AMERICAN JOURNAL OF PHARMTECH RESEARCH
Journal home page: http://www.ajptr.com/

Electroporation- Novel Delivery System

Pravin P. Naik[1]*, Sandeep O. Waghulde[1], Pritam V. Juwatkar[1], Ashwin A. Kotamkar[1], Nilesh Gorde[1], Sanjana Kshetri[1],
1. Konkan Gyanpeeth Rahul Dharkar College of Pharmacy & Research Institute, Karjat.

ABSTRACT

Electroporation is novel delivery system and Electrochemotherapy(ECT) is a novel treatment which consists of a combination of a chemotherapeutic agent and pulsed electric fields. Two types of Electroporation are reversible and irreversible Electroporation. This is relatively new treatment modality relies on the physical effects of locally applied electric fields to temporarily destabilize cell membranes in the presence of a drug to allow increased uptake of the agent into the cytosol. Clinical trial data suggests that irreversible electroporation may become an important and innovative tool in the armamentarium of surgeons treating cancer. Electrochemotherapy has been used effectively in preclinical and clinical studies. The therapy was shown to be effective regardless of histologic type of tumor including head and neck squamous cell carcinoma. Considering the proven safety in several different clinical applications electroporation should be viewed as a clinical platform technology with wide perspectives for use in ECT, gene therapy and DNA vaccination. In this review we gathered the data of the clinical trials that have been published so far.

Keywords: Electroporation (EP), Electrochemotherapy (ECT), Magneto electric nanoparticles, Cancer etc.

*Corresponding Author Email: pravin.aazcom@gmail.com
Received 17 March 2015, Accepted 01 April 2015

Please cite this article as: Naik PP *et al.*, Electroporation- Novel Delivery System. American Journal of PharmTech Research 2015.

INTRODUCTION

In spite of the advantages of the transdermal delivery, only a small percentage of drugs can be delivered transdermally due to the barrier properties of the skin: only small potent lipophilic drugs can be delivered at therapeutic rates by passive diffusion. Moreover, transport of most drugs across the skin is very slow and lag-times to reach steady state fluxes are in hours. Achievement of a therapeutically effective drug level, is therefore, difficult without enhancing skin permeation. A number of approaches have been developed to enhance and control transport across the skin, and expand the range of drugs delivered. These involve chemical and physical methods, based on two strategies: increasing skin permeability and/or providing a driving force acting on the drug[1,2]. Electroporation or electropermeabilization, is a significant increase in the electrical conductivity and permeability of the cell plasma membrane caused by an externally applied electric field.

Two types[3]

Reversible electroporation (RE): Up to a certain degree of damage induced by nanoporation, the cell can be completely repaired and survive. This is called reversible electroporation or just electroporation.

Irreversible electroporation (IRE): After a certain degree of damage to the cell membranes by electroporation, healthy and cancerous cells are irreversibly damaged. They die by apoptosis, which is unique to this ablation technique, in opposition to all other ablation systems which induce necrosis either by heat or radiation. Process by which IRE causes cell death is unclear.

Parameters controlling drug delivery by electroporation

Electrical parameters: Electrical pulses are characterized by their electri-cal parameters: waveform (exponential decay orsquare wave), voltage (50 –1500 V), duration (few As to ms), and interval between pulses (few s to min). These electrical parameters can be optimized, depend-ing on experiment requirements and clinical application of electroporation[4].

Pulse waveform: Two kinds of pulse generators are usually employed to transport molecules by electroporation: there are generators which can deliver exponentially decaying pulses[7,8,9] or square wave pulses[5,6,10].Both are used for different applications, i.e. drug delivery, electrochemotherapy and gene therapy. Due to its long voltage tail profile, the main potential advantages of exponentially decaying pulses are to maintain or expand the high permeability state of the skin induced by electroporation and to promote the electrophoretic movement. However, because the duration of exponential decay pulses depends on the resistance of the skin and the electro-poration system (electrodes, conducting medium), the reproducibility of the pulse conditions for clinical use

could be a problem. In contrast, voltage and duration of square wave pulses remain constant whatever the skin or drug reservoir. Hence, square wave pulses should be used to have a better control and repro-ducibility of drug transport. Until recently, the square wave pulses were exclusively used for electrochemo-therapy and DNA electrotransfer, whereas exponen-tially decaying pulses were restricted to transdermal drug delivery to take advantage of the long voltage tail.

Pulse voltage, duration, number and rate: Control on drug transport by skin electro-poration can be achieved by controlling the pulse voltage, duration, number and rate. The effect of varying these electrical parameters on transdermal transport has been extensively studied in vitro[7,8,11,12,13-15] Because significant voltage drop occurs within the electroporation system, the transdermal voltage is only a fraction (ca. 10 – 50%) of the voltage applied across the electrodes, depending of the relative resistance of the skin and the drug reservoir. Flux rate always enhances when electrical pulse conditions strengthen: when the number, the voltage or the duration of the pulses increase, the transder-mal drug transport increased (see Figs. 1 and 3). With increasing voltage of pulses, the transdermal flux increases but less steeply at high voltages[7,12]. When the pulse duration and pulse numbers increases the drug transport often linearly increases[8]. Increasing the pulse rate increases transdermal flux as well[8,16,13]. The electrical parameters influence transdermal flux but also onset time for transport, which decreases with increasing pulse duration and rate, but is inde-pendent of voltage[16,13].

Pulsing protocol: Two different types of pulsing protocols are usually reported in the literature. They can mainly be distinguished by the pulse duration: (i) numerous (>100), short duration (1 – 2 ms), high voltage pulses; and (ii) a low number (<20), long duration (70– 1000 ms), medium voltage pulses. In the case of exponentially decaying pulses, at the same total electrical charge transported through the skin, a few long pulses allowed generally higher molecular transport than many short pulses[4].

Electrode design: The design of the electrodes is still a critical issue, both in terms of efficacy of drug transport and tolerance. The early research on skin electroporation was performed in vitro with electrodes placed on both sides of the skin. If insights on transdermal drug delivery were gained, the position and design of the electrodes were not representative of in vivo condi-tions. Various electrodes and reservoir systems, e.g. plate electrodes with a skin fold[16,17], meander electrodes[17], have been designed for in vivo applications. The efficacy of drug transport is influenced by the electrode design because the distribution and intensity of the electrical field in the skin are affected[18,19]. The simplest configuration to generate a more or less uniform electric

field is parallel plate electrodes in the form of calipers. However, underlying nerves and muscles could be subjected to electrical stimulus and superficial skin burning could be observed. The meander electrodes consist of an array of interweaving electrode fingers, allowing the electric field to be mostly localized within the super-ficial layers of the skin, thereby avoiding undesirable effects in underlying tissues. As the reaction is extremely fast, inert electrodes, e.g. platinum, are preferred to active electrodes, e.g. silver/silver chloride electrodes. As oxydoreduction occurs at the electrodes, hydrogen and hydroxyl ions are produced and could lead to a pH shift in the reservoir.

Physicochemical properties of the drug: In addition to the electrical parameters of the pulses, the physico-chemical properties of drug can affect the transdermal drug delivery by electroporation.

Charge: Because the electrophoretic movement is the main mechanism of transport for charged molecules through a highly permeabilized skin by electropora-tion, the pK_a of the drug and the pH of the delivery solution are essential parameters influencing the elec-tric charges of the molecule to deliver. Increasing the charge of the permeant enhances its transport. Hence, the pH of the drug reservoir, which affects drug ionization, will influence the efficacy of drug delivery. The transport of neutral molecules is also enhanced by electroporation, due to passive diffusion through the permeabilized skin[20]. This transport of neutral molecules is lower, compared to the transport of charged molecules during pulses. At physiological conditions, the skin is negatively charged, presenting a better permselectivity to the cations. However, due to the short duration of current application, the contribution of electroosmosis is lim-ited[20], suggesting that the skin permselectivity is not as important as for iontophoretic transport.

Lipophilicity: The influence of the partition coefficient of the permeant has not been systematically investigated. In contrast to passive diffusion, increasing the lipophi-licity of the drug tends to decrease the enhancement ratio. The transdermal fluxes of nalbuphine and lipo-philic prodrugs were enhanced by electroporation, as compared to passive diffusion. The total amount of nalbuphine and its prodrugs were similar but the enhancement ratio decreased as the lipophilicity in-creased[15]

Molecularweight: Another physicochemical property of the drug influencing the transdermal transport by skin electro-poration is its molecular weight. Using FITC dextran of increasing molecular weight, Lombry et al.[21] showed that significant transport and intracellular penetration in the skin were detected after high voltage pulse application (Figure. 4). The greater the molecular size, the lower the transdermal transport. The absence of molecular weight cut off (at least up to 40 kDa) suggested that electroporation could be useful for macromolecule delivery.

Formulation of drug reservoir: Because the drug concentration affects the trans-dermal transport of drug by electroporation, the choice of drug concentration in the reservoir could allow control on drug delivery. The higher the drug concentration, the higher the transport. However, a non-linear relationship between the quantity of drug delivered in the skin and the drug concentration of the reservoir has been reported[11,22].

Table 1: Parameters affecting drug transport by electroporation (adapted from Pre´at and Vanbever [23])

Parameters	Increase in	Effect
Electrical parameters	Pulse voltage	+
	Pulse number	+
	Pulse length	+
Physicochemical properties of drug	Charge	+
	Molecular weight	_
	Conformation	?
	Lipophilicity	_
Formulation of drug reservoir	Competitive ions	_
	Ionization (pH)	+
	Viscosity	_

+, Positive effect; _, negative effect.

The current theory is as follows[24]

When an electrical field of more than 0.5 V/nm is applied to the resting trans-membrane potential, it is proposed that water enters the cell during this dielectric breakdown. Hydrophilic pores are formed. After the application of an electrical field, water molecules line up in single file and penetrate the hydrophobic center of the bilayer lipid membrane; These water channels continue to grow in length and diameter and expand into water-filled pores, at which point they are stabilized by the lipid head groups that move from the membrane-water interface to the middle of the bilayer. This entire process can occur within a few nanoseconds.

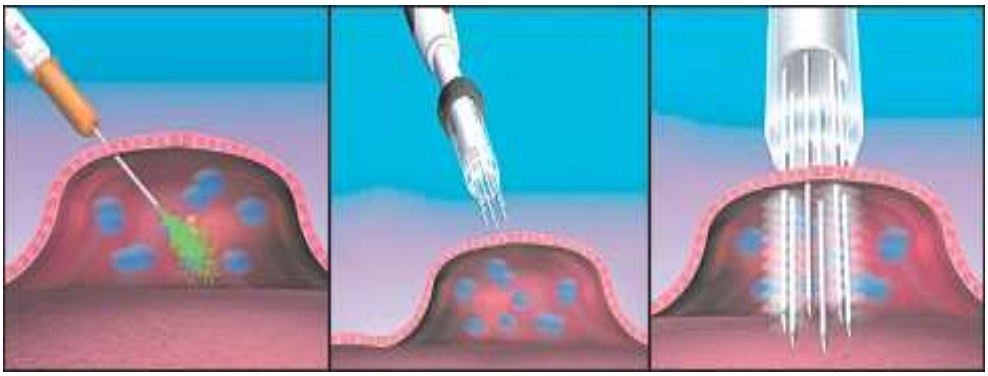

Figure 1: Application of electroporator in to diseased area

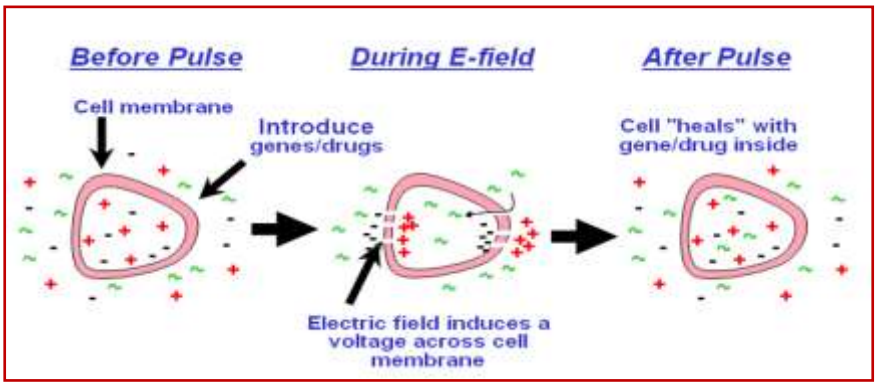

Figure 2: Absorption of drug into cell during E- field

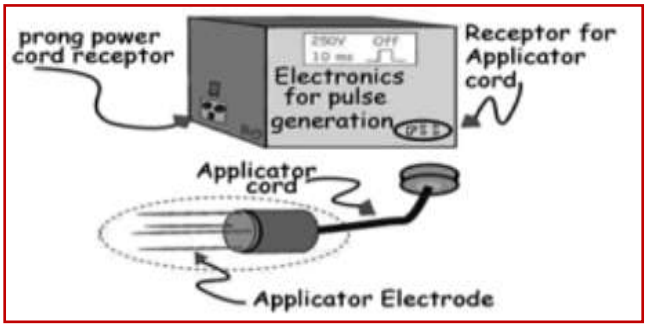

Figure 3 (a): Pulse Generator and applicator

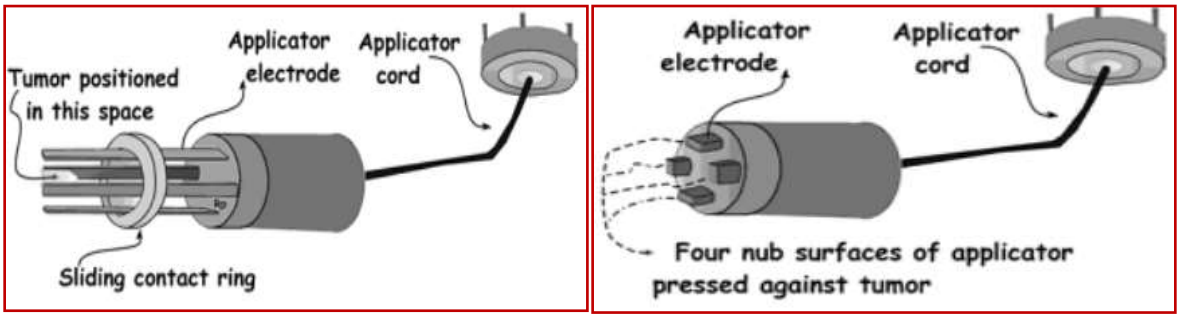

Figure 3 (b): Different types of applicator

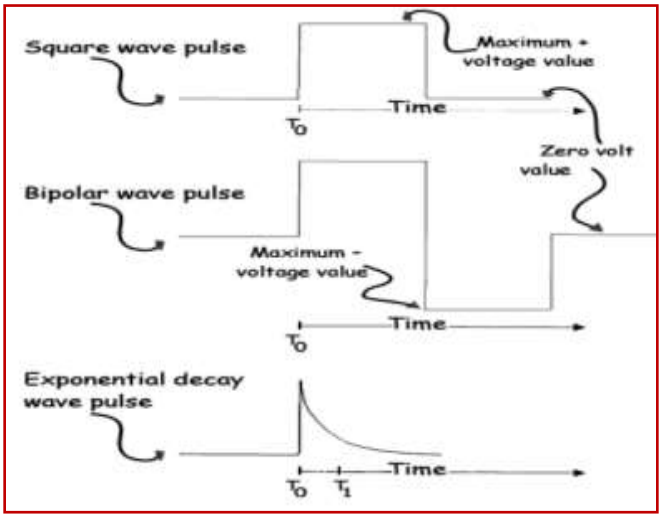

Figure 4: Different types of pulse generated during generation of electric impulses.

Recent technique

1. **Non-thermal irreversible electroporation (N-TIRE)[25]:** This procedure is done using small electrodes (about 1mm in diameter), placed either inside or surrounding the target tissue to apply short, repetitive bursts of electricity at a predetermined voltage and frequency. These bursts of electricity increase the resting transmembrane potential (TMP). When the electricity applied to the tissue is above the electric field threshold of the target tissue, the cells become permanently permeable from the formation of nanopores. As a result, the cells are unable to repair the damage and die due to a loss of homeostasis.N-TIRE is unique to other tumor ablation techniques in that it does not create thermal damage to the tissue around it.

2. **High-frequency irreversible electroporation (H-FIRE)[26]:** This technique uses electrodes to apply bipolar bursts of electricity at a high frequency, as opposed to unipolar bursts of electricity at a low frequency. This type of procedure has the same tumor ablation success as N-TIRE. However, it has one distinct advantage, H-FIRE does not cause muscle contraction in the patient and therefore there is no need for a paralytic agent.

Side effects

In patients with smaller tumors, Slight discomfort is common when the electric pulses are delivered under local anesthesia. This is because the pulses cause the muscles just under the skin to contract. If you are treated using a general anesthetic, you will not feel these contractions at all. In patients with larger tumors, wound formation and there is also a risk of infection, in which case treatment with antibiotics may be necessary. The wound is treated according to standard guidelines for wound care.

Clinical Application

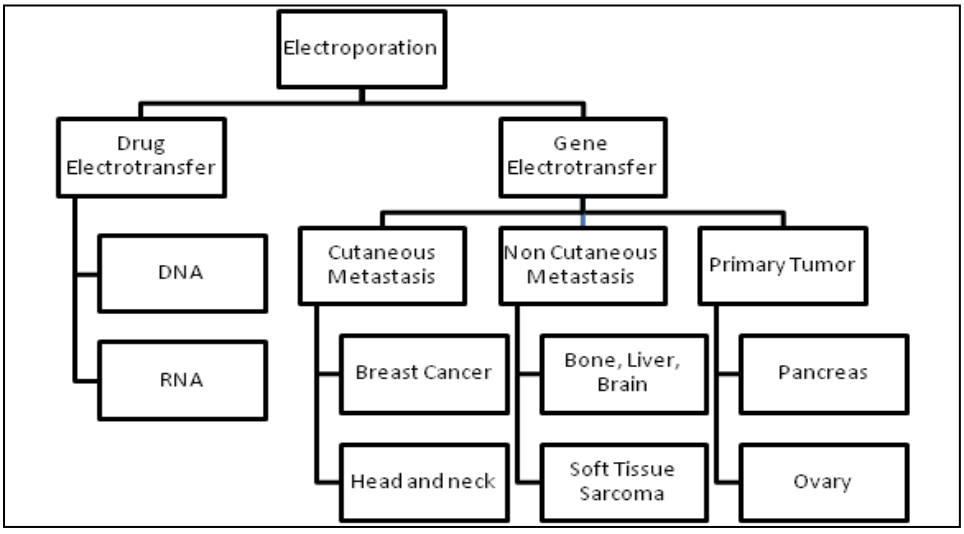

Figure 5: Perspective Clinical Applications of Electroporation.

1. The first research looking at how electroporation might be used on human cells was conducted in 2003.
2. Tissue Ablation with Irreversible Electroporation in 2005 study suggested that irreversible electroporation may become an important and innovative tool in the armamentarium of surgeons treating cancer.
3. The first successful treatment of malignant cutaneous tumors implanted in mice was completed in 2007.
4. In oral tongue cancer[27] the ECT showed good functional outcome for speech and eating in 2011.
5. The nanotechnology capable of high-specificity targeted delivery of anti-neoplastic drugs would be a significant breakthrough in Cancer in general and Ovarian Cancer in particular. The difference in the membrane electric properties between the tumor and healthy cells and the capability of magneto-electric nanoparticles[28] (MENs) to serve as nanosized converters of remote magnetic field energy into the MENs' intrinsic electric field energy. This capability allows to remotely control the membrane electric fields and consequently trigger high-specificity drug uptake through creation of localized nano-electroporation sites. In in-vitro studies on human ovarian carcinoma cell (SKOV-3) and healthy cell (HOMEC) lines, applied a 30-Oed.c. field to trigger high-specificity uptake of paclitaxel (PTX) loaded on 30-nm CoFe2O4@BaTiO3 MENs. The drug penetrated through the membrane and completely eradicated the tumor within 24 hours without affecting the normal cells.

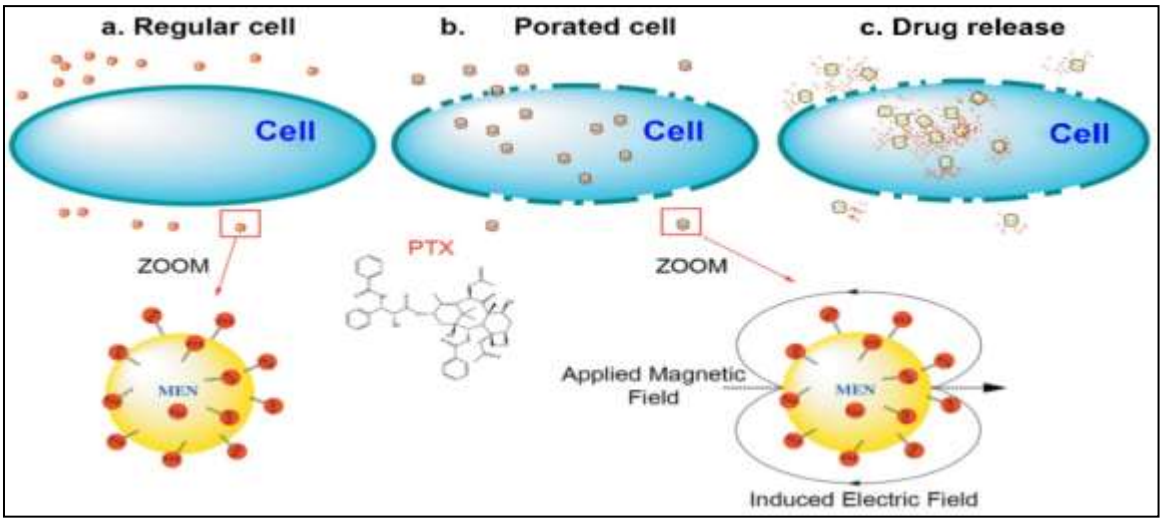

Figure 6: Magneto electric nanoparticles control electric field and increase specific drug uptake

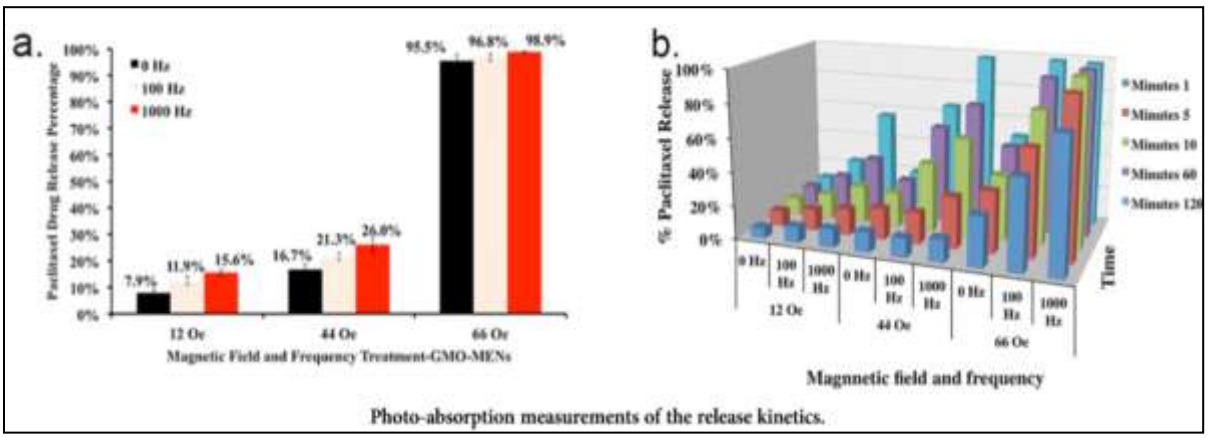

Figure 6: Magnetic field verses drug release percentage.

6. IRE has proven effective in treating human cancer, with surgeons at Johns Hopkins and other institutions now using the technology to treatpancreatic cancer[29] previously thought to be unresectable in 2014.
7. DRUG VS DRUG PLUS EP (2014)[30]:

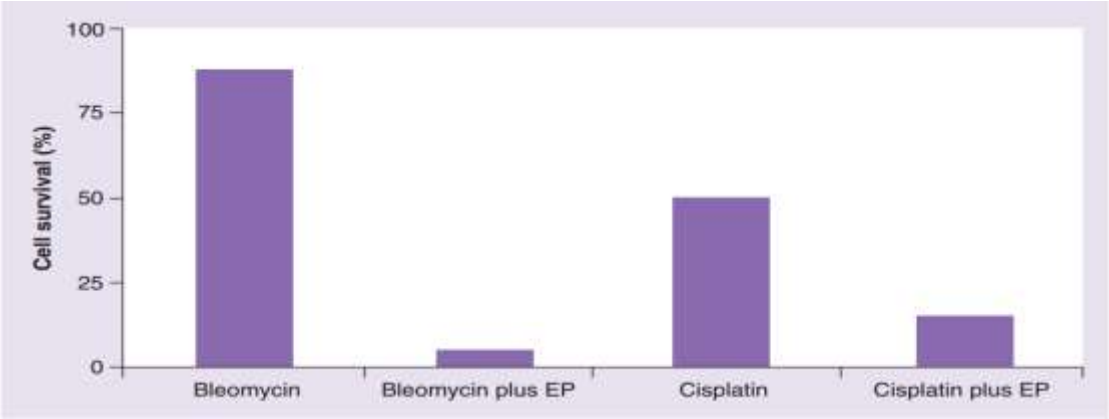

Figure 7: DCF3 cell survival cultured in the presence of bleomycin 10^{-8} M or cisplatin 15 µM and exposed to electroporation

Electrochemotherapy (ECT) is the result of a combined treatment associating chemotherapeutic drugs and local application of electric pulses to induce cell membrane electroporation (EP). In vitro bleomycin cytotoxicity increased up to 1000-fold and that of cisplatin by 80-fold. Subsequently, prospective randomized studies clearly showed that the combined treatment was more effective than the two components used separately in the treatment of metastatic tumor nodules in patients.

Manufacturer of Electroporation Equipments

Figure 8: Angiodynamics

Angiodynamics is manufacturer of Naniknife^{TM} bult on treatent based on IRE. Applicator output: 1-6, Number of Pulses: 10-100, Pulse amplitude: 100-3000V,Pulse Length: 20-100 µs,Max. current: 50A. Percutaneous and laproscopic treatment of prostate, liver, lung, kidney, bone. Targeting Hepatocellular carcinoma clinical trial 2010.

Figure 9: IEGA

IEGA is manufacturer of CliniporatorTM built for ECT and gene elctrotransfer .

Applicator output: 1, **Number of Pulses**: 1-20, **Pulse amplitude**: 100-1000V,**Pulse Length**: 30-5000 µs,**Max. current**: 16 A

Treatment of skin tumor lesions, cutaneous and subcutaneous applications. Also for deeper lying tissues.

CONCLUSION

Electroporation is an efficient method for enhancing drug delivery in vitro and in vivo, and expands the range of compounds delivered. Combined with other enhancing methods, electroporation can provide adequate delivery according to the treatment. Pulse protocol and electrode design need to be optimized to reduce the adverse effects. A high response to treatment rate (approximately 90%) accompanied by a low local relapse rate (10.6%) may be achieved after

one ECT procedure. In addition, a positive effect of ECT on the patients' quality of life was demonstrated. No serious adverse events or deaths related to ECT have been reported.

REFERENCES

1. Folvari M. Non-invasive administration of drugs through the skin: challenges in delivery system design. P.S.T.T. 2000: 3(12): 417 – 425.
2. Barry B.W. Novel mechanisms and devices to enable success-ful transdermal drug delivery. Eur. J. Pharm. Sci. 2001:14(2):101 – 114.
3. Korohoda W, Grys M, Madeja Z.Reversible and irreversible electroporation of cell suspensions flowing through a localized DC electric field. Cell. Mole. bio.lett. 2013:18(1):102-119.
4. Vanbever R, LeBoulenge E, Preat V. Transdermal delivery of fentanyl by electroporation: I. Influence of electrical factors. Pharm. Res. 1996:13:559– 565.
5. Mir L, Orlowski S. Mechanisms of electrochemotherapy. Adv. Drug Deliv. Rev.1999:35(1): 107–118.
6. Heller R, Gilbert R, Jaroszeski MJ. Clinical applications of electrochemotherapy. Adv. Drug Deliv. Rev. 1999:35(1):119–129.
7. Prausnitz MR, Bose VG, Langer R, Weaver JC. Electroporation of mammalian skin: a mechanism to enhance transdermal drug delivery. Proc. Natl. Acad. Sci. USA 1993 :90(22):10504-8.
8. Vanbever R, Lecouturier N, Pre´at V. Transdermal delivery of metoprolol by electroporation. Pharm. Res. 1994:11(11): 1657– 1662.
9. Pliquett U, Weaver JC. Transport of a charged molecule across the human epidermis due to electroporation. J. Control. Release.1996:38:1 – 10.
10. Denet AR, Pre´at V. Transdermal delivery of timolol by electroporation through human skin. J. Control. Release. 2003:88(2):253–262.
11. Regnier V, Le Doan T, Preat V. Parameters controlling topical delivery of oligonucleotides by electroporation. J. Drug Targeting. 1998:5:275–289.
12. Vanbever R, LeBoulenge E, Preat V. Transdermal delivery of fentanyl by electroporation: I. Influence of electrical factors. Pharm. Res. 1996:13:559– 565.
13. Prausnitz MR, Pliquett U, Langer R, Weaver JC. Rapid temporal control of transdermal drug delivery by electroporation. Pharm. Res.1994:11:1834– 1837.

14. Jadoul A, Lecouturier N, Mesens J, Caers W, Pre´at V. Electrically enhanced transdermal delivery of alnitidan. J. Control. Release.1998:54:265– 272.
15. Sung KC, Fang JY, Wang JJ, Hu OY. Transdermal delivery of nalbuphine and its prodrugs by electroporation. Eur. J. Pharm. Sci. 2003:18:63– 70.
16. Dujardin N, Van der Smissen P, Preat V. Topical gene transfer into rat skin using electroporation. Pharm. Res. 2001:18:61–66.
17. Zhang L,Rabussay DP., Clinical evaluation of safety and human tolerance of electrical sensation induced by electric fields with non-invasive electrodes, Bioelectrochemistry 2002: 233–236.
18. Mori K, Hasegawa T, Sato S, Sugibayashi K. Effect of electric field on the enhanced skin permeation of drugs by electroporation. J. Control. Release. 2003:90:171– 179.
19. Fukushima S, Kishimoto S, Horai S, Miyawaki K, Kamiyabu S, Kamata Y, Yamaoka Y, Takeuchi Y. Transdermal drug delivery by electroporation applied on the stratum corneum of rat using stamp-type electrode and frog-type electrode in vitro. Biol. Pharm. Bull. 2001:24: 1027– 1031.
20. Vanbever R, Leroy MA, Pre´at V. Transdermal permeation of neutral molecules by electroporation. J. Control. Release. 1998:54:243– 250.
21. Lombry C,Dujardin N, Preat V. Transdermal delivery of macromolecules using skin electroporation. Pharm. Res. 2000:17:32–37.
22. Vanbever R, Preat V. Factors affecting transdermal delivery of metoprolol by electroporation. Bioelectrochem. Bioenerg. 1995:38:223– 228.
23. Pre´at V, Vanbever R. Skin electroporation for transdermal and topical drug delivery, in: R.H. Guy, J. Hadgraft (Eds.), Transdermal Drug Delivery.Marcel Dekker, New York, 2002:123: 227– 254.
24. Kee ST, GehlJ, LeeEW. “Clinical Aspects of Electroporation”. 2011.
25. Garcia PA, Rossmeisl JH, Davalos RV. Electrical conductivity changes during irreversible electroporation treatment of brain cancer. Annual International Conference of the IEEE Engineering in Medicine and Biology Society:2011:739–42
26. Arena CB, Sano MB, Rossmeisl JH, Caldwell JL, Garcia PA, Rylander MD, Rafael V.High-frequency irreversible electroporation (H-FIRE) for non-thermal ablation without muscle contraction. BioMedical Engineering OnLine.2011:10:102
27. Landstrom FJ. Electroporation therapy for T1 and T2 oral tongue cancer.Acta tolaryngol. 2011

28. Rakesh G, Magneto-electric Nanoparticles to Enable Field-controlled High-Specificity Drug Delivery to Eradicate Ovarian Cancer Cells , Scientific Reports, article number 2953, 2013-10-16
29. "A Potential Boon for Pancreatic Cancer Patients". Johns Hopkins Surgery: News From the Johns Hopkins Department of Surgery. 2014-06-23.
30. Ruggero C, Mattia R,Matteo C.Locally enhanced chemotherapy by electroporation: clinical experiences and perspective of use of electrochemotherapy, Future Oncol. 2014:10(5):877–890.