Relation between serum xenobiotic-induced receptor activities and sperm DNA damage and apoptotic markers in Europeans and Inuits

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BACKGROUND

Persistent organic pollutants (POPs) can interfere with the hormone activities and suspected to have the potential to introduce male reproductive disorders. The maturation and differentiation of male germ cells during spermatogenesis are physically regulated by the hormonal system [1] and fine-tuned apoptotic mechanisms [2]. We have demonstrated that determination of the integrated xenobiotic serum activity as an exposure marker of POP mixtures is an effective tool for estimation of the bio-effect on the estrogen receptor (ER), androgen receptor (AR) and aryl hydrocarbon receptor (AhR) functions [3,4,5] .

AIM

To explore possible relations between the integrated xenobiotic-induced receptor activities of serum POP extracts and sperm DNA damage and apoptotic markers (pro-apoptotic marker Fas and anti-apoptotic marker Bcl-xL).

STUDY POPULATION

This study was a part of the EU supported project INUENDO including randomly selected voulunteers: 54 male Inuits from Greenland (Sisimiut and Tasiilaq) and 208 male Europeans (69 from Warsaw (Poland), 81 from Sweden, 58 from Kharkiv (Ukraine).

CONCLUSIONS

Inuits had lower serum xenoestrogenic, dioxin-like but higher serum xenoandrogenic activity than *Europeans*.

Inuits had a lower sperm DNA damage level than *Europeans*.

Inuits elicited negative correlations between sperm DNA damage and xenobiotic-induced receptor activities.

Europeans elicited positive correlations between sperm DNA damage and serum xenobiotic-induced receptor activities.

Further studies are needed to elucidate whether altered receptor activities in concert with genetic and /or nutrient factors may have protecting effects on sperm DNA damage of the Greenlandic Inuits.

METHODS

Serum extractions: 1) Solid phase extraction (SPE) and high-performance liquid chromatography (HPLC) were used to obtain the actual POP mixture free of endogenous hormones to determine xenoestrogen (XER/XERcomp) and xenoandrogen (XAR/XARcomp) receptor transactivity [3,5,6]. 2). Ethanol-hexane extraction followed by clean-up on Florisil + Na_2SO_4 [4] was used to obtain the lipophilic POPs for measurement of AhR mediated dioxin-like activity (AhRag/AhRcomp). **Xenobiotic induced receptor activities** : CALUX bio-systems were used to determine the ER-, AR- and AhR- mediated transactivity [3,4,5] applying the recombinant cell lines MVLN, CHO-K1 and Hepa1.12cR, respectively.

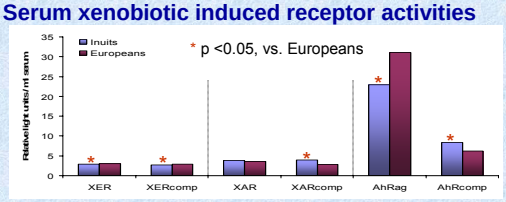
Sperm DNA damage: Terminal deoxynucleotidyl transferase-driven dUTP nick labeling assay (TUNEL) [7].

Sperm apoptotic markers: immune-fluorescence method [7].

Statistical analysis: One-way ANOVA and Student's t-test were used to compare the differences of variables among populations; Spearman's rank and Pearson correlation analysis to evaluate the correlation of variables.

RESULTS & DISCUSSION

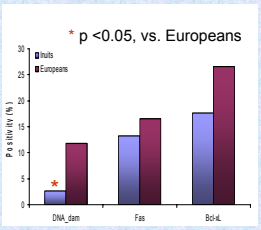
Serum xenobiotic induced receptor activities



❖The xenobiotic agonistic activity on the receptors (XER, XAR and AhRag) were determined by exposure of the cells to serum extract alone. The male Inuits had significantly lower median level of XER, AhRag than the male Europeans.

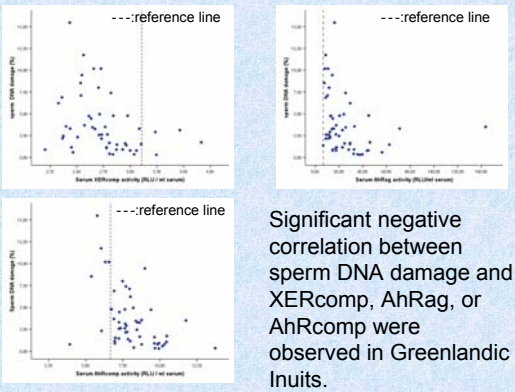
❖The competitive activity of serum extract on ligand-induced receptor activities were determined by co-exposure of serum extracts with typical receptor ligands 17β-estradiol or methyltrienolone or 2,3,7,8-tetrachlorodibenzo-p-dioxin (XERcomp, XARcomp and AhRcomp).
Greenlandic Inuits had significantly higher level of XARcomp and AhRcomp but lower XERcomp than that of Europeans.

Sperm DNA fragmentation and apoptotic markers



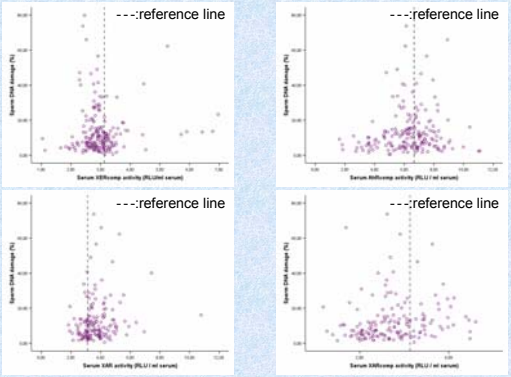
European men showed significantly higher level of sperm DNA damage than Greenlandic Inuits. European men showed a trend of higher level of apoptotic markers compared to Inuits.

Correlation of serum xenobiotic-induced receptor activities and sperm DNA damage in *Inuits*



Significant negative correlation between sperm DNA damage and XERcomp, AhRag, or AhRcomp were observed in Greenlandic Inuits.

Correlation of serum xenobiotic-induced receptor activities and sperm DNA damage in *Europeans*



Significant positive correlations between sperm DNA damage and serum xenobiotic induced receptor activities were observed in Europeans.

References: [1]Liu. (2005).Arch Androl 51, 77-92. [2]Oldereid et al.(2001). Mol. Hum. Reprod. 7, 403-408. [3] Bonefeld-Jorgensen et al.(2006). Environ. Health 5, 12. [4] Long et al.(2006). Environ Health 5,14. [5] Krüger et al. (2007), Environ Health Perspect.in press. [6] Hjelmborg et al. (2006). Anal. Bioanal Chem. 385, 875-887. [7] Stronati et al. (2006). Reproduction. 132, 949-958