Combined in-utero and juvenile exposure of mice to arsenate and atrazine in drinking water modulates the gene expression and clonogenicity of myeloid progenitors in bone marrow.

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Introduction

Increasing evidence proves that human fetuses are exposed to multiple risk factors and major concerns have been expressed towards exposure to potential endocrine modulating chemicals at early stage of life and during growth.

Understanding that exposures occur as mixture of chemicals and that they converge on other inherent and environmental risk-modulating factors, there is a need to develop experimental models to assess the effects of exposure to multiple chemicals during different stage of life.

In the present study, the clonogenicity of myeloid progenitors (CFU-GM) and the modulation of gene expression of 1197 cancer-related genes (DNA macroarrays) in bone marrow were used to investigate in male and female young mice the combined effects of continuous exposure to arsenate and atrazine in drinking water.

Materials and Methods

✓***Experimental animals:*** male and female CD-1 mice, 4 months of age.

✓ ***Treatment:*** Female adult mice were treated with arsenate in drinking water (1 mg As/L) for 10 days before mating and during the gestation. Separate groups of arsenic exposed males and females offspring were exposed for 4 months to 1mg Atr/L of atrazine (Atr), to 1 mg As/L of additional arsenate (As) or to atrazine and arsenate together in drinking water (As+Atr). Control mice without any treatment were also analysed (Ctrl).

✓ ***Total RNA Extraction:*** from bone marrow using RNeasy Qiagen kit.

✓ ***Retrotranscription and cDNA Labeling:*** Super Script III Reverse Transcriptase (Invitrogen), ^{33}P-dATP (Amersham), Mouse Cancer 1.2 CDS primer mix (Atlas™, Clontech, U.S.A.) and 1 µg total RNA.

✓ ***cDNA Hybridization*** on Mouse Cancer 1.2 Array (Atlas™, Clontec, U.S.A.) membranes (16 hours at 50°C).

✓ ***Image Acquisition:*** Cyclone instrument (Packard Camberra Instruments, U.S.A.) after exposure for 21 hours on a phosphor-image screen (Packard).

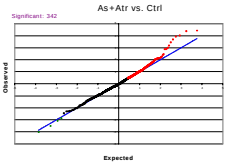
✓***Image Analysis:*** Atlas Image software (Atlas™). The pixel intensity of each spot were normalized as percentage of total pixels on the membrane.

✓ ***Data Analysis:*** by Significance Analysis of Microarrays (SAM).

✓ ***Selection Criteria: 1)*** changes must be ≥ 20% higher/lower than expression in proper control;
2) radioactivity of each spot must exceed 0.1 % of total radioactivity on membrane;
3) these criteria have to be met in at least 3 out of the 4 samples.

Modulated genes in male mice

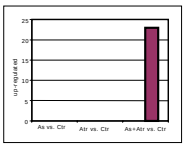
Significance Analysis of Microarrays



List of significant genes

Gene Name	Gene Family	As vs. Ctrl	Atraz vs. Ctrl	As+Atr vs. Ctrl
integrin alpha M	adhesion cell-cell receptors	-	-	up
Eph receptor B2	adhesion cell-cell receptors	-	-	up
Eph receptor B4	adhesion cell-cell receptors	-	-	up
CD14 antigen	adhesion cell-cell receptors	-	-	up
heat shock protein 2	HSP	-	-	up
fibroblast growth factor receptor 4	receptors (G.F&Chemokines)	-	-	up
platelet derived growth factor receptor, beta polypeptide	receptors (G.F&Chemokines)	-	-	up
CD28 antigen	receptors (G.F&Chemokines)	-	-	up
interleukin 1 receptor, type I	receptors (IL&IFN)	-	-	up
adenosine A2a receptor	receptors	-	-	up
epidermal growth factor	G.F.,Cytok.,Chemokines adhesion cell-cell receptors	-	-	up
fibroblast growth factor 10	G.F.,Cytok.,Chemokines	-	-	up
growth differentiation factor 5	G.F.,Cytok.,Chemokines	-	-	up
macrophage stimulating 1 (hepatocyte growth factor-like)	G.F.,Cytok.,Chemokines	-	-	up
chemokine (C motif) ligand 1	G.F.,Cytok.,Chemokines	-	-	up
transforming growth factor, beta 1	G.F.,Cytok.,Chemokines	-	-	up
protein kinase C, epsilon	intracell modulators	-	-	up
histidine triad nucleotide binding protein	intracell modulators	-	-	up
a disintegrin and metalloproteinase domain 12 (meltrin alpha)	protein turnover adhesion cell-cell receptors	-	-	up
procollagen, type IX, alpha 2	cytoskeleton/motility	-	-	up
RAD54 like (S. cerevisiae)	DNA repair DNA recombination	-	-	up
ubiquitin B	protein turnover stress response	-	-	up
actin, beta, cytoplasmic	cytoskeleton/motility	-	-	up

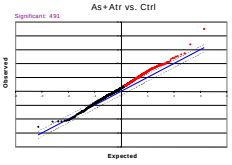
Number of significant genes



In male mice the exposure to the only arsenate (As) or to atrazine (Atr) did not result in significant changes on the gene expression in bone marrow cells, whereas, co-exposure to arsenic and atrazine (As+Atr) resulted in a significant up-modulation of gene expression. In these treated mice 342 genes were up-modulated but 23 in a significant manner according to our selection criteria (see Material and Methods).
Six of these up-regulated genes code for the adhesion cell-cell receptors, and 8 genes for the biosynthesis of chemokines and cytokines.

Modulated genes in female mice

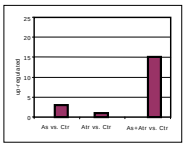
Significance Analysis of Microarrays



List of significant genes

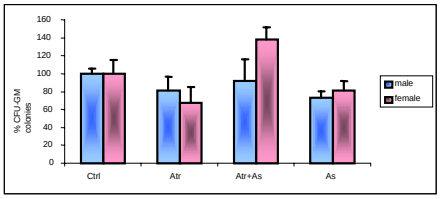
Gene Name	Gene Family	As vs Ctr	Atraz vs Ctr	As+Atr vs Ctr
cyclin E1	cell cycle	-	-	up
integrin alpha M	adhesion cell-cell receptors	-	-	up
desmoglein 2	adhesion cell-cell receptors	-	-	up
Eph receptor B4	adhesion cell-cell receptors	-	-	up
matrilin 1, cartilage matrix protein 1	matrix	-	-	up
CD3 antigen, zeta polypeptide	receptors immune system	-	-	up
macrophage stimulating 1 (hepatocyte growth factor-like)	G.F.,Cytok.,Chemokines	up	-	-
transforming growth factor, beta 1	G.F.,Cytok.,Chemokines	-	-	up
vascular endothelial growth factor B	G.F.,Cytok.,Chemokines	-	-	up
eruglass related MMTV integration site 2a	G.F.,Cytok.,Chemokines progression/tumor suppressors	-	-	up
secreted frizzled-related sequence protein 2	signaling/ extracell transporters/carriers	up	-	up
integrin alpha L	adhesion cell-matrix receptors	-	-	up
integrin beta 1 binding protein 1	adhesion cell-matrixreceptors	up	-	-
serine (or cysteine) proteinase inhibitor, clade E, member 1	protein turnover	-	up	-
klefin A3	protein turnover	-	-	up
protein tyrosine phosphatase, receptor type, A	receptors	-	-	up
actin, gamma, cytoplasmic	cytoskeleton/motility	-	-	up
actin, beta, cytoplasmic	cytoskeleton/motility	-	-	up

Number of significant genes



In female mice, the co-exposure to arsenic and atrazine (As+Atr) resulted in a significant up-modulation of gene expression. In particular, in these exposed female mice 491 genes was up-modulated compared to the control, 15 in a significant manner according to the selection criteria used. These selection criteria allowed us to identify 3 genes with higher expression in As-treated and one up-modulated gene in Atr-treated female mice. No down-modulated genes were detected.
The functional role exerted by these genes were in cell adhesion (5 genes) and in the biosynthesis of chemokines and cytokines (4 genes). The others up-modulated gene code for protein turnover, cytoskeleton motility and cell cycle.

Clonogenicity of myeloid progenitors (CFU-GM)



The use of a methylcellulose colony-forming unit-granulocyte/macrophage (CFU-GM) assay was used in this study to evaluate the haematotoxicity of atrazine and arsenate in mice.
In male mice the percentage of CFU-GM weakly decreased after exposure to individual compounds, while the co-exposure did not change the clonogenicity of the progenitors.
In females the percentage of CFU-GM decreased significantly after atrazine exposure, did not change with arsenic treatment, but dramatically increased after the combined administration.

Conclusions

The results of this study indicate that in-utero and juvenile co-exposure of mice to atrazine and arsenate in drinking water significantly modulates the transcriptional activation of genes in bone marrow cells. In both the male and female mice the up-modulated genes code prevalently for the biosynthesis of chemokines, cytokines and for adhesion receptor proteins. However, only few of them (4) were common to the two gender, suggesting remarkable sex differences.

These differences were also evident in results from the CFU-GM assay. In female mice the clonogenic potential of myeloid progenitors was also significantly stimulated following the exposure to arsenic and atrazine, supporting a co-operative role of these two chemicals in promoting cell proliferation and clonogenicity. This effect was not as so significantly induced in male, suggesting that the gene pathway regulating the clonogenic capability is sex related and differently modulated.

Interestingly, the effects on the stimulation of clonogenicity and modulation of gene expression in bone marrow were only observed in mice co-exposed to arsenic and atrazine and were not as evident in mice exposed in drinking water to arsenic or atrazine alone, supporting the needs for extensive studies on mixture of chemicals or in combined exposure to multiple chemicals.

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