Cellular bioenergetics in chicken embryo fibroblast (CEF) and immortal CEF (DF-1) cells.

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ABSTRACT

As CEF and spontaneously immortalized CEF (DF-1) cells differ in growth rate, mitochondrial function and response to oxidative stress, studies were designed to assess bioenergetics in CEF and DF-1 cells using an XF24 flux analyzer (Seahorse Biosciences, Billerica, MA). Whereas CEF cells exhibited increased O_2 consumption (OCR) and extracellular acidification (ECAR) rates with seeding densities of 50 K, 100 K and 150 K cells per well, indicating an overall increase in cell energetics, DF-1 cells increased ECAR without changing OCR when seeding density exceeded 100K which suggests an increased reliance on glycolysis for energy production. Subsequent studies utilized 100 K cell seeding density for both CEF and DF-1 cells incubated in media containing glucose with or without pyruvate (1, 5, and 10 mM). After establishing basal OCR, cells were treated sequentially with oligomycin (1 μg/ml), FCCP (150 nM), and antimycin A (5 μM) to assess OCR linked to ATP synthesis, proton leak, reserve capacity and non-mitochondrial use (see Hill et al., 2009 Biochem J. 424:99-107). The results suggest that compared to CEF cells, DF-1 cells have lower proton leak, lower non mitochondrial oxygen consumption and higher reserve capacity when incubated with glucose alone. In CEF cells, adding pyruvate in the media stimulated ATP-linked OCR that was associated with decreased reserve capacity and decreased non-mitochondrial OCR. In contrast, DF-1 cells appeared to decrease ATP-linked oxygen consumption and increase reserve capacity in the presence of pyruvate plus glucose in comparison to cells receiving glucose alone. Regardless of energy sources in the media, DF-1 cells exhibited higher reserve capacity and ATP-linked OCR and lower proton leak and non-mitochondrial OCR compared to CEF cells. Studies to assess CEF and DF-1 bioenergetics in response to oxidative stress are in progress.

INTRODUCTION

DF-1 cells are chicken embryo fibroblast (CEF) cells that became spontaneously immortalized (i.e. without viral or chemical treatment) (Schaefer-Klein et al., 1998). The more rapid growth of DF-1 cells compared to CEF cells (and other immortal CEF cell lines) was associated with increased mitochondrial transcription and ATP production (Kim et al., 2001). DF-1 cells exhibit higher MnSOD and lower catalase expression compared to CEF cells. Also, antimycin A increased necrotic death in DF-1 cells that was associated with increased levels of hydrogen peroxide and superoxide anion (You et al., 2004). Thus, DF-1 cells apparently exhibit increased susceptibility to oxidative stress compared to CEF cells.

OBJECTIVE

The major objectives of this study was to compare cellular bioenergetics of CEF and DF-1 cells, and to assess bioenergetic responses to oxidative stress induced by a secondary lipid peroxidation product,4-hydroxynonenal (HNE).

MATERIALS AND METHODS

CEF and DF-1 Cells were grown in DMEM media with 25 mM glucose,10% fetal bovine serum, 14 mM L-glutamine, and 1% antibiotics (penicillin □streptomycin). All CEF studies were conducted on cells between passage number 7 to 9.

Bioenergetics were assessed with an XF24 Flux Analyzer (Seahorse Biosciences, North Billerica, MA) using procedures similar to those reported by Hill et al. (2009) with modifications. The Flux Analyzer measures oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) in specialized microplates. For all bioenergetic studies, the culture medium was changed to an unbuffered DMEM media containing 25 mM glucose 60 min prior to the initiation of OCR and ECAR measurements.

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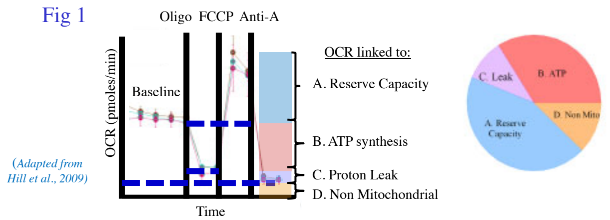
Materials and Methods (cont.)

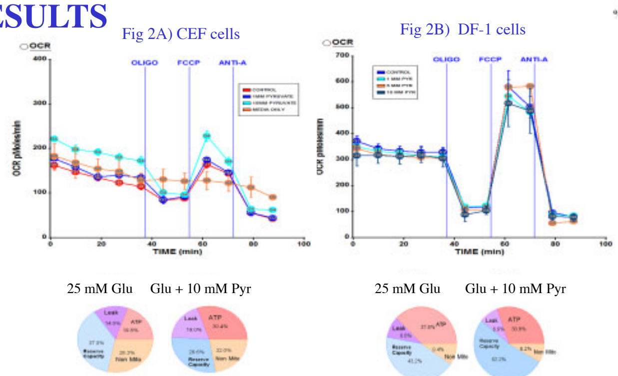
OCR response to oligomycin (Oligo), FCCP, and Antimycin-A (Anti-A), enables assessment of basal OCR, OCR linked to mitochondrial ATP production, OCR linked to proton leak, maximal respiratory capacity, O_2 consumption reserve capacity and non-mitochondrial OCR (Fig. 1) .



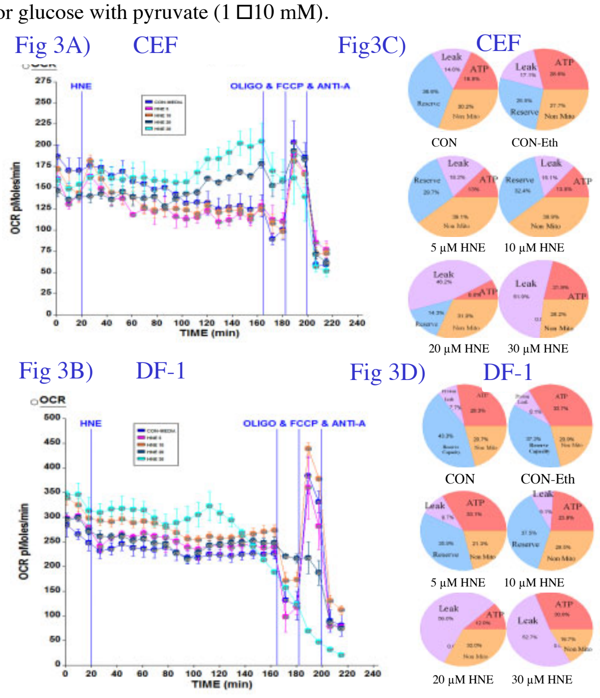
Optimization of Conditions:

Initial studies were conducted to optimize the cell seeding density, and concentrations of oligomycin and FCCP (data not shown).

RESULTS

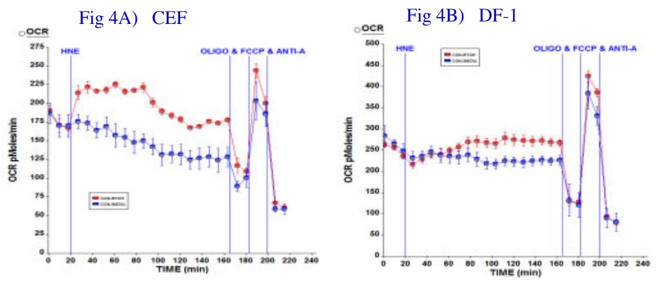


Exp. 1: Bioenergetics of CEF and DF-1 cells (Fig. 2): CEF s (2A) exhibited greater proton leak, non-mitochondrial O_2 consumption and lower reserve capacity compared to DF-1 cells (2B) with glucose (25 mM) alone or glucose with pyruvate (1 □10 mM).

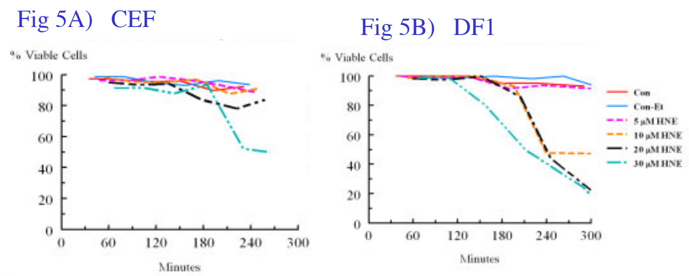


Exp. 2: Assessment of bioenergetics in response to 4-HNE (Fig. 3). Time course changes in OCR in response to 4-HNE (0 □30 μM) in CEF (A) and DF-1 (B) cells. Cells were treated with Oligomycin (Oligo, 1 μg/mL), FCCP (150 nM), and Antimycin A (Anti-A, 5 μM) to assess O_2 consumption related to ATP synthesis, proton leak, reserve capacity and non-mitochondrial sources in CEF (C) and DF-1 (D) cells.

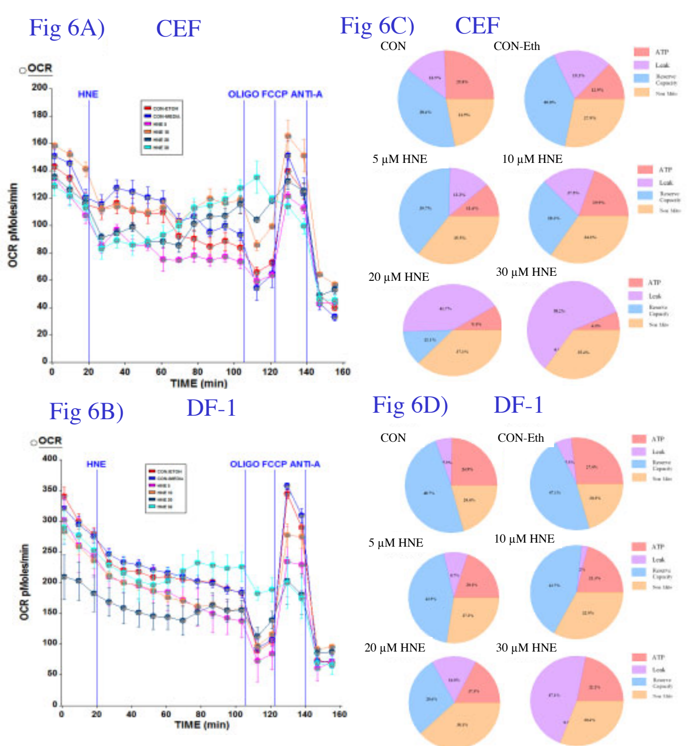
Results (cont.)



Exp. 2 (cont.) Fig. 4. OCR was different between media only control and media plus ethanol vehicle (3% final volume) in CEF (A) and DF-1 (B) cells. Since there could be a synergistic effect of ethanol and HNE, Exp. 3 was conducted (see Fig. 5 and 6 below).



Exp. 3: Effect of 4-HNE (with less than 0.05% ethanol vehicle final volume) on viability of CEF and DF-1 cells. (Fig. 5). Percent viability was determined by trypan blue exclusion in CEF (A) and DF-1 (B) cells. It is apparent that DF-1 cells are more susceptible to oxidative stress caused by 4-HNE than are CEF cells.



Exp. 3: Bioenergetics of CEF and DF-1 cells to 4-HNE (Fig. 6). Time course changes in OCR in response to 0 to 30 μM 4-HNE are shown for CEF (A) and DF-1 (B) cells. After 100 min, cells were treated sequentially with Oligo, FCCP and Anti-A (See Exp. 2). Bioenergetic assessment of O_2 consumption (%) related to ATP synthesis, proton leak, reserve capacity, and non-mitochondrial sources in CEF cells (C) and DF-1 (D) cells. (Note: ethanol vehicle was < 0.05% of final volume.)

SUMMARY

1) Under control conditions DF-1 cells exhibited higher reserve capacity, lower proton leak, and lower non-mitochondrial O_2 consumption (Fig. 2).

2) DF-1 cells are more susceptible to 4-HNE toxicity than CEF as indicated by an inability of DF-1 cells to maintain;
   a) coupled mitochondria above 10 μM HNE (Exp. 2, Fig. 3),
   b) cell viability over 4 h with 10, 20 or 30 μM 4-HNE (Exp. 3, Fig. 5).

3) With 0.05% ethanol vehicle, CEF and DF-1 cells maintained mitochondrial respiratory reserve capacity to 20 μM HNE, but not at 30 μM levels (Fig. 6).

4) These findings provide additional insight into DF-1 cells rapid growth and greater susceptibility to oxidative stress than CEF cells (Kim et al., 2001; You et al., 2004).

REFERENCES

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4) You, S. B-W. Kong, S-Y. Jeon, D. N. Foster, and H. Kim. 2004. Deregulation of catalase, but not MnSOD, is associated with necrotic death of p53-defective DF-1 cells under Antimycin-A-induced oxidative stress. Mol. Cells: 18:220-229.

Additional: Global Gene Expression

Fig. 7. Gene network: Cancer, Cardiovascular System Development and Function, Organismal Development:

Up-Reg in DF-1
Down-Reg in DF-1

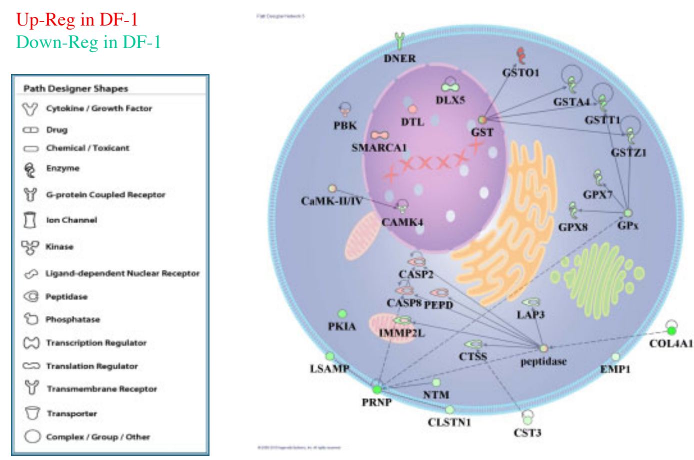

Fig. 7. We have initiated global gene expression studies in CEF and DF-1 cells (no 4-HNE treatment). Gene expression was determined with a 44K microarray (Agilent) and verified by qRT-PCR (not shown). The gene network was generated by Pathway Designer of Ingenuity Pathway Analysis (http://www.ingenuity.com). A list of genes in this network is attached below. DF-1 cells exhibited down-regulation of several GSHTs and GSHPxs. These findings concurs with increased susceptibility to oxidative stress in this study and previous findings (e.g. You et al., 2004). The role that up-regulation of caspases (CASP2, CASPS) which should increase apoptosis may play in immortal DF-1 cells is not clear unless these enzymes remain in an inactive form. From Buri et al. (2005. Mol. Biol. Cell 16:2926-33), down-regulation of IMMP2L (inner mitochondrial membrane protease) could be hypothesized to prevent or delay apoptosis. In addition, VDAC was also down-regulated in DF-1 cells (data not shown).