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Estrogenic activity of cadmium, copper and zinc in the yeast estrogen screen

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ABSTRACT

Heavy metals are increasingly studied due to their apparent ability to disrupt signaling pathways of living organisms including humans. Among various mechanisms of action, metals are suspected of exerting estrogenic activity in human and wildlife. In this study, a wide range of concentration of cadmium, copper, lead, mercury and zinc (from 95.4 pM to 1 mM) alone or in combination with the natural estrogen, 17-β estradiol, has been tested using the yeast estrogen screen, an estrogen receptor dependent transcriptional expression assay. No direct trans-activation of the estrogen-responsive element could be measured with any of the concentration of the metals tested. Nevertheless, cadmium, copper and zinc were able to potentiate the estradiol-induced response in a dose-dependent manner. Significant stimulation was obtained from 10 nM cadmium, 100 nM copper and 2 nM zinc. Maximum response led to decrease of the estradiol EC50 by a factor 10. This study indicates that cadmium, copper and zinc can act as potential endocrine disrupters by modulating the estrogenic activity of endogenous hormones.

1. Introduction

Heavy metals are widely dispersed into the environment and a major concern in the modern industrial context in terms of pollution. They exert their toxicity by reacting with various biological molecules which then lose their ability to function properly. They have multiple ways of action and a wide range of effects have been described in living organisms. Consequences for populations can be of high concern because metallic compounds can affect reproduction and fertility, the immune and the neuronal systems and may lead to carcinogenesis (Chang, 1996). During the last decade, particular attention has been put on mechanism of toxicity in relation with alteration of the endocrine system. Despite that the focus has been mainly put on organic compounds, metallic compounds are increasingly studied for their ability to disrupt signalization pathways of various living organisms including humans (Leblond and Hontela, 1999; McLachlan, 2001).

Among various mechanisms of action, metals are suspected of exerting estrogenic activity in human and wildlife. Martin et al. (2003) demonstrated estrogen-like activity of several divalent metals including copper, lead and mercury in MCF7 breast cancer cells. Cadmium and organic tin also demonstrated estrogenicity in similar assays (Choe et al., 2003). Cadmium is currently the most documented metal regarding direct ability to induce estrogen-dependant responses. It can bind the rainbow trout estrogen receptor alpha and lead to the release of the natural estrogen, 17-β-estradiol (E2) from the estrogen receptor (Nesatyy et al., 2006). Garcia-Morales et al. (1994) observed a cadmium-induced growth in the human breast cancer cell line MCF7. Using human breast cancer cell-lines, Stoica et al. (2000) showed that cadmium is able to activate estrogen receptor by binding to the hormone binding domain of the receptor. Garcia-Morales et al. (1994) and Choe et al. (2003) found that cadmium, at concentrations of 10 and 100 nM, is able to induce MCF7 cells proliferation. Choe et al. (2003) also observed induction of an estrogen receptor dependent transcriptional expression assay (MCF7-ERE cell line) at 10–10,000 nM cadmium concentrations. These results were confirmed by Wilson et al. (2004) on T47D human breast cancer cells expressing estrogen-responsive luciferase. In this study, cadmium showed a dose-dependent estrogenic activity at concentrations from 10 fM to 10 μM which was inhibited in the presence of anti-estrogens.

Nevertheless, conflicting results have been reported. Le Guével et al. (2000) used yeast cells transfected with the rainbow trout estradiol receptor and observed that cadmium alone was unable to activate the receptor. They also noticed that 10 μM cadmium inhibited E2-mediated estrogen receptor activity. Silva et al. (2006) and Soto et al. (1995), using the yeast estrogen screen (YES) and E-screen assays (MCF7 cells E2-dependent proliferation assay), concluded to a lack of estrogen receptor activity of cadmium in both tests. However, the same study also reported that an association of 1–10 μM cadmium and E2 tended to decrease estrogenic response when compared to E2 alone.

Because conflicting results may be due to differences in sensitivity and concentrations of both metals or E2 used, this study

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aimed at testing a wide range of different metal concentrations and their combination with E2. The metals, cadmium, copper, lead, mercury and zinc, are common pollutants in the environment and major sources of concern for both human and wildlife health. All metals shared common chemical properties and a similar oxidative state. The yeast estrogen screen is one of the most widely used in vitro tests and has demonstrated its efficiency for evaluating estrogenicity of various organic compounds such as natural or synthetic steroids and xenoestrogens (Routledge and Sumpter, 1996).

2. Material and methods

2.1. Metals solutions

$ZnCl_2$, $CuCl_2$, $PbCl_2$, $HgCl_2$ and $CdCl_2$ were obtained from Sigma–Aldrich. Solutions were prepared in ultrapure water. For each metal, from an initial 10 mM solution, 21 concentrations ranging from 10 mM to 954 pM (details in Fig. 2) were obtained by serial dilution in water. Metals solutions were sterilized by filtration using a 0.22 μm filter then transferred to sterile vials.

2.2. YES assay

The yeast estrogen screen was conducted according to Routledge and Sumpter (1996). In this assay, a genetically modified yeast strain harbors a human estrogen receptor expression cassette and a reporter construct. Interaction of an estrogenic substance with the estrogen receptor causes a conformational change in the receptor, enabling the estrogen receptor complex to bind the estrogen-responsive element and to trigger the expression of a β-galactosidase. The enzyme metabolizes chlorophenol-red β-D-galactopyranoside, a chromogenic substrate, to chlorophenol-red that is measured by absorbance at 540 nm. Absorbance at 620 nm allows measurements of yeast growth in each well. Corrected absorbance was calculated as follows: Cor Abs = $Abs_{540\ nm}$ − ($Abs_{620\ nm}$ − $Abs_{620\ nm}$ blank).

Two set of experiments were conducted. First, each divalent metal were tested in the YES screen, then a combination between metals and 17-β-estradiol was used. About 20 μl of each metals solutions (or 20 μl water for blank and "estradiol alone" wells) were transferred to the wells. Then, 10 μl of each estradiol dilution (or 10 μl ethanol for blank and "metal alone" wells) were added. Ethanol was then allowed to evaporate at room temperature in a sterile cabinet. Finally, 180 μl of assay medium and yeast were added to the wells. Final estradiol concentrations in wells ranged from 10 pM to 10 nM and metals concentrations ranged from 1 mM to 95.4 pM. Each metal dilution has been tested alone (without estradiol), and in association with each estradiol concentration. Serial dilution dilutions of estradiol in ethanol were used as reference. Plates were incubated 48 and 72 h at 30 °C before absorbance reading.

2.3. Yeast growth

Impact of metals on yeast growth was evaluated by recording the absorbance at 620 nm of 20 mL yeast cultures in 50 mL tubes. Cultures were made using the growth medium of the yeast estrogen screen (assay medium without chromogenic substrate). A range of metal concentrations (details in Table 1) was added to the medium and absorbance at 620 nm was recorded every 3 h for 40 h after addition of the inoculums. When absorbance was over 0.5, dilutions were made and measured absorbance was corrected by the dilution factor. Maximum growth rate was calculated for each metal concentration.

Table 1
Maximum yeast growth rate measured in presence of several divalent metals in the YES test medium (mean ± standard error, $n = 3$).

Metal	Concentration (M)	Maximum growth rate (mOD h^{-1})
None	–	9.4 ± 2.2
$HgCl_2$	1.E−03	0
	1.E−06	0
	1.E−09	0
$PbCl_2$	1.E−06	10.4 ± 3
	1.E−09	9.5 ± 1.6
$ZnCl_2$	1.E−03	313.8 ± 2.8
	1.E−06	320.4 ± 9.2
	1.E−09	106.6 ± 49.2
$CuCl_2$	5.E−04	7.6 ± 3.3
	1.25E−04	21.8 ± 11.6
	1.56E−04	14.7 ± 3.6
$CdCl_2$	1.E−05	0
	1.E−06	14.7 ± 0.5
	5.E−07	17.7 ± 0.4
	1.E−07	8.9 ± 0.6
	1.E−08	11 ± 5.3
	1.E−09	13.5 ± 3.7

2.4. Statistics

All YES assay experiments were performed twice. ANOVA were used to test for dose effect of metals on the YES assay and on yeast growth using the Statistica software.

3. Results

3.1. Estrogenic activity of metals

17-β-Estradiol (E2) induced a dose-dependent activation of the human estradiol receptor (hER). Median effect concentration (EC50) recorded after 72 h of incubation was between 100 and 200 pM and maximal corrected OD was 2.0. These results are in good agreement with previous published results (Routledge and Sumpter, 1996, 1997; Beresford et al., 2000; Rajapakse et al., 2002; Silva et al., 2002; Peck et al., 2004). Measurements performed after 48 h of incubation resulted in a mean EC50 of 1.25 nM. When the assay was run with $ZnCl_2$, $CuCl_2$, $PbCl_2$, $HgCl_2$ or $CdCl_2$, no effect of any divalent metal could be assessed (Fig. 1). Concentrations ranging from 95.4 pM to 1 mM of copper, lead, mercury and cadmium did not significantly increase galactosidase activity. Only, zinc caused an increase of corrected OD which was correlated with an increased turbidity reflecting yeast density in the considered wells.

3.2. Potentiation of E2 activity by metals

Joint action of metals and E2 on the hER was assessed. Twenty-one concentrations (from 95.4 pM to 1 mM) of each divalent metal were tested in combination with each of the 11 E2 concentrations used as reference (from 10 mM to 10 μM). No significant effect of any lead concentration in the incubation medium on E2-induced EC50 was observed (Fig. 2). Similarly, maximum estrogenic effect was not modified significantly with any tested concentration of mercury ranging from 1 mM to 122nM. On the contrary, all concentrations over 122 nM $HgCl_2$ were extremely toxic to the yeast cells resulting in the absence of yeast growth and no increase in measured OD.

Zinc showed a clear impact on the response of the YES assay (Fig. 2). Presence of zinc in the incubating medium caused a shift of the E2-induced EC50 in a dose-dependent manner. The