



IN VITRO ASSESSMENT OF GAMMA IRRADIATION EFFECTS ON REGULATORY T CELLS OF LYMPHOCYTE CULTURE IN LYMPHOPROLIFERATIVE DISEASES

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Purpose of research - to assess the sensitivity of regulatory T (Treg) cells of lymphocyte culture to gamma radiation in lymphoproliferative diseases by in vitro tests.

Materials and methods

Primary lymphocyte cultures from peripheral blood of 5 patients between 53 and 70 years of age were examined. Four samples were taken from patients with chronic lymphocytic leukaemia and one sample was taken from a patient with CD5-negative B-cell lymphoma. The cells were irradiated with 2 Gy, 4 Gy and 8 Gy at a dose rate of 0.98 Gy/min using a Rokus-AM gamma-ray machine for external beam irradiation. Lymphocytes were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum, gentamicin (400 mkg/ml) and 100 IU/ml of IL-2. Mononuclear cells were incubated at a mean concentration of $1.2 \cdot 10^9$ cells/l for 1, 3 and 5 days at 37°C. Treg cells were evaluated according to expression of membrane molecules CD45+CD4+CD25+CD127low/- (FACS Canto II, BD Biosciences). Non-irradiated lymphocytes served as a control group. For the comparison of arithmetic means, the single-factor dispersion analysis was performed using STATISTICA 8.0 software.

Main results

In the non-irradiated control group, 68% of Treg cells survived in cell culture over the 5-day observation period. Over the same time period, 48% of lymphocytes survived. When Treg cells and lymphocytes were exposed to a dose of 2 Gy, their survival rates decreased to 58% and 42%, respectively. Similar survival rates resulted from exposure to radiation doses of 4 Gy and 8 Gy. Thus, the viability of Treg cells was reduced to a lesser degree than that of lymphocytes and was, on average, 18% higher than within the general lymphocyte population (Table 1). These data suggested that Treg cells were more resistant to radiation. The analysis of dose-dependent changes in the percent and absolute numbers of Treg cells demonstrated that the percentage of Treg cells slightly increased with elevation of radiation dose meeting the threshold for statistical significance of 8 Gy (from $10.2 \pm 3.9\%$ of non-irradiated cells to $13.3 \pm 4.4\%$ of cells irradiated with 8 Gy; $p < 0.05$). There was no significant reduction of Treg cells due to a wide range of data in the subgroups (F3/55df = 0.17; $p = 0.91$), which pointed to heterogeneity in cell cultures regarding comparison parameters. The mean absolute number of Treg cells in all groups added up to $0.028 \cdot 10^9$ cells/L (25th percentile, 75th percentile – 0.008 and $0.044 \cdot 10^9$ cells/L, respectively, Table 2).

Table 1. Viability of lymphocytes and regulatory T-cells in the culture of non-irradiated lymphocytes (control) and irradiated at a dose of 2G, 4G, 8G.

№ п/п	Dose, Gy	Viability of lymphocytes , %	Viability of Treg cells , %
1	Initially, without irradiation (0 day)	100	100
2	0	48	68
3	2	42	58
4	4	37	55
5	8	36	53
6	Mean	41	59

Table 2. Relative and absolute number of regulatory T-cells in the culture of non-irradiated lymphocytes (control) and irradiated at a dose of 2G, 4G, 8 Gy

№ п/п	Dose, Gy	Regulatory T-cells, %		Regulatory T-cells, * 10^9 cells/l	
		M \pm σ	n	M \pm σ	n
1	0	10,2 \pm 3,9	15	0,031 \pm 0,027	15
2	2	11,4 \pm 5,2	15	0,026 \pm 0,032	15
3	4	12,3 \pm 3,7	15	0,025 \pm 0,025	15
4	8	13,3 \pm 4,4*	14	0,025 \pm 0,021	14
5	all group	11,8 \pm 4,4	59	0,028 \pm 0,026	59

Conclusion

During the specified cultivation period after irradiation with 2, 4 and 8 Gy, the viability of Treg cells appeared to be higher than that of the general lymphocyte population. Dose-response analysis of the absolute number of suppressor regulatory T lymphocytes did not reveal any significant changes in the range of delivered radiation doses. A small increase in their percentage occurred following irradiation with 8 Gy. The obtained data indicate that Treg cells are more radioresistant than other types of lymphocytes.

References

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