Table 1. Values of the MI and SCE-per-cell frequency in human peripheral blood lymphocyte cultures treated with insulin

Concentration of insulin	MI	SCE range	Mean value of SCE	SE	X_k
negative control	4.73	3 – 9	6.27	0.29	100
positive control (MNNG)	3.54	7 – 29	13.77***	1.06	219.62
10^{-10} M	5.63	4 – 10	6.33	0.26	100.96
10^{-9} M	6.16	5 – 12	6.67	0.34	106.38
0.75×10^{-8} M	8.12**	3 – 9	6.23	0.29	99.36
2.5×10^{-8} M	6.65*	3 – 11	6.57	0.39	104.78
2.5×10^{-7} M	5.23	3 – 10	6.40	0.34	102.07
2.5×10^{-6} M	5.04	4 – 11	6.50	0.29	103.67
0.75×10^{-5} M	5.53	4 – 10	6.63	0.33	105.74
10^{-4} M	4.88	2 – 11	6.47	0.30	103.19

*P < 0.05; **P < 0.01; ***P < 0.001 (Student's t-test)

SE – standard error

X_k – percentage of the mean value of SCE of the negative control

Table 2. Evaluation of the effects of insulin in cytokinesis-blocked micronucleus assay on human peripheral blood lymphocytes

Concentration of insulin	BN cells scored	Distribution of BN cells according to the No. of MN				MN/cell (%)
		0	1	2	3	
negative control	3216	3163	49	4	0	17.7
positive control (MNNG)	3057	2963	79	12	3	36.6***
10^{-10} M	3038	2985	47	6	0	19.4
10^{-9} M	3112	3068	39	4	1	16.1
0.75×10^{-8} M	3089	3036	48	5	0	18.8
2.5×10^{-8} M	3240	3178	54	8	0	21.6
2.5×10^{-7} M	3336	3288	44	4	0	15.6
2.5×10^{-6} M	3018	2970	41	7	0	18.2
0.75×10^{-5} M	3060	3001	50	8	1	22.5
10^{-4} M	3125	3065	57	3	0	20.2

BN – binucleated; MN – micronuclei

***P < 0.001 (χ^2 test)

was observed after the treatment with positive control, so that the average value was 13.77 ± 1.06.

The cytokinesis-blocked micronucleus assay is used to detect acentric chromosome fragments or whole chromosomes left upon nucleus division and visible as small additional nuclei in the cytoplasm. Hence, the appearance of micronuclei should point out to clastogenic and/or aneugenic effects. In this investigation, insulin has not significantly changed the percentage of micronucleated lymphocytes, whereas MNNG caused a significant (P < 0.001) increase in comparison to the negative control (Table 2).

The data from the present study demonstrate that under the experimental conditions described, there were no indications of genotoxic properties of human recombinant insulin. It should be mentioned, however, that insulin in plateau-phase A549 human lung carcinoma cells induces signaling events which affect the repair of radiation-induced DNA double-strand breaks, possibly due to changes in chromatin conformation (Jayanth et al., 1995). Moreover, insulin-like growth factors I and II increase clastogenic effects of radiomimetic agent bleomycin, and IGF-I itself was even capable to increase the percentage of spontaneous chromosome aberrations in cultured human lymphocytes (Cianfarani et al., 1998). In light of these experimental data, it seems that further investigations are needed to elucidate the complexity of signal transduction and consequent changes in the cell nucleus in response to insulin.

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