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DIFFERENTIAL INHIBITORY EFFECT OF OK-1035 ON DNA REPAIR IN L5178Y-R AND L5178Y-S SUBLINES WITH FUNCTIONAL OR DEFECTIVE REPAIR OF DOUBLE STRAND BREAKS

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Radiosensitive L5178Y-S subline and its parental, more radioresistant L5178Y-R subline differ in DNA double strand break (DSB) rejoining [1]. DSB rejoining is impaired both in G1 and G2 LY-S cells [1], as determined by non-denaturing filter elution; the slow DSB rejoining is paralleled by slow chromatid break repair estimated from PCC. According to [2] in G1 phase the repair is carried out by a DNA-PK-dependent system. The molecular defect in LY-S cells remains undefined; it is also possible that there is an auxiliary to DNA-PK protein factor indispensable for the repair activity that is lacking in this cell subline.

OK-1035, 3-cyano-5-(-4-pyridyl)-6-hydrazonomethyl-2-pyridone, has been characterized as a specific inhibitor of DNA-dependent protein kinase, DNA-PK [3]. It caused a 50% inhibition of DNA-PK activity at 8 μM, a concentration about 50 times lower than those required for inhibition of other kinases.

We applied the comet assay, a sensitive method of DNA damage determination allowing to examine its distribution in the cell population subjected to the damaging agent. At alkaline pH the effect of both single and double strand breaks on tail moment value is estimated. The level of initial DNA damage measured at DNA-denaturing pH is the same in both LY sublines: the mean tail moment values ±SD, are 92.93 ±10.39 for LY-R cells and 94.93 ±12.94 for LY-S cells. The control cells differ: the respective values for LY-R and LY-S cells are 9.62 ±2.84 and 3.52 ±0.1, reflecting the susceptibility to lysis conditions as well as possible endogenous (oxidative) damage level.

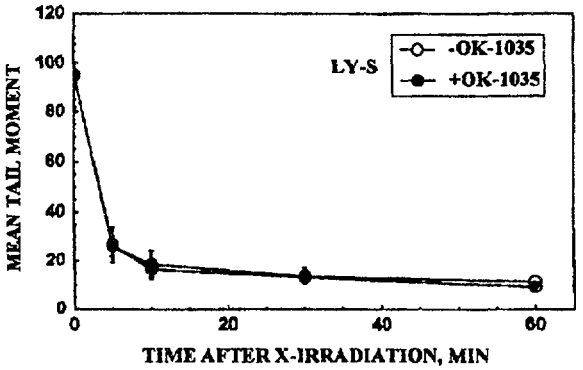

Fig.1. DNA repair measured with the comet assay (tail moment as damage measure) in the absence or presence of DNA-PK inhibitor, OK-1035 (2 mM), in X-irradiated with 8 Gy LY-S (radiosensitive) cells.

As shown in Fig.1, in LY-S cells the repair of X-ray-induced damage proceed identically in the presence or absence of 2 mM OK-1035. In contrast, the inhibitor considerably slows the repair in LY-R cells (Fig.2). These results support the assumption, that the DSB repair defect identified by Włodek and Hittelman [1] is due to a lack of function of DNA-PK in X-irradiated LY-S cells. Since the presence of the Ku subunits of the enzyme in LY-S cell extracts has been confirmed (D. Włodek, P. Jeggo, private communications) it is possible that the defect lays in its activation in the irradiated cells, due to lack of an auxiliary protein or the DNA damage sensor.

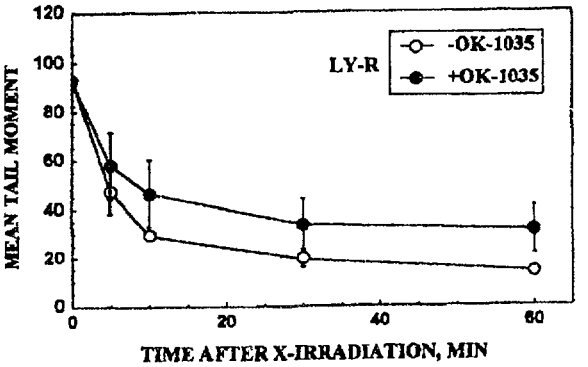

Fig.2. DNA repair measured with the comet assay in the absence or presence of DNA-PK inhibitor, OK-1035 (2 mM), in X-irradiated (8 Gy) LY-R (radioresistant) cells.

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DNA REPAIR IN ADAPTED HUMAN LYMPHOCYTES: COMPARISON OF THE COMET AND SANDWICH ELISA METHODS

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We have previously described the role of calcium ions in the induction of the adaptive response [1] and assumed that the adaptation is dependent on transduction of intracellular signal(s). We have attempted to find correlation between DNA repair kinetics and adaptive response estimated from micronuclei frequency.

Incubation of human lymphocytes in hydrogen peroxide (10 μM H_2O_2, 30 min) evoked a ca 30% decrease in the frequency of micronuclei upon sub-

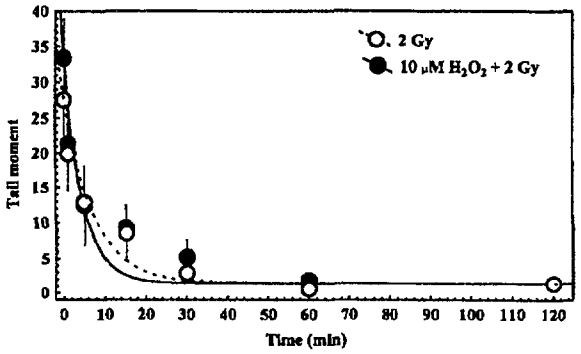

Fig.1. DNA damage repair measured by the comet assay in adapted (10 μM H_2O_2) and non-adapted human lymphocytes. Data computer-fitted (least square method) to the equation y=a*exp(-bt)+c, where a is the initial repairable damage, b is reciprocal of the time constant and c is the residual damage. Bars denote standard deviation.

sequent X-irradiation (2 Gy). The response was reflected in a lower micronuclei frequency but no change in DNA repair rate was observed as measured by the comet assay directly after the challenge dose. Initial DNA damage and its repair were measured with alkaline comet assay immediately after giving the challenge dose (on ice). Lower damage revealed at the chromosomal level in the adapted lymphocytes was unrelated to DNA repair rate. The kinetics of DNA repair in the lymphocytes adapted by 10 μM H_2O_2 treatment and non-adapted cells were similar (Fig.1). We applied also sandwich ELISA [2,3] for

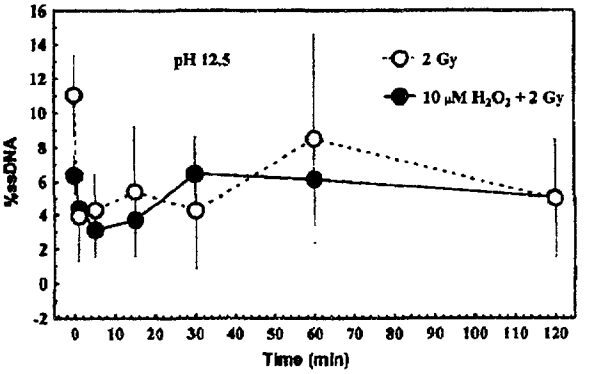

Fig.2. Repair of DNA damage measured by sandwich ELISA in human blood lymphocytes adapted (10 μM H_2O_2, 30 min) and non-adapted. % ssDNA in X-irradiated (2 Gy) lymphocytes detected after 6-min alkali treatment (pH 12.4). The data represent mean results for 2 blood samples, split from the same donor and divided over 4 microtiter plates. The error bars represent the SEM.

DNA strand breaks to study radio-adaptation of human lymphocytes. Preliminary results show no differences in repair kinetics between the adapted and non-adapted cells (Fig.2). These results point to the need of a different approach to solve the problem: a very sensitive method specific for double strand breaks should be applied.

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VALIDATION OF AN IMMUNOCHEMICAL ASSAY FOR THE DETECTION OF DNA DAMAGE AS A TOOL FOR BIOLOGICAL DOSIMETRY OF HUMAN EXPOSURE TO IONIZING RADIATION

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For an accurate assessment of the extent of damage, induced by exposure to ionizing radiation, a complementary approach that combines physical dosimetry and biological indicators of damage is necessary. To this purpose the Nutrition and Food Research Institute (TNO, Rijswijk, the Netherlands) is developing