Lipofection of the human T lymphoblastic leukaemia (T-ALL) cell line Jurkat

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Aim of the experiment was to compare the transfection efficiency of several liposomes in a human lymphoid cell line (Jurkat) by using the green fluorescent protein (EGFP) as a reporter gene.

Materials and methods:

For the lipofection procedure we used Metafectene-Pro (Biontex Laboratories), Dharmafect 1-2-3-4 (Dharmacon RNA Technologies), Lipofectamine 2000 (Invitrogen). As reporter plasmid, to detect the transfection efficiency, we employed pCMV-EGFP, a plasmid which has been generated in our laboratory by cloning the reporter gene EGFP in the vector pRRLsin.PPTS.hCMV.pre under the control of the CMV promoter. Jurkat cell line was purchased from ATCC and cultured in RPMI 1640 medium (Euroclone Life Sciences Division, Pero, Italy) supplemented with 10% FCS (Life Technologies, Gaithersbuurg, MD), 1% HEPES (10mM, Cambrex Bioscience, Verviers, Belgium) and L-Glutamine 2mM. During the lipofection cells were mantained in OptiMEM medium (OptiMEM+ Glutamax, Gibco, Invitrogen). The cells were maintained at 37°C in a humidified 5% CO_2/95% air atmosphere.

Experimental procedures / transfection protocol

One day before transfection Jurkat cells were plated in 1 ml of RPMI 1640 medium supplemented with 10% FCS, 1% HEPES and L-Glutamine 2mM in a 12-well culture plate at the concentration of 100.000 cells per well. On the day of transfection, cells were washed in OptiMEM (OptiMEM+ Glutamax, Gibco, Invitrogen; a serum-free medium) and seeded in a volume of 0.9 ml of the same medium. The lipofection was performed with different reagents in order to test the efficiency of different procedures in transferring the reporter plasmid pCMV-EGFP to our cell line.

Metafectene-Pro was complexed with the pCMV-EGFP plasmid at the reagent:DNA ratio of 3 µl:1.5 µg DNA. Complexes were prepared by mixing 3 µl of Metafectene-Pro diluited in 50 µl of OptiMEM with 1.5 µg of plasmid diluited in the same volume of medium. The mixture was incubated at room temperature for 20 minutes and then 100 µl of the solution was added drop by drop to each well of the

culture plate. At the same time, Lipofectamin, Dharmafect 1, Dharmafect 2, Dharmafect 3 and Dharmafect 4 were complexed with the pCMV-EGFP plasmid at the reagent: DNA ratio respectively of 4 µl:1.5 µg for Lipofectamin and 2.5 µl:1.5 µg for the 4 Dharmafect reagents. Complexes were prepared by mixing the appropriate amount of lipofection reagent diluited in 100 µl of Optimem with 1.5 µg of plasmid diluited in the same volume of medium. As described above for Metafectene-Pro, the mixture was incubated at room temperature for 20 minutes and then 0.2 ml of the solution was added drop by drop to each well of the culture plate. After 5 hours, the transfection mixture was removed and replaced with RPMI 1640 supplemented with 10% FCS, 1% HEPES, L-Glutamine 2mM medium and 1% antibiotics-antimycotic mix (Gibco-BRL, Grand Island, NY). After 48 hours the cells were analysed at the cytofluorimeter for the expression of the reporter gene EGFP in order to estimate the efficiency of the transfection procedure.

Results and discussion:

The lipofection was performed with different reagents in order to test the efficiency of different procedures in transferring the reporter plasmid pCMV-EGFP to our cell line.

As the cytofluorimetric analysis shows, with Metafectene-Pro we obtained the highest percentage of transfected cells (33%) measured as the percentage of cells EGFP-positive (Fig.1). In comparison, with all the other reagents tested we couldn't obtain more than 16% of transfection efficiency. Moreover with some reagents (Dharmafect 1) we observed some cytotoxicity measured as an alteration of the morphologic cytofluorimetric parameters FS-SS and evident at the microscopic observation of the cells. On the contrary, with Metafectene-Pro we didn't detect any cyotoxic effects both the day after transfection and later on (Fig.2).

Conclusion / summary:

Jurkat is a human ATCC cell line derived from a T-ALL patient. Like most of the cells which grow in suspension, Jurkat are in general more difficult to transfect than cell lines growing in adherence. On the whole we can conclude that Metafectene-Pro in our system gave the best results in terms of transfection efficiency. The percentage of cells transfected with Metafectene-Pro (33%) was sufficiently high to detect the expression of the reporter gene luciferase by a luminometer in subsequent experiments (not shown).

Appendix: Tables and/or figures:

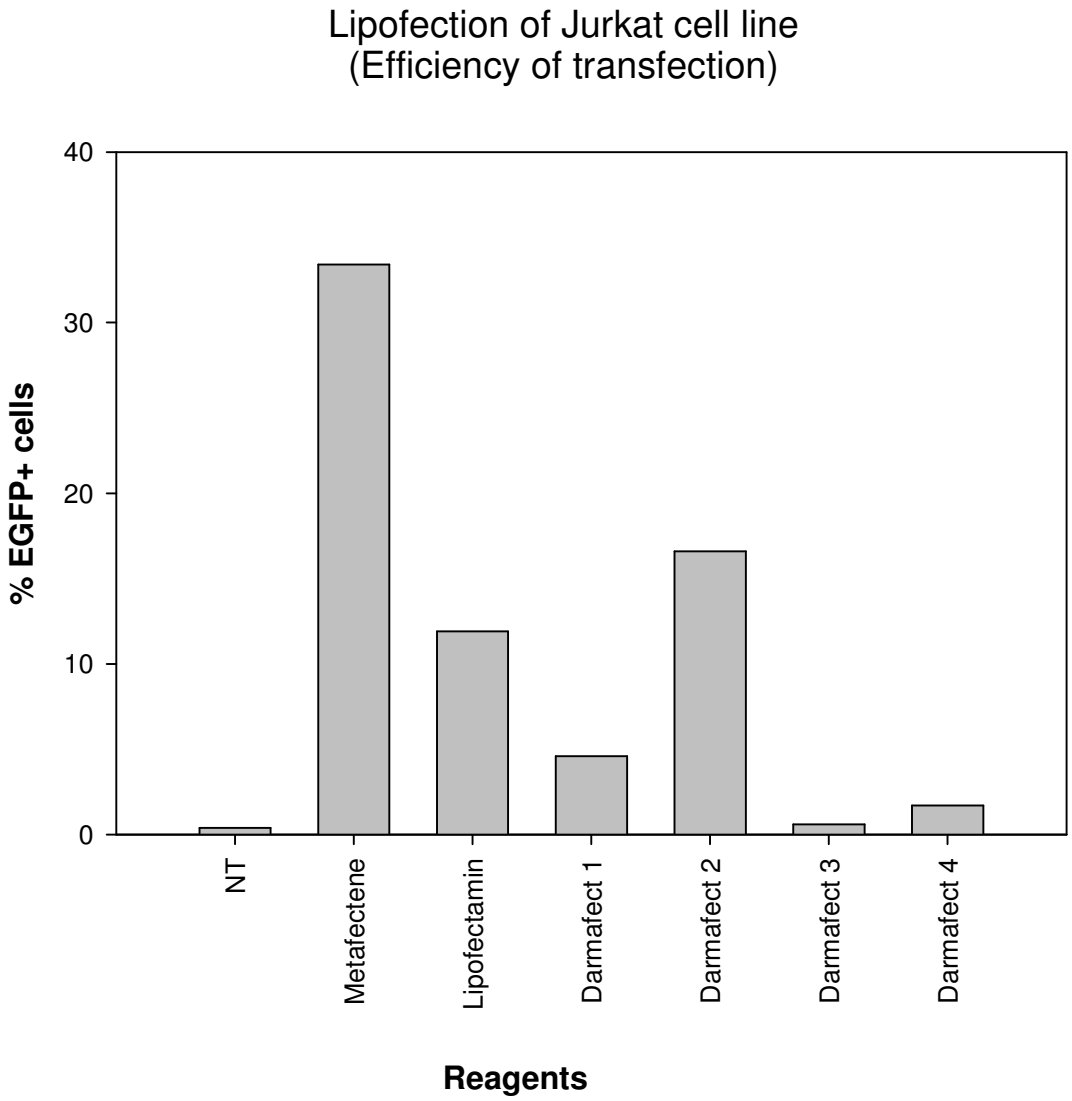

Fig.1. Cytofluorimetric analysis of the percentage of cells EGFP-positive after lipofection with different reagents.

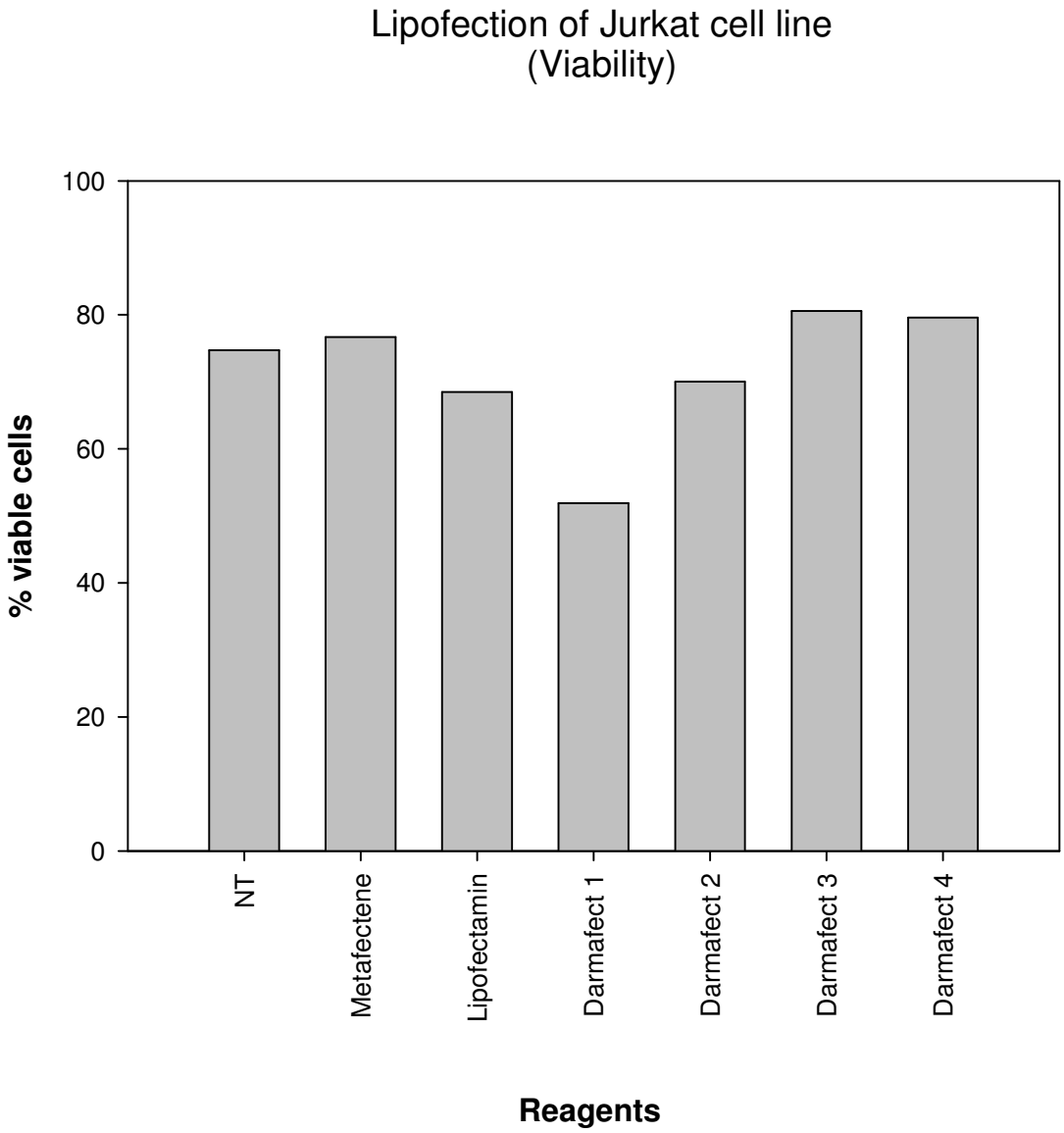

Fig.2. Percentage of viable cells based the morphologic citofluorimetric parameters FS and SS estimated 48 hours after transfection of Jurkat cell line with different lipofection reagents.

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