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A semi-mechanistic integrated toxicokinetic–toxicodynamic (TK/TD) model for arsenic(III) in hepatocytes

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HIGHLIGHTS

- We present a theoretical model coupling kinetics/dynamics of arsenic in hepatocytes.
- The modeled antioxidant mechanism is based on a novel pathway of Nrf2 activation.
- The model estimations are assessed with data of DNA damage in human hepatocytes.
- The analysis highlights the importance of feedback loops to antioxidant response

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ABSTRACT

Background: A systems engineering approach is presented for describing the kinetics and dynamics that are elicited upon arsenic exposure of human hepatocytes. The mathematical model proposed here tracks the cellular reaction network of inorganic and organic arsenic compounds present in the hepatocyte and analyzes the production of toxicologically potent by-products and the signaling they induce in hepatocytes.

Methods and results: The present modeling effort integrates for the first time a cellular-level semi-mechanistic toxicokinetic (TK) model of arsenic in human hepatocytes with a cellular-level toxicodynamic (TD) model describing the arsenic-induced reactive oxygen species (ROS) burst, the antioxidant response, and the oxidative DNA damage repair process. The antioxidant response mechanism is described based on the Keap1-independent Nuclear Factor-erythroid 2-related factor 2 (Nrf2) signaling cascade and accounts for the upregulation of detoxifying enzymes. The ROS-induced DNA damage is simulated by coupling the TK/TD formulation with a model describing the multistep pathway of oxidative DNA repair. The predictions of the model are assessed against experimental data of arsenite-induced genotoxic damage to human hepatocytes; thereby capturing in silico the mode of the experimental dose–response curve.

Conclusions: The integrated cellular-level TK/TD model presented here provides significant insight into the underlying regulatory mechanism of Nrf2-regulated antioxidant response due to arsenic exposure. While computational simulations are in a fair good agreement with relevant experimental data, further analysis of the system unravels the role of a dynamic interplay among the feedback loops of the system in controlling the ROS upregulation and DNA damage response. This TK/TD framework that uses arsenic as an example can be further extended to other toxic or pharmaceutical agents.

1. Background

Arsenic is one of the most common environmental contaminants and carcinogens (IARC, 1987; IARC, 2003). In vitro exposure to arsenicals leads to oxidative stress, chromosomal aberrations, and inhibition of DNA repair (Kligerman and Tennant, 2007; Qin et al., 2008a, 2008b; Soriano et al., 2008), which are phenomena

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tightly linked to oxidative DNA damage and cancer phenotype (Kojima et al., 2009). Trivalent arsenicals bind to sulfhydryl groups (–SH) of proteins and interfere with a spectrum of signaling pathways regulating cell growth, proliferation, apoptosis and survival (Kitchin and Wallace, 2008). The cellular adaptive response to oxidative stress agents such as arsenic typically leads to the activation of the redox sensitive transcription factor Nuclear Factor-erythroid 2-related factor 2 (Nrf2) (Bloom et al., 2002; Kumagai and Sumi, 2007). This transcription factor is considered to be the orchestrator of the cellular antioxidant defense system (Kong et al., 2001b; Wu et al., 2011). Under homeostatic conditions Nrf2 predominantly localizes in the cytoplasm bound to an inactive complex with the Kelch-like ECH-associated protein 1 (Keap-1). This is a cysteine rich protein which facilitates Nrf2 ubiquitination and degradation (Kobayashi et al., 2006; Kobayashi and Yamamoto, 2005). The challenge of oxidative stress triggers Nrf2 nuclear translocation; in the nucleus it heterodimerizes with a Maf protein and binds to the antioxidant response element (ARE) or the electrophile response element (EpRE), commencing upregulation of various cytoprotective genes (Fig. 1) (Kensler et al., 2007).

Two mechanisms have been proposed to describe the Nrf2 activation: (a) Keap-1 is the redox sensor and therefore the Nrf2 release is Keap-1 dependent, and (b) Nrf2 itself is the redox sensor. The former scenario relies on Keap-1 high cysteine content, and on the fact that experimental evidence supports the hypothesis that the cellular redox sensor is endowed with highly reactive –SH groups present on protein domains (Dinkova-Kostova et al., 2002, 2005). Although it is well known that trivalent arsenicals react with vicinal thiols, it has been experimentally demonstrated that arsenic does not disrupt Keap-1/Nrf2 association in the cytoplasm (He et al., 2006; Wang et al., 2008), posing an argument against the Keap-1 dependent pathway interpretation. Recently, Kong and co-workers proposed a novel mechanism of Nrf2 activation, in which a specific motif of the transcription factor NES_{TA} itself is redox sensitive and its inactivation constitutes the driving force for nuclear retention and localization of Nrf2 (Li et al., 2006). Specifically, under oxidative stress conditions, the reactive cysteines embedded in this motif react with electrophiles and disable its function (Fig. 2). Upregulation of the antioxidant mechanism, leading to increased cellular GSH levels, may favor the restoration of NES_{TA} motif activity and the inhibition of the Nrf2 nucleus translocation (Li and Kong, 2009). Therefore there is a "Force Balance" between ROS and GSH that dictates the localization of Nrf2 transcription factor.

In this study, the recently proposed cellular-level TK model for arsenic exposure in human hepatocytes (Stamatelos et al., 2011) is integrated with a TD model focusing on oxidative stress and concomitant DNA damage. This mathematical formulation involves the description of the basic steps of reactive oxygen species (ROS) generation, antioxidant response via the recruitment of Nrf2, a transcription factor that regulates many cytoprotective genes (Kong et al., 2001a), the oxidative genotoxic

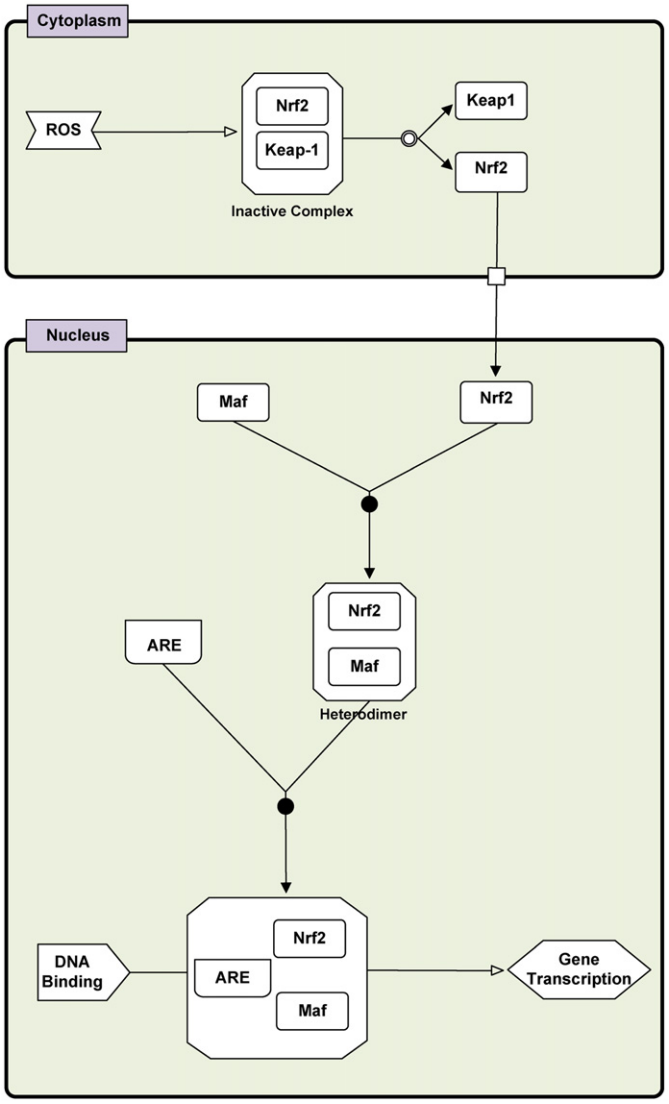

Fig. 1. General scheme of the induction of gene expression through the Keap-1/Nrf2/ARE signaling pathway. ROS increase induces the dissociation of Nrf2 with Keap-1, leading to translocation of Nrf2 to the nucleus. Heterodimerization of Nrf2 with Maf and its binding to ARE leads to transactivation of ARE-inducible genes (adapted from Kensler et al., 2007). The network of biochemical interactions is represented using the Systems Biology Graphical Notation (SBGN) (www.sbgn.org) (Le Novere et al., 2009).

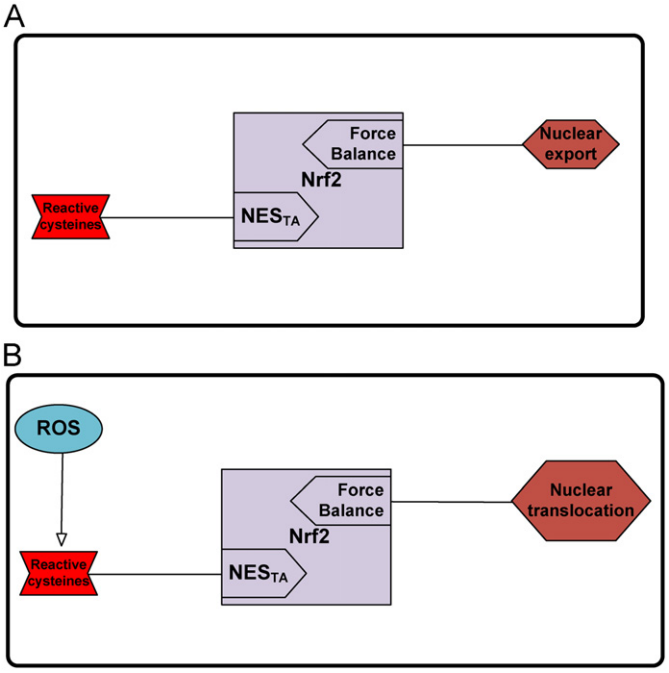

Fig. 2. A new Keap-1 independent model for Nrf2 activation by ROS. The identified NES_{TA} motif contains reactive cysteines and when challenged by oxidative stress is inactivated, leading to Nrf2 nuclear translocation (adapted from Li et al. (Li and Kong, 2009)). Upregulation of the antioxidant mechanism via GSH increase alters the "Force Balance" leading to inhibition of Nrf2 nuclear translocation. The network of biochemical interactions is represented using SBGN formalism (www.sbgn.org) (Le Novere et al., 2009).