Ecotoxicology and Environmental Safety 72 (2009) 273–279

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

A new respirometric endpoint-based biosensor to assess the relative toxicity of chemicals on immobilized human cells

Roberto Dragone [a,*], Chiara Frazzoli [b], Claudio Grappelli [c], Luigi Campanella [d]

[a] Institute for Complex Systems, Consiglio Nazionale delle Ricerche, P.le Aldo Moro 5, 00185 Rome, Italy
[b] Department of Food Safety and Veterinary Public Health and WHO/FAO Collaborating Centre for Veterinary Public Health, Istituto Superiore di Sanità, V.le Regina Elena 299, 00161 Rome, Italy
[c] Department of Genetics and Molecular Biology, University of Rome "La Sapienza", P.le Aldo Moro 5, 00185 Rome, Italy
[d] Department of Chemistry, University "La Sapienza", P.le Aldo Moro 5, 00185 Rome, Italy

ARTICLE INFO

Article history:
Received 19 May 2007
Received in revised form
24 January 2008
Accepted 24 February 2008
Available online 21 May 2008

Keywords:
Biosensors
Biomarker
Mitochondrial dysfunction
Toxicity test
Human cells

ABSTRACT

Several functional and biochemical parameters have been proposed as biomarkers of effect of environmental pollutants. A rapid biosensor working with immobilized human U-937 cells was developed and applied to environmentally relevant chemicals with different structures and toxicological pathways, i.e. benzalkonium chloride, clofibric acid, diclofenac, mercury nitrate, ofloxacin, and sodium dodecyl sulphate. Respiration of cells was relied upon as a comprehensive biochemical effect for screening purposes. Analytical parameter ($\Delta ppmO_2$) and toxicological index (respiratory inhibition, $\delta\%$) measured after 1 h of exposure were utilized for dose–response relationship study. Results (toxicity rating scales based on $\delta_{50}\%$ and steepness) were compared with those obtained by the same approach previously optimized on *Saccharomyces cerevisiae*. The toxicity rating scale obtained by the biomarker based on human mitochondrial and cell metabolic activities compared well with previous scale obtained on yeast cells and with available *in-vivo* acute toxicity indexes; respiration was confirmed as toxicological endpoint reliably measurable by the biosensor.

1. Introduction

The development of novel, sensitive and efficient *in-vitro* bioassays and strategies is recommended in order to monitor human exposure to a broad-range of chemical agents as well as rapidly determine potential implications of these exposures to human health (Spielmann et al., 2006). Biosensors have many advantages, such as simple and low-cost instrumentation, fast response times, and high sample throughput. A biosensor is an analytical device consisting of a chemical sensor and a biologically active detection system: the reaction of the biological system with the analyte produces a change in the parameter which, in its turn, is measured by an appropriate transducer (Hictchman, 1978). To identify threshold values for exposure through foods and/or environment via dose–response studies, biosensors based on yeast cells has been developed and widely applied (Haubenstricker et al., 1990; Campanella et al., 1995, 1996, 2000; Hollis et al., 2000; Frazzoli et al., 2007); biosensors based on mammalian cells have been also developed (Riley et al., 2003; Chin and Kwun, 2007). Object of this study was the development of a respirometric biosensor based on human cells to further reduce uncertainty factors in the assessment of toxicity on humans. The proven wide field of applicability of the respirometric biosensor on yeast model (Campanella et al., 1995, 1996, 2000; Frazzoli et al., 2007) suggests respiration as reliable endpoint for application also on human cells. In fact, cellular respiratory chain, as well as the associated electron transport in mitochondria, are targeted by several groups of chemicals, e.g. a number of different pesticides (Leroux and Delorme, 1997), heavy metals (Gustavson et al., 2002; Strydom et al., 2006; Frazzoli et al., 2007), drugs (Ishikawa et al., 2006; Tao et al., 2006; Scatena et al., 2007), and metabolites (Weinbach and Ebert, 1985). Cellular respiratory rates are normally determined by metabolic activity, covering more than 20 enzymes. Respiration has been studied to determine whether any change in oxygen consumption occurs under optimal nutritional conditions and normal O_2 tension (155–159 Torr, at barometric pressure of 760 Torr). The Henry's law ($p_a = \chi_a k_a$) describes the *equilibrium* between the O_2 partial pressure (p_a) and the dissolved O_2 molar fraction (χ_a). When temperature is set, the k_a value is constant. Physical–chemical properties of oxygen allow its diffusion through the lipid layers of the cellular membrane. Respiration may be inhibited by the exposure to chemicals able to affect cell membrane, mitochondrial functions key points in the metabolic chain.

* Corresponding author. Fax: +39 649913725.
E-mail address: roberto.dragone@uniroma1.it (R. Dragone).

0147-6513/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.ecoenv.2008.02.011

In the present study, we investigated the ability of a set of chemicals with different structures (Table 1) to inhibit respiration of human cells, through the O_2 measurement during the metabolism of a relevant nutrient (glucose) in open system (at 37 °C). The dose–response curves were constructed to determine the slope (potency of the toxicant) and the 50% inhibition of respiration value (δ_{50}).

The biosensor was assembled by coupling a Clark-type O-sensing electrode (used as amperometric transducer of the cellular respiratory activity) to the biological medium, i.e. a disk containing cells immobilized by inclusion in agarose. A protocol was developed to properly prepare the biological media; in particular, human myelomonocytic U-937 cell line derived from a patient with histocytic lymphoma (Sundstrom and Nilsson, 1976) was used. This cell line was chosen because it grows in suspension medium and trypsinization, which can stress the cells and possibly interfere with respiration, is avoided. Moreover, large amounts of cells can be easily obtained by suspension culture. Chemicals selected for investigation included environmentally relevant and widely used substances: clofibric acid, diclofenac, and ofloxacin were selected as active pharmaceutical ingredients (APIs) able to bioconcentrate in hospital effluents, as well as in urban sewage sludge (Zuccato et al., 2000; Ferrari et al., 2004). As regards contaminants, physical, chemical, and biological properties of heavy metals allow them to persist in the body and cause chronic effects; among heavy metals, mercury represents an "hot" metal and its double charged ion (e.g. $Hg(NO_3)_2$) presents good aptitude to bind S atoms in proteins and interfere with essential enzymatic activity (ATSDR, 1999). Further, the exposure to widely and increasingly used surfactants with different mechanisms (Grant and Acosta, 1996) was considered, and both cationic (benzalkonium chloride) and anionic (sodium dodecyl sulphate) agents were assayed.

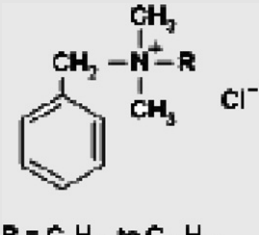
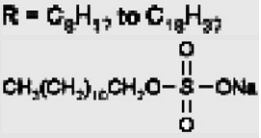
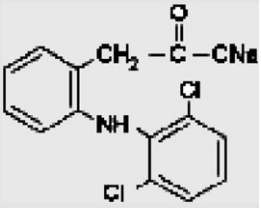
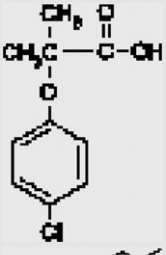
As mentioned, results were compared with those previously obtained on *Saccharomyces cerevisiae* yeast cells and information on the acute toxicity on laboratory animals, e.g. LD_{50} values (median lethal dose), was derived from Material Safety Data Sheet (MSDSs) on these compounds.

2. Materials and methods

2.1. Reagents

Diclofenac sodium salt (CAS no. 15307-79-6, Sigma-Aldrich) is the active principle used in a variety of drugs, such as Dealgic, Deflamat, Diclocular, Diclofan, Diclofenac Hexan, Dicloftil, Dicloreum, Dolaut, Fenadol, Flogofenac, Forgenac, and

Table 1
LD_{50} values, formula, MW, and structure of the chemicals under study, as obtained from the MSDSs

Chemical	LD_{50} (mg/kg)	Formula	MW (g/mol)	Structure
Mercury nitrate monohydrate	ORL-RAT 26	$Hg(NO_3)_2 \cdot H_2O$	342.62	
Benzalkonium chloride	ORL-RAT 240	$C_{24}H_{42}NCl$	350.65	
Sodium dodecyl sulphate	ORL-RAT 1300	$C_{12}H_{25}NaO_4S$	288.38	
Diclofenac	ORL-RAT 53	$C_{14}H_{10}Cl_2NO_2Na$	318.13	
Clofibric acid	ORL-RAT 897	$C_{10}H_{11}ClO_3$	214.65	
Ofloxacin	ORL-RAT 3500 (Exocin)	$C_{18}H_{20}FN_3O_4$	361.37