Evaluation of Immunotoxic Effects of Arsenic and Other Trace Elements on Human Peripheral Blood Monocytes

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Running title; Evaluation of metallic immunotoxicity in monocytes

Abstract In this study, we examined whether arsenic has any toxicological effects on the differentiation of human peripheral blood monocytes into macrophages by macrophage colony stimulating factor (M-CSF) *in vitro* compared with that of other trace elements (metallic compounds), and found that trivalent inorganic arsenite sensitively inhibited the M-CSF-induced *in vitro* maturation of monocytes into macrophages at very low concentrations, nM levels, although other metallic compounds, including chromium, selenium, cadmium, mercury, zinc, nickel, copper, cobalt, manganese and other human pentavalent arsenic metabolites, such as inorganic arsenate, monomethylarsonic acid and dimethylarsinic acid showed cytolethality in monocytes at μ-mM levels [1]. This work may have implications in arsenic-induced chronic inflammatory poisoning in humans.

Key words : arsenic, arsenite, trace element, metal, monocyte, macrophage, immunotoxicity

Introduction

Humans encounter arsenic in drinking water from wells drilled into arsenic-rich strata. Endemic areas of overt arsenic poisoning have occurred in several countries in Asia and the Americas generally through the consumption of contaminated well water. In clinical investigations, it has been demonstrated that arsenic induces an abnormal inflammatory-like immunotoxicity in humans. The various severe inflammatory clinical observations, such as hepatomegaly, hardening of the liver, splenomegaly and cardiovascular diseases were found in arsenic poisoning patients [2-4], thus, we concluded that investigations of the effects of arsenic on immune effector cells would be of great value in enhancing our understanding of the mechanism of the toxic effects of inorganic arsenic in humans because there is little information about their effects on the immune system. Human peripheral blood monocytes are the important immature immune effector cells that are precursors of mature cells of macrophage lineage in the immune system. Monocytes can differentiate into macrophages by the colony stimulating factors (CSFs) [5]. In this study, we examined whether arsenic has any biological and/or toxicological effects on the differentiation of human monocytes into macrophages *in vitro* by macrophage CSF (M-CSF) stimulation compared with that of other trace elements (metallic compounds), and found that a major environmental arsenic pollutant, trivalent inorganic arsenite, sensitively inhibited the M-CSF-induced *in vitro* maturation of monocytes into macrophages at very low concentrations, nM levels [1].

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Materials and methods

Reagents

Sodium arsenite (As^{3+}) and sodium arsenate (As^{5+}) were purchased from Wako Pure Chemicals Co. (Osaka, Japan). Monomethylarsonic acid ($MMAs^{5+}$) was purchased from Trichemical Co. (Yamanashi, Japan). Dimethylarsinic acid ($DMAs^{5+}$) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Sodium selenite (Se^{4+}), sodium

selenate (Se^{6+}), mercury (II) chloride (Hg^{2+}), cadmium chloride (Cd^{2+}), nickel (II) chloride (Ni^{2+}), manganese (II) chloride (Mn^{2+}), zinc chloride (Zn^{2+}), cobalt (II) chloride (Co^{2+}) and copper (II) sulfate (Cu^{2+}) were purchased from Wako. Recombinant human (rh) M-CSF was purchased from Genzyme Tech Co. (Boston, MA, USA).

Preparation and culture of human peripheral blood monocytes

Human peripheral blood monocytes were obtained from normal healthy volunteers by centrifugation on a Lymphoprep (Nycomed, Oslo, Norway) gradient [5]. Monocytes were separated with the anti-human CD14 monoclonal antibody (mAb)-coated MicroBeads using MACS single-use separation columns (Miltenyi Biotec, Bergisch Gladbach, Germany). Purified monocytes were suspended in RPMI 1640 medium (Sigma) supplemented with 10% heat-inactivated fetal calf serum and antibiotics (100 U/ml penicillin G and 100 mg/ml streptomycin). Monocytes plated in flat-bottomed 96-well tissue culture plates (2 x 10^4/well) were cultured with 1000 U/ml rhM-CSF in the presence or absence of metallic compounds. Cultures were maintained in a humidified atmosphere of 5% CO_2/95% air at 37 °C. Monocytes which were incubated with rhM-CSF for 7 days, were differentiated into adhesive M type macrophages (M-Mp) [5].

Assay for cell viability

Cell viability (cell metabolic activity) was determined by the AlamarBlue (AB) assay, that was similar to the MTT assay [6].

Results

Figure 1 shows the cytotoxic effects of various arsenic compounds and other metallic compounds on the viability of human peripheral blood monocyte-derived M-Mp [expressed as lethal concentration *in vitro* in 50% of a population (LC_{50}) value], or on the M-CSF-induced differentiation of human peripheral blood monocytes [expressed as inhibitory concentration *in vitro* in 50% of a population (IC_{50}) value]. Inorganic arsenite showed a simple cytolethality in monocyte-derived M-Mp at μM levels; its LC_{50} value was 7.0 μM, and it significantly inhibited the differentiation of monocytes into M-Mp at nM levels; its IC_{50} value was 60.0 nM. The ratio of its LC_{50}/IC_{50} was 116.7. For the other arsenic compounds, such as a pentavalent inorganic arsenic, arsenate, and pentavalent human methylated arsenic metabolites,

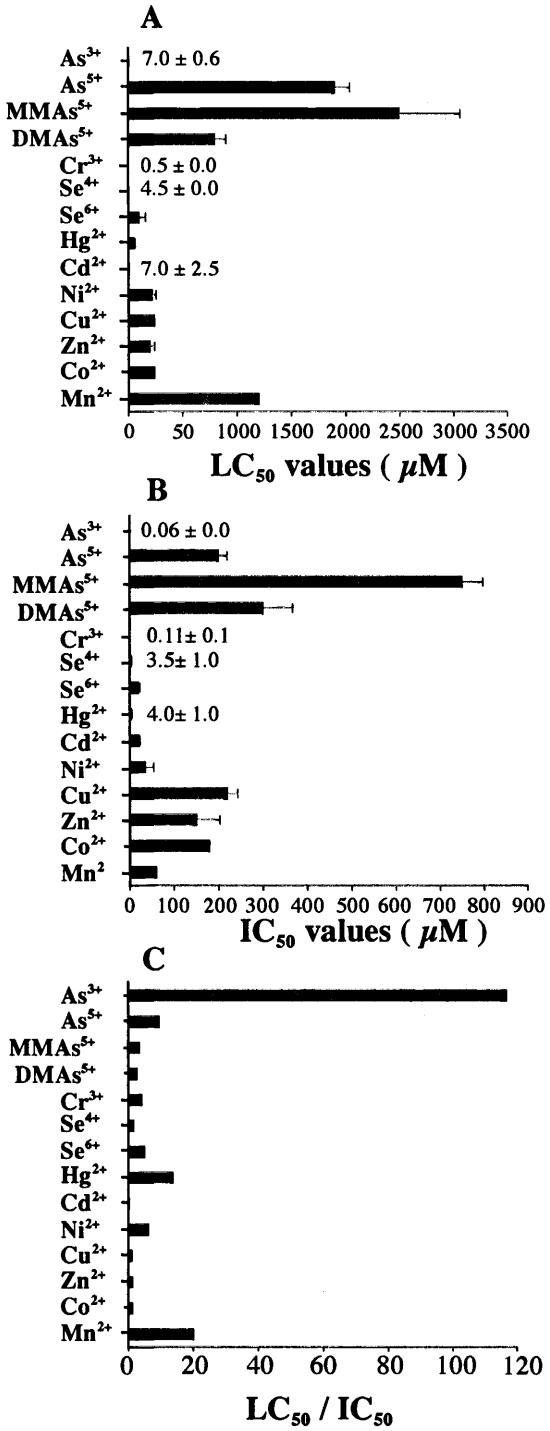

Figure 1. A: Human peripheral blood monocytes were incubated with 1000 U/ml M-CSF for 7 days at 37 °C, and the cells (M-Mp) were further exposed to various concentrations of metallic compounds for an additional 48 h at 37 °C. Cell viability was then measured by AB assay, and LC_{50} values were determined. Results are expressed as arithmetic mean ± SEM of three separate experiments performed in triplicate (n = 9). B: Human peripheral blood monocytes were incubated with 1000 U/ml M-CSF in the presence of various concentrations of metallic compounds for 7 days at 37 °C. After the incubation, cell metabolic activity was then measured by AB assay, and IC_{50} values were determined. Results are expressed as arithmetic mean ± SEM of three separate experiments performed in triplicate (n = 9). C: The ratio of values of LC_{50}/IC_{50}.

monomethylarsonic acid and dimethylarsinic acid, they showed a significant cytolethality in monocyte-derived M-Mp at mM levels, and they inhibited the M-CSF-induced differentiation of monocytes into M-Mp at μM levels. The ratios of their LC_{50}/IC_{50} were 2.7 – 9.5. We subsequently examined the cytolethality of other inorganic metallic compounds, which have been well known as human essential trace elements and/or environmental pollutants, in monocytes. As shown in Figure 1, the rank order of their cytolethality was chromium (III) > selenite > cadmium (II) > mercury (II) >> selenate > zinc (II) = nickel (II) = copper (II) = cobalt (II) >> manganese (II). These inorganic metallic compounds showed a significant cytolethality at μM levels, and most of them more sensitively affected monocytes differentiating into M-Mp than in monocyte-derived M-Mp; their LC_{50}/IC_{50} ratios were 1.6-20.0, although cadmium, copper (II), zinc (II) and cobalt (II) showed a similar cytolethality in both types of monocytes at μM levels [1].

Discussion

In this study, we demonstrated that the bioassay using the maturation of human peripheral blood monocytes into a macrophage lineage *in vitro* is sensitive and useful for measuring the unknown immunotoxic effects of chemical compounds in humans, and a major environmental arsenic pollutant, inorganic arsenite, especially affects the differentiation of monocytes into macrophages; its LC_{50}/IC_{50} ratio was the highest of all the metallic compounds. Arsenite showed a simple cytolethality on matured M-Mp at μM levels, and significantly inhibited the monocyte maturation into M-Mp by M-CSF at nM levels. Inorganic arsenic has caused severe chronic poisoning in Asia and the Americas through the consumption of contaminated well water. Clinical investigations have demonstrated that inorganic arsenic-induced abnormal inflammatory-like immunotoxicity in arsenic poisoning patients is an intermediate clinical stage before fatal carcinogenesis [2-4]. It has been reported that the mean blood concentration of the total arsenic of chronic arsenic poisoning patients from Asia, who continued to drink well water containing high concentrations of inorganic arsenic, was nM levels [4,7], and many cases of inflammatory observations were actually found in these patients [3,4]. Arsenite-induced immunotoxicity described here may play a key role in triggering the immunosuppression and the subsequent carcinogenesis in chronic arsenic poisoning patients. Further clinical research studies will be needed to clarify why inflammatory observations are seen in arsenic poisoning patients.

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