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Short communication

Possible involvement of nuclei in cadmium-induced modifications of cultured cells

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Abstract. Reduced metaphase number and shortening of metaphase chromosomes were detected in McCoy cells exposed to 100 μM $CdSO_4$ (maximal exposure time: 7 h). One hour exposure to ^{109}Cd was enough to label the cell nucleus. This possibly suggests an early nuclear involvement in Cd-induced cell damage.

Key words: Cadmium – Cytoskeleton – Nuclei – McCoy cells

Introduction

Cadmium-induced cell toxicity is currently supposed to be mediated via lipid peroxidation (Gabor et al. 1978; Pritchard 1979) or via Na-K-ATP-ase inhibition (Lai et al. 1980). On the other hand, in cadmium-exposed rat hepatocytes intracellular potassium levels were found to be unchanged, suggesting that toxic effects were not caused by lipid peroxidation, since cell membrane integrity was maintained in these experiments (Stacey et al. 1980).

Cadmium is also able to interfere with subcellular structures, such as microtubules (MTs) and microfilaments (MFs) (Fighetti et al. 1983).

In this work we examine the effects of cadmium on the cytoskeletal and nuclear pattern of cultured McCoy cells in "short-time experiments".

Materials and methods

Cell culture and cadmium exposure. Mouse fibroblasts (McCoy) were grown as described (Fighetti et al. 1983). Medium containing 100 μM Cd^{2+} was added to semiconfluent cultures (final count: $1-4 \times 10^5$ cell/0.5 ml medium/slide) for up to 8 h. Hourly viability, cytoskeleton and nuclei modifications were scored.

Indirect immunofluorescence. To examine MTs and MFs of the same cell, a double labelling immunofluorescence technique was used, as previously described (Fighetti et al. 1983). Phalloidin was used to stain MFs, because of its described specific reaction with F-Actin (Wulf et al. 1979).

Chromosome staining. Cells were rinsed in 75 mM KCl (15 min, 37° C), prefixed with a few drops of methanol: acetic acid 3:1 (prepared just before use) for 10 min at room tem-

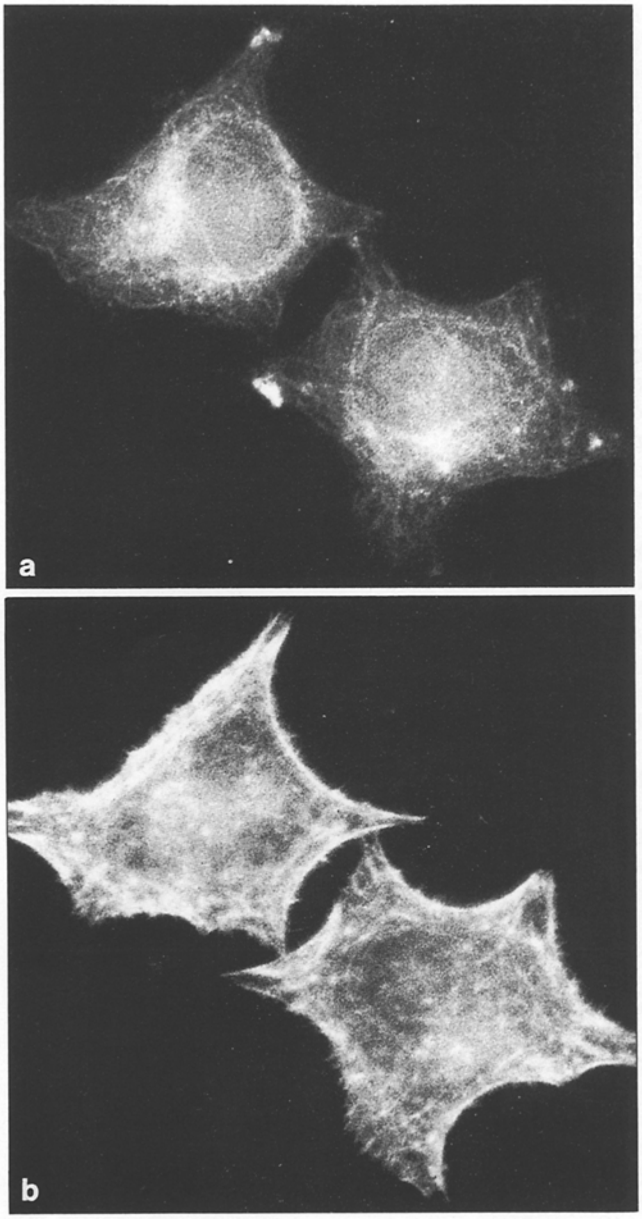

Fig. 1. Untreated McCoy cell. Double immunofluorescence showing MTs **(a)** and MFs **(b)** inside the same cell. Very light fluorescence. In **(b)** the focus is on the cell surface (MFs normal to the cell membrane) (4000 x, reduced to 60%)

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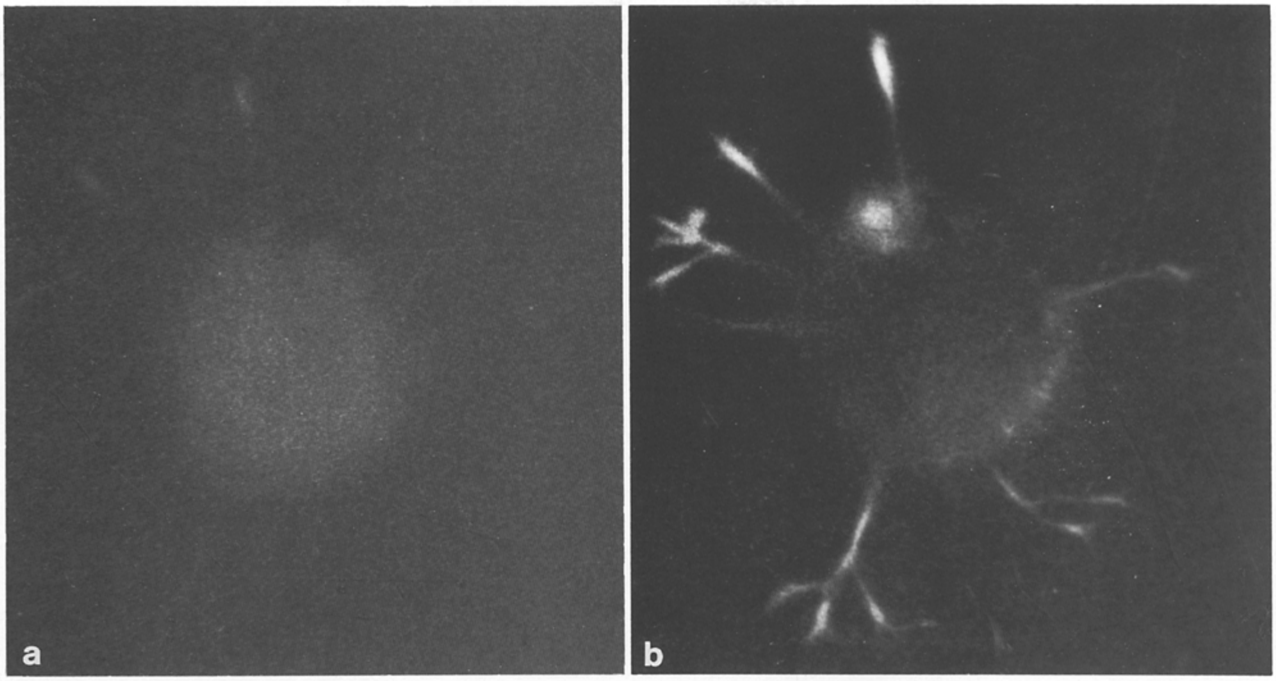

Fig. 2. 7 h-treated cell. The cell is viable. It belongs to the new cell population produced by Cd. MTs (a) are very thin but visible inside cytoplasm and filopodia, MFs (b) are very bright and mostly concentrate in the filopodia and at their bases (5000 x)

perature, fixed again with the same solution (0.5 ml/sample) for 10 min at room temperature and air dried. Finally, cells were stained with 10% Giemsa stain (MERCK) in 0.9% saline for 20 min at room temperature. Only metaphase plates were scored as mitotic.

Nuclei isolation. Cells were processed as described (Enger et al. 1983) with a few modifications.

Cadmium uptake. For uptake experiments, ^{109}Cd (CUS.1 Amersham) was used. Culture medium was prepared with 100 μM $CdSO_4$ (final concentration). After 30 min at 37° C, 5% CO_2, 95% air, ^{109}Cd (0.68 μCi/6 × 10^6 cells) was added. To study the nuclear binding of cadmium before cytoskeletal modifications began, a 3-h incubation was performed. Sample radioactivity of isolated nuclei was determined by a β-counter Beckman LS 1800, in the ^{14}C-window.

Results and discussion

After 7 h exposure to cadmium, the cell viability (Trypan blue exclusion test) was almost normal (over 90% surviving cell). In unexposed cells, interphase MTs show heavy fluorescence (Fig. 1 a). MFs are arranged all over the cytoplasm, with bright fluorescence especially on the cell periphery (Fig. 1 b). After 7 h cadmium exposure, the fluorescence of MTs (Fig. 2 a) is very soft and it can be seen only by careful focusing. Perhaps MTs depolymerize and repolymerize in the new position as we suppose MFs do. At this time (7 h), MFs are arranged in very long, bright arms, with concentrated fluorescence at their bases (Fig. 2 b). Notably, no morphological variations were present at 6 h; they started at 7 h, dramatically increasing at 8 h. The cell toxicity appears not to be dose related. Only at 40 μM Cd^{2+} and at 100 μM were the cells viable up to 13 h. Cadmium 40 μM did not affect the cell shape, but 100 μM cadmium induced a change in both the nuclear shape and the chromosomal pattern at 6 h and a subsequent cytoskeletal and morphological modification at 7 h. We saw dramatic nuclear changes between 6 and 7 h treatment. After a 6-h incubation with cadmium we found 25% reduction of metaphases (0.61% against 2.43% in control samples). Almost no variations were observed either 7 h (0.60%) or 8 h (0.56%).

Chromosomes were shorter and thinner than control. An example is shown in Fig. 3 (a: control; b: treated cell).

Cadmium uptake

Though most of the radioactivity was lost in the medium and in the washing buffer, it was possible to monitor considerable binding of ^{109}cadmium to the nuclei (6.7% after 1 h, 4.6% after 2 h and 3.2% after 3 h treatment). We would like to emphasize the highest label of nuclei in the 1st h of experiment.

The observed morphological modifications, although presumably not cadmium specific, are far from being understood. Many hypotheses have been advanced in the last 20 years, with particular regard to Cd-induced cytoplasmic perturbances. What we found in vitro is not only a cytoskeleton modification but also a depression of mitotic events, probably related to the nuclear cadmium uptake. It is not a metaphase arrest, but it is a stop signal before mitosis. This signal could be compulsory for cytoskeletal perturbations.

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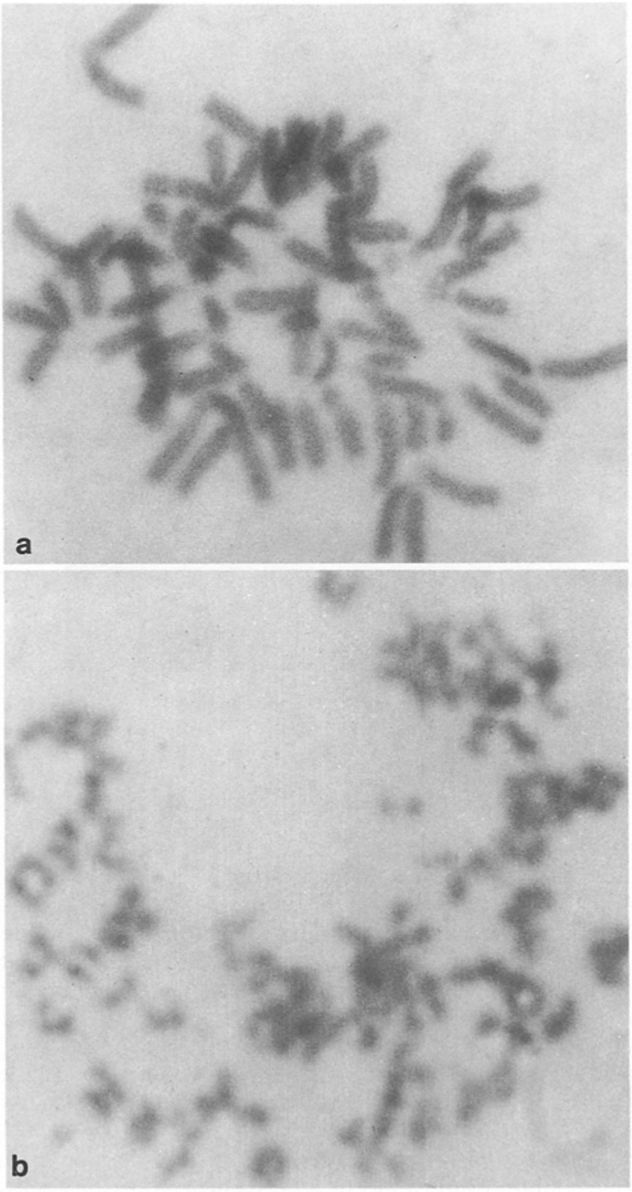

Fig. 3. Metaphases: Giemsa stain. **(A)** Untreated cell; **(B)** 6 h-treated cell; this chromosomal modification happens 1 h before the cell body contraction commences (13900 x, reduced to **(A)** 58%, **(B)** 76%)

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