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ELECTROPORATION IN CANCER TREATMENT: CLINICAL APPLICATIONS AND CHALLENGES

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Abstract:

Objective: *Electroporation (EP) is a relatively new modality in cancer treatment with promising outcomes. EP has shown significant efficacy in different domain of cancer treatment as independent or in combination with conventional techniques. The main objective of this study was to comprehensively review applications of EP for cancer treatment as an adjunctive or alternative treatment. The main focus of the review is radiosensitizing effect of EP.*

Methods: *The databases of PubMed, Embase, Cochrane library, Web of Science, Biomed central, Science Direct, and Google scholar were searched using the set terms. The search key words based on the MeSH heading included "electroporation" OR "electrochemotherapy" AND "irradiation" OR "radiosensitizing". The obtained results were screened for the title and abstract by two authors and the relevant papers were reviewed for further details.*

Results: *The main fields of EP applications in cancer treatment are targeted drug delivery, electrochemotherapy, and radiotherapy. One of the main applications of EP is radiosensitizing effect of different cancerous cell lines to chemotherapy and radiotherapy. A single session EP (consisting a train of pulses or even single pulse) before radiotherapy can significantly enhance the tumor response. Moreover, EP enhances efficacy of different chemotherapeutic agents. The main reason of these phenomena is inducing radiosensitivity to ionizing radiation and sensitivity to chemotherapeutic agents. In addition, enhancing the production of reactive oxygen species has been reported as the main mechanism of action of enhancing treatment efficacy. This combined treatment modality was effective in different cell lines with variety range of radiosensitivity.*

Conclusion: *EP alone or in combination with other agent including gold nanoparticles can enhance the efficacy of radiotherapy and also the chemotherapy. Further studies are needed to develop more efficient EP protocols and also combined treatments. The current evidence shows we can expect EP based techniques can be translated into the clinic setting.*

Keywords: electroporation, cancer treatment, radiosensitizing effect

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INTRODUCTION:

Cancer is the most common cause of mortality worldwide. Radiotherapy and chemotherapy are two important treatment options for management of loco-regional tumors[1]. Each of these techniques has some major side effects. Combinations of radiotherapy and chemotherapy along with the recent advances in treatment planning in radiotherapy have resulted in better management of cancer through more efficient antitumor treatments. However, the mortality rates because of loco-regional tumors have been steadily increasing during the last decade. Therefore, developing new approaches or improvements in the current modalities is necessary. Developing targeted drug delivery modalities along with developing efficient radiosensitizing agents are the two main avenues in this regard. More localized and targeted drug delivery enhances targeted uptake of the anticancer agents into the tumor cells. Similarly, radiosensitizer agents increase tumor specific sensitization to radiotherapy while maintaining low dose to surrounding healthy tissue which consequently increases the tumor treatment efficacy. Electroporation (EP) has shown promising outcomes and potentials as both targeted drug delivery system and radiosensitizing technique. Several studies have shown that EP increases chemotherapeutic agent uptake in the tumor cells which in turn enhances their intracellular accumulation and consequently the radiosensitizing effect [2]. EP is a technique that induces a dramatic increase in cell membrane permeability to ions and macromolecules through applying short, intense electric field pulses [3-5]. The use of EP to transport non-permeant chemotherapy drugs is called electrochemotherapy (ECT). In other word, combination of EP with chemotherapeutic drugs is called ECT [6, 7]. ECT is a localized treatment of cancer through which short and intense electric pulses to improve transmembrane transfer of molecules that are otherwise nonpermeant such as highly cytotoxic drugs. Using this approach, hydrophilic drugs such as cisplatin and bleomycin can enter across the membrane to directly reach the cytosol of cells to kill tumors. ECT enhances the accumulation of non-permeant or poorly permeant anticancer drugs with high intrinsic cytotoxicity in tumor cells is enhanced by ECT [8-15]. Therefore, the radiosensitizing effect of drugs is enhanced and the required radiation dose to kill a cell or trigger a certain biological interaction is decreased. The other approach to increase the radiotherapy efficacy or tumor response to antitumor agents is combined application of EP and ECT with other agents such as nanoparticles. Gold nanoparticles (NPs) have shown promising effects in this regard. Combined EP-gold NPs showed synergistic radiosensitizing effects to radiotherapy [16]. The present study was aimed to comprehensively review the recent advances EP for cancer treatment as well as its clinical applications and challenges. The main focus of this review is radiosensitizing effect of EP alone or in combination with other modalities in cancer treatments using radiotherapy and chemotherapy.

METHODS:

The scientific records were retrieved by a systematic search of multiple bibliographic databases up to Feb 25, 2017 including Medline, Embase, Cochrane library, web of science, Biomed central, Science Direct, Google scholar, and additional resources. The language of search was restricted to English. The search key words were "electroporation", "cancer treatment", "electrochemotherapy", "clinical applications", and "radiosensitizing". The titles and abstracts of all the retrieved records in the searching stage were carefully reviewed by two authors and the relevant records with full texts were used for further assessments. The reference lists of the identified records were also manually checked and the additional eligible studies for full review. Any published or unpublished clinical or preclinical study on the effects of EP alone or in combination with other modalities in any cancerous cell lines was included. The studies should have investigated tumor response using at least one of the following measures: survival fraction, tumor growth delay, tumor doubling time, and dose enhancement factor. Studies were excluded if they were abstract, letters, guidelines, or patent (Fig. 1). Because of the immense amount of the records and discrepancies in the methodologies, research design and the subjects the authors decided to perform comprehensively review on the current literature.

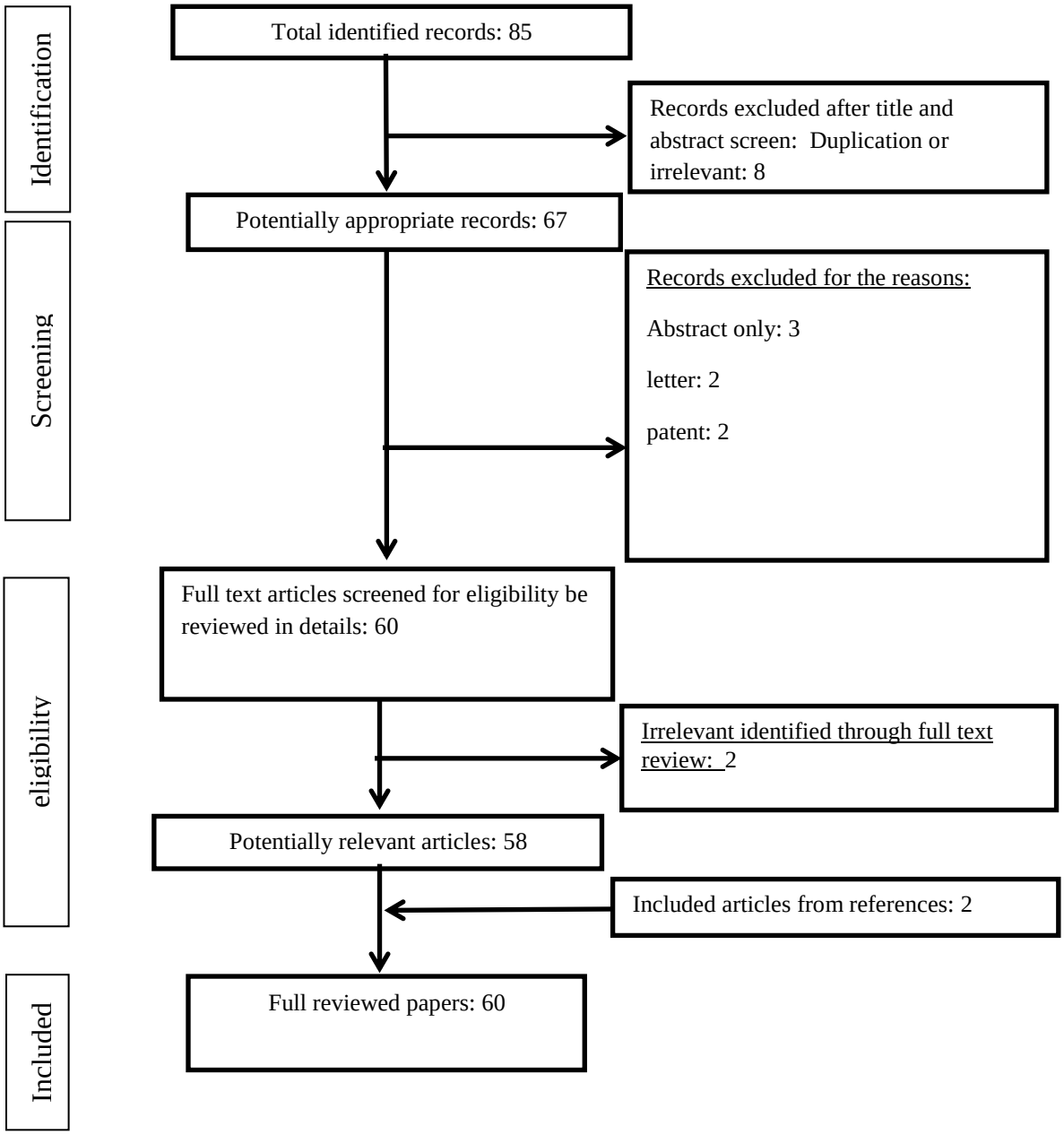

Figure1: The flowchart of the study design process

RESULTS:

After the applying eligibility criteria, total of 60 papers were full reviewed. The main findings of the studies were discussed in the following sections: recent advances in EP technique for cancer treatment as well as its clinical applications and challenges. The main focus of this review is on the radiosensitizing effect of EP in radiotherapy and in electrochemotherapy which is discussed in more details.

Electroporation in radiotherapy

The membrane of a living cell plays pivotal role in maintaining the functionality of a cell and the related organism. The main focus of therapeutic modalities in various disorders is on the membrane. The 5 nm-thick membrane is made up of phospholipid bilayer molecules, whose polar ends project into the cytoplasm and external medium and non polar ends into the middle of the bilayer [17]. This cell membrane acts as a barrier that impedes

the free movements of ions, molecules, and foreign agents through cell membrane separating cytoplasm and external medium. However, using an intense and short enough external electric pulse can transiently increase the permeability of membrane which allow movement of big molecules of therapeutic agent [18-21]. The pore's size and its duration depend on several parameters including electric pulse shape, field strength, pulse duration, pulse repetition frequency, extracellular media content, and resting transmembrane voltage [22-29]. The medical applications of EP include gene electrotransfer [30], transdermal drug delivery, tumor and tissue ablation, and the electrochemotherapy of tumors[31, 32]. Extensive in vitro or in vivo studies have demonstrated that ECT is efficient, safe, inexpensive, and localized treatment without significant side effects that can be used for various types of superficial [10, 11, 15, 33-41] and also for deep seated tumors [42]. There are two types of EP based on the duration of induced pore and viability of the exposed cell: reversible EP (REP) and irreversible EP (IEP). REP is a biophysical process that through the application of short, intense electric pulses [43-45]. During REP the permeability of cell membrane is transiently increased and then the cell returns to its normal state and the induced pore will disappear. In contrary, in REP the intensity of electric pulse is high enough to destroy the membrane structure and to kill the cell. In contrast to REP used in ECT, the IEP, introduced in 2005, has been used for tumor ablation in cancer treatment [46] [47, 48]. IEP works on the basis of utilizing strong electrical fields instead of moderate intensity to induce transient pore in membrane or deposit heat [49, 50]. Neal et al demonstrated that human breast cancer tumors orthotopically implanted in the mouse can be successfully treated using IEP. In addition, IEP is an alternative modality with more benefits and less side effects for surgical resection in different cancers such as breast conserving therapy [32]. The recent in vivo studies on electropermeabilization have demonstrated a shift towards the combining this phenomenon with other techniques such as sonoporation[51, 52], immunotherapy [53], and nanoparticles [16, 54].

The radiosensitizing effects of EP and ECT have been demonstrated in different cell lines including Ehrlich ascites carcinoma (EAC) [36, 55], SCK mammary carcinoma[55], LPB sarcoma [56, 57], murine mammary adenocarcinoma CaNT[58], and SA-1 sarcoma[58]. squamous cell carcinoma cells (SCCVII) [59], solid tumor fibrosarcoma [60], and in spontaneous mouse mammary tumor[61].

EP can modulate the intrinsic functional characterization of the target cells and increase the oxidative burst in the target cells [62, 63]. Gabriel et al reported that EP can induce oxidative jump and generate reactive-oxygen species (ROS) in CHO cells[64]. The electro-induced oxidative jump can be appeared when the applied electric field intensity is higher than critical threshold value that controlled by duration of pulse. The critical value of 0.44 V/cm is necessary to simultaneously trigger the electropermeabilization and electro-induced ROS production [65]. The electro-induced ROS generation is non-homogenous and restricted to the electropermeabilized site of the cell membrane [66]. Therefore, this technique has a potential to be combined by other radiosensitizing agents and/or ionizing radiation to increase lethal damage of irradiation. The efficiency of the combined modality of EP-radiotherapy is determined by quality of radiation, electric pulse parameters, and time interval between electroporation and radiotherapy. West et al for the first time reported the radiosensitizing effects of EP to radiotherapy on CHO cell line [67]. They studies three time intervals of EP-radiotherapy of immediately, 1 h, and 24 h. They observed using EP immediately before irradiation enhanced the efficacy of radiotherapy, dose enhancement factor, of 1.19 times. The time interval was an important factor for inducing radiosensitizing effect as application of EP at 1h or 24 h before irradiation resulted in no radiosensitizing effect.

Studies have shown that EP can induce radiosensitizing effects to orthovoltage x-rays in the energy ranges of 200–500 kV [36] [68]. In this regard, Sersa et al reported that EP enhanced the effect of 220KV irradiation in tumor-bearing animals [36]. They investigated whether EP can increase the radiosensitizing effect of cisplatin as a chemotherapeutic agent. The interesting finding of this study was that delivering of electric pulses prior to irradiation and in the absence of cisplatin enhanced the efficacy of irradiation. Similar results were reported by Kranjc et al [68]. Irradiation of LPB sarcoma cells pretreated by electric pulses enhanced the cytotoxicity of radiation (DEF=1.25).

Shil et al demonstrated that EP significantly increased the ROS generation in the Ehrlich Ascites Carcinoma (EAC) cells. Similarly, the vivo experiments, on the 7th day post treatment showed that the average tumor volume of EP-radiotherapy group significantly (51%) reduced compared with the control group[69].

Electroporation in Chemotherapy

ECT as a localized treatment of cancer enhances transmembrane transfer of nonpermeant molecules such as highly cytotoxic drugs using EP. This technique has shown significant effects on hydrophilic drugs such as cisplatin and bleomycin which are the main chemotherapy agents. ECT increased the accumulation of high intrinsic

cytotoxicity anticancer drugs which are non-permeant or poorly permeant to tumor cells [8-15]. Therefore, ECT enhances the radiosensitizing effects of drugs which in turn reduce the required radiation dose to kill a cancerous cell.

Electrical parameters are the crucial factors affecting the tumor viability[70, 71]. Usually, 8 square shape pulses pulse duration of 100 µs and a repetition frequency of 1 Hz and intensity of 1000-1300 V/cm were applied in a single session of ECT.

Sersa et al for the first time reported the radiosensitizing effect of ECT [36]. They reported that EP enhanced the radiosensitizing effect of cisplatin in EAT tumor cell line. Administration of cisplatin alone before irradiation increased the tumor response from 27% to 73%. Using EP alone prior to radiation increased the response to 54%. Using ECT resulted in 92% tumor response[36].

Maxin et al (2004) observed that EP can increase radiosensitizing effect of tirapazamine (TPZ). They studied the effects of TPZ, EP and irradiation (ECT group) and reported that that ECT treatment was more effective in large tumors than small tumors[59]. Shil et al. studied the possible combined radiosensitizing effects of EP to γ-radiation and doxorubicin (DOX) as a hydrophilic chemotherapy drug on murine fibrosarcoma and reported 2 times higher effect of ECT compared with the control group[60]. Raeisi et al (2012) suggested that ECT can be used for sensitizing of large tumors to Cobalt-60 γ-rays.

There are two basic requirements for effective ECT including sufficient electric field distribution[72] and appropriate drug concentration [73]. The standard ECT protocol is performed with 8 pulses of 100 µs duration and repetition frequency of 100 Hz. The most important parameter is electric field strength that can be set ranging from 1000 to 1300 V/cm [74]. Furthermore, the drugs should be administered prior to EP [75]. The optimum time interval between EP and systemic injection of drug ranges 1 to 10 min in human tumors [76]. This interval ranges 8 to 28 min for intratumoral administration[35, 77].

EP is an appropriate delivery system for only drugs that are poorly or non-permeant with high intracellular cytotoxicity[78]. Two chemotherapeutic drugs of Bleomycin and cisplatin are the promising candidates for ECT[12, 79]. EP can enhance the cytotoxicity of bleomycin and cisplatin by factor of 1000 and 100, respectively [7, 43]. In addition to these two drugs, other drugs such as doxorubicin, 5-FU, tirapazamine, and actinomycin have been used in combination with EP [80]. The mechanism of action of these drugs is induction of single and double strand breaks in DNA molecule [81].

This radiosensitivity induced by EP is associated with formation of free radicals (reactive oxygen species) in tumor cells [65]. Reviewing the findings of previous in vitro and in vivo studies, the mechanisms responsible for radiosensitizing effect of EP can be divided into two groups: Increased drug uptake in the cells induced by EP phenomenon and generation of reactive oxygen species by electric pulses[66] [82].

CONCLUSION:

A single session EP, consisting of a train of pulses or even single pulse, before radiotherapy can significantly enhance the tumor response. Moreover, EP enhances efficacy of different chemotherapeutic agents. The main reason of these phenomena is inducing radiosensitivity to ionizing radiation and sensitivity to chemotherapeutic agents. In addition, enhancing the production of reactive oxygen species has been reported as the main mechanism of action of enhancing treatment efficacy. This combined treatment modality was effective in different cell lines with variety range of radiosensitivity. Application of EP alone or in combination with other agents including gold NPs can enhance the efficacy of radiotherapy and also the chemotherapy. Further studies are needed to develop more efficient EP protocols and also combined treatments. The current evidence indicates a good perspective of clinical applications for EP.

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