar shape, which is able to cross through vessels as narrow as 1 μm in diameter[11].

In clinical practice, erythrocyte deformability is influenced by several factors, such as temperature, osmotic pressure, immunological factors, biological factors, K^+, Mg^{2+} and Ca^{2+} ions, composition and structural changes of the membrane and aging. Viewed by scanning and transmission electron microscopy, the cytoplasm of red blood cells appears homogeneous. Erythrocytes have cell membranes, but do not have either a nucleus or typical intracellular organelles. However, the structure of erythrocytes, evaluated by scanning electron microscopy and transmission electron microscopy, shows processes of different sizes unevenly distributed on the surface, the cells are not smooth and some may even be fragmented.

We studied the dose effect of irradiation on ultra-structural changes of erythrocytes and the changes of cell morphology and electrolytes during storage. In this study, we found that γ-ray irradiation could cause significant changes of Na^+, K^+ and Cl^- ion

concentrations in plasma, as well as alterations to erythrocyte morphology (Table I). Our study also showed that different doses of γ-irradiation could cause various degrees of damage to the erythrocyte membrane. The changes in K^+ concentration were more substantial than those of the Na^+ and Cl^- ions after irradiation and were dose-dependent. As shown in Table I, there was a regular downward trend of pH (F-test, $P<0.01$), and different irradiation doses and different storage times were both important factors.

After irradiation, the proportion of normal erythrocytes decreased, while that of abnormal ones increased. The abnormal erythrocytes were mainly echinocytes, sphero-echinocytes and degenerated shapes. (Table II and Figures 1, 2, 3, 4, 5, 6). The acanthocytes were characterised by an irregular surface with thorny-shaped projections. These cells are usually seen in subjects with liver disorders, haemolytic anaemia and acanthocytosis (70-80%). Normal erythrocytes may be transformed into acanthocytes in improperly prepared slices.

This study showed that the morphology of erythrocytes changed markedly after irradiation. As the dose of irradiation was increased, the proportions of echinocytes, sphero-echinocytes and erythrocytes with a degenerated shape increased, illustrating that the changes of ultra-structure are dose-dependent.

Electron microscopic studies showed a significant changes in red blood cell shape during blood storage. Simpson reported that red cells appear to change shape in response to alterations in their environment both *in vitro* and *in vivo*. Analysis of micrographs showed that the cells had changed shape. In the peripheral blood of healthy people, 52.8% erythrocytes are bi-concave, which is close to the percentage on the 21st day of storage (radiation dose 25-35 Gy)[12,13]. Tamara *et al.* examined stored erythrocytes by scanning electron microscopy and found a significant transformation of red blood cell shape during blood storage. The number of abnormally shaped red blood cells continued to increase as the storage period increased, and by the 42nd day of storage, the percentage of abnormally shaped red blood cells exceeded 76%[10].

After irradiation, the concentrations of free K^+ and Na^+ in plasma changed. The increase of free K^+ reflected the leakage of K^+ from erythrocytes. In this study the concentration of free K^+ in the plasma increased rapidly in 1 week after irradiation, reaching much higher levels than in the control group. This result shows that irradiation can cause membrane damage. After 21 days of storage (following 35 Gy irradiation), besides significant morphological changes, the concentration of K^+ increased from 4.78 mmol/L to 20.89 mmol/L. This value was higher than the normal physiological level, but within the national standard for the concentration of K^+ in whole blood (less than 21 mmol/L), so it was acceptable. In contrast, the concentration of Na^+ decreased to different degrees after irradiation. The gradual rise of the K^+ concentration in plasma was a reflection of excretion of the K^+ from red blood cells. This study showed that different doses of γ-ray irradiation cause varying degrees of damage to the red blood cell membrane.

We, therefore, inferred the irradiation-induced damage to erythrocytes from morphological changes. ^{137}Cs γ-ray irradiation can cause ultra-structural damage to erythrocytes, such as changes of protein structure, alterations of deformability, modifications of permeability and cell membrane rupture, which are manifested as morphological abnormalities, and significant changes of electrolyte concentrations and pH.

When the radiation dose was low and the storage time was short, erythrocytes were mainly normal in morphology, with a small percentage of echinocytes. As the radiation dose and storage period increased, more and more cells transformed into echinocytes, sphero-echinocytes and degenerated shaped cells. If the radiation dose was too high and the exposure time is too long, most cells became abnormally shaped. Scanning electron microscopy shows the cell surface structure and transmission electron microscopy reveals the cross-section of cell structure. In our experience of electron microscope photographs, the scanning electron microscopy photographs were clearer and more intuitively interpreted than the transmission electron microscopy ones.

The effect of irradiation is directly correlated with the dose. The selection of an appropriate dose of γ-ray irradiation that eliminates the risk of transfusion-associated Graft-versus-Host disease while preserving the quality of the transfused product remains a debated issue[14]. At present, there is no unified international standard. The American Association of Blood Banks recently recommended that "In regard to components, exposure of x-rays or gamma rays at a minimum

dose of 25 Gy targeting the central portion of the irradiation canister or irradiation field can prevent the proliferation of T lymphocytes"[15], the Council of Europe recommended 25-50 Gy[16], as did Britain[17].

The mechanism of action of irradiation has not been fully elucidated, although several authors have reported that ^{137}Cs γ-ray irradiation is able to induce apoptosis of lymphocytes. γ-irradiation can initially induce the expression of Fas and FasL as a defence strategy of the cells and, interestingly, T-cell lymphoblastic lymphomas exhibit a clear reduction of the expression levels of both proteins[18]. Lymphocyte apoptosis is regulated by a number of gene products which promote cell death or extend cell survival. Fas, a cell surface antigen, belongs to the tumour necrosis factor receptor/nerve growth factor receptor superfamily and mediates apoptosis in a wide variety of cell types after being cross-linked by Fas ligand (FasL), which is a type II transmembrane protein belonging to the tumour necrosis factor family[19]. γ-rays can up-regulate the expression of Fas and FasL on lymphocytes. When FasL binds with Fas, it can transmit a "death signal" to cells, and induce apoptosis of Fas-expressing cells within a few hours. The cells then lose their capacity for proliferation and differentiation, while immunological rejection and attacks are not possible *in vivo*, even when the lymphocytes are alive. γ-ray irradiation is, therefore, considered an effective prevention method in storing blood products. In this study, six different doses (10 Gy, 15 Gy, 25 Gy, 35 Gy, 45 Gy and 55 Gy) of γ-rays were applied, and the expression of Fas and FasL on lymphocytes in the red blood cell products was measured. The result shows that after exposure to ^{137}Cs γ-ray, the expression of Fas and FasL on lymphocytes was significantly different from that in the control group, and the levels of expression were positively correlated with the dose of irradiation (r=0.95, 0.96). However, there was no significant difference in the Fas/FasL ratio (P>0.05), and the differences between irradiated groups were not related to dose.

With the optimal irradiation dose, lymphocytes can be inactivated while other cells retain normal functions. According to our experimental results and the United States and Europe standards, we recommend 35 Gy as the dose of radiation and 21 days as storage duration (lymphocytes can be inactivated effectively and the normal functions of erythrocytes are retained).

We verified the harmful effect of ^{137}Cs γ-rays on erythrocytes based on a set of experiments. Further research is needed to reveal the toxicological mechanism and more experiments are also required to determine the safe ranges of irradiation intensity, exposure time and storage time after irradiation. These results indicate a need for a re-evaluation of the significance of studies using γ-ray irradiated erythrocytes and for investigation into the mechanisms by which erythrocyte shape is changed after irradiation of blood.

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