# The Effect of 2-Chlorodeoxyadenosine on Endogenous Respiration of a Human Lymphoma Cell line (U-937)

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## Introduction

2-Chlorodeoxyadenosine (2-CdA) is a new purine analogue which is very effective in the treatment of lymphoid neoplasms [1]. In contrast to most antineoplastic agents, 2-CdA is capable to kill resting and dividing lymphocytes and monocytes [2,3]. As early as 4 hours after exposure to 2-CdA, strand breaks begin to accumulate in the DNA of lymphocytes, presumably because the accumulation of deoxynucleotides in the cells interferes with some aspect of DNA repair [4]. A loss of cellular nicotinamide adenine dinucleotide ($NAD^+$) occurs, very likely because of activation of the enzyme poly(ADP-ribose)-synthetase by the ends of single strands of DNA, initiating a sequence of apoptosis [5].

Cellular $NAD^+$ serves simultaneously as an electron donor in the respiratory chain. Therefore we studied aerobic respiration in a human histiocytic lymphoma cell line (U-937) incubated with 2-CdA. Effects on respiration by 2-CdA may be expected due to the presumable $NAD^+$-depletion, but also due to unknown secondary mechanisms of cytotoxic action [6].

## Materials and Methods

Peripheral blood mononuclear cells (MNC) were isolated according to standard methods [7], T-lymphocytes by using the sheep erythrocyte rosetting technique [8]. The medium for cell culture and respirometric experiments was RPMI 1640 (Sigma Chem. Co. Ltd., Irvine, UK), supplemented with 10% FCS, 300 mg/ml glutamine, 100 U/ml penicillin, 0.1 mg/ml streptomycin and 0.25 $\mu$g/ml amphotericin B.

U-937 cells were incubated at a density of $10^6$ cells/ml with or without 300 nM 2-CdA for six hours. For respirometric measurements we used a two-channel respirometer (OROBOROS® *Oxygraph*; Paar, Graz) with a Clark type oxygen sensor. All experiments were performed within one hour after isolation ($5 \cdot 10^6$ cells in each chamber of 2 ml volume; 37 °C; stirrer speed 350 rpm). The same incubation medium (=cell culture medium) was used after centrifugation for 2-CdA-incubated cells as for controls. After initial purging of the gas phase above the medium with 5% $CO_2$/20% $O_2$, the chamber was closed. Viability of cells was determined before and after respirometric measurement by trypan-blue exclusion.

## Results and Discussion

Endogenous respiratory flux of U-937 cell cultures, expressed per cell, was in the same range as that of isolated T-lymphocytes. The latter tended to be more active, however, than mononuclear blood cells, as observed in a preliminary investigation (not shown). Over experimental periods up to 60 min, constant

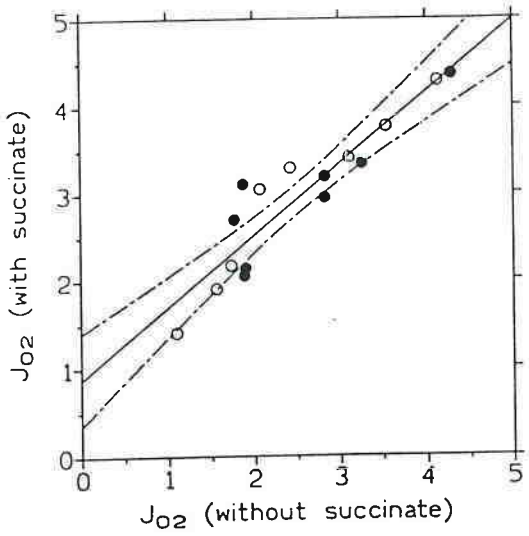


Fig. 1. Respiratory oxygen flux, $J_{O_2}$ [pmol·s$^{-1}$·10$^{-6}$ cells], without succinate and after stimulation with succinate (10 mM), in controls (○, without 2-CdA) and CdA-treated cells (●). The dashed lines show the 95% C.L. of the linear regression.

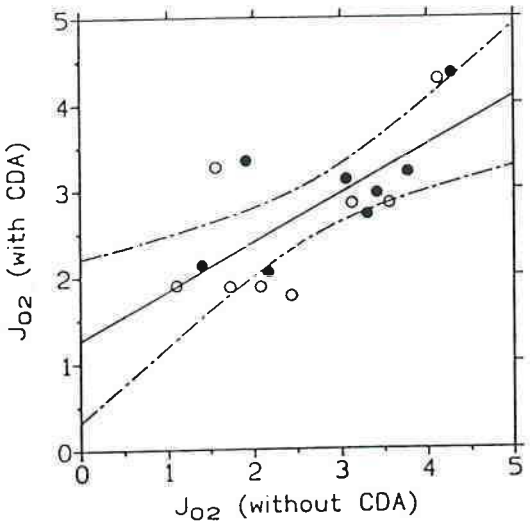


Fig. 2. Respiratory oxygen flux, $J_{O_2}$ [pmol·s$^{-1}$·10$^{-6}$ cells], without 2-CdA and after 6-h incubation with 2-CdA, in controls (○, without succinate) and after addition of succinate (●, 10 mM). The dashed lines show the 95% C.L. of the linear regression.

levels of oxygen flux were observed. Cell viability was 91% on average after respirometric experiments. No significant differences in viability were observed in 2-CdA-treated (6 h) and untreated cells, neither before nor after the experiments, in agreement with reports in the literature: Even at 3- to 30-fold higher 2-CdA-concentrations, viability declines only after 24 h exposure, as judged by dye exclusion as a measure of cell membrane integrity [4].

To test cell membrane integrity at a sublethal level, we measured the response of respiration to the external addition of succinate. As seen in Fig. 1, succinate led to a stimulation of oxygen flux, $J_{O_2}$. The intercept of the linear regression between succinate-stimulated and unstimulated cells was significantly different from zero, whereas the slope did not differ from 1. This indicates an additive rather than proportional stimulation of $J_{O_2}$ by succinate. The stimulatory effect was identical between 2-CdA-treated (●) and untreated cells (○).

The quantitative measure of cellular respiration *in vitro* yields a potentially more sensitive test of sublethal toxicological effects in comparison to measures of cell viability [9]. When plotting oxygen flux in 2-CdA-treated versus untreated cells (Fig. 2), a trend towards increased respiration was apparent, particularly when fluxes of the controls (without 2-CdA) were low. This effect was significant only when combining the measurements without (○) and with (●) succinate (Fig. 2), and corroboration of the relationship requires further experiments. Support for the combined analysis (Fig. 2) stems from the fact that no significant difference of succinate stimulation was observed between 2-CdA-treated and untreated cells (Fig. 1).

The possible stimulation of respiration by a toxicological effect of 2-CdA might be due to uncoupling of oxidative phosphorylation. This hypothesis was

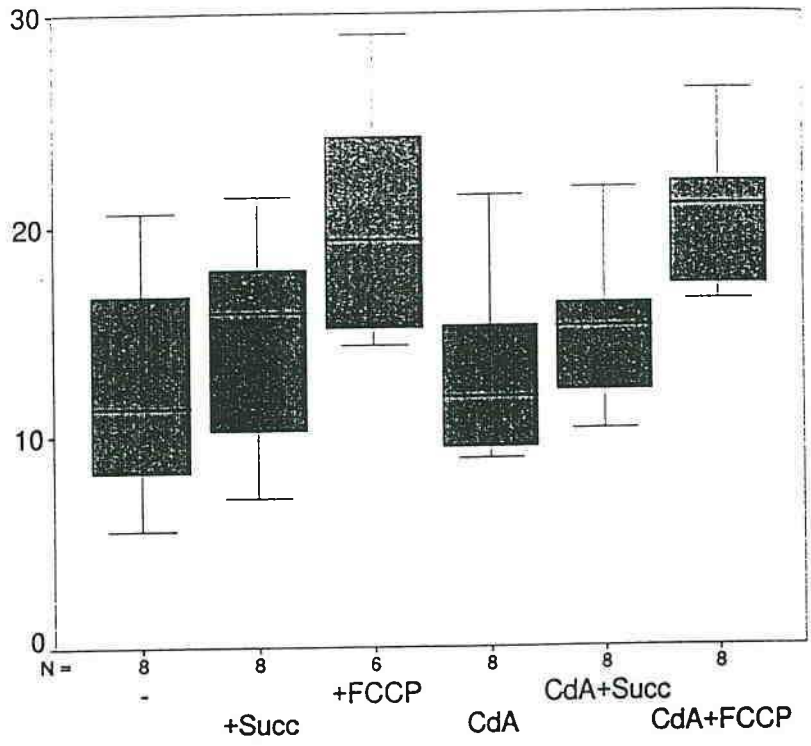


Fig. 3. Oxygen flux, $J_{O_2}$ [pmol·$s^{-1}$·$10^{-6}$ cells]; endogenous (-); after 6 h of 2-CdA-incubation (CdA); after addition of succinate (+ Suc, CdA + Suc); after addition of FCCP, 0.2 $\mu$M ( + FCCP, CdA + FCCP ). The * between columns indicates a difference at $p<0.05$. Median (bold line), 25-75% percentiles (shaded bars), range (bars).

tested and rejected by titrating the uncoupler FCCP to the respiring intact cells. Uncoupling increased significantly the respiratory flux of 2-CdA-treated cells (Fig. 3), although the effect of uncoupling was relatively small in both control and 2-CdA-exposed cells.

Our results show that high-resolution respirometry of mononuclear blood cells can potentially be used for the characterization of toxicological effects on endogenous aerobic metabolism. Stimulation of oxygen flux by 2-CdA is very small (if significant at all) within six hours of high dose incubation. Since cell death occurs at the earliest after 24 hours of 2-CdA incubation, a study of the complete time course of the sublethal respiratory response up to the loss of viability will provide more information on the toxicological mechanism of 2-CdA action.

## ACKNOWLEDGEMENTS

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