Claims

1. A method for screening and selection of potential drug candidates or for testing a person's sensitivity to a substance with effect on mitochondria, the method comprising

i) subjecting a sample of human blood components containing mitochondria to high-resolution respirometry,

ii) contacting the cell sample with a substance that increases the leakage of the inner mitochondrial membrane to protons,

iii) adding a test sample comprising a test substance in a vehicle,

iv) comparing the oxygen consumption before and after addition of the test sample and comparing the oxygen consumption of the test sample with the oxygen consumption of a control sample of the vehicle, wherein a decrease in oxygen consumption indicates a negative effect on the mitochondria,

v) contacting the sample from iii) with an inhibitor of mitochondrial complex I-function,

and

vi) contacting the sample from v) with an inhibitor of mitochondrial complex III-function.

2. A method for investigating mitochondrial effects of drug candidates in clinical trials or in treatment regimens, the method comprising

i) subjecting a sample of human blood components containing mitochondria to high-resolution respirometry, wherein the sample of cells is from a person subjected to a clinical study or to a treatment regimen, and wherein a test substance has been administered to the person during the clinical study or treatment regimen

ii) contacting the cell sample with a substance that increases the leakage of the inner mitochondrial membrane to protons,

iii) comparing the oxygen consumption of the sample from the person subjected to the clinical study or to a treatment regimen with the oxygen consumption of a control sample, wherein a decrease in oxygen consumption indicates a negative effect on the mitochondria,

iv) contacting the sample from iii) with an inhibitor of mitochondrial complex I-function,

and

v) contacting the sample from iv) with an inhibitor of mitochondrial complex III-function.

3. A method according to claim 1 or 2, wherein the high-resolution respirometry is performed at an oxygen concentrations in the range of 400 – 25 µM O_2 at a constant temperature of 37°C.

4. A method according to any of the preceding claims, wherein the substance that increases the leakage of the inner mitochondria membrane to protons is added to obtain maximal capacity of the electron transport system of the mitochondria present in the sample,

5. A method according to any of the preceding claims, wherein the substance that increases the leakage of the inner mitochondria membrane to protons is selected from carbonyl cyanide p-(trifluoromethoxy) phenylhydrazone (FCCP), carbonyl cyanide m-chlorophenylhydrazone (CCCP), 2,4-Dinitrophenol (DNP) and other protonophores, and mixtures thereof.

6. A method according to any of the preceding claims, wherein the substance that increases the leakage of the inner mitochondria membrane to protons is added at a concentration in a range of from 0,1 to 10 µM such as from 0.5 to 5 µM, from 1 to 3 µM or about 2 µM.

7. A method according to any of the preceding claims, wherein the substance that increases the leakage of the inner mitochondria membrane to protons is FCCP.

8. A method according to any of claims 1, 3-6, wherein the vehicle is an organic solvent such as e.g. ethanol, isopronol, methanol, DMSO (dimethylsulfoxide), DMF (dimethylformamide), or DMA (dimethylacetamide).

9. A method according to any of claims 1, 3-8, wherein the test sample is in liquid form and the test sample contains a known concentration of the test substance.

10. A method according to claim 9, wherein the test sample is added in stepwise increasing concentrations.

11. A method according to claim 10, wherein the stepwise addition of test sample results in final concentrations of 1 µM to 10 mM of the test sample.

12. A method according to any of claims 1, 3-11, wherein the control sample in iii) is identical to the test sample but without content of test substance.

13. A method according to any of the preceding claims, wherein the inhibitor of mitochondrial complex I-function is added to elucidate the cellular respiration dependent on oxidation of complex II-substrates.

14. A method according to any of the preceding claims, wherein the inhibitor of mitochondrial complex I-function is added at a concentration in a range of from 0,1 to 10 µM such as from 0.5 to 5 µM, from 1 to 3 µM or about 2 µM.

15. A method according to any of the preceding claims, wherein the inhibitor of mitochondrial complex I-function is rotenone.

16. A method according to any of the preceding claims, wherein the inhibitor of mitochondrial complex III-function is added to determine any non-mitochondrial oxygen-consuming activity, such as auto-oxidation of said sample,

17. A method according to any of the preceding claims, wherein the inhibitor of mitochondrial complex III-function is added at a final concentration of from about 0.1 to 10 µg/ml such as from about 0.5 to 5 µg/ml, from about 0.75 to 2 µg/ml or 1 µg/ml.

18. A method according to any of the preceding claims, wherein the inhibitor of mitochondrial complex III-function is antimycin A.

19. A method according to any of claims 2-18, wherein the control sample is from a control group.

20. A method according to any of claims 2-19, wherein the control sample is taken before any treatment starts.

21. A method according to any of claims 1, 3-18 for screening of drug candidates.

22. A method according to any of claims 1-20 for testing a subject's sensitivity to a drug substance or a drug candidate.

23. A method according to any of claims 2-20 for evaluating mitochondrial toxicity of a test substance in clinical trials.

24. A method according to any of claims 1-20 for analysing the effect of a substance on mitochondrial function.

25. A method according to claim 22, wherein the sample of cells is isolated from said subject.

26. A method according to any of claims 1-20 for evaluating compound capable of stimulating mitochondrial respiration and ATP production.

27. A method for screening and selecting potential drug candidates or for testing the sensitivity of a person to a substance with effect on mitochondria, the method comprising
i) subjecting a sample of human blood components containing mitochondria from a human blood sample to high-resolution respirometry,
ii) contacting the sample of cell to a substance that inhibits complex I respiration,
iii) adding a test sample containing a test substance in a vehicle,
iv) adding a substance that permeabilizes the plasma membrane,
v) adding a reference substance to the sample obtained in iv) wherein the reference substance is a complex II-linked substrate, and
vi) adding a substance that inhibits complex III respiration, and
comparing the oxygen consumption obtained with the oxygen consumption obtained when the method is carried out using the reference substance in step iii) and thus omitting addition of a test sample.

28. A method according to claim27, wherein the substance that permeabilizes the plasma membrane is digitonin.

29. A method according to claim 28, wherein digitonin is added in a concentration of from 5 µg/ml to about 250 µg/ml.

30. A method according to any of claims 27-29, wherein the reference substance is succinate.

31. A method according to claim 30, wherein the final concentration of the reference substance is from about 0.1 to about 20 mM.

32. A method according to any of claims 27-31, wherein the details for the individual steps i) – vi) are as defined in any one of claims 3, 8, 9, 10, 11, 13, 14, 15, 16, 17, 18, 21, 24, and/or 26.