Carcinogen induced inflammation and pancreatic cancer

1687

Sandeep Kadekar [1], Ilona Silins[1], Anna Korhonen[2], Johan Högberg[1], Kristian Dreij[1] and Ulla Stenius[1]

[1]Institute of Environmental Medicine, Karolinska Institutet, Sweden [2]Computer Laboratory, University of Cambridge, UK

INTRODUCTION

Pancreatic cancer is the fifth major cause of cancer death in developed countries. It has a low 5-year survival rate (5%). Younger men have been reported to be more sensitive for pancreatic cancer than women, suggesting a role of hormones in pancreatic cancer development. Among factors reported as important for pancreas cancer development are oxidative stress and inflammation.

We used data from the National Toxicology Program (NTP) database to investigate chemicals causing pancreatic cancer in 2 year cancer bioassays in rats. We made a surprising finding that all chemicals tested by NTP and that caused pancreatic tumours in exocrine pancreas were affecting male rats only. We used a previously developed literature classification tool (http://**www.cl.cam.ac.uk/~alk23/crab/crab.html**. ***BMC Bioinformatics* 10:303, 2009**) to investigate modes of action (MOA) for these chemicals. The results showed that literature on chemicals causing exocrine pancreas cancer in male rats provided much evidence on mutations, inflammation and oxidative stress. We investigated for evidence of inflammation by differences in Ca^{2+}-mediated inflammatory responses in a pancreatic cell line Panc-1.

NTP database: Chemicals causing pancreatic cancer in males but not in females

Chemicals	Total number of PubMed abstracts
2-Amino-5-nitrophenol	13
Benzyl acetate	88
Butyl benzyl phthalate	202
Chlorendic acid	16
Cinnamyl anthranilate	15
Dichlorvos	1215
2-Mercaptobenzothiazole	176
Roxarsone	106
2,4 & 2,6-Toluene-diisocyanate	855
1,2,3-Trichloropropane	52

Figure 1. Chemicals classified by NTP to be associated with site-specific tumor induction in exocrine pancreas are listed. Total number of PubMed abstracts are shown.

Overview of CRAB technology

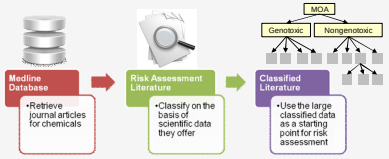

Figure 2. Analysis of abstracts by the text mining tool. The tool is used for classification of evidence found in written text in the scientific literature. In this study all abstracts listed in Fig 1 were classified as shown in Fig 3 within a few min.

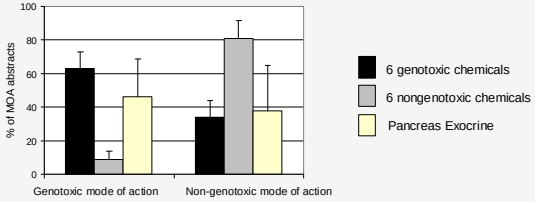

Figure 3. The percent of abstracts containing evidence for genotoxic or nongenotoxic MOAs for chemicals causing exocrine pancreas cancer and for 6 well-known genotoxic and 6 well-known non-genotoxic chemicals.

Effects of chemicals on Ca^{2+} levels in cells

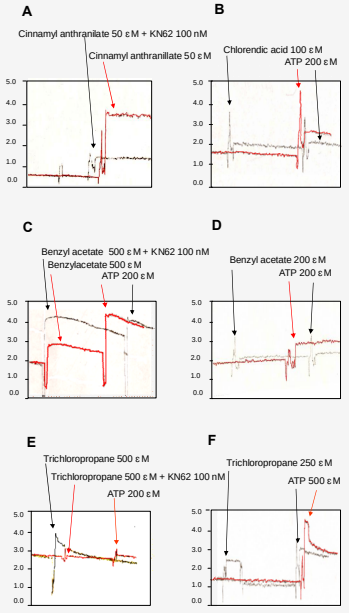

Figure 4. Pancreatic carcinogenic chemicals increase intracellular [Ca^{2+}] in Panc-1 cells via P2X7 receptors. Panc-1 cells were treated with chemicals after pre-treating with KN62 100 nM (P2X7 receptor inhibitor) for 10 min and also chemical alone at different concentrations. *An increase in intracellular calcium levels indicates activation of calcium signaling pathway which can lead to inflammatory responses.*

Activation of calcineurin

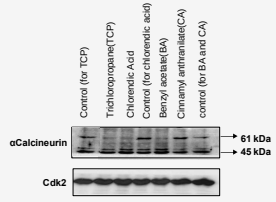

Figure 5. Calcineurin was activated by Trichloropropane(TCP), Chlorendic acid (CA) and Benzyl acetate (BA). Panc-1 cells were treated with 10 ε M for 15 min. Changes in active (45-48 kDa) and inactive (61kDa) subunits of Alpha calcineurin were induced. *Activation of calcineurin leads to nuclear localization of NFATc1.*

SUMMARY AND CONCLUSIONS

- The text mining tool helped us to rapidly sort chemicals according to genotoxic/non-genotoxic MOA.
- All the pancreatic carcinogens increased intracellular [Ca^{2+}] levels in Panc-1 cells and the effect was inhibited by the P2X7 inhibitor KN62. The chemicals also reduced the effects of ATP on [Ca^{2+}] suggesting a role of P2X7 in mediating the effects.
- Alpha calcineurin was activated by some pancreatic carcinogens and this activation could be due to increased [Ca^{2+}] as calcineurin is activated by [Ca^{2+}].
- Some of the pancreatic carcinogens increased nuclear localization of NFATc1 at 30 min. NFATc1 is activated by calcineurin. NFATc1 activates genes are involved in inflammatory responses.
- Fox01/03a phosphorylation was increased by one of the pancreatic carcinogens in the presence of testosterone. Phosphorylation of Fox01/03a leads to cell survival. Damaged cells targeted for apoptosis may survive and proliferate in the presence of testosterone.
- Inflammatory response genes (Interleukin 8, TNF-Ü, TGF-ß and ENPP2) were up-regulated at 6 hours and down-regulated at 24 hours for most of the chemicals. Testosterone prolonged the response for two of the inflammatory markers (TNF-Ü, TGF-ß) at 24 hours.
- Our results suggest that testosterone is pro-inflammatory in the presence of pancreatic carcinogens.
- Our results indicate that multiple mechanisms are involved. Further studies are required to better understand MOA for these pancreatic carcinogens and why only males were affected.

Nuclear translocation of NFATc1

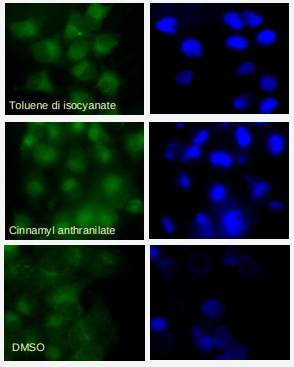

Figure 6. Panc-1 cells were stained for NFATc1. Panc-1 cells were treated with 10 ε M of Toluene-di-isocyanate, Cinnamyl anthranilate and DMSO for 30 min and stained for NFATc1. *Nuclear localization of NFATc1 can lead to activation of inflammatory response genes.*

Effect of chemicals on Fox01/3a phosphorylations

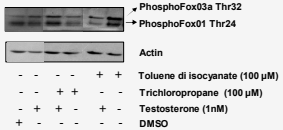

Figure 7. Testosterone affects the phosphorylation of Fox01/3a by test substances. Panc-1 cells were pre-incubated with testosterone (1nM) for 24 hours and then treated with Toluene di-isocyanate (100 ε M), Trichloropropane (100 ε M) for 24 hours. *Phosphorylated Fox01/03 promotes cell survival.*

Pancreatic carcinogens upregulate inflammatory markers

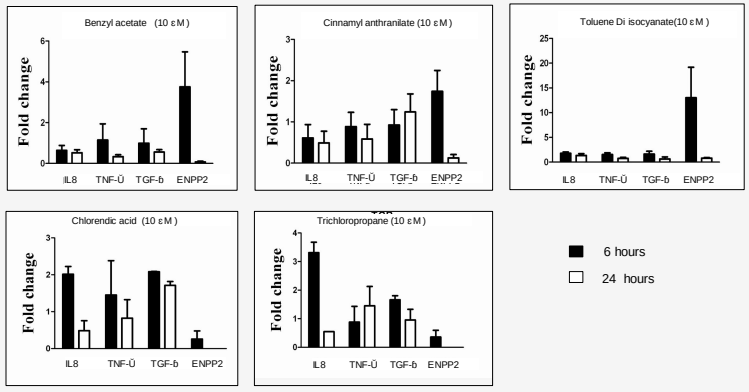

Figure 8. Effect of pancreatic carcinogens on inflammatory markers at 6 and 24 hours. Quantitative Real Time PCR was performed. Panc-1 cells were treated with Benzyl acetate, Cinnamyl anthranilate and Toluene di isocyanate, Chlorendic acid and Trichloropropane (10 ε M) for 6 hours and 24 hours. Primers for Interleukin 8, TNF-Ü, TGF-ß and ENPP2 were used.

Testosterone prolongs the inflammatory response of pancreatic carcinogens

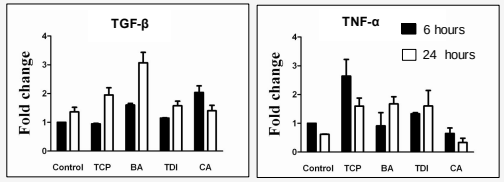

Figure 9. Effect of pancreatic carcinogens on inflammatory markers at 6 and 24 hours in the presence of testosterone. Quantitative Real Time PCR was performed using primers for TNF-Ü and TGF-ß. Panc-1 cells were treated with Trichloropropane(TCP), Benzyl acetate(BA), Cinnamyl anthranilate(CA) and Toluene di isocyanate(TDI) (10 ε M) for 6 hours and 24 hours. Cells were preincubated with testosterone (1 nM) for 24 hours.

Contact:
Sandeep Kadekar, Institute of Environmental Medicine, Karolinska Institutet,Nobels väg 13, 171 77 Stockholm, Sweden
Email: sandeep.kadekar@ki.se