Transfection of DU-145 cells with Metafectene

Barbara Comuzzi and Zoran Culig, Department of Urology, University of Innsbruck, Austria.

DU-145 is an adeherent cell line derived from a prostate cancer brain methastatic lesion.
The cells were cultured in 175 cm^2 flasks with RPMI supplemented with 10% FCS and 1% antibiotics (Penicillin/Streptomicin) at 37° C in 5% CO_2. When the confluence (ca 80%) was reached, cells were tripsinized and seeded in new bottles to allow them to growth exponentially.
Next day the cells were plated in 12 well plates (85.000 cells per well in 1.5 ml complete medium) and transfected after 24 hours of incubation . The transfection was performed employing 0.8 ug/well of the androgen-inducible reporter gene ARE_2TATACAT and 0.08 ug/well of wild-type Andogen Receptor (AR) cDNA, as DU145 do not express it. Two mixtures were prepared (each of them in a polypropylene Falcon tube): 50 uL of RPMI (serum and antibiotics free) with 10.6 ug DNA and 50 uL of the same medium with 32 uL of Metafectene (lipid-DNA ratio of 3 uL:1 ug). DNA solutions and Metafectene were gently vortexed before use and kept at room temperature. The two solutions were mixed gently and incubated at room temperature for 20 min to allow the DNA-lipid complex to be formed. After incubation, 5900 uL of medium (serum and antibiotics free) were added to the mixtures and medium from the cells removed and replaced with 500 uL per well of this solution. After 3 h of incubation at 37°C, 500 uL of 6% FCS RPMI, with antibiotics, were added to each well containing the cells transfected.
After 24 h the cells were treated with 10 pM, 100 pM and 1 nM of syntetic androgen methyltrienolone (R1881). Every solution was prepared in RPMI supplemented with 3% FCS.
After 24 h of incubation, Chloramphenicol Acetyl Transferase (CAT) assay was performed to measure reporter gene activity.

Table 1.

	Transfection reagent 1			**Metafectene**		
		mean 1	**mean 2**		**mean 1**	**mean 2**
	1074.4			1160.1		
pls	1151.4	1100.9	1100.9	(1407.5)	1214.5	1118.0
	1076.9			1075.9		
	2077.4			9629.6		
10 pM	(3541.2)	2750.9	2355.7	9148.1	9506.0	9506.0
	2634.0			9740.3		
	2226.8			9820.6		
100 pM	2603.8	2318.6	2318.6	11083.1	10411.7	10411.7
	2125.2			10331.4		
	(1894.8)			(7470.7)		
1 nM	2254.1	2277.8	2469.4	(5791.4)	9277.7	14571.0
	2684.6			14571.0		

pls= cells only transfected
10pM, 100pM, 1nM = concentrations of R1881 employed

mean 1 is the mean calculated considering all the values, **mean 2** is the mean withouth consideration of the values in brackets.
Transfection reagent 1 = reagent normally used by us

Discussion

As shown in Table 1 reporter gene activity in the cells transfected with METAFECTENE and treated with the highest concentration (1 nM) of R1881 reached value of 14571 cpm. Reporter gene activity in cells only transfected and not treated, were considered the "basal level" (1118 cpm). Comparing these two data we can assume that, in our conditions, there is a maximal 13 folds induction. With the reagent used by us in a parallel experiment, we got an induction of 2.24 folds. We can conclude that in our experiment METAFECTENE showed a maximal induction 5 times higher compared to that obtained with the reagent we normally use .

The table shows that also the intermediate stimulatory concentrations (10 and 100 pM) had higher induction (8.5 and 9.3 folds, respectively) compared to the intermediate inductions obtained with the other reagent (2 folds in both cases). Moreover, while in the METAFECTENE transfection the counts obtained for 10 and 100 pM can be clearly distinguished, the transfection made with the other reagent didn´t give clear concentration-dependent.