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Benzene metabolites block gap junction intercellular communication Role in hematotoxicity and leukemia?

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Abstract

A metabolite of benzene, *trans*,*trans*-muconaldehyde (MUC) was found to be a strong inhibitor of gap junction intercellular communication (GJIC) with potency similar to that of chlordane. Hydroquinone and the MUC metabolite OH–M–CHO were also strong inhibitors of GJIC. The other MUC metabolites tested, CHO–M–COOH and OH–M–COOH had weak effects on GJIC, while COOH–M–COOH had no effect. Benzene showed no effect on GJIC. The relative potency of the metabolites on GJIC is similar to what is observed with regard to hematotoxic effects. The effect of MUC on GJIC took place in parallel with a strong cellular loss of connexin 43. Substances found to inhibit connexin 43 dependent GJIC have been shown to disrupt normal hematopoietic development. The finding that benzene metabolites interfere with gap junction functionality, and especially the loss of connexin 43 induced by MUC, should be considered concerning the mechanism of benzene-induced hematotoxicity.

Keywords: Benzene metabolites; *trans*,*trans*-Muconaldehyde; Connexin 43; Gap junction intercellular communication

1. Introduction

Inhibition of gap junction intercellular communication (GJIC) is suggested as predictor of tumor promoters and non-genotoxic carcinogens [1,2]. Substances with the ability to inhibit connexin 43 dependent GJIC have also been observed to interfere with normal hematopoietic development [3]. Chronic exposure to benzene may lead to bone marrow depression and development of leukemia. The mechanism is unknown, although metabolism of benzene is required for the induced toxicological effects. We have investigated the effect of the benzene metabolites *trans*,*trans*-muconaldehyde (MUC), hydroquinone (HQ) and four MUC metabolites on GJIC.

2. Results

GJIC was measured by scrape loading and image analysis in rat liver epithelial cells IAR6.1 [4].

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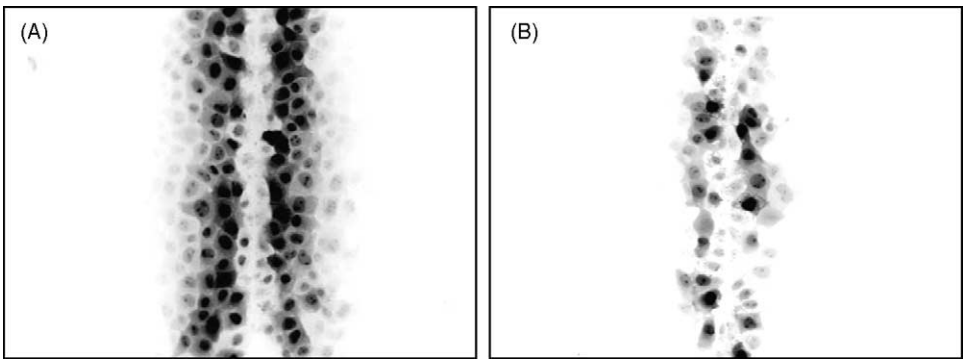

Fig. 1. Measurement of GJIC by scrape loading and image analysis. (A) IAR6.1 cells control and (B) exposure to substance resulting to about 60% inhibition of GJIC.

Fig. 1 shows the extent of Lucifer Yellow coupling in unexposed cells (A), and in cells exposed to a substance resulting in about 60% inhibition of GJIC (B). Fig. 2 shows different response patterns for inhibition of GJIC. The phorbol ester TPA inhibited GJIC transiently while inhibition by chlordane was rapid and sustained. MUC also resulted in sustained inhibition but with slow onset. Western blots show that exposure to MUC resulted in rapid loss of connexin 43, the connexin shown to be responsible for GJIC in the IAR6.1 cells (Fig. 2) [5]. The tumor promoting phorbol ester TPA showed transient induction of connexin 43 phosphorylation, while only small effects were observed after exposure to chlordane.

Benzene, tested up to 20 mM concentration, had little or no effect on GJIC (data not shown). Structures of the MUC metabolites studied are shown in Fig. 3. Fig. 4 shows GJIC in IAR6.1 cells exposed for 5 h. Chlordane, a known tumor promoter and inhibitor of GJIC, was used as positive control. MUC was found to be a strong inhibitor of GJIC, and the most potent inhibitor of the benzene metabolites tested, followed by hydroquinone (HQ) and the OH–M–CHO metabolite. The CHO–M–COOH and OH–M–COOH metabolites had a weak inhibitory effect on GJIC, while the COOH–M–COOH metabolite had no inhibitory effect.

3. Discussion

Previous studies have shown that metabolites of benzene induce different types of chromosomal aberrations [6,7]. We have demonstrated that these metabolites are also potent inhibitors of gap junction intercellular communication. Aberrant regulation of GJIC has been associated with several human pathological conditions

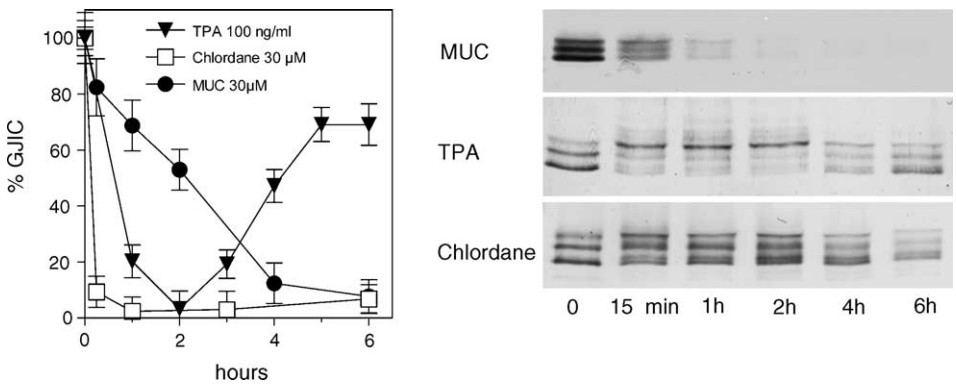

Fig. 2. Different response patterns for inhibition of GJIC (left) and connexin 43 Western blots (right). MUC exposure results in rapid loss of connexin 43.

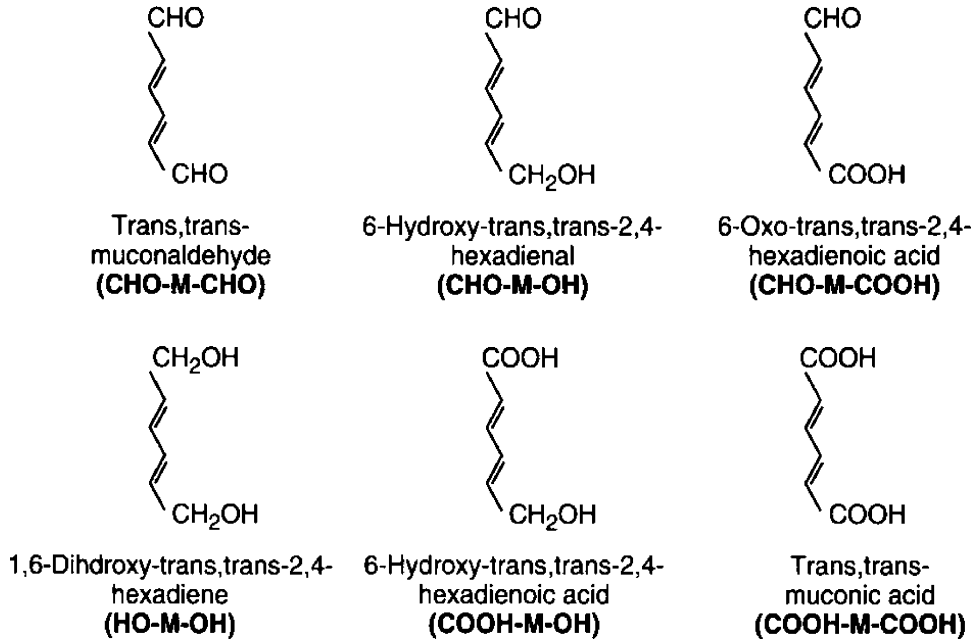

Fig. 3. *trans,trans*-Muconaldehyde (MUC) and its metabolites.

[8], and compounds with the ability to block GJIC have been linked to cancer induction and effects on inflammatory processes as well as development [9–12].

Exposure of 4–6 h duration was needed to obtain maximum effect of MUC on GJIC. This is different from other substances such as TPA, EGF and chlordane, where maximum effect is obtained already after 15–30 min exposure [5]. The effect of MUC on GJIC may therefore be related to the observed induced-depletion of connexin 43 from the cells.

Benzene exposure has been associated with leukemia and bone marrow repression [3]. Eighty to hundred-fold increase in connexin 43 has been observed in bone marrow during establishment and regeneration of the hematopoietic system, indicating that gap junction function could be critical for initiation of blood cell formation [13,14]. Decreased connexin 43 expression during embryogenesis has been shown to compromise the terminal stages of primary T and B lymphopoiesis [13]. Functional connexin 43 dependent coupling in bone marrow and thymic stromal cells have

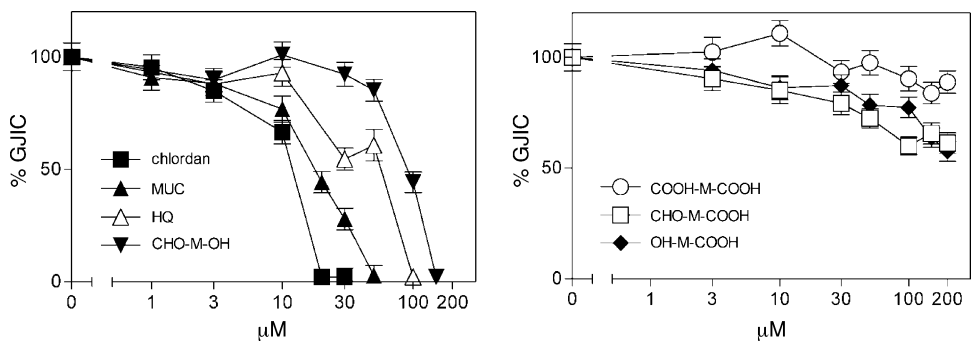

Fig. 4. Effect of chlordane, HQ, MUC and different MUC metabolites on GJIC. The cells were exposed for 5 h to different concentrations of the substances prior to determination of GJIC by scrape loading.

suggested that groups of stromal cells in the bone marrow and thymus affect hematopoiesis [15]. Inhibitors of GJIC have been observed to block such stromal support [14]. Together, this indicates a possible role of GJIC inhibitory effects of benzene metabolites in benzene-induced myelogenic pathological conditions.

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