Transfection of mouse neuroblastoma N18TG2 cells with Metafectene Pro reagent

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A number of reagents that give reasonably good transfection efficiency of mouse neuroblastoma cells have been developed; however, these reagents usually display a toxic action. Here we report experiments to investigate the transfection efficacy and cytotoxicity of the Metafectene Pro on mouse neuroblastoma N18TG2 cell line and on the derived 2/4 clone.
2/4 cells are able to synthesize acetylcholine, as they were isolated following N18TG2 transfection with a choline acetyltransferase construct (Bignami F. et al.,1997; De Jaco A. et al., 2002).

Materials and methods

Materials

Metafectene PRO, a polycationic liposomal trasfection reagent, was obtained from Biontex Laboratories GmbH (Munich, Germany).
The plasmid, pEGFP-N3 vector encoding GFP fluorescent protein was used for evaluating transfection efficiency.

Cells

Mouse neuroblastoma N18TG2 cells were maintained in DMEM supplemented with L-glutamine (2mM), penicillin (100 U/ml), streptomycin (100 μg/ml) (Sigma) and 10% heat-inactivated FCS.

Mouse neuroblastoma 2/4 cells were grown in DMEM 15 mM Hepes, 14.28 mM $NaHCO_3$, geneticine (0.2 mg/ml), L-glutamine (2mM), penicillin (100 U/ml), streptomycin (100 μg/ml) (Sigma) and 10% heat-inactivated FCS.

Transfection

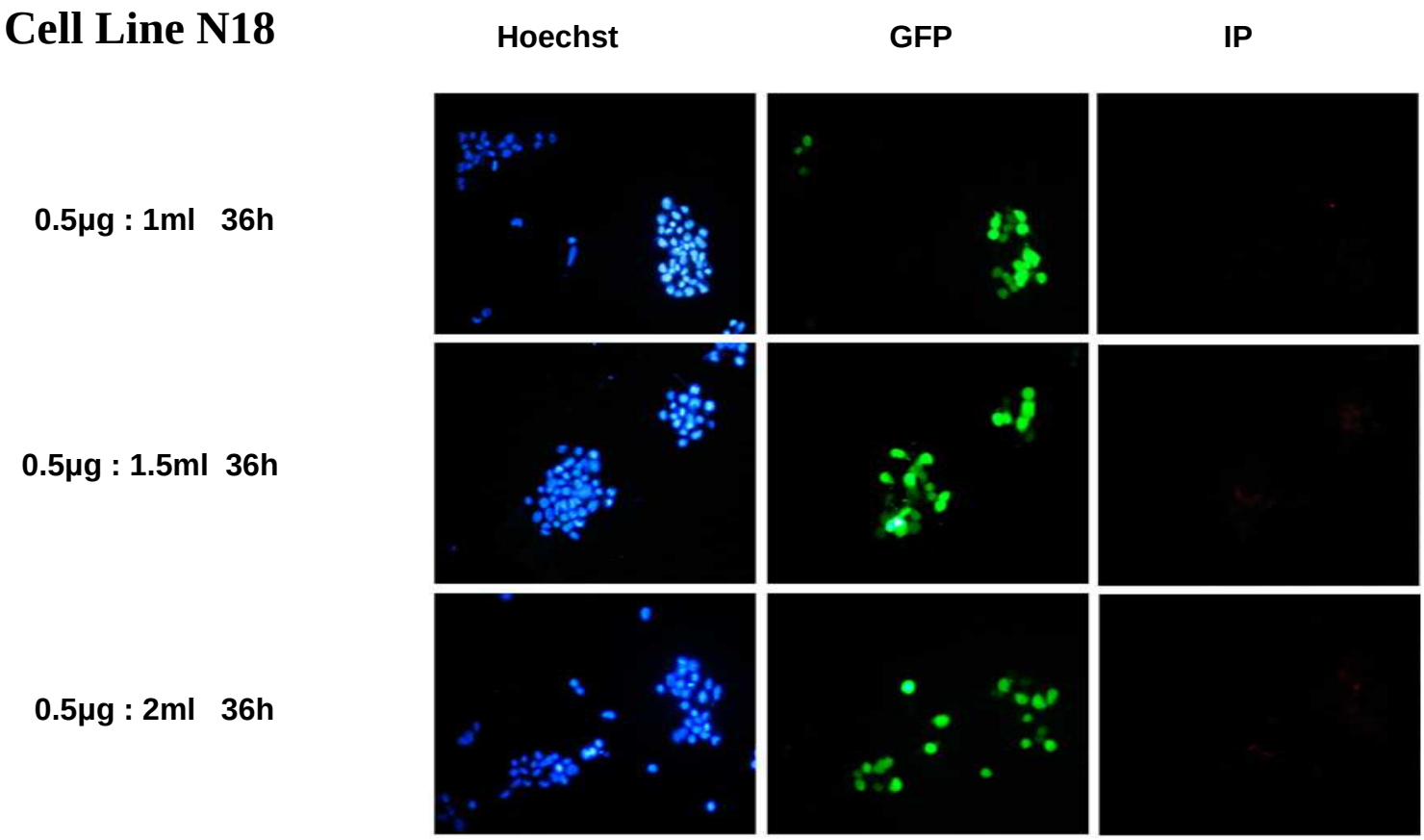
For transfection experiments cells were seeded in 24–well microplate (8×10^4 cells/well); one day later growth medium was replaced with 0.5 ml complete fresh medium, in each well; and after 2 hours trasfection was performed. Metafectene PRO was complexed with DNA as follows :

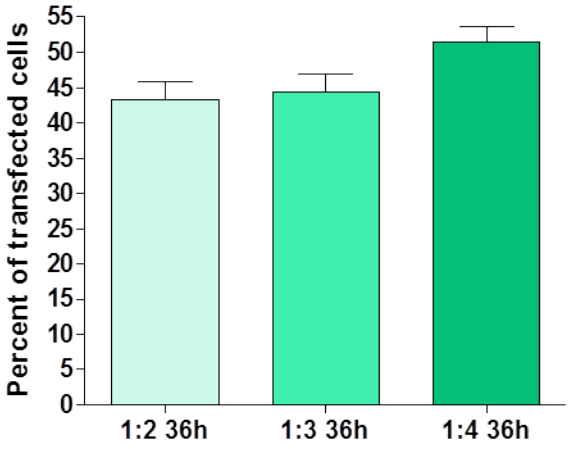
pEGFP-N3 plasmid:Metafectene 0.5μg : 1μl , 0.5 μg : 1.5 μl or 0.5 μg : 2μl .

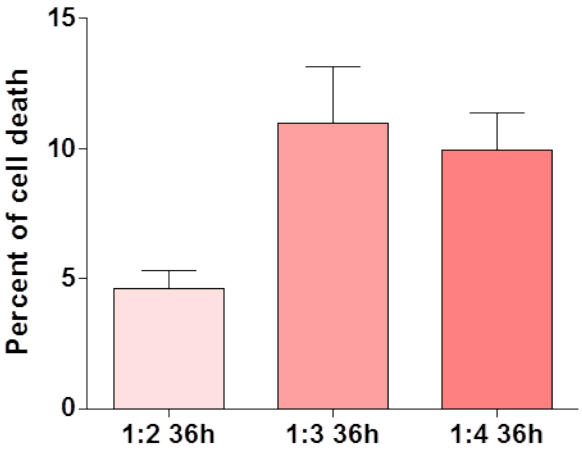
The indicated volume of Metafectene PRO was added to 30 μl of PBS while the plasmid DNA was diluted in a volume of 30 μl PBS. Both solutions were thoroughly mixed and incubated for 20 min at RT. The metafectene PRO-DNA complex was added to the cultures, which were kept for 12 and/or 36 h at 37°C. Fluorescence analyses were performed 36 h after addition of the transfection mixture.
Transfection efficiency was monitored by GFP fluorescence evaluating the number of transfected cells (by GFP fluorescence) total cells (Hoechst-staining); the percentage of cell death was determined by propidium iodide staining.

Results

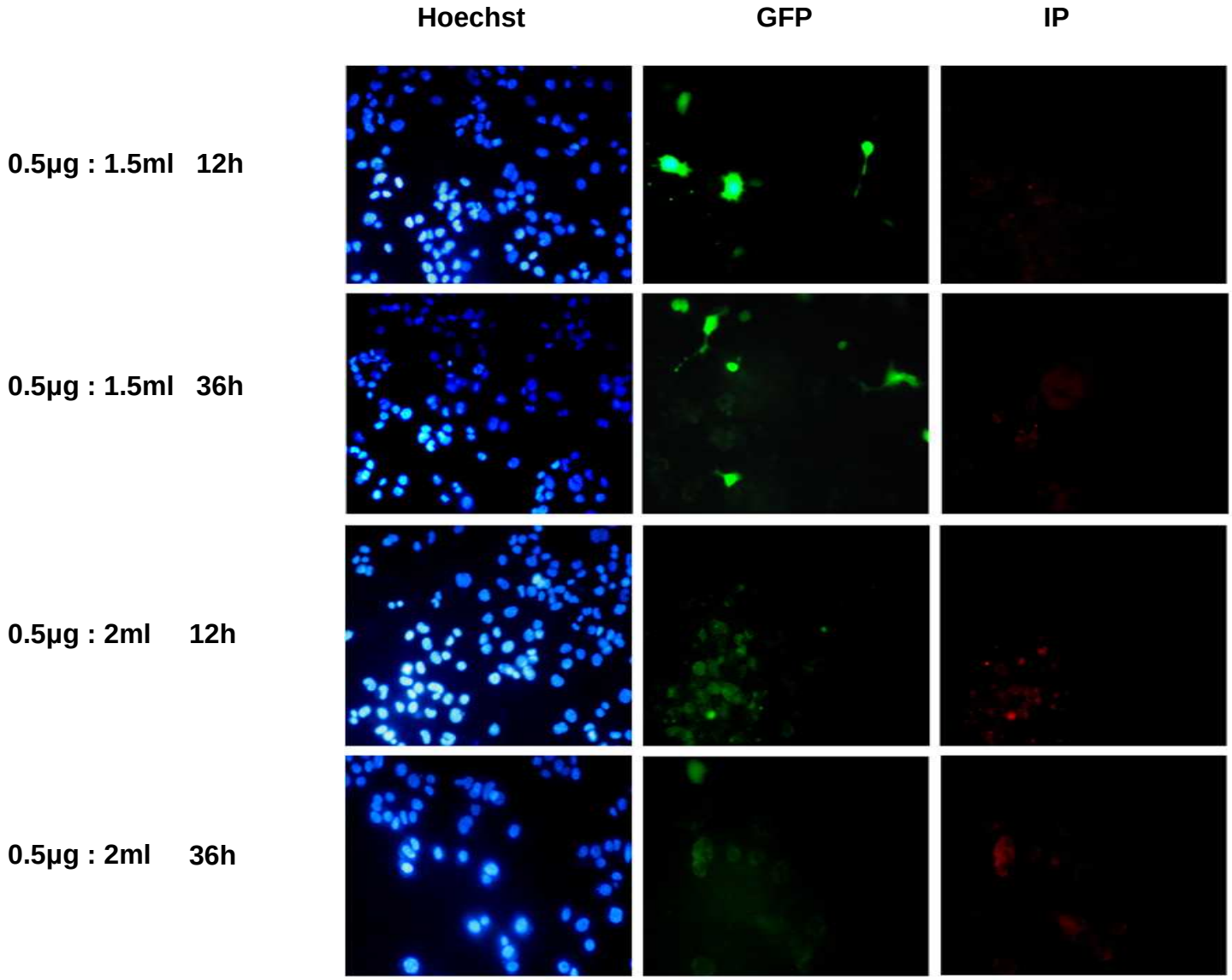
Results shown are the mean of 3 independent experiments.

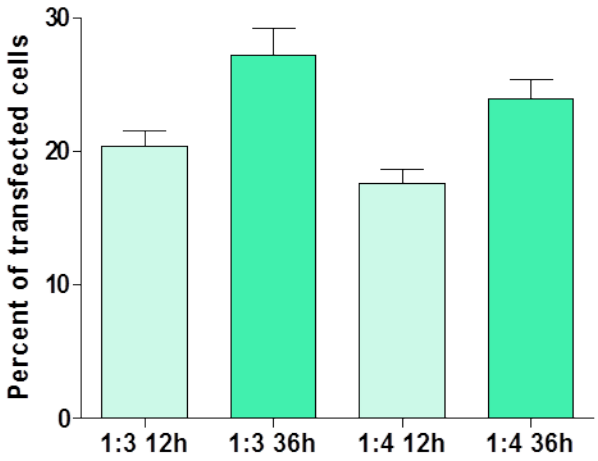


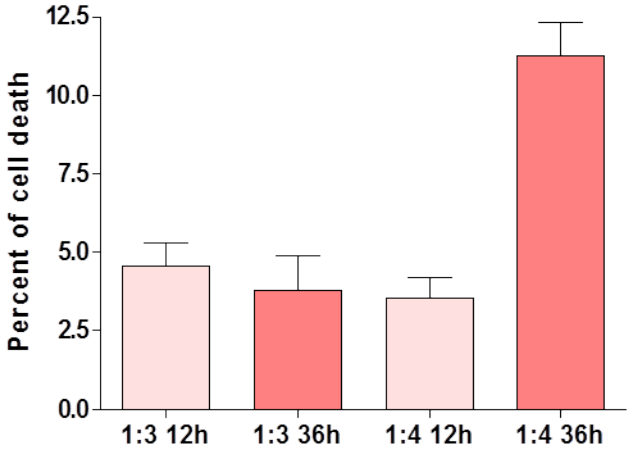




Cell Line 2/4


Hoechst
GFP
IP
0.5µg : 1.5ml 12h
0.5µg : 1.5ml 36h
0.5µg : 2ml 12h
0.5µg : 2ml 36h


Percent of transfected cells
30
20
10
0
1:3 12h
1:3 36h
1:4 12h
1:4 36h


Percent of cell death
12.5
10.0
7.5
5.0
2.5
0.0
1:3 12h
1:3 36h
1:4 12h
1:4 36h

Conclusion

Metafectene PRO successfully transfected mouse neuroblastoma cell lines N18TG2 and 2/4.
Considering both transfection efficiency and cytotoxicity we concluded that the optimal DNA / reagent ratio was 0.5 μg : 2 μl for N18TG2 cells and 0.5 μg : 1.5 μl for 2/4 cells.