BIOLOGIJA. 2004. Nr. 4. P. 50–53

Benzene-induced cytotoxicity and apoptosis in TrHBMEC and HL-60 cell lines

D. Bironaite[3]*, D. Siegel[1], B. B. Weksler[2], D. Ross[1]

[1] Dept. of Pharmaceutical Sciences, Box C238, School of Pharmacy, UCHSC, 4200 East 9th Ave, Denver, CO, USA
[2] Cornell University, Coll. Med., New York, NY
[3] Dept. of Developmental Biology, Institute of Biochemistry, Mokslininku 12, 08662 Vilnius, Lithuania

Benzene is a ubiquitous pollutant and known human leukemogen. The mechanism(s) by which benzene causes leukemia still remain unknown. It is generally accepted that in order to exert its cytotoxicity, benzene has to be metabolically activated to hydroquinone (HQ), catechol (Cat.), 1, 2, 4-benzenetriol (BT) and phenol (Phe). Both cell lines after the stimulation with benzene metabolites produce eIL-8, which has been reported to have proapoptotic and cytotoxic effects in HL-60 and TrHBMEC lines, respectively. The slight inhibition of apoptosis by catalase, IL-8 and CXCR1 monoclonal antibodies supports the hypothesis about a complex mechanism of cell toxicity, induced apoptosis and possible excitation of leukemia by hydroxylated benzene metabolites.

Key words: HL-60, apoptosis, leukemic cells, cytotoxicity, quinones, ROS

INTRODUCTION

Chronic exposure to benzene can be associated with bone marrow toxicity and leukemias [1] in human or causing malignancy of different organs in rats and mices [2]. Once absorbed, benzene is metabolized into a series of hydroxylated metabolites, including phenol, hydroxyquinone, catechol, and benzenetriol [3]. Benzene metabolites can be further bioactivated by myeloperoxidases and other hemeprotein peroxidases to reactive semiquinones and quinones, which can lead to the formation of reactive oxygen species (ROS) [4]. Such a complex of production of primary and secondary multimetabolites of benzene damages not only hemopoietic cells but also causes the dysfunction of bone marrow stromal cells [5]. Endothelial cells form a physical but interactive barrier between blood cells and tissues [6]. Recent studies have shown that chemokine eIL-8 (endothelial cell derived CXCL8), the main neutrophil chemotactic and activation factor, could be involved into apoptosis induction in most blood progenitor and leukemic cell lines [7]. For a normal hematopoiesis, production of cytokines is a strictly controlled process and could be induced by the inflammatory cytokines such as IL-1, tumor necrosis factor (TNF-α) or other extracellular stimuli such as oxidative stress [8]. Moreover, it was found that the acute myelogenous leukemia cells constitutively produce eCXCL-8, which binds to two distinct receptors, CXCR1 and CXCR2, with a similar high affinity [9]. The stimulation of these receptors may activate different intracellular downstream pathways and play an important physiological role in the apoptosis of leukemic cells. With this in mind, in our study we examined the stimulation of eIL-8 production by phenolic benzene metabolites in the TrHBMEC cell line and promyelocytic leukemic cell line, HL-60, as a model system for the future studies of leukemogenic disorders caused by benzene exposure.

Abbreviations: HQ, hydroquinone; Cat. Catechol; BT, benzenetriol; Phe, phenol; TrHMBEC, transformed human bone marrow endothelial cell line; eIL-8 or eCXCL-8, endothelial interleukin 8; PI, propidium iodide; PSG, penicillin, streptomycin, L-glutamate; Yo-Pro-1, fluorescent dye; Mab, monoclonal antibody.

MATERIALS AND METHODS

Chemicals and cell culture

Benzene metabolites hydroquinone, catechol, 1, 2, 4-benzenetriol and phenol were obtained from Sigma-Aldrich Inc. (St. Louis, MO). IL-8/CXCL8 Mab, CXCR-1 (IL-8 RA) Mab, recombinant human IL-8 and eIL-8 (endothelial cell-derived IL-8) were ob-

* Corresponding author. E-mail: daiva@bchi.lt

tained from R & D (Minneapolis, MN). Human IL-8 ELISA KIT II for quantitation of human IL-8 was obtained from BD Biosciences Pharmingen (Sad Diego, CA). TrHBMEC cells were obtained and grown according to Weksler [10]. HL-60 cells were cultured in RPMI 1640 supplemented with 10% FBS and 1% PSG at 37 °C in a humidified atmosphere (5% CO_2/air). Viability was measured by Trypan blue dye extrusion test. Trypan blue dye penetrates damaged cell membranes, whereas healthy cell membranes stay impermeable. The amount of IL-8 protein secreted in extracellular media was determined using the OptEIA Human IL-8 ELISA Kit II (BD Biosciences) according to the manufacturer.

Detection of apoptosis

Cells were stained with Acridine Orange/Propidium Iodide (AO/PI), Ethidium Bromide/Propidium Iodide (EB/PI) or Hoechst 33342/PI and visualized using a fluorescence microscope. All three combinations of dyes were used for the measurement of intracellular apoptotic changes with a fluorescence microscope. Samples in triplicate were scored as viable, viable apoptotic, necrotic, nonviable apoptotic or cells without chromatin. A Yo-Pro-1/PI assay was done using a flowcytometer according to the manufacturer's protocol (Molecular Probes Inc. Eugene, Oreg.). This method is based on the detection of changes in cell permeability with Yo-Pro-1 dye, a green-fluorescent nucleic acid which is permeant and stains to apoptotic cells but not to live cells. Necrotic cells are labeled with red-fluorescent propidium iodide.

Statistical methods

All data, except results in Fig. 1 A and 2 A, are expressed as mean ± SEM of at least three independent experiments. Means were compared by a one-way ANOVA (nonparametric) Neuman–Keuls multipal comparison test. Statistical analyses were performed using the PRISM (version 3) software package (San Diego, CA).

RESULTS

Expression of eIL-8 and cytotoxicity of benzene metabolites in TrHBMEC cell line

Our results showed that 100 μM of both HQ and Cat increased eIL-8 levels in cell growth media up to 2.5–3 times compared to control (Fig. 1 A). 100 μM of Cat and BT was 1.45 and 3.22 times more cytotoxic to TrHBMEC cells than 100 μM of HQ after 48-h of treatment, respectively (Fig. 1 B). BT in the 48-h treatment did not show any stimulation of eIL-8 production (Fig. 1 A). Catalase completely protected TrHBMEC viability after BT exposure (data not shown).

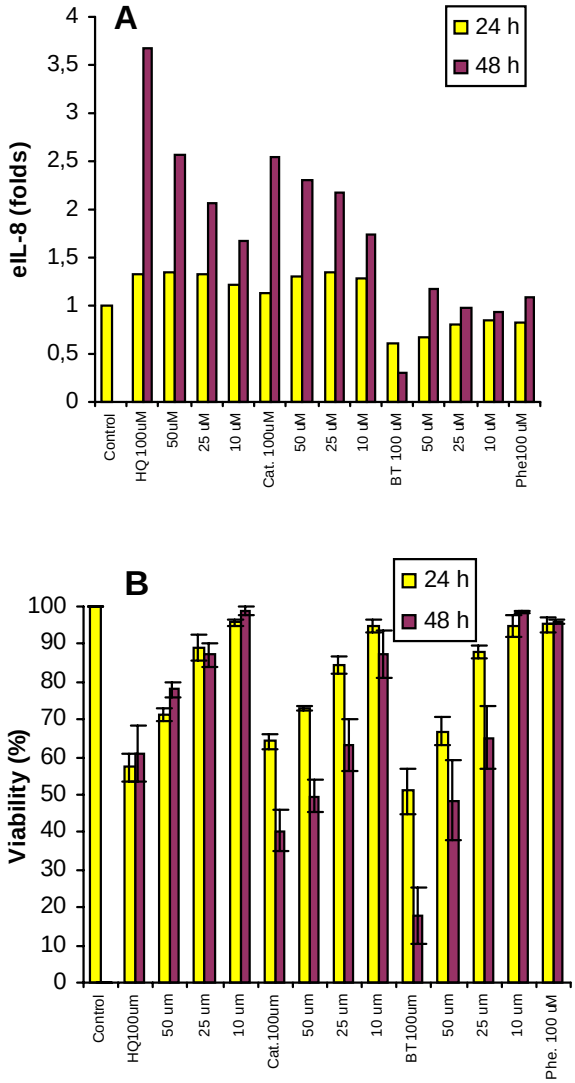

Fig. 1. Stimulation of eIL-8 and induction of cytotoxicity in TrHBMEC line by hydroquinone (HQ), catechol (Cat.), benzenetriol (BT) and phenol (Phe). A – folds of stimulation of eIL-8. Data are presented as a mean of two independent experiments. B– cytotoxicity results are presented as a mean ± SE (n = 3–5)

Detection of apoptosis induced by benzene metabolites in HL-60 cell line

Induction of apoptosis by HQ, Cat., and BT in HL-60 cell line was a concentration- and time- dependent process estimated by the measurement of morphological changes using a fluorescent microscope as described in Materials and Methods (Fig. 2 B). Our results showed that HQ and BT were the most potent inducers of apoptosis in HL-60 cell line. Fig 2A shows that all three benzene hydroxylated metabolites significantly increased the eIL-8 level measured by the ELISA method. IL-8 Mab (R & D) and CXCR1 Mab (R & D) slightly inhibited the early induction of apoptosis measured using a flowcytometer and Yo-pro-1/PI assay in HL-60 cells af-

ter 24 h of exposure cells to 50 mM of all metabolites (Fig. 3). Since we used monoclonal antibodies to identify the stimulation of eIL-8 production, we exposed cells to a mild apoptotic stimulus, 50 mM of compounds. Mab and catalase were less protective to the exposure of cells to 100 mM of compounds for 24 h. The most significant effect of catalase was noticed in the HQ treatment (Fig. 3).

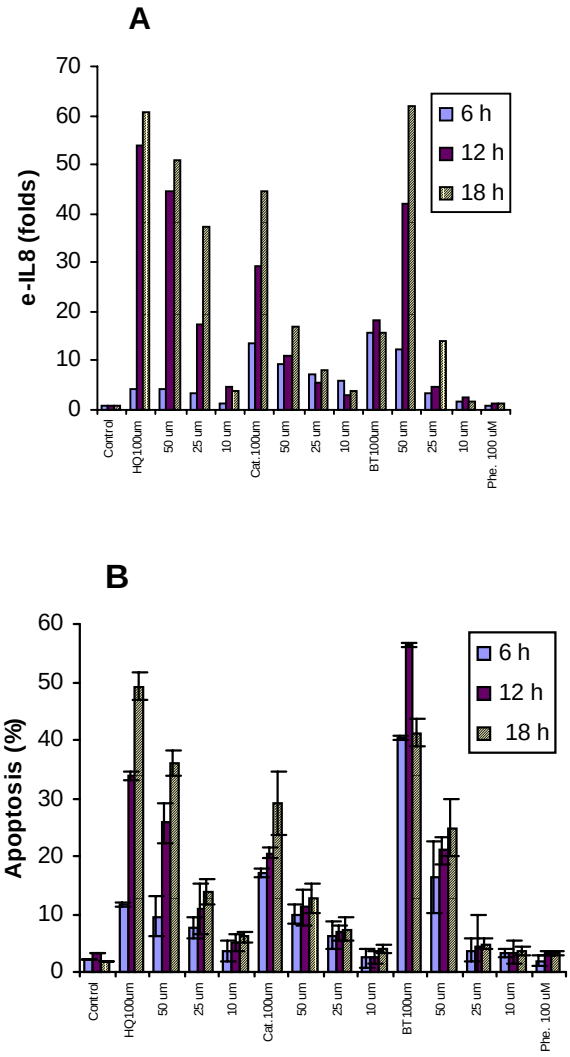

Fig. 2. Stimulation of eIL-8 production and induction of apoptosis in HL-60 cell line by hydroquinone (HQ), catechol (Cat.), benzenetriol (BT) and phenol (Phe). A – folds of stimulation of eIL-8. Results are presented as an average of two independent experiments. B – induction of apoptosis. Apoptosis was estimated with a fluorescence microscope using fluorescent dyes AO/PI. Results are presented as a mean ± SE (n = 5–7)

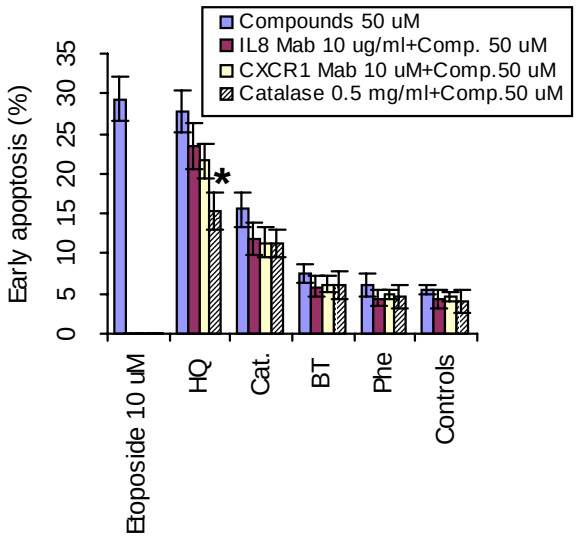

Fig. 3. Induction of early apoptosis in HL-60 cells by benzene metabolites. The early apoptosis was measured with a flowcytometer using fluorescent dyes Yo-Pro-1/PI (Vybrant apoptosis assay kit # 4, Molecular Probes Inc. Eugene, Oreg.) according to manufacturer's suggestions. Data are presented as a mean ± SEM (n = 3, $p < 0.05$). Controls present cells without incubation or incubated only with Mab(s) and catalase. Etoposide presents a positive control

DISCUSSION

In our study, we discussed a few possible ways of causing endothelial and leukemic cell toxicity either to induce apoptosis or a stimulation of other intracellular signaling pathways. The first mechanism involves oxidation of phenols to yield reactive quinones, semiquinones and other ROS with a consequent damage of DNA, which is critical for cell death and carcinogenesis [5]. Additionally to the direct damage of nucleic acids, proteins or induction of apoptosis, ROS can stimulate eIL-8 production [11]. Analysis of the NH_2-terminal sequence revealed that the apoptosis-inducing factor is identical to eIL-8. There is also a possibility that benzene metabolites can directly stimulate both CXCR1 and CXCR2 on HL-60 cells and activate different intracellular signaling pathways such as stimulation of phospholipases C and D [12] or activation of extracellular signal-regulated kinases (ERK1 and/or ERK2) belonging to the family of mitogen-activated protein kinases (MAPKs) [13]. At least two principal pathways of MAPKs activation are known this far: transactivation of tyrosine kinases such as epidermal growth factor receptor (EGFR) and the protein kinase C (PKC)-Raf kinase pathway [14, 15]. In some cases, a role of calcium-sensitive kinase PYK2 has been demonstrated as well [16]. There is also a possibility that benzene metabolites can stimulate production of TNF-a, which modulates expression of various biological molecules [11]. Additionally, CXCL-8 expression could be induced by an oxidative stress directly [11] or through the activation of NF-kB, an essential transcription factor for CXCL8 gene, which eventually increases transcription of CXCL8 gene [17]. In conclusion, the apoptosis-inducing mechanism by benzene metabolites in HL-60 cells is a complex pro-

cess involving a direct damage of DNA, stimulation of GPCRs, CXCR1/2, affection of Ca(II) homeostasis, production of secondary messengers such as chemokine eIL-8, hydrogen peroxide and other kinds of ROS. Additional studies to determine which apoptosis-inducing pathway is limiting after the exposure of leukemic cells to benzene metabolites will be done to better understand the pathogenesis of leukemia.

Received 21 January 2004
Accepted 29 November 2004

References

1. Goldstein BO. J Toxicol Environ Health 1977; 2: 69–105.
2. Farris GH, Robinson SN, Gaido KW, Wong BA, Wong VA, Hahn WP, Shah RS. Fundam Appl Toxicol 1997; 36: 119–29.
3. Ross D. Eur J Haematol (Suppl) 1966; 57: 111–8.
4. Hayes RB, Yin SN, Dosemeci M, Linet M. Am J Med 1977; 40: 117–26.
5. Dorshkind K. Annu Rev Immunol 1990; 8: 111–37.
6. Ohneda O, Bautch VL. British J Haematol 1997; 98: 798–808.
7. Terui Y, Ikeda M, Tomizuka H, Kasahara T, Ohtsuki T, Shimamura S, Miura Y, Hatake K. Bioch Biophys Res Comm 1998; 243: 407–11.
8. Vinante F, Rigo A, Vincenzi C et al. Leukemia 1993; 7: 1552–6.
9. Tobler A, Moser B, Dewald B et al. Blood 1993; 82: 2517–25.
10. Schweitzer KM, Vicart P, Deluis C, Paulin D. Drager AM, Langebhuijsen MMAC, Weksler BB. Labor Invest (Suppl 1) 1997; 76: 25–36.
11. Laksminarayanan V, Beno DWA, Costa RH, Roebuck KA. J Biol Chem 1997; 268: 25569–76.
12. Cohen MV, Baines CP, Downey JM. Annu Rev Physiol 2000; 62: 79–109.
13. Gutkind JS. J Biol Chem 1998; 273: 159–61.
14. Prenzel N, Zwick E, Daub H, Leserer M, Abraham R, Wallasch C, Ullrich A. Nature 2000; 402: 884–8.
15. Cai H, Smola U, Wixler V, Eisenmann-Tappe I, Diaz-Meco MT, Moscat J, Rapp U, Cooper GM. Mol Cell Biol 1997; 17: 732–41.
16. Dikic G, Tokiwa S, Lev SA, Courtneidge J, Schlessinger A. Nature 1996; 383: 1553–60.
17. Janssen-Heininger YM, Poynter ME, Baeuerle PA. Free Radic Biol Med 2000; 28: 1317–27.

D. Bironaitë, D. Siegel, B. B. Weksler, D. Ross

BENZENO INDUKUOTAS CITOTOKSIÐKUMAS IR APOPTOZË TrHBMEC BEI HL-60 LÀSTELIØ LINIJOSE

S a n t r a u k a

Benzenas yra vienas ið daugelio plaèiai paplitusiø aplinkos terðalø, sukeliantis leukemijà. Jo veikimo mechanizmas iki ðiol dar nëra visiðkai aiðkus. Yra þinoma, kad toksinis benzeno poveikis pasireiðkia suaktyvinus jo metabolizmà. Tuomet susidaræ hidroksilinti metaboliniai produktai, tokie kaip hidrochinonas (HQ), katecholas (Cat.), benzenetriolis (BT) ir fenolis (Phe), sukelia apoptozæ HL-60 làstelëse ir yra citotoksiðki TrHBMEC làsteliø linijai. HL-60 làsteliø apoptozës inhibicija katalaze, IL-8 ir CXCR1 monokloniniais anikûniais patvirtina kompleksiná hidroksilintø benzeno metabolitø apoptozës bei citotoksiðkumo veikimo mechanizmà.