

PROJECT FINAL SUMMARY REPORT

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| Title of the project | | |
| Oxidative stress and chronic diseases: Exocyclic DNA adducts as markers for disrupted genomic integrity and risk | | |
| Acronym of the project | | |
| OXEXRISK | | |
| Type of contract | | Total project cost |
| Shared Cost Project | | €1,107,422 |
| Contract number | Duration | EU contribution |
| QLK4-CT-2000-00286 | 30 months | € 950,000 |
| Commencement date | Period covered by the progress report | |
| 1 March 2001 | 1 March - 31 August 2003 | |
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| Key words | | |
| Exocyclic DNA adducts, DNA-repair, oxidative stress, genomic integrity, chronic degenerative diseases | | |
| World wide web address | | |
| http://www.cordis.lu/marketplace | | |
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Objectives:

To increase knowledge on formation and repair of etheno (ϵ)-DNA adducts in pro- and eukaryotic systems

To understand the risk resulting from these adducts in disrupting genomic integrity and causing human diseases.

To focus on exogenous factors (diet) and endogenous processes (lipid peroxidation and estrogen metabolism) leading to ϵ -DNA adducts in humans, rodents, *Drosophila* and *E.coli*.

By analysis of key parameters, such as repair functions, persistence and mutational specificity of ϵ -DNA adducts to define the mutagenic and carcinogenic potential of these miscoding lesions.

Overall: To achieve a better understanding of mechanisms and pathways underlying the formation and persistence of ϵ -DNA adducts, the genotoxic consequences of their formation in the genome and their role in chronic degenerative diseases.

Results and Milestones:

ϵ -DNA adducts as biomarkers of oxidative stress induced by dietary fatty acids and altered estrogen metabolism were studied in relation to human breast cancer. 4-hydroxy estradiol, a metabolite of estradiol implicated in redox cycling and arachidonic acid were found to be synergistic factors in the generation of ϵ -adducts in calf thymus DNA. ϵ -DNA adducts in human breast epithelial cells were higher than those seen in cultured cells. The ratio of 4-OH to 2-OH estradiol in serum was found to be variable ranging from 1 - 190.

A method for M_1dG -DNA adduct (formed from malondialdehyde) has been developed and applied to human WBC and breast samples. ϵ -DNA adducts in colon cancer in relation to genomic instability and repair mechanisms of these adducts in target tissues from human breast and colon cancer patients are being elucidated. Progress in obtaining a large collection of colorectal samples was made and preliminary data on microsatellite instability and immunohistochemistry for mismatch repair proteins were obtained. Biochemical assays for repair of ϵC and ϵA in oligonucleotides showed a very wide variation in activity in colorectal tumor, normal and proximal tissues. The tendency of higher repair activities in tumor tissue than in normal colon epithelium were observed.

Pathways of formation and repair involved in maintenance of physiological levels of ϵ -DNA adducts are being investigated; transgenic rodents with modified repair genes were used to determine the biological consequences of ϵ -DNA adducts. Work with a number of knock-out mice strains has progressed. One finding was that nucleotide excision repair (NER) via XRCC seems not to be involved in repair of ϵA

and ϵ C.

Human (phage display) antibodies specific for ϵ A in oligonucleotides have been generated and are undergoing characterization. Evidence was obtained that ϵ A is not a transcription blocking lesion. 4-Hydroxy-nonenal (HNE) adducts in DNA were found to be highly recombinogenic in single-stranded phage. Lipid peroxidation products such as HNE are known to produce etheno adducts in DNA are also able to generate much more complex adducts that may undergo further chemical modifications depending on the chemical composition of the reaction mixtures. 4-Hydroxy-nonenal (HNE) adducts in DNA were found to be replication blocking lesions of high recombinogenic potential in single-stranded phage. In *E. coli* these long-chain adducts are eliminated from double-stranded DNA by nucleotide excision repair, and from single-stranded DNA by recombination.

Characterization and purification of new and known DNA repair enzymes involved in the removal of ϵ -DNA adducts has progressed. Enzymes implicated in the repair of ϵ -adducts have been identified. Human and the *E. coli* 3-methyladenine (3-meA)-DNA-glycosylases (ANPG and AlkA proteins, respectively) excise ϵ A residues. Two homologous proteins present in human cells and *E. coli* were identified that remove ϵ C residues by a DNA glycosylase activity. The human enzyme is an activity of the mismatch-specific thymine-DNA glycosylase (hTDG) while the bacterial enzyme is an activity of the mismatch-specific uracil-DNA glycosylase (MUG), i.e. the homologue of hTDG. The repair of ϵ dC has been shown to be enhanced by human AP endonuclease by stimulating the hTDG turnover. Recently, two structurally unrelated proteins, bacterial MUG and human ANPG were found that can both release 1, N^2 - ϵ G from defined oligonucleotides containing a single modified base. Using cell-free extracts from genetically modified *E. coli* and murine embryonic fibroblasts lacking MUG and mANPG activity, respectively, the incision of the 1, N^2 - ϵ G-containing duplex oligonucleotide was shown to be an absolute requirement of MUG or ANPG. Taken together, these observations suggest a possible role for these proteins in counteracting the genotoxic effects of 1, N^2 - ϵ G residues *in vivo*. hMTH1 seems not to be involved in the sanitization of the nucleotide pool containing ϵ -adducted nucleoside triphosphates. The separate pathway of preferential ϵ dCTP dephosphorylation was observed in human lung tissues by biochemical assays. This pathway should be now identified.

The genetic activity profiles of ϵ -adduct forming chemicals were investigated by estimating forward mutation spectra, structural chromosome aberrations and mitotic recombination, utilizing germ cells and somatic cells of *Drosophila melanogaster* as *in vivo* targets. These genetic endpoints were correlated with adduct levels of ϵ dA and ϵ dC in DNA of larvae tissue measured by the immunoaffinity- 32 P-postlabelling method. Etheno DNA-adduct forming compounds are bifunctional by definition. However, comparative data of bifunctional agents, known to form cross-links within the DNA, revealed that ϵ -adducts and not DNA cross-links are involved in mutation induction by ϵ -adduct forming chemicals, vinyl bromide and vinyl carbamate.

Benefits and Beneficiaries:

Exploratory studies to develop assay kits for (high throughput) screening assays for

ϵ -DNA adducts excreted in human urine for possible future industrial exploitation / clinical and epidemiological applications are being performed. Large amounts of repair proteins have been purified and provided to other partners. Syntheses of $N^2,3\epsilon$ -3'-dGMP and $1,N^2\epsilon$ -3'-dGMP markers for measurement of adducts in DNA, as well as syntheses of 4-hydroxy-nonenal for Consortium Partners were done.