Effect of cytomix and sedative drugs on mitochondrial electron transport chain of cultured primary human astrocytes

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Introduction: Septic shock is a major cause of death among patients in intensive care units worldwide. Despite the recent developments and progression in medical research, sepsis remains a challenge. Multiple-organ failure including brain dysfunction (septic encephalopathy) is the predominant cause of death in septic patients. Elevations of cytokine concentrations in the brain have been described in both experimental and clinical studies. Furthermore, mitochondrial dysfunction has also been described in septic encephalopathy. Since also sedative drugs interfere with brain function, they may contribute to septic encephalopathy.

Aim of this project is to evaluate the effects of:
a) Cytomix (mixture of cytokines) relevant in severe sepsis
b) Sedoanalgesic drugs (Propofol, Fentanyl and Midazolam) at clinically relevant concentrations
c) Combination of both cytomix and drugs
on mitochondrial function of primary human astrocytes.

Methods: The primary human astrocytes from 10 donors, were purchased from ScienceCell Research laboratories (Carlsbad, CA, USA) and cultured in poly-L-lysine coated 6- or 96- well plates. Experiments were performed when cells reached 90-100% confluency. Cells were incubated with a) cytomix (TNF-α [10 ng/ml], IFN-γ [10 ng/ml] and IL-1β [10 ng/ml]) for 1, 6, 12, 18, 24 and 36 hours, b) sedoanalgesics (propofol [30 µM], fentanyl [2ng/ml], midazolam [600ng/ml]) alone for 1h, and c) a mixture of drugs (1h pre-incubation) and cytomix (24 hours in combination).
Mitochondrial electron transport chain complexes I and IV, ATP synthase (Figure 1), citrate synthase enzymatic activities and cellular ATP content were measured using commercially available kits according to the manufacturer's instructions.
For the measurements of mitochondrial complex-dependent oxygen consumption, cells were incubated with cytomix for 1, 12 and 24 hours and respiratory rates were determined using XF96 Extracellular Flux Analyzer (Seahorse Bioscience, North Billerica, MA) and the titration experiments were performed using the high resolution oxygraph (Oxygraph-2k, Oroboros Instruments, Innsbruck, Austria)(data not shown). Cells were permeabilized with digitonin, supplemented by the complex-I related substrates, glutamate and malate. Afterwards, ADP was added to examine complex I, ADP-stimulated oxygen consumption. For complex I + II-dependent oxygen consumption succinate (complex II substrate) was added. Then, oligomycin was added to inhibit ATP synthase and finally, the uncoupler carbonyl cyanide-4-(trifluoromethoxy) phenylhydrazone (FCCP) was added to obtain maximal cellular respiratory capacity.

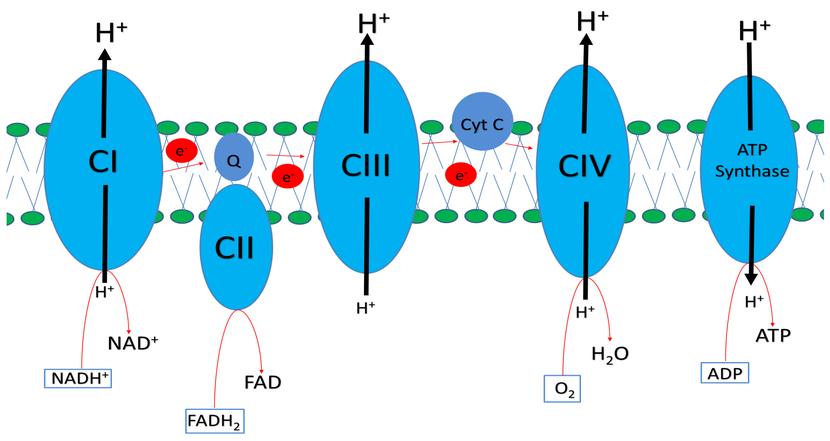

Figure 1. Mitochondrial electron trasnsport chain (complex I-IV) and ATP synthase

Results: Cytomix (24 hours incubation) induced a significant increase in complex I enzymatic activity (controls: 24 ± 12 vs. cytomix-treated cells: 34 ± 12, paired t-test p=0.041, n=10) (Figure 2), whereas complex IV, ATP synthase and citrate synthase enzymatic activities were not affected. Incubation with sedative and analgesic drugs alone or the combination with cytomix (24 hours) had no effects on mitochondrial enzymatic activities (n=10) (Figure 3).

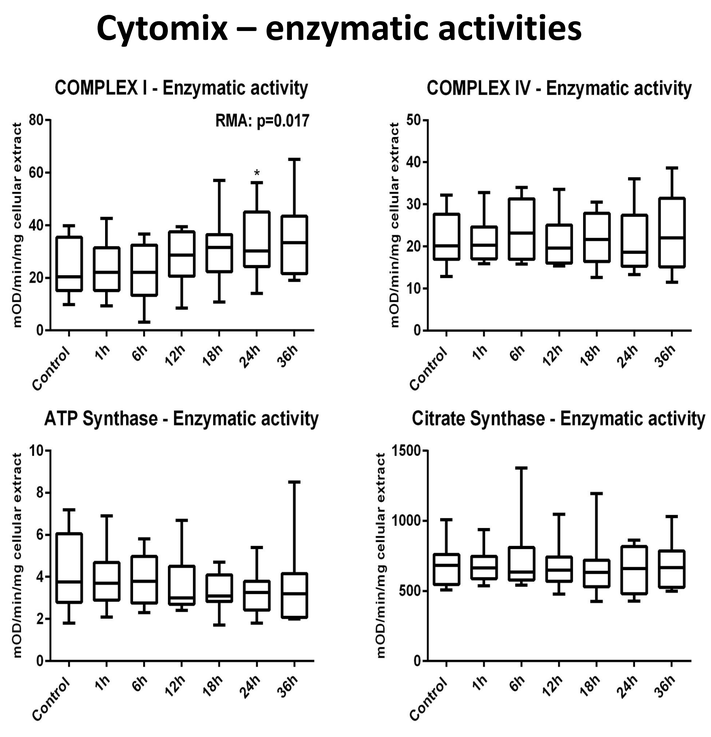

Figure 2. Effect of cytomix (TNF-α [10 ng/ml], IFN-γ [10 ng/ml] and IL-1β [10 ng/ml]) on mitochondrial electron transport chain complexes I and IV, ATP synthase and citrate synthase enzymatic activities in time-dependent manner (1, 6, 12, 18, 24 and 36 hours) (n=10).

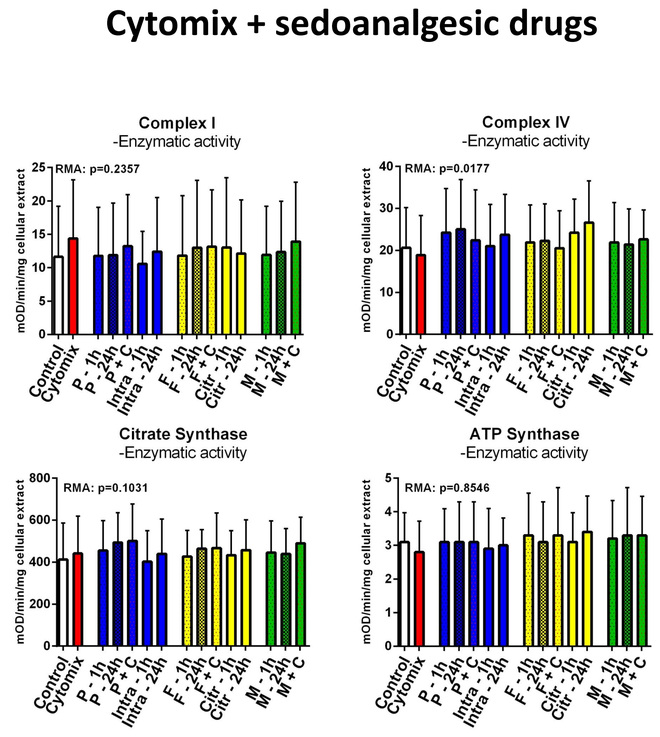

Figure 3. Mitochondrial enzymatic activities and citrate synthase activity after incubation with sedoanalgesic drugs (P=propofol, F=fentanyl, M=midazolam) alone, their solvents (Intra=intralipid for propofol and citr=citrate for Fentanyl) and in combination with cytomix (n=10).

Preliminary results suggest a tendency of decreased cellular ATP content after incubation with cytomix for 24 hours (n=4), whereas propofol and midazolam alone had no effect. Fentanyl (1 hour incubation) tended to increase cellular ATP content (Figure 4).

Preliminary respirometry experiments (n=3) suggest that cytomix does not affect complex I- and II-dependent oxygen consumption, however the maximal respiratory capacity after 12 and 24 hours of incubation with cytomix increased compared to 1 hour incubation and controls (Figure 5).

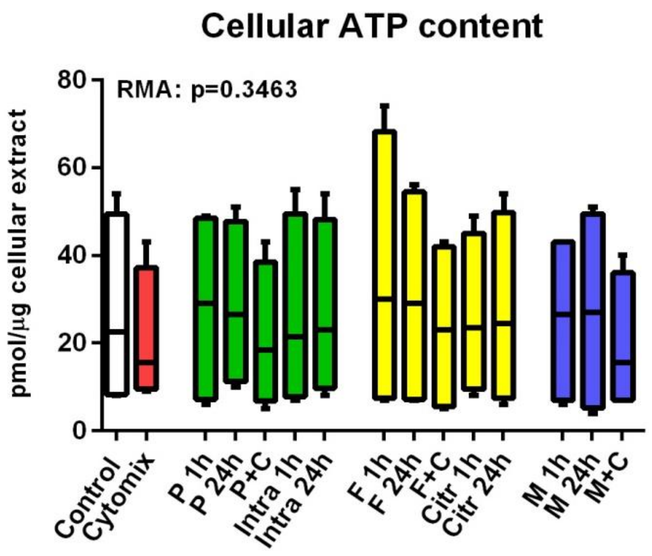

Figure 4. Cellular ATP content after incubation with cytomix (C), propofol (P), Inralipid (intra), fentanyl (F), citrate (Citr) and midazolam (M) for 1 and 24 hours, both alone and in combination with drugs (n=4).

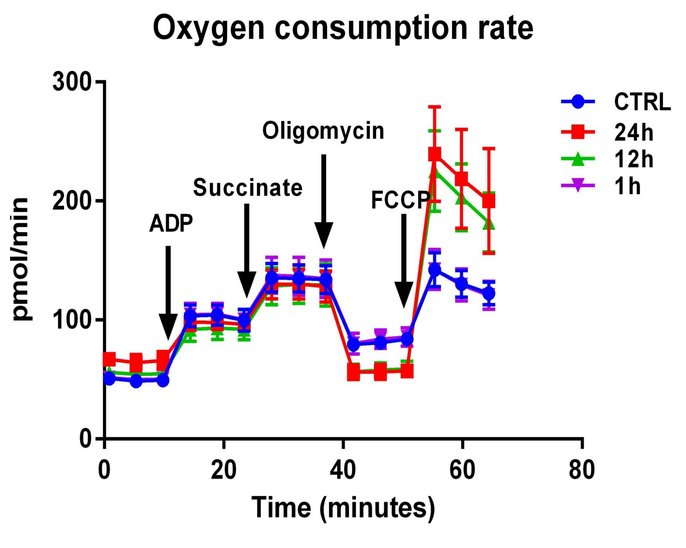

Figure 5. A representative figure for complex-dependent mitochondrial oxygen consumption, performed using XF96 Extracellular Flux Analyzer, after incubation with cytomix for 1, 12 and 24 hours.

Conclusion: Preliminary data suggest that incubation of primary human astrocytes with cytomix increases complex I enzymatic activity in a time-dependent manner. However incubation of astrocytes with clinically relevant concentrations of propofol, fentanyl and midazolam alone or in combination with cytomix had no significant effects on enzymatic activities of mitochondrial complexes. Additional in vitro and in vivo experiments will be performed in order to investigate the effect of cytomix and drugs on mitochondrial bioenergetics.

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