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Effect of Ni(II), Cd(II) and Al(III) on human fibroblast bioenergetics, a preliminary comparative study

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Abstract Exposure to Ni, Cd or Al leads to different health issues depending on the dose and the exposure frequency. Their mechanism of action is poorly known, but as metals, they may have some points in common. The aim of this work was to compare the impact on cell bioenergetic of these metals using a common cell model: a normal human dermal fibroblast (NHDF) in primary culture. To study cell bioenergetics which "concerns energy conservation and conversion processes in a living cell" as defined by Demirel and Sandler, two technics are combined: oximetry and microcalorimetry. The heat flow measured by microcalorimetry reflects cell metabolism and more generally glucose catabolism (the only nutriment brought to the NHDF). Cell respiration is measured by oximetry and shows the impact on the mitochondria, the energy factory of the cell. Without incubation, Cd inhibits thermogenesis and cell respiration, Ni has no effect, and Al inhibits cell respiration but not thermogenesis. After 24 h of contact at 40 µM, NHDF died with Cd but seemed over-activated with Al and Ni (thermogenesis and cell respiration increased).

Keywords Human fibroblasts · Bioenergy · Thermogenesis · Cell respiration · Microcalorimetry · Metals

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Introduction

Metal exposure has become more and more recognized as a source of pathology and has become a global public health issue throughout the world. The presence of these potentially toxic components in their natural state in our environment leads to different means of contamination. One of the most known is ingestion by drinking water or eating food [1]. This observation led legal authorities to put it under surveillance and ask for regular investigations [2, 3]. Thanks to these measures, scientists can now determine the exposure rate of the population and are able to estimate the acceptable doses of exposure for each metal.

While the mechanism of action remains poorly known, their effects on the human body are well documented [4]. Constant exposure to metals such as nickel (Ni), cadmium (Cd) or aluminum (Al) can lead to an accumulation in the organism and is responsible for chronic pathologies, even if the level of exposure is relatively low. Thus, for Cd whose target organ is the kidney, this well-known phenomenon leads to tubular and glomerular dysfunction and then to renal insufficiency accompanied by bone disorders [5, 6]. Considered as responsible for professional diseases since 1973, cadmium has been classified in group 1 carcinogenic agent by the IARC [7]. In the same way, Ni, which we handle daily when we use coins, jewels or zippers, is now recognized as one of the most allergenic metals [8]. Moreover, besides leading to contact dermatitis, this metal is also classified as a potentially carcinogenic substance for humans by the IARC [9] and a chronic exposure to nickel is registered as a risk factor for lung cancer in the professional illness table. Finally, Al, found in cooking recipients, water, cosmetics or drugs such as vaccines, is said to be responsible for dialysis encephalopathy, osteoporosis and osteomalacia [10]. It accumulates in bones and the

brain bringing about memory and learning disorders but also inducing neurodegenerative pathologies [11–13].

Many studies have been carried out on different cellular types [14, 15], in particular cell lineage such as human osteoblast-like (osteosarcoma) cell line MG-63 or HCT-116 cells from human colorectal carcinoma. Very different study techniques were implemented, so in this context, it was difficult to fully understand the mechanism of action of these metals. That is why making a comparative study of them on a human cell culture such as dermal fibroblast seemed relevant. According to previous reports, we chose to start this work by the analysis of cell bioenergetics [16] using microcalorimetry [17–19] and oximetry methods [20], which allow the measurement of the energy flow inside the cells. The energy is stocked in the cells in the form of ATP; cell bioenergy is therefore represented by the production and consumption of this molecule.

Materials and methods

Reagents

The metal powders used were of high purity (99.9 %): Cadmium chloride, Cd(II), and nickel chloride hexahydrate, Ni(II), were from Aldrich®, and aluminum chloride anhydrous crystallized, Al(III), was from Fluka® analytical.

Primary normal human dermal fibroblasts (NHDF) were purchased from Lonza®. Fibroblast basal medium-2 (FBM-2) supplemented with a growth kit containing 10 mL of fetal bovine serum, 0.5 mL of insulin, 0.5 mL of gentamicin sulfate amphotericin-B (GA-1000) and 0.5 mL of r-human fibroblast growth factor-B were also from Lonza®. During the harvest, NHDF were treated by trypsin–EDTA (1×) 0,05 % purchased from Gibco®.

Sodium dodecylsulfate (SDS) in aqueous solution (at 10 %) came from Sigma-Aldrich®.

Cell culture

Fibroblasts were routinely cultured in culture medium (FBM-2) in sterile boxes for cell culture with an area of 75 cm^2 (T75) and maintained at 37 °C and 5 % CO_2. When cells were at confluence, FBM-2 was discarded and cells were treated with the trypsin solution for 2 min at 37 °C. The cell suspension obtained was diluted in FBM-2 and introduced into new T75 boxes. NHDF were then used for all experiments between passages 5 and 8.

For the experiments, after trypsin treatment, the cell suspension was transferred into a Falcon tube and centrifuged for 5 min at 1600 rpm. The pellet was suspended in 5 mL of FBM-2, and an aliquot of this suspension was made in order to use the *Beckman coulter Z2 particle count and size analyzer* to determine the number of cells. Finally, the suspension was diluted with the culture medium in order to obtain 1 million cells per milliliter.

Thermal analysis

Cell thermogenesis was measured with a microcalorimeter diatherm passive *ThermoMetric® 2277 thermal activity monitor*, at 37 °C. The microcalorimeter was frequently dynamically calibrated in the course of the study. Power–time curves were thus corrected automatically and registered with the Tian equation used in Digitam 4.1 software (thermometric, Sweden). Identical volumes (900 µL) of the fibroblast suspension and pure medium culture were introduced in the measuring and in the reference chamber, respectively. Chambers were then gradually introduced into the device, and a mechanical and constant agitation at 60 rpm ensured the homogeneity of the suspension. Thermal power (P, watt) was continuously registered, and data acquisition was automatically made by Digitam 4.1 software. The effect of the metal was evaluated by injection into the cell suspension of a concentrated metal aqueous solution using a programmable syringe pump (Thermometric 612 Lund syringe pump 2) in order to obtain final concentrations from 0 to 500 µM of Cd and Ni and from 0 to 2 µM of Al. To determine NHDF thermal power (thermogenesis), a volume of the SDS solution (70 µL) was injected to kill the cells (leading to the baseline of the experiment).

A typical curve is illustrated in Fig. 1. This can be described in three parts: First the heat flow leads to a constant power value (A) corresponding to the expenditure energy of the system. Second the heat peak is due to the dilution of the injected metal solution. After that, a stable power value is reached (B). At the end, after a new heat peak, due to the SDS dilution, a third power value plateau is observed (C).

Fibroblast thermogenesis was calculated by the difference between C and A, and the value obtained was divided

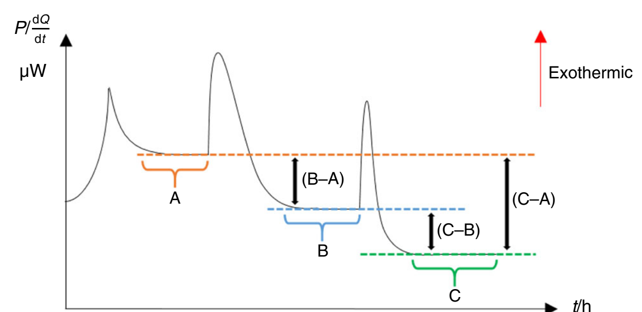

Fig. 1 For example, a typical power–time *curve* of NHDF. *a*, *b* and *c* steps correspond to the addition of NHDF, metal and SDS, respectively

by the number of cells contained in the measuring chamber in order to express cell thermogenesis as pW/cell. The effect of the metal was determined by the difference in the heat flow value plateau between *B* and *A*. The result was divided by the corresponding cell thermogenesis ($C - A$) for the purpose of expressing the effect as percentage of inhibition (I %) according to Eq (1):

$$I(\%) = \frac{B - A}{C - A} \times 100 \quad (1)$$

Oxygen uptake rate measurement

Cell oxygen consumption was monitored by a Clark-type polarographic oxygen electrode system called Oxygraph (*Hansatech® Instrument Ltd*), and data were processed by the software *Oxygraph Plus V1.01*. Electrode assembly was done as detailed by D.von Heimburg et al. [21]. Before taking the measurement, the electrode was calibrated based on Truesdale and Downing [22], who showed that dissolved oxygen in distilled water was between 210 and 230 nmol L^{-1}, at 37 °C and under atmospheric pressure, after degassing with N_2 until equilibrium was reached. Then, the fibroblast suspension (700 µL) was introduced in a closed measuring chamber at 37 °C with continuous stirring at 300 rpm. After 5 min, aliquots of concentrated metal solution were sequentially introduced (at intervals of 5 min) in order to obtain final concentrations from 0 to 500 µM of Cd and Ni and 0 to 5 µM of Al. As shown in Fig. 2, oxygen concentration variations were recorded and cell respiration was determined by measuring the slope of oxygen uptake against time. Cell respiration was thus expressed as nmol O_2/s/10^6 cell. The effect of the metal was determined by the difference between the slope of oxygen uptake with (S_m) and without metal (S_i), and results were expressed as percentage of inhibition according to Eq. 2:

$$I(\%) = \frac{S_i - S_m}{S_i} \times 100 \quad (2)$$

Additional experiments

In a second set of experiments, fibroblasts were incubated with metal (40 µM final concentration) for 24 h. After that, thermogenesis was determined by the difference between plateau values observed before and after the addition of SDS. The value found was compared to the average fibroblast thermogenesis value of 15 ± 5 pW/cell reported by Liu [23]. For cell respiration, oxygen concentration was recorded and the value of oxygen uptake was compared to the average consumption of 10^6 fibroblasts in suspension, 0.032 ± 0.002 nmol O_2/s/10^6 cell, as previously established [24].

Statistical analysis

Result comparison was made by Student's *t* test, and $p < 0.05$ was considered as significant.

Results and discussion

Thermal analysis

Among the metals studied in our experimental conditions, Cd alone altered fibroblast thermogenesis. As shown in Fig. 3, this inhibition was concentration dependant. To determine the inhibitory power of Cd, data were processed according to Eq (3):

$$I(\%) = \frac{[M]}{IC_{50} + [M]} \times 100 \quad (3)$$

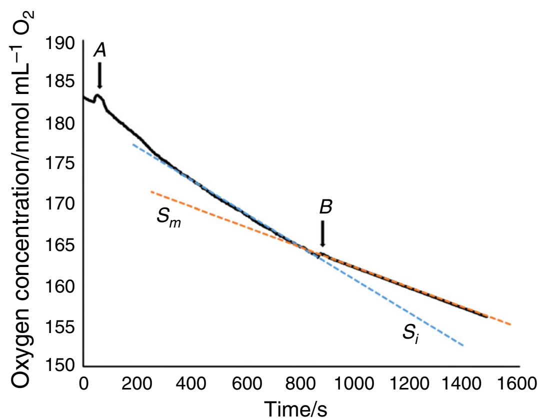

Fig. 2 For example, a typical oxygen concentration *curve* generated by NHDF suspension. *Arrows a* and *b* indicate the addition of cell suspension and metal, respectively. The slope of the *curve* after *a* and *b* measures the oxygen uptake with or without metal

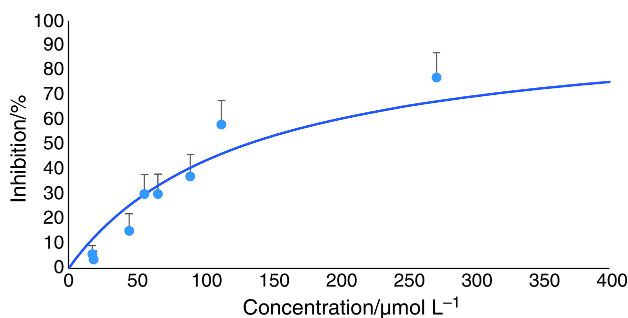

Fig. 3 Inhibition rate of heat release in the presence of different Cd concentrations. *Circles* represent the mean value of five independent experiments, and *error bars* represent the corresponding standard error. The *solid line* is the best fit obtained for the inhibition curve

where I was the inhibitory effect, $[M]$ metal concentration and IC_{50} molar concentration which inhibits half of thermogenesis.

The best fit obtained (using the least-square method) led to an IC_{50} of 132 ± 18 µM. This value was well supported by MTT assay, a biological cytotoxicity test (IC_{50} = 189 ± 44 µM, data not shown).

As reported by Maskow and Paufler [25], cell thermogenesis is mainly due to glucose catabolism, the main source of cell energy [26, 27]. Results demonstrated that Cd induced an alteration of glucose utilization by fibroblasts, whereas Ni and Al appeared to have no effect.

Oxygen uptake rate

As was the case in thermal analysis, Ni had no effect on cell respiration contrary to Al and Cd, which inhibited fibroblast oxygen uptake. As illustrated in Fig. 4, both metals reduced cell respiration related to their concentration. To quantify this effect, the data were processed as described for thermogenesis using the cell inhibition equation. Thus, IC_{50} as 3.1 ± 0.3 and 2.8 ± 0.5 µM were obtained for Cd and Al, respectively. These values, not statistically different, highlight that Cd and Al altered cell respiration and so the mitochondrial function. At this stage, it is impossible to differentiate any mechanism of action.

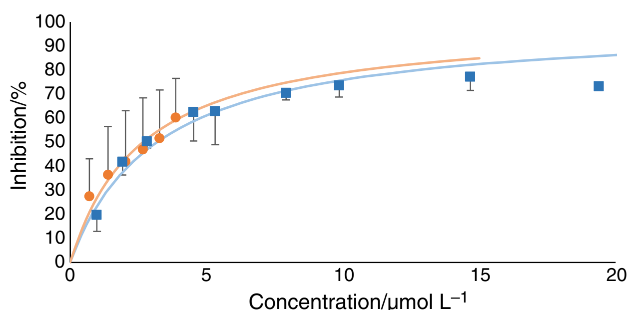

Fig. 4 Inhibition rate of oxygen uptake in the presence of different metal concentrations. *Circles* and *squares* represent the experimental values of Al and Cd, respectively. The *solid lines* represent the fit. Data are expressed as mean values ± standard error of 10 independent experiments

Now, as previously reported, glucose catabolism can be coupled with oxygen uptake [24]. Indeed, let us recall that considering the enthalpy of glucose combustion $\Delta H°c$ (Glc) = −468 kJ mol^{-1} of O_2 [28]. The thermal value (p) can be expressed as: $p = \frac{d(O_2)}{dt} \times |\Delta H°c(\mathrm{Glc})|$. Considering an oxygen uptake of $\frac{d(O_2)}{dt} = 0.032 \pm 0.002$ nmol O_2/s/10^6 cell, theoretical thermogenesis can be calculated as: $p = 0.032 \times 468 = 15\,\mathrm{pW/cell}$.

In this condition, overall results lead to the assumption that each metal, Ni, Cd and Al, acts on the glucose catabolism and the mitochondrial function according to a specific mechanism of action. If the metals were inhibiting oxygen consumption, which is directly linked to glucose catabolism, cell respiration and cell thermogenesis would decrease proportionally and similar IC_{50} would be found, but this is not the case in this study. Indeed, Al thermogenesis is not affected in the concentration range used, and IC_{50} = 2.8 ± 0.5 µM for respiration. For Cd, IC_{50} = 132 ± 18 and 3.1 ± 0.3 µM for thermogenesis and respiration, respectively. This strongly suggests that even if Cd and Al have possible toxic effects on glucose catabolism coupled with respiration, they may act by a specific pathway remaining to be elucidated.

To comfort this assertion, the effect of the metals was determined on thermogenesis and cell respiration after a previous incubation of 24 h. The results of this set of experiments are reported in Table 1. Ni, Cd and Al were used at the same concentration of 40 µM corresponding to the maximum of Al solubility, in our experimental conditions. Cd led to fibroblast death, and both Al and Ni increased thermogenesis and cell respiration by comparison with the control.

This original observation indicates that Al and Ni stimulate NHDF bioenergetics. Hence, the exposure time plays a role in cellular response. As found in other works [29–31], it is possible that metals, by stress effects, modify the global metabolism of fibroblasts. This phenomenon might reflect an adaptation of the cells to an aggressive agent as an attempt of survival. In this context, a new study might be designed to refine these findings.

Table 1 Heat released and oxygen uptake of NHDF exposed 24 h to Ni and Al

	Ni	Al	Literature data[a]
Thermogenesis pW/cell	25 ± 2	25 ± 3	15 ± 5
Oxygen uptake nmol O_2/s/10^6 cell	0.064 ± 0.010	0.088 ± 0.012	0.032 ± 0.002
	$n = 5$	$n = 8$	

Data are expressed as mean values ± standard error of $n = 5$ and $n = 8$ independent experiments for Ni and Al, respectively

[a] [18, 19]

Conclusions

Microcalorimetry and oximetry are suitable and effective tools to study human fibroblast bioenergetics and its alteration by toxic compounds such as Ni, Cd and Al, the object of the present work. In the concentration range used, Cd is the most toxic, acting both on glucose catabolism and cell respiration, whereas Al inhibits only respiration and Ni has no observed effects. Surprisingly, an extended contact induces a reverse effect leading to an increase in glucose metabolism and cell respiration. This original finding highlights the complexity of the mechanism involved. Moreover, it might emphasize a strong change for NHDF metabolism corresponding to the cell response to survive in hostile environments. Metabolomics studies are in progress to support this hypothesis. Finally, this preliminary study could be at the origin of a large research field taking into account the different factors and responses between cell cultures and a whole organism which would require confirmation by a number of experiments.

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