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EFFECT OF IONIZING RADIATION ON PLATELET FUNCTION IN VITRO

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The use of extracorporeal irradiation in the treatment of chronic lymphatic leukemia and for immunosuppression in organ transplantation underlines the importance of knowing the sensitivity of blood cells to radiation.

Contradictory data in the literature cloud the issue of the effects of ionizing radiation on platelet function. WERNER (1943) reported that direct exposure of platelets to ionizing radiation (12 Gy) led to impairment of clot retraction. On the contrary, HARTMAN & CONLEY (1953) showed that platelet-rich plasma exposed directly to a larger dose of roentgen rays (100 Gy) did not abolish clot retraction. Similarly, no conclusive results exist on the effect of ionizing radiation on other platelet function tests and no related report has appeared since 1966 (HOLLARD et coll.). Therefore, the effect of ionizing radiation on platelet function was investigated by analysing its effect on platelet aggregation, ADP release, clot retraction and platelet factor-3 availability.

Material and Methods

Preparation of platelet-rich plasma. Venous blood was drawn (into plastic syringes) from healthy volunteers who had not taken any medication for at least 2 weeks before blood donations. The blood was mixed with sodium citrate 3.2 per cent (9: 1 v/v). Siliconized or plastic tubes and pipettes were used throughout all experiments. In order to obtain platelet-rich plasma (PRP) or platelet-poor plasma (PPP) the blood-citrate mixture was centrifuged at 250×g for 15 min or 1 500×g for 15 min, respectively. The final concentration of platelets in the PRP was adjusted to 300×10^9/l for the assays of platelet aggregation, ADP release and platelet factor-3 availability and to 80×10^9/l for the clot retraction test, by diluting PRP with PPP.

Irradiation of platelets. The PRP and PPP were irradiated by a commercially available machine (Monopay, Siemens) operating at 60 kV and 8 mA with 0.2 mm Cu filtration. Low energy radiation was chosen in order to maximize the absorbed dose; the depth of platelet suspension to be irradiated on each plastic plate was only 5 to 10 mm.

PRP and PPP were transferred onto plastic plates (5 cm diameter) at room temperature and irradiated. The distance from the focus to the surface of platelet suspension was kept constant at 5 cm.

Common doses in radiation therapy, i.e. 1, 4, 10, 20 and 50 Gy, were used.

Platelet function was examined within 2 hours of irradiation of the samples. Platelet function tests were performed simultaneously on irradiated and non-irradiated (control) platelet plasma. Platelet counts were done using the method of BRECHER & CRONKITE (1950).

Platelet aggregation was assayed using an aggregation meter (EEL type 169) connected with a pen-recorder, as described by O'BRIEN (1962). Maximum aggregation was indicated by the maximum increase in light transmission which appeared within 5 min after addition of the aggregation

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Table 1

Platelet aggregation by ADP before and after irradiation in vitro, expressed as percentage (mean ± SD)

Dose (Gy)	ADP 1 μmol		ADP 2 μmol		ADP 4 μmol	
	Before	After	Before	After	Before	After
1	45.0 ±31.32	30.50±24.35	34.40±20.44	29.0 ±15.57	56.25±13.84	49.0 ±25.25
4	26.65±14.82	27.29±13.06	34.55±15.04	40.64±22.46	53.91±23.83	52.33±24.28
10	29.29±23.87	29.71±23.99	32.4 ±26.34	30.60±21.55	46.0 ±27.24	44.75±29.34
20	17.20± 9.42	22.6 ±17.94	39.20±26.57	38.80±24.15	39.67±29.13	40.33±11.50
50	21.0 ±11.49	21.25± 6.18	26.60± 5.86	27.20± 7.01	35.67±10.41	41.67±15.28

No statistically signficant difference from zero.

Table 2

Platelet aggregation by adrenaline before and after platelet irradiation in vitro, expressed as percentage (mean ± SD)

Dose (Gy)	Adrenaline 10 μg/ml	
	Before	After
1	62.0 ±12.22	70.35±12.72
4	59.25±19.43	66.88±15.83
10	60.75±14.08	65.50±10.79
20	43.50± 4.95	43.0 ± 7.07
50	40.26±26.90	40.40±25.85

No statistically significant difference from zero.

Table 3

Platelet aggregation by collagen before and after platelet irradiation in vitro, expressed as percentage (mean ± SD)

Dose (Gy)	Collagen 20 μg/ml	
	Before	After
1	76.8 ± 8.64	73.5 ± 9.8
4	84.25±11.03	80.75±14.39
10	69.8 ±11.78	60.60±14.76
20	68.20±43.0	68.60±17.43
50	54.50±11.84	60.75±19.36

No statistically significant difference from zero.

agent. For 0 and 100 per cent aggregation the transmission of light with PRP and PPP was measured.

Material used for aggregation was: (1) adenosine diphosphate (ADP, Sigma Chemical Company, St. Louis, USA) in final concentrations of 1 μmol, 2 μmol and 4 μmol, (2) adrenaline in a final concentration of 5 μg/ml, and (3) collagen (chemic Munchem collagen reagent 'Horm') in a final concentration of 20 μg/ml.

Platelet release of ADP was tested using the method described by WEISS (1967). Platelet factor-3 availability was estimated according to the method of SPAET & CITRON (1965).

Clot retraction was tested as follows: One ml of PRP at a final concentration of 80×10^9 platelets/l was placed into a siliconized tube containing a wire coil. Then, 0.1 ml of water soluble thrombin at a concentration of 600 units/ml was added and plasma clotting occurred in less than 10 seconds. After incubation for one hour in a water bath at 37°C, the coil with the retracted clot was removed and the remaining serum measured and expressed as a percentage of the initial volume of PRP.

All these assays of platelet function were made on 6 different volunteers for each radiation dose.

Results

The effects of ionizing radiation on platelet aggregation appear in Tables 1 to 3. The platelet aggregation was unaffected by doses of 1, 4, 10, 20 and 50 Gy. A minor decrease in ADP-induced platelet aggregation of PRP given low doses (1 Gy) was noted (Table 1) but was not statistically significant. Similarly, aggregation of platelets by adrenaline and collagen showed no statistically significant differences between irradiated and control platelet plasma.

No statistically significant differences in ADP platelet release, platelet factor-3 availability or clot retraction were observed between irradiated and non-irradiated platelet plasma at doses of 1 to 50 Gy

Table 4

Platelet aggregation by ADP release, following platelet aggregation by collagen. Results before and after irradiation in vitro (mean ± SD)

Dose (Gy)	Percentage of platelet aggregation	
	Before	After
1	8.75± 5.56	9.67±8.0
4	15.57± 5.13	15.67±5.51
10	23.8 ±13.24	19.40±3.97
20	8.1 ± 4.46	8.6 ±3.19
50	14.5 ± 7.12	15.0 ±6.13

No statistically significant difference from zero.

Table 5

Availability of platelet factor-3 before and after platelet irradiation in vitro, expressed as clot time (mean ± SD)

Dose (Gy)	Clot time (seconds)	
	Before	After
4	34.93±3.3	34.37±2.92
10	32.5 ±3.57	32.7 ±3.9
20	36.6 ±5.6	33.9 ±7.29
50	35.0 ±4.5	35.6 ±5.6

No statistically significant difference from zero.

Table 6

Clot retraction expressed as percentage of serum released from retracted clot, before and after platelet irradiation in vitro (mean ± SD)

Dose (Gy)	Per cent of serum	
	Before	After
4	60.32± 0.66	60.6 ± 1.18
10	66.79±31.25	73.03±12.00
20	63.0 ±16.78	60.88±12.62
50	70.18± 9.15	68.17±14.93

No statistically significant difference from zero.

(Tables 4–6). The analysis of the results was done with the paired t-tests and no statistically significant difference from zero was found (Tables 1–6).

Discussion

The first report on the effect of ionizing radiation on platelet function in vitro was published by WERNER. He showed that clot retraction was reduced after in vitro exposure to roentgen radiation of 12 Gy. By contrast, HARTMAN & CONLEY demonstrated no disturbance in clot retraction in platelet plasma irradiated with 100 Gy. A more recent and thorough investigation of the effect of roentgen irradiation on platelet function, in vitro, was reported by CAEN et coll. (1964, 1965) who analysed the effect of low doses of ionizing radiation (0.5–8 Gy) on platelet adhesion on glass, and clot retraction and platelet aggregation with small doses of ADP. They found that clot retraction and platelet adhesion on glass were unaffected by irradiation. However, a small and not statistically significant reduction of platelet aggregation was noticed. They also noted a spontaneous aggregation of platelets (without the presence of ADP) after irradiation of the platelet plasma with small doses of ionizing radiation (1–8 Gy). They postulated that reduced intracellular platelet ADP underlies their finding. Finally, HOLLARD et coll. using the thromboplastin generation test showed a reduction in availability of platelet factor-3 in irradiated platelets after very high doses of radiation (5 000–75 000 Gy).

In the present series, doses common in radiation therapy, ranging from 1 to 50 Gy, were used and more sensitive and specific platelet function tests were utilized. It was concluded that platelet aggregation was largely unaffected by doses of 1 to 50 Gy of ionizing radiation. This confirms the results of CAEN et coll. (1965) though they used lower doses (0.5–8 Gy). In the present experiments the spontaneous platelet aggregation described by CAEN et coll. was not reproduced. This result was not confirmed by using an aggregometer or an optical microscope.

The platelet ADP release test was found to be unaffected (statistically significant) for doses of 1 to 50 Gy and no previous report seems to exist on this.

No statistically significant difference between irradiated and non-irradiated platelet as measured by platelet clot retraction was found. This is in agreement with the report of HARTMAN & CONLEY though they used higher doses of radiation (100 Gy).

Similarly, platelet factor-3 availability was found

to be unaffected (statistically significant) for doses of 1 to 50 Gy. Disagreement of the result in the present investigation with that reported by HOLLARD et coll. can be explained by differences in methodology (thromboplastine generation test) and in radiation doses; they used very high doses of radiation (5 000–75 000 Gy).

The absence of measurable effects on platelet function in vitro suggests that extracorporeal irradiation possibly does not adversely affect platelet function in vivo and that observed disturbances in platelet function in cases of partial or whole-body irradiation are probably due to the effects of irradiating the platelet stem cells in the bone marrow, rather than a direct effect on the circulating platelets.

SUMMARY

The effect of ionizing radiation on platelet function was investigated in vitro. Platelet-rich plasma (300×10^9/l) was irradiated with doses of 1, 4, 10, 20 and 50 Gy. Platelet function tests were performed on both irradiated and control (non-irradiated) platelet samples. The platelet function tests were (1) platelet aggregation by ADP (1, 2, 4 μmol final concentration), adrenaline and collagen, (2) ADP-release from platelets, (3) clot retraction and (4) platelet factor-3 availability. It was found that roentgen irradiation of platelets in vitro did not affect these platelet function tests.

REFERENCES

BRECHER G. and CRONKITE E. P.: Morphology and enumeration of human blood platelets. J. appl. Physiol. 3 (1950), 365.

CAEN J., MICHAEL H., BOURDON R., VAINER H. et BERNARD S.: Effet des radiations in vitro sur les plaquettes sanguines. Nouv. Rev. franç. Hémat. 4 (1964), 324.

—— SINAKOS Z., COUSIN C., DUPERTUIS D., BOURDON R. et BERNARD S.: Effet des radiations in vitro sur les plaquettes sanguines d'homme et de rat. Nouv. Rev. franç. Hémat. 5 (1965), 780.

HARTMAN R. and CONLEY L.: Clot retraction as a measure of platelet function. Effects of certain experimental conditions on platelets in vitro. Bull. Johns Hopk. Hosp. 93 (1963), 355.

HOLLARD D., SERVOZ-GAVIN M. et DARNAULT J.: Effets de l'irradiation à hautes doses sur la fonction plaquettaire in vitro. Hémostase 6 (1966), 57.

O'BRIEN J. R.: Platelet aggregation. II. Some results from a new method of study. J. clin. Path. 15 (1962), 452.

SPAET T. H. and CITRON J.: Studies on platelet factor-3 availability. Brit. J. Haemat. 11 (1965), 269.

WEISS H. J.: Platelet aggregation, adhesion and adenosine diphosphate release in thrombopathia (platelet factor-3 deficiency). Amer. J. Med. 43 (1967), 570.

WERNER H.: Die spontane Retraktion des Blutkuchens. Klin. Wschr. 22 (1943), 414.