USING THE COMET ASSAY TO ASSESS BASAL DNA DAMAGE IN RELATION TO EXPOSURE TO FOOD-CHAIN CONTAMINANTS

Abdelhafid Laidaoui[1,2] and Pierre Ayotte[1,2,3]

1 Département de médecine sociale et préventive, Université Laval; 2 Unité de recherche en santé publique, CHUL-CHUQ;
3 Laboratoire des biomarqueurs, Direction de la toxicologie humaine, INSPQ, Québec, QC, Canada G1E 7G9

ArcticNet
ᐅᑭᐅᖅᑕᖅᑐᑦ ᑐᑭᓯᐊᖅᑎᑦᑎᓂᖅ

Introduction

- Inuit people have expressed concern regarding a possible increase in cancer risk that could result from long term exposure to food-chain contaminants. However, epidemiological studies on cancer are difficult to conduct in Arctic populations due to the small number of cases
- To circumvent this problem, we proposed to measure biomarkers of genetic damage and examine possible relations between this damage and lifestyle, nutritional and environmental factors
- Using the sister chromatid exchange test as a biomarker of genotoxicity, Wulf and colleagues (1986) reported an association between the number of exchanges per lymphocyte and seal diet, smoking, living district and blood mercury and cadmium concentrations. Seal diet as the most important of the factors examined
- The Comet assay (Collins, 2004) will be used to assess the basal genetic damage in circulating lymphocytes obtained from a sample of 300 Inuit participants of Nunavik, in the course of the Inuit Health Survey

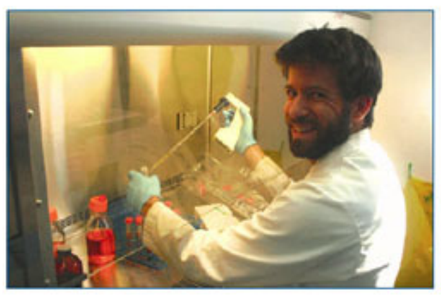
Methods

Population

- During September 2004, more than 1000 Inuit adults were recruited to participate in the Nunavik Health Survey called "Qanuippitaa?"
- Participants were also solicited to participate in the Inuit cohort Study, which include biomarker measurements
- Among those who agreed to participate, we randomly selected 300 participants for the determination of basal DNA damage using the Comet assay

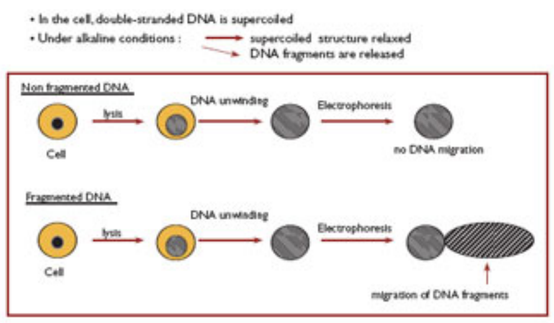
Blood sampling and lymphocyte isolation

- A 6-ml blood sample was collected in a vacutainer tube containing EDTA as the anticoagulant
- The tube was centrifuged and whitish cloudy layer (buffy coat) collected with a transfer pipette
- The mononuclear cell fraction was isolated using a Ficoll gradient
- Cells were washed 3 times with Hanks buffer and suspended in the freezing medium containing 90% fetal calf serum and 10% dimethyl sulfoxide. Cells were slowly frozen and stored in liquid nitrogen.
- Mononuclear cell isolation was performed in the Atlantis Toxicology module



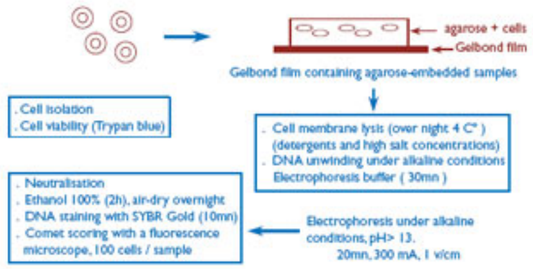
Comet assays

PRINCIPLE

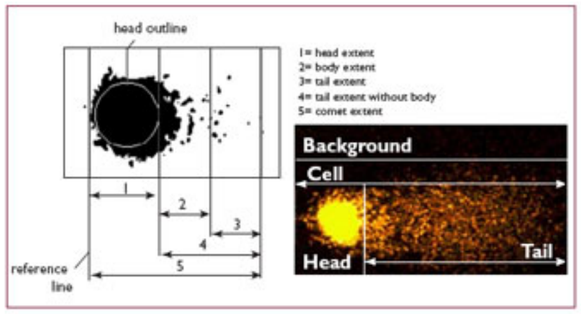


METHODOLOGY

Obtaining comets McNamee protocol

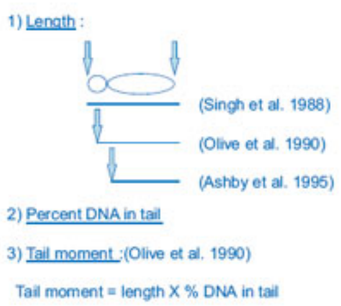


Comet characteristics



Comet parameters are scored using the LAI Comet Assay Analysis System (Loats Associates, Westminster, MD)

Measurements

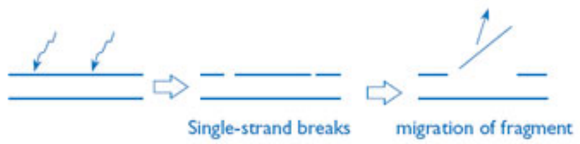


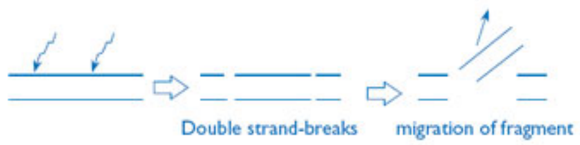
DAMAGE DETECTED

Single and double-strand breaks

Alkaline conditions:

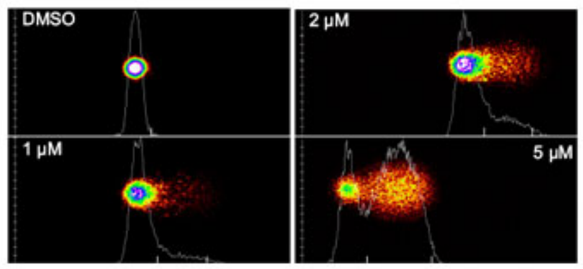
- Detection of both single and double-strand breaks
  plus
- Detection of single-strand breaks arising from "alkali-labile" sites under alkaline conditions





VALIDATION

Damage induced by treating human lymphocytes with benzo(a)pyrene diol-epoxide (BPDE)



Inter-assay variation for control and PBDE-treated human lymphocytes (4 experiments)

Control cells (DMSO)	**PBDE-treated cells**
%DNA tail	%DNA tail
• Mean = 0.83	• Mean = 20.5
• SD = 0.22	• SD = 1.2
• Range = 0.54-1.7	• Range = 20.5-23.1

Data analysis

Multiple linear regression analysis of DNA damage (% DNA in tail)

- Lifestyle habits: smoking, alcohol consumption, diet (questionnaires)
- Pre-existing diseases: diabetes, inflammation
- Exposure to food-chain contaminants
  - Heavy metals in blood (Hg, Cd)
  - Organic compounds (PCBs)
- Nutrients: vitamins, antioxidants, polyunsaturated fatty acids

Time table

- Contaminant and nutrient analyses to be completed in March 2006
- Comet assays performed from July 2005 to February 2006
- Statistical analyses and data interpretation: March 2006-June 2006

References

- Collins AR. The comet assay for DNA damage and repair: principles, applications, and limitations. Mol Biotechnol. 2004;26:249-61
- McNamee JP, Bellier PV, Gajda GB, Miller SM, Lemay EP, Lavallee BF, Marro L, Thansandote A. DNA damage and micronucleus induction in human leukocytes after acute in vitro exposure to a 1.9 GHz continuous-wave radiofrequency field. Radiat Res. 2002;158: 523-33.
- Wulf HC, Kromann N, Kousgaard N, Hansen JC, Niebuhr E, Alboge K. Sister chromatid exchange (SCE) in Greenlandic Eskimos. Dose-response relationship between SCE and seal diet, smoking, and blood cadmium and mercury concentrations. Sci Total Environ. 1986;48:81-94

Acknowledgments

- We are grateful to the Nunavik population for their participation in this research
- Many thanks to Chris Stamler for performing the isolation of mononuclear cells, Suzanne Côté who organised and supervised biological sample collection, and to the Mission Chief, Suzanne Bruneau