Improving *GFP* production utilising venom components to enhance transfection efficiency in Cos 7 cells

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BACKGROUND

Development of new drug molecules can be expensive and time consuming. Finding an appropriate delivery system for each drug has proven to be challenging due to many factors affecting the effectiveness of each method – chemical and physiological properties of drug molecules (Yokoyama, 2005), toxic side effects (Whittlesey *et al*., 2004), immune response, overall drug safety, and many others. Improvement of delivery systems and safety of old drugs has been attempted (Kshirsagar, 2000), together with controlled drug delivery and release profile (Whittlesey *at al*., 2004). Recent discoveries show natural resources such as animal venoms to have a vast pharmacological potential in drug development process (Vonk *et al*., 2011), and could play an important role in the development of novel drug delivery systems.

AIM

Investigation of *Naja naja, Naja atra, Naja sputatrix* and *Naja mossambica* venom effects on calcium-phosphate transfection efficiency in Cos 7 cells.

METHODS

- *E.coli* transformation and DNA purification to bulk up GFP plasmid for transfection experiments.
- Optimisation of calcium-phosphate transfection to determine optimal amount of GFP plasmid for efficient transfection.
- Cytotoxicity assay using $2x10^{2}$ – $2x10^{-8}$ µg/ml of *N. naja, N. atra, N. sputatrix* and *N. mossambica* venom to determine LD50.
- Venom treatment ($2x10$ – $2x10^{-1}$ µg/ml) of Cos 7 cells 15 minutes prior to calcium-phosphate transfection.
- Fluorescent microscopy to visually determine GFP expression.
- SDS-PAGE and Western blot to confirm GFP expression levels.

RESULTS

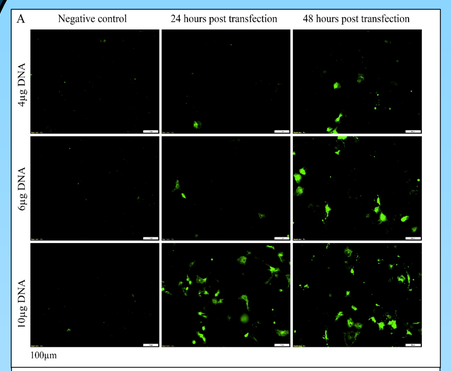

Figure 1: Optimisation of calcium-phosphate transfection. A) Increase in DNA amount and incubation time resulted in increased transfection efficiency. B) Increase in DNA amount resulted in increased GFP expression.

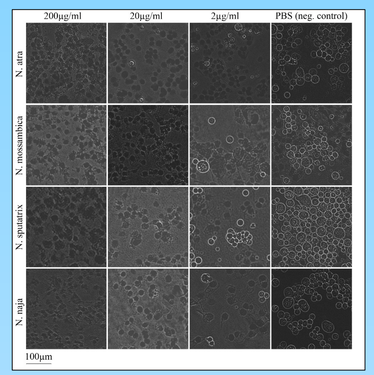

Figure 2: Cytotoxicity assay of Cos 7 cells. 200µg/ml - positive control, PBS - negative control. Lower venom concentration showed higher cell survival.

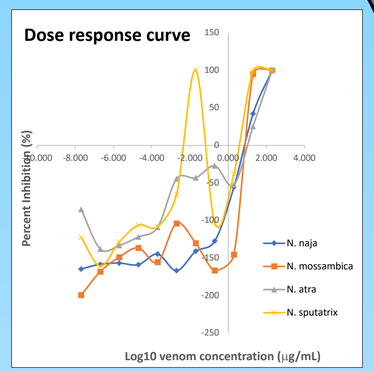

Figure 3: Resazurin assay dose response curve for N. naja, N. sputatrix, N. mossambica, and N. atra.

Figures 2 and 3 show effect of each venom on Cos 7 cells. Visual observation of Cos 7 cells stained with trypan blue suggested that 50% of cells survived when treated with venom concentrations below 2µg/ml. However, LD50 values determined from resazurin assay suggested that 50% of cells survived at 28.18µg/ml, 7.08µg/ml, 12.59µg/ml, and 42.66µg/ml for *N. naja, N. sputatrix, N. mossambica* and *N. atra* respectively.

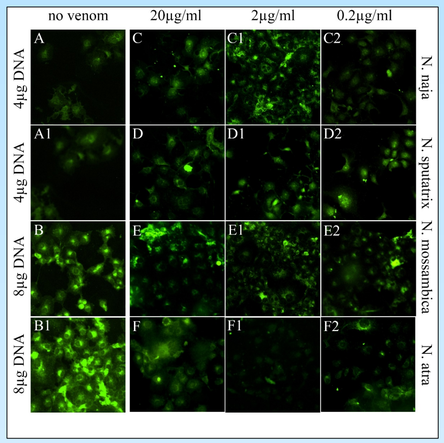

Figure 4: Comparison of GFP fluorescence: A-B: calcium-phosphate transfection without any prior venom treatment (A - 4µg of DNA; B - 8µg of DNA); C-F: Cos 7 cells treated with venom 15 minutes prior calcium-phosphate transfection (4µg of DNA).

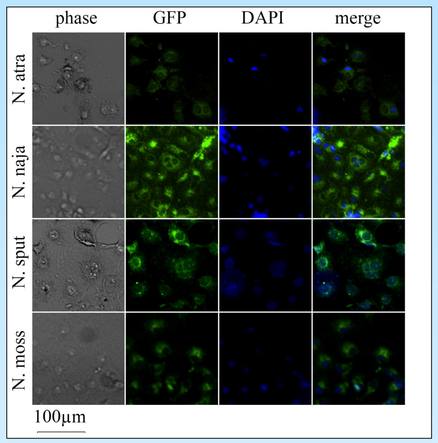

Figure 5: Location of GFP fluorescence at the time of analysis - Golgi apparatus. Cell nucleus stained with DAPI.

CONCLUSION

The results of this study showed that snake venoms with cell wall penetrating properties affected the transfection efficiency in Cos 7 cells. It was noted that when Cos 7 cells were treated with *N. naja* 2µg/ml, *N. mossambica* 20µg/ml and 2µg/ml 15 minutes prior to calcium-phosphate transfection where only 4µg of *GFP* plasmid were used, the amount of *GFP* fluorescence was very similar to the samples where 8µg of *GFP* plasmid were used without any prior treatment with venom. This suggested that venom treatment prior transfection enables easier insertion of foreign DNA into cells.

FUTURE DIRECTIONS

Further study could be done to look at what compounds in the venoms cause the increase of transfection efficiency. The venoms could be fractionated and each fraction tested. Fractions showing increase in transfection efficiency could be analysed by mass spectrometry which would allow for identification of the active compound.

Cytotoxicity assay could be repeated multiple times to determine the precise LD50 for each venom.

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REFERENCES

- Kshirsagar, N.A. (2000) 'Drug delivery systems', *Indian Journal of Pharmacology*. 32, pp. S54-S61.
- Whittlesey, K.J., Shea, L.D. (2004) 'Delivery systems for small molecule drugs, proteins, and DNA: the neuroscience/biomaterial interface', *Experimental Neurology - Elsevier*. 190, pp. 1-16.
- Yokoyama, M. (2005) 'Drug targeting with nano-sized carrier systems', *Journal of Artificial Organs*. 8(2), pp. 77-84.