Effect of low frequency sonophoresis in cetuximab skin penetration: a preliminary study

R. Petrilli¹, J. O. Eloy¹, and R. F. V. Lopez¹

¹School of Pharmaceutical Sciences of Ribeirao Preto, Ribeirao Preto, Sao Paulo, 14040-903, Brazil
raquelp@fcfrp.usp.br

ABSTRACT SUMMARY

Cetuximab is an IgG1 antibody used in the treatment of squamous cell carcinoma (SCC), a malignant tumour from epithelial origin. The use of cetuximab is currently restricted to systemic therapy leading to various side effects which could be avoided by topical administration. In this work, the potential of the low-frequency sonophoresis (LFS) to increase the topical penetration of cetuximab was evaluated in vitro. The skin was pretreated with LFS using poloxamer as a coupling medium until the desired skin electrical resistivity of 1 kΩcm² was reached. Cetuximab skin penetration was evaluated using ELISA method, previously validated by our group. The viability and survival rate of SCC A431 cell line treated with cetuximab were also studied. Cetuximab was able to penetrate the skin only after LFS treatment. The amount recovered from the skin was 3.3 times higher than the one required to kill 50% of A431 cells.

EXPERIMENTAL METHODS

In order to quantify the amount of cetuximab retained within the skin, an ELISA method was developed and validated based on previous studies. Briefly, ELISA was performed in high binding 96 wells flat bottomed plates (Costar TM) coated with EGFR 1.75 μg/mL in PBS followed by blocking with Blocker Blotto: Casein in TBS (20:80 v/v). Then, 100 μL of the skin samples were added and a mix between HRP conjugated and unconjugated antibody 4.0 μg/mL (1:4, v/v) was used as the detection antibody. For spectrophotometric detection, TMB solution was added and the reaction was ended using HCl 1 M. The yellow color was read using a plate reader at 450 nm.

In vitro skin penetration experiments were performed using hairless mice skin mounted in modified Franz diffusion cells. Skin was pretreated by LFS using poloxamer 20% gel as a coupling medium until the skin achieved the 1 kΩcm² electrical resistivity. The ultrasound application was performed at a frequency at 20 kHz, intensity at 7.5 W/cm² and pulses at 5 s on and 5 s off. After this, a dispersion containing 1 mg/mL of cetuximab in PBS or only PBS (control) was put in contact with the skin for 12 h. Cetuximab permeation studies through untreated skin were also performed. After penetration studies, the skin samples were removed from the diffusion cell, washed with deionized water and cetuximab was extracted with water and quantified using the method described before.

Aiming to investigate if the amount of drug that penetrated is enough to be effective, studies in A431 cell line were performed. A431 cell line was obtained from American Type Culture Collection (ATCC, Rockville, MD, USA) and cultivated in Dulbecco’s modified eagle medium containing 10% bovine foetal serum at 37°C in 5% CO₂ atmosphere. When cells reached 80% confluence, they were trypsinized and plated (3000 cells/well) in 96 well plates. Cells were submitted to cetuximab solutions
treatment ranging from $5 \times 10^9$ g/mL to $1 \times 10^4$ g/mL for 72 h in one single dose or with multiple doses every 24 h for 3 days. For survival rate, pretreated cells were washed with PBS and cultivated for another 72 h prior to MTT assay. Kruskall Wallis and Dunns post-test ($p < 0.05$) were used as statistical test.

RESULTS AND DISCUSSION
No interference from skin was observed for cetuximab quantification. Also, cetuximab recovered from the skin untreated with LFS was under the detection limit of the analytical method, probably because of cetuximab macromolecule size which prevented its penetration into the skin. On the other hand, in the skin pretreated with LFS it was possible to quantify $8.5 \times 10^{-5}$ g/cm$^2$ cetuximab.

Fig 1 and 2 show cell viability and survival, respectively, after the treatment with cetuximab. Viable cells in Fig 1 were analyzed after 72 h to determine if they were able to continue grow after treatments (cell survival) or if they were damaged (Fig 2). The treatment of A431 cells with 100 μg/mL cetuximab during 72 h, with a single dose, resulted in cell viability of 73.9 % ($\pm$ 6.8%), whereas the treatment with multiple dose decreased cells viability to 46.4 % ($\pm$ 9.6%), indicating the superiority of the multiple doses scheme to kill A431 cancer cells. LC50 could be determined for the multiple doses group and corresponded to $4.32 \times 10^{-5}$ g/mL (or $2.57 \times 10^{-5}$ g/cm$^2$). For the survival rate experiment, the viability was 30.4 % ($\pm$ 9.7%) and 28.9 % ($\pm$ 12.4 %) for the 100 μg/mL cetuximab for single dose and multiple dose treatment, respectively. No statistical difference was observed between the single or multiple dose groups.

CONCLUSION
Our results demonstrated that cetuximab can penetrate through the skin after pretreatment with LFS, but not through intact, non-treated, skin. Considering the cell viability results, the amount that penetrated through the skin is 3.3 times higher than the LC50 for cetuximab solution. Further studies will be carried out to investigate the effect of delivery systems to improve skin penetration of cetuximab.

REFERENCES

ACKNOWLEDGMENTS
São Paulo Research Foundation (Fapesp grant #2013/15134-2, #2012/05177-3) and CNPq (grant #480962/2013-8).