RAPORT ON THE PERFORMANCE OF THE RESEARCH PROJECT

‘Influence of magnetic field generated by ADR Protect stimulator on the activity on human immunocompetent cells *in vitro*’

The aim of the carried research was an assessment of ADR Protect stimulator on bases of immunotrophic influence of magnetic field, which is produced by stimulator, and, in case of estimation of such an influence, introductory description of its character and range.

The research was experimented due to commission of the producer of ADR-Protect stimulator in period of time between May-October 2001 in Immunological Laboratory of Microwave Protection Division of Military Institute of Hygiene and Epidemiology in Warsaw. It was based on previous establishments of the project and according to accepted range of experimental methods and schedule of research.

Materials and methods

The influence of ADR stimulator on the set of the culture of immunological cells was investigated in two variants of experiments:

1. Before running the cell cultures the culture liquid (RPMI 1640) was exposed to the field of ADR for 15 minutes and then it was used in the procedure of establishment and running of the cell culture. The results obtained from the control culture and the one containing liquid exposed to ADR were compared. There were 10 experiments based on this variant. Every time, for isolation and the culture of immunocompetent cells, peripheral blood of men of good health was used (blood donors in age between 20-26).
2. Two sets of immunological cell culture were set up in two culturing microplates (Nuncoln microplates). Beneath one of them, we placed correctly adjusted magnetic field stimulator ADR for the whole period of culturing (72 hrs). The results obtained from control plates and exposed ones were compared. There were 20 experiments carried on according to this variant. Every time, for isolation and the culture of immunocompetent cells, peripheral blood of men of good health was used (blood donors in age between 20-26).

The cultures of mononuclear cells isolated from the blood of people of good health (blood donors not showing any clinical symptoms of acute or chronical infectious diseases) were run according to the method described previously [1] with the later modification [2].

Every cell culture was run identically in three wells of plates and the final result was presented as a mean value of these three cultures.

Briefly: every time the experimental set consisted of two plates (exposed and control) containing identical microculture kits. Every microculture contained 10^5 of cells in 0.2 ml RPMI 1640 + 15% of inactivated autological serum. Proper triplets of culture were left without stimulation but were stimulated by phitohemaglutinin (PHA HA 16, Murex Biotech, 0.4 μg/culture) or by concanavalin A (Con A, Sigma.8 μg/culture). The cultures were incubated for 72 hours in incubator ASSAB in 37°C and in moist atmosphere with 5% of CO_2 in air. After 24-hour incubation, previously described changes

[1, 2] were made in proper microculture triplets. And 18 hours before the microcultures were due to halt they were marked with 3 H-timidine

(3HTdR, Ammersham, act. Spec. 2 Cu/mM) in dose of 0.4 μC/culture. After the finish of the culture, the measures of the integrated 3HTdR were carried out in scintillating meter Packard Tri-carb 2100 TR.

The mentioned set of culture allowed estimation of:

- spontaneous integration of 3THdR,
- proliferation response to PHA,
- response to Con A, (results were presented as mean of radioactive disintegration number – dpm – x 10^3/culture),
- lymphocyte T suppression activity index (SAT),
- IL-2 receptors saturation index,
- monokins (IL-1ra/IL-1β) influence on lymphocytes proliferation index (index LM).

Obtained results were statistically analysed with the use of parametric test *t* Student and non-parametric test, *twin pair test* by Wilcoxon. Individual differences between exposed to ADR samples and control samples were measured.

Results

Comparison of results from 10 experiments containing cultures run in control culture liquid (RPMI 1640) and in liquid previously exposed to field generated by ADR is presented in table 1 and 2.

In cultures run in medium exposed to field generated by ADR, higher values of spontaneous integration of 3THdR comparing to control cultures were observed (respectively 4.20±2.69 and 1.63±0.81 *10^3 dpm). The differences were statistically

significant, both with reference to mean values and to the number of experiments having result pointing out to the presence of differences. No significant changes in values describing reactivity of lymphocytes to mitogenes (PHA and Con A), P/C index; lymphocyte T suppression activity index (SAT) and degree of IL-2 receptors saturation were observed. Downward trend of LM index (index of lymphocyte integration with monokins) was observed, however, the differences were not statistically significant.

In table 3 and 4 the results of 20 experiments comparing the values obtained in cultures exposed to field generated by ADR during the whole culturing period and in control cultures were presented. In this set of experiments no statistically significant differences in values of investigated parameters were observed. Like in previous set of experiments, an upward trend of values of spontaneous integration of 3THdR and downward trend of LM index (index of lymphocytes integration with monokins) in cultures exposed to ADR influence were observed.

Conclusions

1. Application in human mononuclear blood cells culture of RPMI-1640 medium, exposed to magnetic field of ADR (15 minutes) before its use, causes an increase of spontaneous integration of 3THdR and decrease of LM index values (index of lymphocytes integration with monokins) comparing to control culture. Other assessing parameters remain unchanged (proliferation response to PHA and Con A, SAT index and IL-2 receptors saturation index).

2. Exposition of human mononuclear blood cells culture to ADR field in the whole period of culturing (72 hrs) leads to occurrence of changes similar to the ones present in case of exposition of the same medium (before culturing) to ADR influence (upward trend in spontaneous integration of 3THdR values and decrease of LM index).

3. The carried research indicates that exposition to ADR field has a direct influence on culture medium RPMI 1640 state by causing changes of unknown character. These changes condition derivatively occurrence of previously described functional changes in immunological cells.

4. Observed functional changes in cultured cells might be introductory interpret (needs further experimental proof) as a result of change in monocytes' activity in range of produced monokins which regulate the degree of lymphocytes' proliferation.

5. To exclude or to show the possibilities of direct influence of ADR field on immunological cells it is essential to perform further studies on the experimental model, which would eliminate indirect influences on culture medium.

6. Obtained results can not be directly prescribed to the assessment of cells exposed to ADR field and to draw conclusions describing the kind of these influences on functional state of cells in organism (*in vivo*).

Bibliography

1. Dąbrowski M.P. and coll.: Ann. N.Y. Acad. Sci., 1987, 496, 697-706.
2. Dąbrowski M.P. and coll.: Mediators of Inflamm., 2001, 10, 101-107.

Researches

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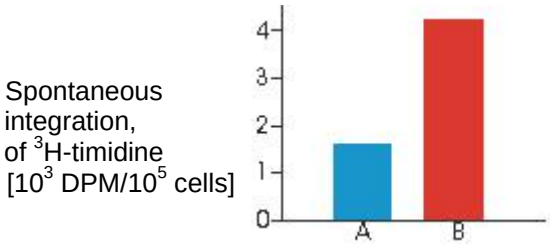
Research Results



Mean results of investigation of mononucleus blood cells' proliferation (immunological cells) in *in vitro* cell culture.
B. Proliferation after 15-minute medium exposure to ADR Protect, which was further used in cell culture.

Table I.

Spontaneous integration of ^{3}H-timidine and response to mitogenes of human mononuclear blood cells (MBC) in 72-hour microculture run in medium exposed to 15-minute influence of ADR Protect plate.

		Spontaneous integration of ^{3}H-timidine without mitogene (*10^{3}DPM/10^{5} cells) **(N=10)**		Response of MBC induced by PHA (*10^{3}DPM/10^{5} cells) **(N=10)**		Response of MBC induced by Con-A (*10^{3}DPM/10^{5} cells) **(N=10)**		**P/C index** (of PHA/Con-A response) **(N=10)**	
		control	**ADR Protect**	**control**	**ADR Protect**	**control**	**ADR Protect**	**control**	**ADR Protect**
Arithmetic mean of results (x)		**1.63**	**4.20**	67.27	66.69	48.96	51.24	1.49	1.32
Standard deviation (SD)		**0.81**	**2.69**	35.04	29.41	25.38	31.63	0.44	0.31
Statistical assessment of differences (p)		**p=0.01**		Not significant		Not significant		Not significant	
Analysis of differences in a group (number of cases)	Above norm	**9**		1		2		1	
	In norm	**1**		8		7		7	
	Below norm	**0**		1		1		2	
Statistic analysis of differences (Wilcoxon test)		**p<0.01**		Not significant		Not significant		Not significant	
Summed up statistic analysis		Increase of value after the influence of ADR PROTECT; difference statistically significant		Lack of differences between samples		Lack of differences between samples		Lack of differences between samples	

Table II.

Immunoregulational parameters (SAT index, LM index, IL-2 receptors saturation index) of human mononuclear blood cells (MBC) in 72-hour microculture *in vitro* run in medium exposed to 15-minute influence of ADR-Protect plate.

		SAT(lymphocyte T suppression activity index) **(N=10)**		LM (monokins (IL-1ra/IL-1β) influence on lymphocytes proliferation index) **(N=10)**		Degree of IL-2 receptors saturation **(N=10)**	
		control	**ADR PROTECT**	**control**	**ADR PROTECT**	**control**	**ADR PROTECT**
Arithmetic mean of results (x)		27.28	24.51	20.22	12.21	81.22	84.33
Standard deviation (SD)		13.37	9.16	17.05	10.01	17.06	10.99
Statistical assessment of differences (p)		Not significant		Not significant (p=0.24)		Not significant	
Analysis of differences in a group (number of cases)	Above norm	2		1		1	
	In norm	6		5		8	
	Below norm	2		4		1	
Statistic analysis of differences (Wilcoxon test)		Not significant		Not significant Mean of differences 3.36±14.97		Not significant	
Summed up statistic analysis		Lack of differences between samples		Downward trend of value after the influence of ADR PROTECT; difference statistically not significant		Lack of differences between samples	

Table III

Spontaneous integration of ^{3}H-timidine and response to mitogenes of human mononuclear blood cells (MBC) in 72-hour microculture run in medium exposed to ADR PROTECT plate during the whole culturing period.

		Spontaneous integration of ^{3}H-timidine without mitogene (*10^{3}DPM/10^{5} cells) **(N=10)**		Response of MBC induced by PHA (*10^{3}DPM/10^{5} cells) **(N=10)**		Response of MBC induced by Con-A (*10^{3}DPM/10^{5} cells) **(N=10)**		**P/C index** (of PHA/Con-A response) **(N=10)**	
		control	**ADR PROTECT**	**control**	**ADR PROTECT**	**control**	**ADR PROTECT**	**control**	**ADR PROTECT**
Arithmetic mean of results (x)		1.62	2.20	69.79	68.29	41.08	36.63	1.86	2.01
Standard deviation (SD)		0.58	1.20	27338	19.28	19.28	16.73	0.63	0.50
Statistical assessment of differences (p)		Not significant p=0.06		Not significant		Not significant		Not significant	
Analysis of differences in a group (number of cases)	Above norm	5		1		1		2	
	In norm	14		18		17		16	
	Below norm	1		1		2		2	
Statistic analysis of differences (Wilcoxon test)		Not significant		Not significant		Not significant		Not significant	
Summed up statistic analysis		Upward trend of value after the influence of ADR PROTECT; difference statistically not significant		Lack of differences between samples		Lack of differences between samples		Lack of differences between samples	

Table IV

Immunoregulational parameters (SAT index, LM index, IL-2 receptors saturation index) of human mononuclear blood cells (MBC) in 72-hour microculture run in medium exposed ADR-Protect plate during the whole culturing period.

		SAT(lymphocyte T suppression activity index) **(N=10)**		LM (monokins (IL-1ra/IL-1β) influence on lymphocytes proliferation index) **(N=10)**		Degree of IL-2 receptors saturation **(N=10)**	
		control	**ADR PROTECT**	**control**	**ADR PROTECT**	**control**	**ADR PROTECT**
Arithmetic mean of results (x)		23.84	18.01	**17.70**	**11.50**	82.9	87.6
Standard deviation (SD)		17.34	13.10	**10.59**	**7.58**	14.7	10.5
Statistical assessment of differences (p)		Not significant		**p<0.05 (p=0.04)**		Not significant	
Analysis of differences in a group (number of cases)	Above norm	4		4		2	
	In norm	11		7		17	
	Below norm	5		9		1	
Statistic analysis of differences (Wilcoxon test)		Not significant		Not significant Mean of differences 6.2±112.2		Not significant	
Summed up statistic analysis		Lack of differences between samples		Downward trend of value after the influence of ADR PROTECT; difference statistically not significant		Lack of differences between samples	