

The study of Gap Junctional Intercellular Communication in keratinocytes as screening of promoter effect induced by industrial and environmental toxic substances

R. ZEFFERINO, G. ELIA*, MARIA LASALVIA, CLAUDIA PICCOLI**, D. BOFFOLI**, N. CAPITANIO**, L. AMBROSI***

Department of Medical and Occupational Sciences, University of Foggia, OO.RR., Foggia

* Salvatore Maugeri Foundation, Environmental Hygiene Centre of Bari, Policlinico

** Department of Biomedical Sciences, University of Foggia, OO.RR., Foggia,

*** Salvatore Maugeri Foundation IRCS, Cassano Murge (BA)

KEY WORDS

Cancer; mercury; trichloroethylene; apoptosis; keratinocytes

SUMMARY

Background: *Disordered functioning of gap junctions between normal and initiated cells has been proposed as one possible mechanism of tumour promotion. Many putative carcinogens such as peroxisome proliferators, are known to activate various signal transduction mechanisms and modulate gap junctional intercellular communication (GJIC). They act as tumour promoters on pre-existing "initiated" cells, rather than as genotoxic initiators.* **Objectives:** *The aim of this article is to provide a screening-tool to evaluate the promoter carcinogen effect of environmental and occupational chemical contaminants, focusing on their ability to alter GJIC.* **Methods:** *GJIC was investigated in serum-free cultured primary human keratinocytes, by directly evaluating the intercellular transfer of a microinjected fluorescent dye (Dye transfer). The expression of caspase 3, which is the ultimate target to be activated of both mitochondrial- and non-mitochondrial-linked pro-apoptotic pathways, was evaluated using Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR).* **Results:** *Mercury chloride (10 nM), mono-methyl Mercury (250 nM) and Trichloroethylene (500 µM) were shown to significantly inhibit GJIC. Conversely di-methyl mercury, lead acetate and epichloridine had no effect on GJIC. All Trans Retinoic Acid completely reversed the inhibitory effect on GJIC induced by HgCl₂ but not that induced by mono-methyl mercury and trichloroethylene. The result of a RT-PCR assay on total RNA cell extract showed that treatment of keratinocytes with 10 nM HgCl₂ resulted in a decrease of the pro-apoptotic caspase 3 expression.* **Conclusions:** *In this work a protocol is designed to study gap junction intercellular communication in primary cultures of human keratinocytes which could be used as a reliable screening tool to test the promoter carcinogen effect of various environmental and occupational contaminants.*