EVALUATION OF METAL TOXICITY ON PRIMARY GONADAL CELLS IN CULTURE BY DIFFERENT METHODS

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INTRODUCTION

The increasing incidence of infertility among the population of reproductive age is a considerable problem that has put a heavy burden on the national public health system. Although the cause of the documented infertility cases is largely unknown, there are more evidences that environmental pollutants are partly responsible.
The aim of our work was to study the direct effects of toxic metals (Ni^{2+} and Co^{2+}) on gonadal cells in primary cultures. In the experiments reported here mouse Leydig and human ovarian granulosa cells were used. Ovarian follicular granulosa cells supply nutrients and create the hormonal milieau (mainly progesterone) during oocyte growth. Leydig cells in the interstitium of testis produce testosterone, which acts in a paracrine manner in the testis to support spermatogenesis. Both types of these gonadal cells maintain the ability to produce high amount of sexualsteroids *in vitro* , and can be stimulated with luteinizing hormone (LH) or its analogues (e.g. human chorionic gonadotropin, hCG).
In addition to our previous functional endpoints (cell viability, sexualsteroid production) the localization of cell adhesion proteins (cadherins, ß-catenin) and cytoskeletal tubulin was also checked by immunofluorescent method. Cell adhesion molecules are cell surface proteins that play critical roles in cell recognition and cell adhesion. In the testis, cellular interactions, including cadherin mediated cell junctions are an important prerequisite for spermatogenesis and sperm maturation. In granulosa cells interaction with extracellular matrix proteins regulates granulosa cell morphology, steroidogenic potential, and gonadotropin responsiveness. The effects of cell-cell and cell-matrix interactions are likely due in part to their ability to promote viability of granulosa cells.
In the light of the evidence that toxic metals can affect many of these processes, it is possible, that cell adhesion molecules may be targets for metal toxicity.

MATERIALS AND METHODS

Culture and exposure of human ovarian granulosa cells

Granulosa cells were obtained from follicular fluid of women undergoing ovulation induction and ovum retrieval for the purpose of in vitro fertilization (IVF). In brief, cells were dispersed by collagenase (400 IU/ml, type IV, Sigma) and separated by gradient centrifugation using Ficoll-Paque (Amersham Pharmacia Biotech AB). The cell suspension was diluted to 22 000 cells/ml in the 1:1 mixture of DME and Ham's F12 media supplemented with 10% Fetal Bovine Serum (FBS; Gibco BRL). Cell cultures were maintained for 48 h at 37°C under a humidified atmosphere of 95% air / 5 % CO_2 while exposed to different concentrations of toxic metal ions (15.625 - 1000 μM Ni^{2+} or 2 - 31.25 μM Co^{2+}). Following the culture, cytotoxicity of Ni^{2+} and Co^{2+} was evaluated. The hCG stimulated progesterone (P) production of cells was measured to check the LH-receptor mediated P response.

Culture and exposure of mouse Leydig cells

For isolation of interstitial (Leydig) cells by mechanical dissociation without enzyme treatment. The interstitial cell suspension was diluted to 10^6 cells/ml in Minimum Essential Medium (MEM; Sigma) supplemented with 10 % FBS. Cell cultures were incubated for 48 h at 34°C under a humidified atmosphere

of 95% air / 5 % CO_2 in the presence or absence of different concentrations of toxic metal ions (15-1000 μM Ni^{2+} or 15-1000 μM Co^{2+}). After incubation, cytotoxicity of Ni^{2+} and Co^{2+} was measured. The hCG stimulated testosterone (T) response was also determined.

Cytotoxicity evaluation

Following the 48-h culture, viability of the treated cells was evaluated by the MTT cytotoxicity assay. This colorimetric assay measures the conversion of a tetrazolium salt (MTT) to blue formazan particles by dehydrogenase enzymes of intact mitochondria of living cells. Formazan was measured at 570 nm against 620 nm reference by an Anthos 2010 microplate reader.

Quantification of sexualsteroids

Determination of progesterone and testosterone directly from aliquots of the culture medium was performed by enzyme linked immunosorbent assay (ELISA). The ELISA kits were purchased from Dialab (Austria).

Immunocytochemistry

For immunocytochemical detection of cadherins, β-catenin and tubulin, cells were incubated with (1:300 diluted) pan-cadherin (Sigma), (1:1000 diluted) anti-β-catenin (Sigma) or (1:250 diluted) antitubulin (Sigma). Anti-mouse FITC developed in rabbit (Sigma) was used as secondary antibody. The samples were evaluated by fluorescent microscopy.

RESULTS AND DISCUSSION

The viability of human granulosa cells was diminished in the presence of 62.5 μM Ni^{2+} or 8 μM Co^{2+}, while the progestrerone production decreased from 15.625 μM Ni^{2+} or 2 μM Co^{2+}. In mouse Leydig cell cultures the cell viability was reduced in the presence of 1000 μM Ni^{2+} or 250 μM Co^{2+}, while the testosterone production declined already from 125 μM Ni^{2+} or 125 μM Co^{2+}. These data suggest that the effect of both metal ions on sexualsteroid production of cells was not probably due to the cell death. The results of immunocytochemical examinations showed that the exposure to Ni^{2+} or Co^{2+} decreased the amounts of cadherins and ß-catenin along the surface of the cell-to-cell contacts. Probably destablized these cell contacts. A possible mechanism of action may be associated with the alterations in cAMP content, and intercellular communication may be decreased by the toxic metal exposure. On the other hand, paralelly with observed alterations of cell shape we found some changes of distribution of microtubuli.
Measuring viability and in vitro sexualsteroid production of gonadal cells supplemented by immunocytochemical examinations of adherent-type cell contact proteins may be sensitive endpoints for preliminary evaluation of reproductive toxicity of metals prior to the in vivo experiments.

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