Transfection of C6 rat glial cells with Metafectene

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Experiment 1. Preliminary testing

C6 rat glial cells were transfected with pGL3-IkBalpha vector (containing luciferase gene under control of Inhibitor kB protein alpha) using either Metafectene or competitor transfection reagent (designated as Lipid L). Briefly, pGL3-IkBalpha plasmid was purified with Promega Wizard DNA purification kit, precipitated with 80% ethanol and reconstituted in DMEM.

1.5 µg of DNA was used for transfection. DNA was mixed with 1.5 µl of Lipid L (w/w ratio is1:2) or 3 µl of Metafectene (v/v ratio is 1:2) in 50 µl of DMEM, incubated 20 minutes at room temperature and added to cells in 12–well plate in plain DMEM medium. Four hours later equal volume of DMEM/10 % FBS was added to the wells and cells were further incubated for ~8 hrs (overnight).

Then cells were washed with plain DMEM twice and fresh DMEM/10%FBS was added to cells. At each medium change samples were taken for measurement of LDH release (Toxicity test).

24 hrs later cells were washed twice with PBS and assayed for luciferase activity.

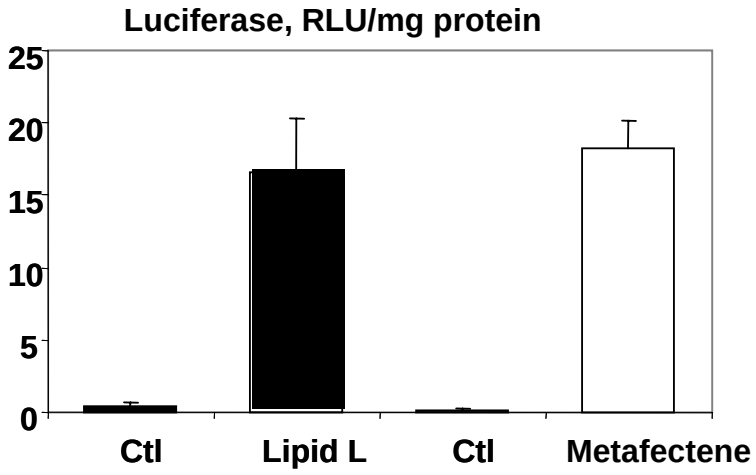
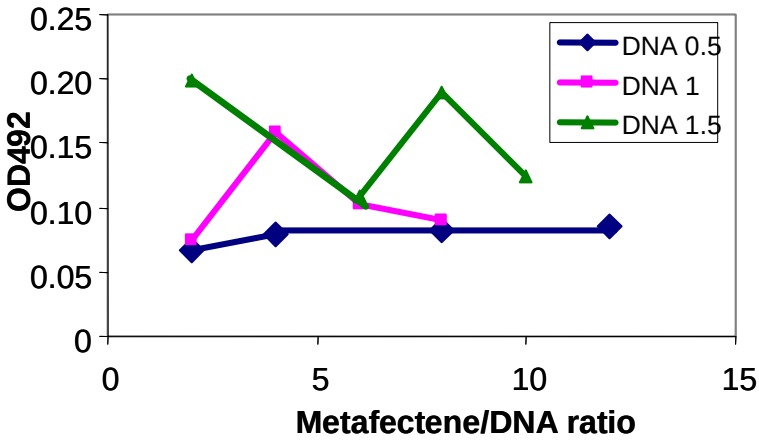


Figure 1. Luciferase activity in C6 cells transfected with pGL3-IkBalpha reporter vector using Lipid L or Metafectene. Data are presented as mean±SD. Chart is a compilation of at least three different experiments.

Conclusion: Luciferase activity in C6 cells transfected with Metafectene is higher, however, statistically insignificant ($p<0.54$). Toxicity of either Lipid L or Metafectene was similar (data not shown).

Experiment 2. Optimization.

C6 cells were grown in 24-well plate until approximately 60% confluent. At this time, cells were washed with plain DMEM and transfected with pGL3-IkBalpha construct according to the manufacturer optimization protocol with modifications. (Modifications included incubation of cells in 0.5 ml/well of plain DMEM/transfection mix for 4 hours and addition of an equal volume of complete DMEM/10%FBS to the wells with further 8 hours (overnight) incubation). Next morning samples of medium were assayed for LDH release. Cells were washed and incubated in fresh DMEM/10%FCS medium for another 24 hrs. Medium then was assayed for LDH release, cells washed with PBS, lysed and assayed for luciferase activity.



Thus, most toxic DNA/Metafectene ratios were 1:4.

Figure 2 LDH release by C6 cells after 4 hrs incubation with DNA/Metafectene mix.

However, the highest luciferase activity was observed at the DNA/Metafectene ratio of 1:8 at the highest DNA concentration taken.

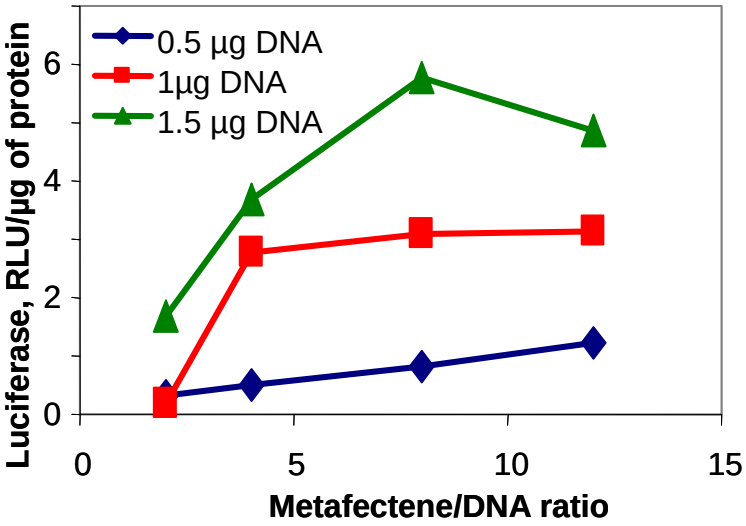


Figure 3. Luciferase activity for the optimization protocol.

Experiment 3. Optimized testing
Lipid L and Metafectene were compared in this experiment using optimal conditions for both substances. C6 cells were transfected using Lipid L/DNA ratio 2.5:1 and Metafectene/DNA ratio 8:1.

Cells were transfected as previously described. LDH release was measured in culture medium after transfections.
Transfected cell cultures were then stimulated with LPS (lypopolysaccaride) at 1µg/ml (LPS stimulation increases IkBa expression) in order to estimate the magnitude of IkBalpha promoter activation. After 4 and 24 hrs of stimulation with LPS cells were lysed and luciferase activity was estimated using Bright-Glo reagent (Promega).

Luciferase activity, raw data, 4 hrs

Metafectene		**Lipid L**	
Ctl	**LPS**	**Ctl**	**LPS**
0.454	0.609	0.085	0.129
0.400	0.678	0.041	0.189
0.432	0.929	0.073	0.123

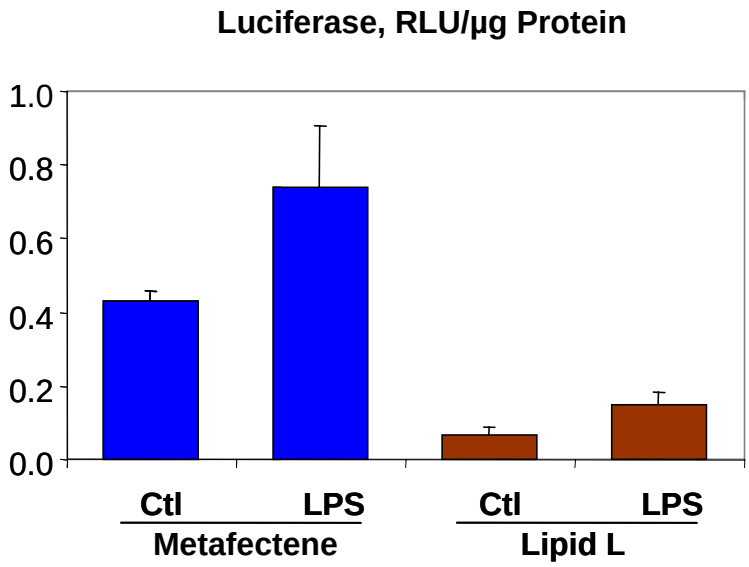


Figure 4. Luciferase activity in C6 cells 4 hrs after LPS stimulation.

Thus transfection efficiency was at least 5-6 times higher for Metafectene compare to Lipid L. However, Metafectene at optimal concentration is more toxic to C6 cells than Lipid L (LDH release was 0.456±0.06 for Metafectene and 0.214±0.002 for Lipid L).

Since transfection efficiency is higher with Metafectene we were able to observe luciferase activity in C6 cells for at least 36 hrs after LPS stimulation, while with Lipid L luciferase activity was barely detectable at 24 hr time point.