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Review

Antitumor effectiveness of electrochemotherapy: A systematic review and meta-analysis

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Abstract

Background: This systematic review has two purposes: to consolidate the current knowledge about clinical effectiveness of electrochemotherapy, a highly effective local therapy for cutaneous and subcutaneous tumors; and to investigate the differences in effectiveness of electrochemotherapy with respect to tumor type, chemotherapeutic drug, and route of drug administration.
Methods: All necessary steps for a systematic review were applied: formulation of research question, systematic search of literature, study selection and data extraction using independent screening process, assessment of risk of bias, and statistical data analysis using two-sided common statistical methods and meta-analysis. Studies were eligible for the review if they provided data about effectiveness of single-session electrochemotherapy of cutaneous or subcutaneous tumors in various treatment conditions.
Results: In total, 44 studies involving 1894 tumors were included in the review. Data analysis confirmed that electrochemotherapy had significantly ($p < .001$) higher effectiveness (by more than 50%) than bleomycin or cisplatin alone. The effectiveness was significantly higher for intratumoral than for intravenous administration of bleomycin ($p < .001$ for CR%, $p = .028$ for OR%). Bleomycin and cisplatin administered intratumorally resulted in equal effectiveness of electrochemotherapy. Electrochemotherapy was more effective in sarcoma than in melanoma or carcinoma tumors.
Conclusions: The results of this review shed new light on effectiveness of electrochemotherapy and can be used for prediction of tumor response to electrochemotherapy with respect to various treatment conditions and should be taken into account for further refinement of electrochemotherapy protocols.
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Keywords: Electroporation; Chemotherapy; Bleomycin; Cisplatin; Treatment effectiveness; Skin neoplasms

Introduction

Electrochemotherapy (ECT) is an antitumor therapy in which administration of a chemotherapeutic drug is followed by local application of electroporation pulses. Electroporation transiently permeabilizes tumor cell membranes, thus enabling diffusion of a chemotherapeutic drug (bleomycin or cisplatin) into the cells and increasing its cytotoxicity.[1,2] Other secondary mechanisms of ECT were also recognized.[3–7] Since the first clinical study in 1990,[8,9] ECT has been reported as highly effective, with complete response rates between 60 and 70% and objective response rates of about 80%,[10–17] especially when the standard operating procedures (SOP) for ECT were followed.[18] ECT is routinely used in treatment of cutaneous and subcutaneous tumors due to high effectiveness, safety, limited toxicity, simplicity, cost-effectiveness, organ-sparing effect, and suitability for repetitive and neoadjuvant treatment.[1,10,11,19–28] New ECT approaches are currently being developed for treatment of deep seated tumors.[29–33]

Effectiveness of ECT depends on extracellular drug concentration at the time of electroporation pulse delivery and on distribution of electric field inside tumor.[34–36] Other

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influential parameters related to patient, tumor and treatment characteristics (such as age; gender; tumor type, size and location; drug type, dose and route of administration; electrode type; protocol of electroporation pulse delivery) probably contribute to variability in tumor response to ECT, but their role has not been sufficiently explored.[11,24,37]

The aim of this systematic review was to consolidate current experience with clinical ECT of cutaneous or subcutaneous tumors from the effectiveness point of view and to provide a transparent and objective framework for discussion on differences in effectiveness of clinical ECT. The main objectives were to evaluate: (a) overall effectiveness of ECT; (b) effectiveness of ECT in comparison to chemotherapeutic alone; (c) differences in effectiveness of ECT with respect to drug type and route of administration; (d) differences in effectiveness of ECT with respect to histological type of tumors.

Materials and methods

Search strategy

A systematic search of 16 bibliographic databases was performed to obtain articles regarding clinical ECT (Fig. 1), using search terms "electrochemotherapy" and "clinical" and time range between 1st January 1991 and 18th October 2011. Language restriction to English was applied. Some references cited in these articles were screened to identify additional potentially eligible studies. Unpublished studies, abstracts, posters, reviews, editorials, lectures and commentaries were not included in this review.

Inclusion criteria for studies

Studies were included in systematic review if they provided:

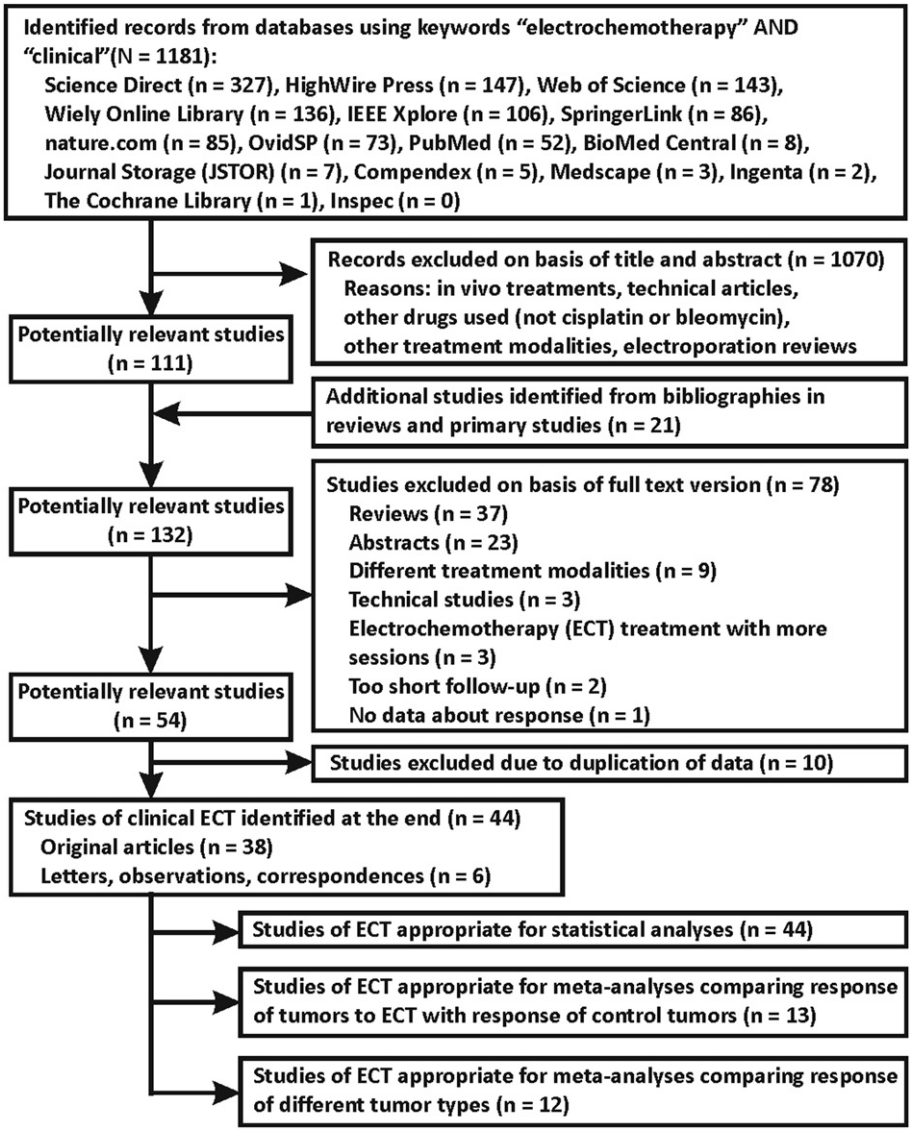

Figure 1. Selection process for the studies included in systematic review.

Table 1A
Summary of the studies and characteristics of tumors included in systematic review. Eligibility of the studies for meta-analysis is denoted in last two columns.

Original data			Data used in evaluation								Eligibility for meta-analysis	
First author, year published (reference)	No. of patients/tumors	Gender of patients M/F	Included no. of patients/tumors	No. of responses (%)			Drug/route	Type of tumor(s)	Response evaluation	Median follow-up in mo. (range)	Control tumors	Tumor types
				CR (%)	PR (%)	NR (%)						
Allegretti, 2001[a] [46]	14/14	9/5	3/3	3(100.0)	0(0.0)	0(0.0)	bleo i.t.	SCC	Biopsy	31 (5.6–36.7)	No	No
Belehradek, 1993[9]	8/42	8/0	5/26	23(88.5)	0(0.0)	3(11.5)	bleo i.v.	SCC	WHO	1.6 (1.0–8.3)	Yes	No
Bloom, 2005[47]	54/69	42/12	54/69	17(24.6)[b]	22(31.9)	30(43.5)	bleo i.t.	SCC	WHO, biopsy	>1 (–)	Yes	No
Burian, 2003[48]	12/12	11/1	12/12	10(83.3)	2(16.7)	0(0.0)	bleo i.t.	SCC	Biopsy	1 (–)	No	No
Byrne, 2005[a] [49]	19/63	11/8	15/53	33(62.3)[b]	4(7.5)	16(30.2)	bleo i.t.	Melanoma	WHO, biopsy	6 (3–6)	Yes	No
Campana, 2009[a] [24]	52/608	20/32	52/267	125(46.8)	126(47.2)	16(6.0)	bleo i.t. or i.v. or combined	Melanoma, breast cancer, sarcoma, SCC, HN cancer	RECIST	1 (–)	No	Yes
Curatolo, 2008[50]	1/–	1/0	1/7[c]	7(100.0)	0(0.0)	0(0.0)	bleo i.v.	Kaposi sarcoma	–	14 (–)	No	No
Curatolo, 2009[51]	1/–	1/0	1/7[c]	7(100.0)	0(0.0)	0(0.0)	bleo i.v.	Merkel cell carcinoma	Biopsy	6 (–)	No	No
Curatolo, 2011[a] [52]	23/532	13/10	18/114[c]	80(70.2)	34(29.8)	0(0.0)	bleo i.v.	Kaposi sarcoma	RECIST	18 (2–50.4)	No	No
Domenge, 1996[34]	7/53	5/2	6/30[c]	7(23.4)	4(13.3)	19(63.3)	bleo i.v.[d]	HN SCC, salivary or breast AC	WHO	– (1–2)	Yes	Yes
Fantini, 2008[53]	1/–	1/0	1/7[c]	7(100.0)	0(0.0)	0(0.0)	bleo i.t. or i.v.	BCC with squamous differentiation	–, biopsy	3 (2–9)	No	No
Garbay, 2006[54]	1/–	1/0	1/7[c]	7(100.0)	0(0.0)	0(0.0)	bleo i.v.	Kaposi sarcoma	WHO, biopsy	28.7 (–)	Yes	No
Gargiulo, 2010[55]	15/15	–/–	15/15	12(80.0)	3(20.0)	0(0.0)	bleo i.v.	BCC, SCC, Bowen disease	WHO	13 (3–24)	No	Yes
Gaudy, 2006[56]	12/30	9/3	9/23	17(74.0)	3(13.0)	3(13.0)	bleo i.t.	Melanoma	WHO	4.8 (2–6)	Yes	No
Gehl, 2006[57]	1/8	1/0	1/7	7(100.0)	0(0.0)	0(0.0)	bleo i.v.	Melanoma	WHO	6 (–)	No	No
Gualdi, 2010[58]	1/3	1/0	1/3	3(100.0)[e]	0(0.0)	0(0.0)	bleo i.t.	Kaposi sarcoma	Biopsy	2 (–)	No	No
Heller, 1996[59]	6/18	3/3	6/18	6(33.3)	7(38.9)	5(28.8)	bleo i.v.	Melanoma, BCC, AC	WHO, biopsy	2.5 (2–5)	Yes	Yes
Heller, 1998[60]	34/143	29/5	34/143	130(90.9)	12(8.4)	1(0.7)	bleo i.t.	Melanoma, BCC, SCC, Kaposi sarcoma	WHO, biopsy	20 (7–28)	Yes	Yes
Kaehler, 2010[61]	1/6	0/1	1/6	6(100.0)[f]	0(0.0)	0(0.0)	bleo i.t.	Melanoma	Biopsy	~4 (–)	No	No
Kis, 2011[14]	9/158	2/7	9/158	37(23.4)	61(38.6)	60(38.0)	bleo i.v.	Melanoma	WHO	7 (2–13)	No	No
Kubota, 1998[62]	1/17	1/0	1/17	14(82.4)	3(17.6)	0(0.0)	bleo i.t.	Transitional cell carcinoma	–	3 (–)	No	No
Kubota, 2005[a] [63]	1/8	0/1	1/8	7(87.5)	1(12.5)	0(0.0)	bleo i.t.	Melanoma	Biopsy	1.6 (–)	No	No
Landstrom, 2010[13]	6/6	3/3	6/6	5(83.3)[g]	0(0.0)	1(16.7)[g]	bleo i.t.	HN BCC and SCC	Biopsy	18.5 (3–24)	No	Yes
Landstrom, 2011[64]	5/5	3/2	5/5	5(100.0)	0(0.0)	0(0.0)	bleo i.t.	HN SCC, AC	Biopsy	24 (24–24)	No	Yes
Larkin, 2007[65]	30/148	–/–	26/111	66(59.5)	24(21.6)	21(18.9)	bleo i.t. or i.v.	Melanoma, AC, SCC, chondrosarcoma	WHO	– (2–12)	No	Yes
Marenco, 2011[a] [66]	1/11	1/0	1/11	8(72.7)	0(0.0)	3(27.3)	bleo i.v.	SCC	–	2 (–)	No	Yes
Marone, 2011[67]	1/–	1/0	1/7[c]	7(100.0)	0(0.0)	0(0.0)	bleo i.v.	Metastatic eccrine porocarcinoma	–	5 (–)	No	No
Marty, 2006[11]	61/290	20/41	41/171	126(73.7)	19(11.1)	26(15.2)	bleo i.t. or i.v. or cispl i.t.	Melanoma, carcinoma, sarcoma	WHO	4.4 (2–12.7)	No	Yes
Matthiessen, 2011[15]	52/196	17/35	24/94	58(61.6)	18(19.2)	18(19.2)	bleo i.t. or i.v.	Melanoma, BCC, SCC, AC, breast cancer	RECIST	– (2–6)	No	No
Mir, 1998[h] [68]	50/291	–/–	8/16[i]	3(18.7)	6(37.5)	7(43.8)	bleo i.v.	Melanoma, HN SCC	WHO?	>1 (–)	No	No
Quaglino, 2008[a] [23]	14/233	8/6	14/233	136(58.4)	80(34.3)	17(7.3)	bleo i.v.	Melanoma	WHO	21 (5–28)	No	No

Rebersek, 2004[37]	6/12	1/5	6/12	4(33.3)	8(66.7)	0(0.0)	cispl i.t.	Breast cancer	WHO	2 (2−6)	Yes	No
Rols, 2000[69]	5/61	2/3	5/61	6(9.8)	19(31.2)	36(59.0)	bleo i.v.	Melanoma, HN SCC	WHO, scanning	1.6 (1−2)	Yes	Yes
Rudolf, 1995[70]	2/24	1/1	2/24	22(91.7)	0(0.0)	2(8.3)	bleo i.v.	Melanoma	WHO	4.1 (3.3−4.9)	Yes	No
Sersa, 1998[71]	4/19	3/1	1/4[j]	4(100.0)	0(0.0)	0(0.0)	cispl i.t.	BCC	WHO	>8 (−)	Yes	Yes
Sersa, 2000[k] [72]	10/82	2/8	10/82	66(80.5)	5(6.1)	11(13.4)	cispl i.t.	Melanoma	WHO	>2 (−)	Yes	No
Sersa, 2003[a] [73]	14/211	−/−	3/10	5(50.0)	2(20.0)	3(30.0)	cispl i.t.	Melanoma	WHO?	2 (1.5−2.7)	No	No
Shimizu, 2003[a] [74]	1/1	0/1	1/1	0(0.0)	1(100.0)	0(0.0)	bleo i.t.	Digital chondrosarcoma	Biopsy	1 (−)	No	No
Snoj, 2005[a] [28]	1/1	1/0	1/1	0(0.0)	0(0.0)	1(100.0)	cispl i.t.	Melanoma	−	1 (−)	No	No
Snoj, 2006[75]	1/19	0/1	1/19	18(94.7)	0(0.0)	1(5.3)	cispl i.t.	Melanoma	−	104 (3−104)	No	No
Snoj, 2007[76]	1/244	0/1	1/7[c]	7(100.0)	0(0.0)	0(0.0)	bleo i.v.	Melanoma	−	9 (−)	No	No
Snoj, 2009[27]	1/1	1/0	1/1	0(0.0)	1(100.0)	0(0.0)	bleo i.v.	Melanoma	−	4.9 (−)	No	No
Tijink, 2006[77]	7/17	4/3	7/17	14(82.4)	3(17.6)	0(0.0)	bleo i.t.	SCC, melanoma, sarcoma, Merkel cell carcinoma	−, MRI, biopsy	12 (1−15)	No	Yes
Whelan, 2006[a,l] [78]	1/1	0/1	1/1	0(0.0)	0(0.0)	1(100.0)	bleo i.t. and i.v.	Breast AC	−	∼2 (−)	No	No
Summary	548/3672	237/202	413/1894	1125(59.4)	468(24.7)	301(15.9)						

CR = complete response; PR = partial response; NR = no response; mo = month; − = no data; bleo = bleomycin; cispl = cisplatin; i.t. = intratumoral, i.v. = intravenous; SCC = squamous cell carcinoma; BCC = basal cell carcinoma; AC = adenocarcinoma; HN = head and neck.

[a] Only data for the first ECT session included.

[b] CR confirmed after 3 months by biopsy.

[c] The number of tumors per patient reduced to 7 if more than 7 tumors per patient with identical response reported.

[d] Bleomycin administered either i.v. or i.a.

[e] CR confirmed after 2 months by biopsy.

[f] CR confirmed after 1 month by biopsy.

[g] The responses confirmed after 2 months by biopsy.

[h] Not included in meta-analysis of tumor types because data is from different studies.

[i] Only 8 patients included because other data have been published in other articles (included are 5 patients with malignant melanoma tumors from the Ljubljana group and 3 patients with head and neck SCC tumors from the Reims group).

[j] Only 1 patient included because data for 2 patients with malignant melanoma tumors were published in Sersa et al., 2000, and 1 patient with BCC tumors was treated with more than one ECT sessions.

[k] The results from this article were updated thus having at least 2 months instead of 1 month follow up (data adopted from Sersa, 2006).

[l] A large nodule.

(1) information about single-session ECT of cutaneous or subcutaneous tumors performed on human patients using bleomycin or cisplatin administered intratumorally or intravenously; and
(2) data for number of patients and tumors, tumor response (evaluated at least 4 weeks after treatment), chemotherapeutic drug, route of drug administration, and tumor type.

Studies were eligible for meta-analysis if they also provided:

(3) data for control tumors (i.e. tumors treated with chemotherapeutic drug only or electroporation pulses only, or no treatment); or
(4) data for at least two different histological types of tumors.

Study selection and data collection

Authors BM, TJ and GS independently examined studies identified with search strategy, performed selection of studies for further evaluation, read their full texts and extracted the relevant data (i.e. author and year of publication, number and gender of patients, number and type of tumors, tumor response, criteria for response assessment, chemotherapeutic drug, route of drug administration, duration of follow-up and the risk of bias). Disagreements between the authors were resolved by discussion. If the same data was reported in several studies, either the first published or the more comprehensive study was used. The authors of studies considered in this review were not contacted to provide additional data. The number of tumors included in the analysis was truncated to seven to prevent overestimation of effectiveness of ECT, when more than seven tumors with the same response were reported for the same patient (the approach adopted from Marty et al.[11]).

Assessment of risk of bias

The risk of bias of included studies was assessed independently by 2 out of 3 authors (BM, TJ, GS) according to the recommendations of the Cochrane Collaboration.[38] Disagreements were discussed until consensus was reached. In addition to standard ratings (low, unclear or high risk of bias), a rating of "not applicable" was introduced for studies not including control tumors when judgment on sequence generation and allocation concealment was not possible. Reviewers were not blinded to the authors, location, funding and acknowledgements of the studies.

Outcome measures

The outcome measure of interest was the response of individual tumors to a single-session ECT (or control treatment). Tumor response in the evaluated studies was determined following WHO or RECIST criteria,[39,40] or by biopsy or scanning. We classified the response of individual tumors as complete response (CR), partial response (PR), no change (NC) or progressive disease (PD) according to the data reported in the studies. In addition, we introduced the objective response (OR; including CR and PR) and the no response (NR; including NC and PD) classifications. The complete and objective response rate (denoted as CR% and OR% respectively) were determined for each study.

Data analysis

Common statistical methods were used to analyze data from studies satisfying the first two criteria described above (Table 1A). The overall CR% and OR% (CR% and OR% columns in Table 2) were calculated separately from the pooled response data of individual tumors classified into various groups (either group of tumors treated with ECT or control tumors, or for various subgroups with respect to the chemotherapeutic drug, the route of drug administration, and the tumor type). Statistical comparison of CR% and OR% values between different (sub)groups was performed using Chi-square test. Differences were considered statistically significant for $p < .05$.

The overall CR% and OR% values result in a summary with equal contribution of all individual tumors. Consequently, the relative "weight" of each study in the overall results is proportional to its size. When applying analysis on data accumulated from studies performed by independent researchers, it is unlikely that the studies are functionally equivalent and of similar size. In such cases, a meta-analysis based on the random-effects model is the preferred method for pooling the data with the most reliable estimate of the summary effect.[38,41] However, only few studies were eligible for meta-analysis in this review; therefore, both approaches were used. Since there is no exact rule for the minimum number of studies to be included in a meta-analysis, we adopted the limit of six studies from some previous reviews.[42–45]

A meta-analysis was used to evaluate the differences in antitumor effectiveness between ECT and chemotherapeutic drug alone and between different tumor types. The risk difference (RD) was used as the measure of the effect, defined as the probability of response in one group minus the probability of response in the other group. The between-study heterogeneity was assessed with the I^2 statistic. Small number of eligible studies prevented the use of funnel plots and subgroup analysis. The software was written in Matlab following published procedures.[38,41]

A sensitivity analysis was performed to investigate the impact of studies with high risk of bias on the results of data analysis. In addition, the influence of the SOP for ECT on reported ECT effectiveness was evaluated by comparing the results of studies before and after year 2006.[18]

Results

Search results

The initial search identified 1181 records after removal of duplicates. The study selection procedure is shown in Fig. 1. Finally, 44 studies were appropriate for systematic review and data analysis (Table 1A). A much smaller subset of these studies was eligible for meta-analysis therefore the results of meta-analysis were treated as supplementary to the results of other statistical methods (Fig. 1, Tables 1B and 1C).

Characteristics of the eligible studies

Characteristics of the studies used for systematic review are listed in Table 1A. In total, 413 patients and 1894 tumors were included. The studies were mostly non-randomized phase I or II studies and case reports. In only two studies tumors were randomized between different treatments but randomization was poorly conducted.[49,56] Studies eligible for meta-analysis comparing response of tumors to ECT with response of control tumors are listed in Table 1B. Among them, 13 studies were included for comparison of effectiveness between ECT and chemotherapy alone. Studies eligible for meta-analysis comparing response of different tumor types to ECT are listed in Table 1C. Among them, 8 studies were suitable for comparison of response to ECT between melanoma and non-melanoma tumors, and 6 studies for comparison of response to ECT between carcinoma and melanoma tumors. The results of risk of bias assessment of all studies are summarized in Fig. 2.

Statistical analysis

ECT had significantly higher effectiveness than treatment with chemotherapeutic drug alone (Table 2). Namely, the overall CR% and OR% for ECT were 59.4% and 84.1% respectively and only 8.0% and 19.9% respectively for the

Table 1B
Summary of studies with any type of control tumors (chemotherapeutic drug only, electroporation pulses only or no treatment) included in meta-analysis comparing response of tumors to ECT with response of control tumors. For other details on these studies see Table 1A.

First author, year published (reference)	No. of patients/tumors	Included no. of patients/tumors	No. of responses (%)			Type of control
			CR (%)	PR (%)	NR (%)	
Belehradek, 1993[9]	1/–	1/7[a]	0(0.0)	0(0.0)	7(100.0)	bleo i.v.
Bloom, 2005[47]	8/37	8/37	0(0.0)	1(2.7)	36(97.3)	bleo i.t.
Byrne, 2005[49]	15/19	15/19	5(26.3)	1(5.3)	13(68.4)	bleo i.t.
Domenge, 1996[34]	2/–	2/7[a]	0(0.0)	0(0.0)	7(100.0)	bleo i.v.[b]
Garbay, 2006[54]	1/–	1/7[a]	0(0.0)	0(0.0)	7(100.0)	bleo i.v.
Gaudy, 2006[56]	9/15	9/15	2(13.3)	6(40.0)	7(46.7)	bleo i.t.
Heller, 1996[59]	6/16	6/16	0(0.0)	0(0.0)	16(100.0)	bleo i.v.
Heller, 1998[60]	3/6	3/6	0(0.0)	0(0.0)	6(100.0)	EP pulses
	8/20	8/20	0(0.0)	1(5.0)	19(95.0)	bleo i.t.
Rebersek, 2004[37]	6/8	6/8	0(0.0)	0(0.0)	8(100.0)	No treatment
	6/6	6/6	0(0.0)	5(83.3)	1(16.7)	cispl i.t.
Rols, 2000[69]	4/–	4/7[a]	0(0.0)	0(0.0)	7(100.0)	bleo i.v.
Rudolf, 1995[70]	2/3	2/3	0(0.0)	0(0.0)	3(100.0)	bleo i.v.
Sersa, 1998[71]	2/5	2/5	0(0.0)	0(0.0)	5(100.0)	No treatment
	1/1	1/1	0(0.0)	0(0.0)	1(100.0)	EP pulses
	2/5	2/5	2(40.0)	2(40.0)	1(20.0)	cispl i.t.
Sersa, 2000[72]	6/22	6/22	0(0.0)	0(0.0)	22(100.0)	No treatment
	2/2	2/2	0(0.0)	0(0.0)	2(100.0)	EP pulses
	10/27	10/27	5(18.5)	5(18.5)	17(63)	cispl i.t.

CR = complete response; PR = partial response; NR = no response; EP = electroporation; – = no data; bleo = bleomycin; cispl = cisplatin; i.t. = intratumoral, i.v. = intravenous.

[a] The number of tumors per patient reduced to 7 if more than 7 tumors per patient with identical response reported.

[b] Bleomycin administered either intravenously or intraarterially.

Table 1C
Summary of studies included in meta-analysis comparing responses of tumors of different histological types. For other details on these studies see Table 1A.

First author, year published (reference)	No. of patients/tumors	Included no. of patients/tumors	No. of responses (%)			Type of tumor
			CR (%)	PR (%)	NR (%)	
Campana, 2009[a] [24]	34/373	34/373	17(50.0)	15(44.1)	2(5.9)	Melanoma
	18/235	18/235	9(50.0)	9(50.0)	0(0.0)	Non-melanoma (breast cancer, sarcoma, SCC, HN cancer)
Domenge, 1996[34]	5/26	4/16	0(0.0)	4(25.0)	12(75.0)	HN SCC
	1/20	1/7[b]	7(100.0)	0(0.0)	0(0.0)	Salivary AC
	1/7	1/7	0(0.0)	0(0.0)	7(100.0)	Breast AC
Gargiulo, 2010[55]	9/9	9/9	7(77.8)	2(22.2)	0(0.0)	SCC
	5/5	5/5	4(80.0)	1(20.0)	0(0.0)	BCC
	1/1	1/1	1(100.0)	0(0.0)	0(0.0)	Bowen disease
Heller, 1996[59]	3/10	3/10	3(30.0)	2(20.0)	5(50.0)	Melanoma
	2/6	2/6	1(16.7)	5(83.3)	0(0.0)	BCC
	1/2	1/2	2(100.0)	0(0.0)	0(0.0)	AC
Heller, 1998[60]	12/84	12/84	75(89.3)	8(9.5)	1(1.2)	Melanoma
	20/54	20/54	51(94.4)	3(5.6)	0(0.0)	BCC
	1/4	1/4	4(100.0)	0(0.0)	0(0.0)	Kaposi sarcoma
	1/1	1/1	0(0.0)	1(100.0)	0(0.0)	SCC
Landstrom, 2010[13]	3/3	3/3	2(66.7)	0(0.0)	1(33.3)	HN SCC
	3/3	3/3	3(100.0)	0(0.0)	0(0.0)	HN BCC
Landstrom, 2011[64]	4/4	4/4	4(100.0)	0(0.0)	0(0.0)	HN SCC
	1/1	1/1	1(100.0)	0(0.0)	0(0.0)	AC
Larkin, 2007[65]	19/103	17/100	63(63.0)	21(21.0)	16(16.0)	AC
	4/36	2/2	0(0.0)	1(50.0)	1(50.0)	Melanoma
	5/6	5/6	3(50.0)	1(16.7)	2(33.3)	SCC
	1/2	1/2	0(0.0)	1(50.0)	1(50.0)	Cervical carcinoma
	1/1	1/1	0(0.0)	0(0.0)	1(100.0)	Synovial chondrosarcoma
Marty, 2006[11]	32/190	20/98	65(66.3)	14(14.3)	19(19.4)	Melanoma
	29/100	21/73	61(83.6)	5(6.8)	7(9.6)	Carcinoma and sarcoma
Rols, 2000[69]	4/55	4/55	1(1.8)	18(32.7)	36(65.5)	Melanoma
	1/6	1/6	5(83.3)	1(16.7)	0(0.0)	HN SCC
Sersa, 1998[71]	2/13	2/13	13(100.0)	0(0.0)	0(0.0)	Melanoma
	1/4	1/4	4(100.0)	0(0.0)	0(0.0)	BCC
Tijink, 2006[77]	4/12	4/12	10(83.3)	2(16.7)	0(0.0)	SCC
	1/3	1/3	3(100.0)	0(0.0)	0(0.0)	Merkel cell carcinoma
	1/1	1/1	1(100.0)	0(0.0)	0(0.0)	Sarcoma
	1/1	1/1	0(0.0)	1(100.0)	0(0.0)	Melanoma

CR = complete response; PR = partial response; NR = no response; SCC = squamous cell carcinoma; BCC = basal cell carcinoma; AC = adenocarcinoma; HN = head and neck.
[a] Tumor responses per patients.
[b] The number of tumors per patient reduced to 7 if more than 7 tumors per patient with identical response reported.

chemotherapeutic drug alone. Treatment with electroporation pulses alone did not have any effect on tumor response. Similarly, the results of meta-analysis showed that ECT significantly increased the probability of CR% and OR% by 55% and 59% on average, respectively, in comparison to application of chemotherapeutic drug alone (Table 3).

A statistical comparison of response between different tumor types (melanoma, carcinoma and sarcoma) was performed separately for each chemotherapeutic drug and route of administration. No significant differences in overall CR% and OR% values were found between tumor types. Therefore the data for different tumor types was pooled for each chemotherapeutic drug and route of drug administration.

The overall CR% and OR% regardless of the drug and route of administration were 62.6% and 82.8% respectively (Table 2). However, effectiveness of ECT depended on the route of drug administration with the overall CR% and OR % significantly higher for bleomycin administered intratumorally (72.7% and 85.8%, respectively) than intravenously (54.9% and 80.7%, respectively). There was no difference in effectiveness of ECT between bleomycin or cisplatin administered intratumorally.

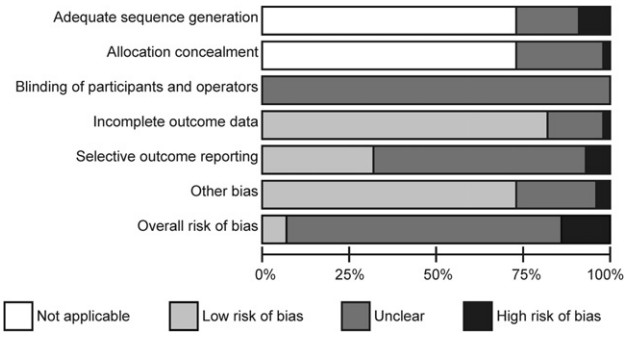

Figure 2. Assessment of risk of bias for the studies included in systematic review.

A statistical comparison of response between different chemotherapeutic drugs and routes of drug administration was performed separately for each tumor type. No significant differences in overall CR% and OR% values were found between different drugs and routes of administration. Therefore the data for different chemotherapeutic drugs and routes of administration was pooled for each tumor type.

The overall CR% and OR% regardless of tumor type were 59.4% and 84.1% respectively (Table 2). However, effectiveness of ECT depended on the tumor types with the overall CR% and OR% significantly higher for non-melanoma (67.0% and 86.4%, respectively) than melanoma tumors (56.8% and 80.6%, respectively). Sarcoma tumors showed significantly better overall CR% and OR% than carcinoma tumors. Among different subtypes of carcinoma tumors, basal cell carcinoma tumors had significantly better response than melanoma tumors.

Results of meta-analysis comparing effectiveness of ECT for different tumor types showed significantly increased probability of CR and OR by 33% and 17%, respectively, for non-melanoma tumors in comparison to melanoma tumors ($p = .013$ for CR%, $p < .035$ for OR %) and significantly increased probability of CR% by 40% but insignificantly increased probability of OR% by

Table 2
The overall complete response rate (CR%) and objective response rate (OR%) were calculated from response data of individual tumors pooling individual tumor data of all studies together. CR% and OR% were calculated separately for tumors that: (1) served as controls; (2) were treated with ECT using different types of drug and routes of administration; (3) were of different histological types. Note that the sum of the numbers of articles, patients and tumors for subgroups is not necessarily equal to the value reported for all types because some studies, patients and tumors are included in several subgroups and some are not included in subgroups due to inseparable data. The numbers and letters in superscript are used to identify pairs of values (CR% or OR%) with statistically significant difference between them. The numbers in superscript indicate statistically significant differences between different subgroups within each group of studies. The letters in superscript indicate statistically significant differences between responses of tumors receiving ECT and responses of tumors receiving chemotherapeutic drug only. The statistical significances of Chi-square tests are listed in footnote of this table.

	No. of studies	No. of patients	No. of nodules	Overall response	
				CR%	OR%
Studies with control groups combined with respect to type of control					
All types	13	74	220	6.4	15.9
All drugs	13	74	176	8.0^{a}	$19.9^{3,e}$
Bleomycin i.v.	6	16	47	$0^{1,b}$	$0^{4,5,f}$
Bleomycin i.t.	4	40	91	7.7^{c}	$17.6^{4,6,7,g}$
Cisplatin i.t.	3	18	38	$18.4^{1,2,d}$	$50.0^{5,6,8,h}$
EP pulses	3	6	9	0	0^{8}
No treatment	3	14	35	0^{2}	$0^{3,7}$
Studies with ECT groups combined with respect to type of drug and route of administration					
All types	40	392	1421	62.6^{a}	82.8^{e}
Bleomycin i.v.	19	137	835	$54.9^{9,10,b}$	$80.7^{11,f}$
Bleomycin i.t.	16	192	414	$72.7^{9,c}$	$85.8^{11,g}$
Cisplatin i.t.	7	63	172	$75.6^{10,d}$	85.5^{h}
Studies with ECT groups combined with respect to tumor type					
All types	44	413	1894	59.4	84.1
Melanoma	22	150	922	$56.8^{12,13,14,15,16}$	$80.6^{27,28,29,30,31}$
Non-melanoma	29	239	663	67.0^{12}	86.4^{27}
Carcinoma	22	175	434	$62.7^{13,17,18}$	$81.1^{32,33}$
SCC	15	109	188	$49.5^{19,20,21}$	$69.7^{28,34,35,36,37}$
BCC	6	32	79	$88.6^{14,19,22,23,24}$	$100.0^{29,34,38}$
AC	6	28	130	$59.2^{22,25,26}$	$81.5^{35,38,39,40}$
Sarcoma	8	25	138	$73.9^{15,17,20,23,25}$	$99.3^{30,32,36,39}$
Kaposi sarcoma	5	22	135	$74.8^{16,18,21,24,26}$	$100.0^{31,33,37,40}$
All studies with ECT groups combined					
	44	413	1894	59.4	84.1

CR% = complete response rate, OR% = objective response rate; EP = electroporation; i.v. = intravenous; i.t. = intratumoral; SCC = squamous cell carcinoma; BCC = basal cell carcinoma; AC = adenocarcinoma; statistical significances of Chi-square test: ^{1,4,27} .002, ^{2,7} .008, ^{3} .004, $^{5,6,9,10,12,14-16,19-22,29-34,36-40,a-h}$ <.001, ^{8} .006, ^{11} .028, ^{13} .036, ^{17} .017, ^{18,23} .010, ^{24} .015, ^{25} .011, ^{26} .007, ^{28} .001, ^{35} .020.

Table 3
Summary of the results of meta-analysis comparing effectiveness of ECT with respect to control group and effectiveness of ECT between different tumor types.

Comparison	No. of studies	No. of patients	No. of nodules	CR			OR		
				RD (CI_{low},CI_{up})	p(RD)	I^2	RD (CI_{low},CI_{up})	p(RD)	I^2
ECT vs. tumor controls receiving drug only	13	155	730	0.55 (0.33,0.77)	<.001	97.66	0.59 (0.44,0.74)	<.001	91.27
ECT in melanoma vs. non-melanoma tumors	8	175	592	−0.33 (−0.58,−0.07)	.013	95.65	−0.17 (−0.33,−0.01)	.035	95.37
ECT in melanoma vs. carcinoma tumors	6	79	363	−0.40 (−0.73,−0.07)	.018	96.50	−0.24 (−0.60,0.12)	.200	97.18

CR = complete response; OR = objective response; RD = summary risk difference for studies included in meta-analysis; CI_{low} and CI_{up} = the lower and upper confidence interval of RD, respectively; p(RD) = statistical significance of RD; I^2 = between-study heterogeneity.

24% for carcinoma tumors in comparison to melanoma tumors ($p = .018$ for CR%, $p < .200$ for OR%; Table 3).

Sensitivity analysis

For the sensitivity analysis, six studies with an overall high risk of bias rating (Belehradek, 1993; Rudolf, 1995; Domenge, 1996; Rols, 2000; Curatolo, 2008, Campana, 2009) were removed from the statistical analysis.[9,24,34,50,69,70] All partial and overall responses to ECT were in general statistically insignificantly different from those reported in Table 2. Similarly, only minor changes in results were revealed for meta-analysis when comparing the response of tumors to ECT with response to chemotherapeutic drug only. Namely, increased probability of CR by 56% (CI of RD between 0.31 and 0.81) and of OR by 56% (CI of RD between 0.36 and 0.75) was obtained (compare to data in Table 3).

Additional sensitivity analysis was performed with respect to the year of study's publishing. When only studies published after publication of the SOP in 2006 were considered (25 studies, 1192 tumors), the overall CR% and OR% were 59.7% and 87.8% respectively (practically the same as CR% and OR% of 59.4% and 84.1% respectively in Table 2). When only studies published before 2006 were considered (19 studies, 592 tumors), the overall CR% and OR% were 61.1% and 77.4%, respectively. When comparing CR% and OR% of studies published before and after the ESOPE study, the difference for OR% was significant but the difference for CR% was not ($p < .001$ for OR%, $p = .565$ for CR%).

Discussion

Several clinical reviews have reported on effectiveness of ECT, but no systematic and comprehensive summary of effectiveness of clinical ECT has been published to date. In this systematic review, local effectiveness of a single-session ECT across all eligible studies was estimated as complete and objective response rate (denoted as CR% and OR% respectively) of 59.4% and 84.1% respectively (Table 2). The reviews of studies conducted before publication of the SOP for ECT reported similar values (CR% and OR% of 64% and 83%, respectively),[10,12] whereas later reviews reported only effectiveness of ECT for each study without appropriate synthesis of the data[17,19,20,26,79] with exception of two recent reviews summarizing effectiveness of ECT for melanoma and adenocarcinoma tumors.[14,17] We also separately determined the overall effectiveness of ECT for the studies conducted before (CR% and OR% of 61.1% and 77.4%, respectively) and after SOP publication (CR% and OR% of 59.7% and 87.8%, respectively). The OR% increased significantly after publication of the SOP, possibly as the result of adopting the SOP in the newer studies.

ECT has significantly higher effectiveness than chemotherapy alone (Table 2). Overall CR% and OR% of 8.0% and 19.9%, respectively, were achieved in control tumors treated with chemotherapeutic drug alone (bleomycin or cisplatin) applied at the same cumulative doses as in ECT (Table 2) without taking the intrinsic differences in effectiveness of bleomycin and cisplatin at used doses into account. Namely, bleomycin alone has a very low antitumor effect while cisplatin alone is moderately effective even without electroporation pulses.[80–82] From Table 2 it follows that ECT drastically improved the effectiveness of chemotherapy in general (CR% and OR% increased on average by around 55% and 63% respectively). Similar conclusions can be reached based on meta-analysis (Table 3). These results confirm that cytotoxicity of bleomycin and cisplatin is vastly increased when electroporation pulses are delivered to tumors in presence of sufficiently high extracellular concentration of chemotherapeutic drug.[2,83] The increase in effectiveness of ECT in comparison to chemotherapeutic drug alone in our study is higher than in study by Sersa et al.[72] (increase or OR% by 40% for cisplatin), probably because we pooled the results for cisplatin and bleomycin together. The uptake of bleomycin by the cells is known to be more potentiated by electroporation pulses than the uptake of cisplatin.[82]

However, in this review no significant difference between overall CR% or OR% was found between bleomycin or cisplatin administered intratumorally, which is also in agreement with the ESOPE study results.[11] On the other hand, ECT with intratumoral administration of bleomycin or cisplatin revealed significantly higher overall CR% value than ECT with intravenous administration of bleomycin (Table 2). Advantages of intratumoral versus intravenous

administration have been suggested in early studies,[12] but no significant differences in effectiveness of ECT were found between intravenous and intratumoral administration of the drug in the ESOPE study.[11] Lower effectiveness of ECT with bleomycin given intravenously could be explained by insufficient volume coverage with the proper concentration of the drug in the tumor due to heterogeneous distribution of blood flow in tumors, or by insufficient interstitial drug concentration at the time of electroporation pulse delivery.[4,11] The cytotoxic activity of bleomycin and cisplatin is concentration- and time-dependent.[81,84,85] The SOP recommendations regarding the treatment window for application of electroporation pulses after administration of the drug were followed in most of the studies included in our review.[18,34] According to Front et al, the concentration of bleomycin in interstitial fluid around tumor is high enough for efficient ECT treatment for considerably longer period than suggested in the SOP for ECT.[86] Consequently, the insufficient interstitial drug concentration in the tumors due to improper timing of electroporation pulse delivery is an unlikely cause for lower effectiveness of ECT with intravenous bleomycin. On the other hand, the interstitial drug concentration in tumors cannot be predicted from the administered dose due to large variability in tumor drug uptake[86,87] and because of heterogeneous distribution of tumor blood flow within tumors, with the periphery usually being better perfused than the center.[4] Since the cytotoxicity of the drug depends on the extracellular concentration of the drug in the tumor, it is this parameter and not the administered dose of the drug that should be considered when planning effective ECT with intravenous bleomycin,[86] for example by utilizing some noninvasive means for assessment of chemotherapy drug concentrations in tumor.[88,89]

Significant differences in effectiveness of ECT between different tumor types were observed with melanoma tumors having in general lower CR% and OR% in comparison to all non-melanoma tumors combined, or carcinoma and sarcoma tumors separately (Tables 2 and 3). In general, sarcomas also responded significantly better than all carcinomas combined (Table 2). However, among all types of tumors, basal cell carcinomas (BCC) had the highest and squamous cell carcinomas (SCC) the lowest overall CR% and OR% (Table 2). Therefore the often repeated statement about equal clinical effectiveness of ECT regardless of tumor type appears to be unjustified. In some early studies in mice, different response rates to ECT were observed for different types of tumors with the best antitumor response being observed for fibrosarcomas[90,91] and the differences in intrinsic sensitivity of tumor cells to bleomycin were reported.[91] In some clinical studies, higher effectiveness of ECT was noticed in non-melanoma than melanoma tumors, but due to statistical insignificance the effectiveness of ECT was reported to be the same for all tumor types.[11,17] Differences in the response rate were pointed out by Mir et al, with BCC and SCC tumors having higher and lower response to ECT respectively than melanomas.[68] Additionally, the highest effectiveness of ECT in patients with BCC regardless of drug type and administration was reported in several other studies.[13,53,55,59,60,71,92] The difference in response between BCC and SCC tumors might be their different sizes; the SCC tumors were usually significantly bigger than BCC tumors and would therefore require repeated ECT treatments.

In meta-analysis of data from the eligible studies, some methodological factors were identified that are contributing to high heterogeneity (i.e. I^2 statistic) of included studies (Table 3), such as: differences in characteristics of patients; stage of the disease; size of tumors; protocols of electroporation pulse delivery; and inconsistent reporting of time of tumor response. Unfortunately, meta-analysis encompassing these confounding factors and thus estimating their influence on effectiveness of ECT is currently not possible because of too few studies eligible for meta-analysis published to-date.

Conclusions

The overall effectiveness of ECT in clinical setting and the differences in effectiveness of ECT of cutaneous and subcutaneous tumors due to heterogeneous treatment conditions (i.e. tumor type, drug type, route of drug administration) were systematically addressed for the first time. The identified differences could be used for a refined prediction of response to ECT of different tumor types, drug used and route of drug administration. This information should be taken into account for refinement and individualization of ECT treatment to further improve its effectiveness in cutaneous and subcutaneous tumors and to develop procedures for ECT of deep seated tumors.

Conflict of interest

The authors declare no potential conflicts of interest. Damijan Miklavcic holds patents of which some have been licensed to IGEA SpA; the producer of a clinical device used in some of the studies considered in this systematic review.

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