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Selenite is a potent cytotoxic agent for human primary AML cells

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ABSTRACT

Selenite is a potent inhibitor of malignant cell growth. Although the cytotoxic effects have been extensively investigated *in vitro*, there are only a limited number of studies using primary tumor cells with concomitant comparison to conventional drugs. An *ex vivo* model with primary cells from 39 consecutive patients with acute myeloid leukemia (AML) were exposed to a panel of conventional cytotoxic drugs, and the effects on viability were compared to those of clinically achievable concentrations of selenite. Selenite at 5 μM caused the lowest mean survival of primary tumor cells in the panel of all tested drugs (28.95% CI 18.60–39.30%). The cells showed a significant ($p < 0.05$) correlation in the resistance to all tested conventional AML drugs whereas selenite did not, indicating sensitivity to selenite also in multi drug resistant cells. Exposure to selenite also resulted in an increased mRNA expression of the antioxidant proteins TrxR1 and Grx, while staining for TrxR1 showed decreased protein levels. The results strongly suggest a great potential for selenite in the treatment of multi drug resistant AML.

1. Introduction

Acute myeloid leukemia (AML) is the most common form of acute leukemia in adults [1]. The overall incidence per 100,000 people is 3.4 cases in the whole population, 1.2 at the age of 30, and more than 20 at the age of 80 years [2]. The median age at diagnosis is 70 years. Despite improvements in supportive care and abundant trials of novel and conventional agents during the past 30 years, the outcome for the majority of patients remains poor. Conventional chemotherapy for AML is generally divided into remission induction and post remission therapy. Overall, approximately 60–80% of younger adults but only 45–55% of older patients with AML will achieve a complete remission [3]. The median survival in the older population is 8–12 months and less than 15% of the patients obtain a sustained remission for 3 years or more. Among the reasons for the poor outcome in elderly patients are increased treatment mortality from chemotherapy, higher incidence of unfavorable cytogenetics and increased expression of the multidrug resistance gene MDR1 and other proteins that confer resistance to chemotherapy [4]. There is an urgent need for new drugs in the treatment of AML, drugs that are more specific to leukemic cells and that confer less toxic side effects, especially in the older population.

Selenium is an essential trace element in mammals, important for many cellular processes [5]. Recognized for its cancer preventive properties in clinical trials [6,7], selenium has in recent years also evolved as a potential chemotherapeutic agent [8]. The effect of selenium is strictly concentration dependent, with antioxidant properties at low to moderate levels, primarily because it is a constituent of several redox active selenoenzymes [9]. At supranutritional doses, selenium compounds inhibit cell growth and have prooxidant effects [10,11]. Higher concentrations

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of mainly inorganic forms of selenium compounds, e.g. selenite (SeO_3^{2-}), lead to acute toxicity due to DNA strand breaks [12]. Several reports show that the prooxidant and toxic effects induced by selenium compounds occur at lower doses in malignant cells compared to benign and non neoplastic cells [13,14] and thereby giving a large therapeutic potential in cancer treatment. The detailed mechanism for the tumor specific induction of apoptosis by selenium has not been completely understood although several pathways have been proposed [15–18].

Thioredoxin reductase (TrxR) is an essential selenoenzyme. TrxR is part of the thioredoxin system comprising thioredoxin (Trx), TrxR and NADPH [19–22]. Mammalian TrxR's are homodimeric flavoenzymes with a penultimate C-terminal selenocystein essential for the catalytic activity [23–26]. The regulation of TrxR1 is very complex [27] and involves expression of more than 20 different mRNA variants, all differing in the 5′ end [28]. These different transcript variants may result in at least five different protein isoforms [29–31]. The function and regulation of these isoforms still remains elusive. TrxR has a broad substrate specificity and reduces not only protein disulfides but also low molecular compounds [22]. TrxR1 also reduces several selenium compounds and is therefore important in the metabolism of selenium [32,33]. Increased levels of Trx and TrxR have been associated with cancer development and drug resistance and have in recent years developed as promising novel drug targets [34,35].

In this study, we investigated the sensitivity of primary AML cells to selenite and compare the effects of selenium to that of conventional AML drugs. Furthermore, we study the expression and role of redox enzymes in selenite cytotoxicity and the induction of apoptosis.

2. Materials and methods

2.1. Chemicals

Sodium selenite (Na_2SeO_3), dithiothreitol (DTT), Tris–HCl, EDTA, HEPES, bovine serum albumin (BSA), Glutathione (GSH) and NADPH were all purchased from Sigma. Baker's yeast glutathione reductase was obtained from Fluka. Daunorubicin (Cerubidin®) was obtained from Sanofi-aventis AB, Paris, France, idarubicin (Zavedos®) and amsacrine (Amekrin®) from Pfizer Inc. New York, NY, USA. Ara-C (Arabine®) was purchased from Mayne Pharma (Nordic) AB, Stockholm, Sweden and fludarabin (Fludara®) from Schering AGBerlin, Germany. Chlorodeoxyadenosin (Kladribin®) was obtained from Johnson & Johnson, New Brunswick, NJ, USA, mitoxantrone (Mitoxantron Meda®), from Meda AB, Solna, Sweden, and Etoposid (Eposin®) was purchased from Teva Sweden AB, Helsingborg, Sweden, metrizoate-Ficoll (Lymphoprep Fresenius Kabi Norge AS for Axis-Shield PoC AS, Norway.

2.2. Primary AML samples

Fresh leukemic cells from bone marrow and/or peripheral blood from 39 untreated AML patients (for information about the patients see Table 1) were collected in heparinzed test tubes and separated on Metrizoate-Ficoll. Leukemic cells (>90% pure) were further washed in RPMI 1640 with Glutamax-I (GIBCO BRL). All patients were karyotyped and grouped into a favorable, intermediate or the poor cytogenetic risk group. The favorable group included patients with t(15;17), t(8;21) and inv(16)/t(16;16). Patients defined as poor risk had either −5, −5q, −7, t(9;22), abn(3q) or a complex karyotype defined as three or more cytogenetic aberrations. Patients not belonging to the favorable or the poor risk group were defined as intermediate risk patients. Three patients had AML secondary to either MDS (myelodysplastic syndrome) or myeloproliferative disease and two patients had AML related to previous treatment with chemotherapy. All experiments involving human material were approved by the regional ethical committee.

Table 1
Patient characteristics.

Characteristic	Value
Number of patients	39
Age (years) – mean (range)	70 (35–88)
Gender – male/female	22 (56%)/17 (44%)
Secondary AML	
Therapy related	2 (5%)
Previous hematologic disorder	3 (8%)
Karyotype	
Favorable	2 (5%)
Intermediate	29 (74%)
Adverse	8 (20%)
FAB[a] type	
M0	7 (18%)
M1	4 (10%)
M2	6 (15%)
M3	1 (3%)
M4	8 (21%)
M5	2 (5%)
Unclassified	11 (28%)
FLT3-ITD[b] – positive/negative/NA	4 (10%)/15 (38%)/20 (25%)

[a] French–American–British classification system.
[b] FMS-like tyrosine kinase 3-Internal tandem duplication.

2.3. ATP viability assay, drug panel

Cells were seeded at 2.2×10^5 cells/mL in 1.8 mL RPMI Glutamax-I supplemented with 10% fetal calf serum (FCS) (GIBCO BRL) and 0.2 mL of the tested drug diluted in PBS. The cytostatic drugs and selenite were added in duplicates at final concentrations as follow: Ara-C 0.5 μM, fludarabin 2 μM, chlorodeoxyadenosin 50 nM, amsacrine 1 μM, daunorubicin 0.2 μM, idarubicin 0.05 μM, mitoxantron 0.1 μM, etoposide 20 μM, selenite 2.5 and 5 μM. Cells were treated continuously for 4 days with each drug. In the case of daunorubicin, idarubicin, mitoxantron and etoposide the cells were incubated in the dark for 1 h in shaking-waterbath at 37 °C, spinned down ($400 \times g$ for 7 min) and re-suspended in 2 mL fresh medium. After for 4 days of cultivation in a humified incubator (5% CO_2, 37 °C) cellular ATP was extracted by 1.25% trichloroacetic acid (TCA) for 5 min at RT. Samples were either analyzed directly or after storage in −20 °C. The incubations were designed to mimic