A Rapid Method of Estimating Damage and Repair of DNA

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The number of found factors damaging cell DNA increases recently. To understand the impact of these new factors it is necessary to have simple method of estimating the impact level. The Comet assay is useful for this purpose but its general limitation is the use of gamma radiation breaking DNA molecules in the places where damage appears under the effect of various factors. In this project we developed alternative method utilizing ozone with concentration 200 to 1000 ug/l instead of gamma radiation. The diffusion level of DNA comet tail under 10 min impact of ozone with 900 ug/l concentration was 12.4±0.8% being close to that under gamma radiation of Co-60 with dose of 3 Grays (12.1±0.8%). Besides the impact time was shortened 18 times. The samples of peripheral blood were studied to develop this method for volunteers exposed by smoking, acclimation, hypernutrition, solarium visiting and food restriction, as well as the control group. To analyze cells of capillary blood we prepared slides in agarose gel. DNA decay was carried out by slide processing in PBS with pH about 7.4 to 7.5 preliminary saturated by ozone-oxygen mixture with 900 ug/l ozone concentration for 5 min. The reliability of results was estimated using the parametric statistics. The statistically significant decreasing of DNA damage level from 30% to 2.5% was found in the row noted above. This can be explained by the decreasing of the express concentration of free radicals corresponding to the impact. Then we studied DNA repair and found that smoking and acclimation factors also result in least repair levels: 73.5% and 83% correspondingly. Thus the proposed method allows detecting rapidly the dangerous factors affecting DNA damage and estimating the resistance of human organism to these factors.

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