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Phenotypic anchoring of arsenic and cadmium toxicity in three hepatic-related cell systems reveals compound- and cell-specific selective up-regulation of stress protein expression: Implications for fingerprint profiling of cytotoxicity

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Abstract

Exposure of cells to toxic chemicals is known to up-regulate the expression of a number of stress proteins (SPs), including metallothionein (MT) and members of the heat shock protein (HSP) family, and this response may allow the development of a fingerprint profile to identify mechanisms of toxicity in an in vitro toxicology setting. To test this hypothesis, three hepatic-derived cell culture systems (rat hepatoma FGC4 cell line, rat hepatocytes, human hepatoma HepG2 cell line) were exposed to cadmium (as $CdCl_2$) and arsenic (as $NaAsO_2$), two compounds believed to exert their toxicity through an oxidative stress mechanism, under conditions of phenotypic anchoring defined as minimal and mild toxicity (approximately 5 and 25% reduction in neutral red uptake, respectively). The expression of six SPs – MT, HSP25/27, HSP40, HSP60, HSP70, and HSP90 – was then determined by ELISA. Expression of four of these SPs – MT, HSP25/27, HSP40 and HSP70 – was up-regulated in at least one experimental condition. However, the patterns of expression of these four SPs varied across the experimental conditions, according to differences in toxicant concentration and/or level of toxicity, cell-type and toxicant itself. This lack of uniformity in response of a focussed set of mechanistically defensible targets suggests that similar problems may emerge when using more global approaches based on genomics and proteomics, in which problems of redundancy in targets and uncertain mechanistic relevance will be greater.

Keywords: Cytotoxicity; Stress proteins; Metallothionein; Heat shock proteins; Cadmium; Arsenic; Phenotypic anchoring; FGC4 cells; Rat hepatocytes; HepG2 cells

1. Introduction

Exposure of cells to a variety of stresses, including chemical stress, elicits an up-regulation of a num-

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ber of cytoprotective systems [1], amongst which the heat shock response is one of the most studied [2,3]. In this response, the synthesis of a number of proteins (heat shock proteins, HSPs) is up-regulated; these proteins play a role in maintaining protein structure/function by acting as chaperones to sites of degradation and as facilitators of folding [4]. HSPs represent a highly conserved family of proteins of differing molecular weights [5]. Numerous studies have documented an increase in levels of individual members of the HSP family following exposure of cells to toxic chemicals (as recent examples, see [6–8]), but there are few studies in which multiple HSPs have been investigated. Stress proteins in addition to the HSPs may be up-regulated following chemical stress; one such is metallothionein (MT), which is reported to be up-regulated following treatment of cells with metals and other xenobiotics [9–11].

Current drug development strategies aim to filter out new chemical entities with adverse toxicology profiles at an early stage of development, taking advantage of large-scale screening in cultured cells and genomics/proteomics technology to achieve this [12–14]. However, there is a drive to simplify this process, using more focussed subsets of genomic/proteomic endpoints [15]. For this approach to be successful, these endpoints should fulfil a number of criteria: coherent and mechanistically linked; concentration-dependent up-regulation, preferably with good induction at low levels of toxicity; similar responses in different cell-types; and, similar magnitudes of responses with chemicals exerting toxicity through similar mechanisms.

SPs represent an attractive option for this mechanistically focussed strategy. In this study, we investigated the effect of arsenic and cadmium on levels of SPs in rat hepatocytes and cells of a rat and human hepatoma cell line, FGC4 and HepG2, respectively. The SPs chosen were MT, HSP25/27, HSP40, HSP60, HSP70 and HSP90 (HSP27 is the human orthologue of rat HSP25). Arsenic and cadmium were chosen as the test compounds as they (a) have previously been shown to increase SP levels in various cell-types [16–20], and (b) appear to mediate toxicity through a common oxidative stress mechanism [21–24]. Experimental conditions were established such that the effect of arsenic and cadmium could be evaluated at similar levels of toxicity (‘phenotypic anchoring’). The results indicate that arsenic and cadmium elicit selective changes within the SPs studied, the magnitudes of which differed between the two toxicants and the cell-types.

Table 1
Primary antibody specifications and dilutions used

Stress protein	Specification and supplier	Dilution
MT	Mouse monoclonal IgG, 100 μg at 1 mg/ml Stressgen, Cat.# SPA-550E	1:2000
HSP25	Rabbit polyclonal, 100 μl Stressgen, Cat.# SPA-801E	1:1000
HSP27	Rabbit polyclonal, 200 μl Stressgen, Cat.# SPA-803	1:2000
HSP40	Mouse monoclonal, 100 μg Stressgen, Cat.# SPA-450E	1:1000
HSP60	Mouse monoclonal, 200 μg at 1.3 mg/ml MBL International, Cat.# SR-B807	1:4000
HSP70	Mouse monoclonal, 200 μg/ml Santa Cruz Biotechnology, Cat.# sc-24	1:1000
HSP90	Mouse monoclonal, 200 μg/ml Santa Cruz Biotechnology, Cat.# sc-13119	1:2000

Suppliers: MBL International, Woburn, USA; Santa Cruz Biotechnology, Santa Cruz, USA; Stressgen, Victoria, Canada.

2. Materials and methods

2.1. Materials

Fungizone and gentamicin were purchased from E.R. Squibb and Sons Ltd. (Hounslow, UK) and Hoechst Marion Roussel (Uxbridge, UK), respectively. Nutrient mixture F-12 Ham (N8641), minimum essential medium Eagle (M2279), Williams’ medium E (W1878), fetal bovine serum (F7524), 100× MEM non-essential amino acid solution (M7145), calf skin collagen solution (C8919), 7.5% albumin solution (A8412), 10× trypsin–EDTA solution (T4174), 0.25% trypsin–EDTA solution (T4049), Dulbecco’s phosphate buffered saline (D8537), L-glutamine (G7513), phenylmethylsulfonyl fluoride (P7626) and protease inhibitor cocktail (P8340) were purchased from Sigma–Aldrich Company Ltd. (Poole, UK). Cadmium chloride ($CdCl_2$) and sodium (meta)arsenite ($NaAsO_2$) were obtained from BDH Laboratory Supplies (Poole, UK) and Fluka Chemicals (Gillingham, Dorset, UK), respectively. Tissue-culture treated Costar plastic 6-well plates were obtained from Corning Inc., NY, USA.

Details of primary and secondary antibodies used for ELISA are given in Tables 1 and 2, respectively. Chemiluminescence kit (Amersham ECL Plus™) for detection of Western blots was obtained from GE Healthcare (Little Chalfont, UK).