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Direct delivery of chemotherapeutic agents for the treatment of hepatomas and sarcomas in rat models

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The combination of a chemotherapeutic agent and electric pulses has been termed electrochemotherapy (ECT). This procedure is based on the premise that electric pulses can increase the uptake of molecules through the cell membrane due to permeabilization of the membrane through a process called electroporation. This procedure has been successful in increasing the effectiveness of anti-tumor agents (electrochemotherapy; ECT). Response rates of >80% have been obtained in both animal and human trials for several types of skin malignancies using ECT with bleomycin. This study was initiated to determine if ECT could be used to effectively treat internal tumors such as hepatomas in an animal model and human rhabdomyosarcomas in athymic rats. Bleomycin, cisplatin, doxorubicin, 5-fluorouracil, and taxol were used in conjunction with electric pulses. Following an intra tumor injection of a single drug, electric pulses were administered directly to the tumor. For the hepatoma model, ECT worked the best with cisplatin and bleomycin, yielding complete response rates of about 70%. The other drugs used to treat hepatomas were ineffective. Bleomycin combined with electric pulses resulted in a 100% response rate for sarcoma; response rates with cisplatin and doxorubicin were low. These studies indicate that ECT is a technically feasible procedure for visceral tumors and soft tissue sarcomas.

Key words: liver neoplasms, experimental; rhabdomyosarcoma; electroporation; bleomycin; cisplatin; doxorubicin; fluorouracil

Introduction

The delivery of drugs to cancerous tissue is an important modality in the potential treatment of various tumors. Most anti-tumor drugs have an intracellular mode of action. However, for many of these drugs, the cell membrane is often times a significant barrier which reduces the effectiveness by restricting intracellular access. As a result, it is essential to find a mechanism to deliver the drugs through the cell membrane more efficiently. Therefore, it is possible to increase the therapeutic potential of these drugs by increasing the permeability of the tumor cell membranes.

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Electric pulses can be used to temporarily and reversibly permeabilize cell membranes.[1-4] The transient alteration of the cell membranes permeability using electric pulses is known as electroporation. Over the past twenty years electric fields have been used successfully as a method of targeting molecules to tissues,[5,6] electrofusing cells to tissues[7-9] and increasing the uptake of certain drugs by cells.[10,11]

Recently, work has been performed demonstrating that electroporation could be used to enhance the effectiveness of chemotherapeutic agents. This combination of electric pulses and anti-tumor agents is known as electrochemotherapy (ECT).[12, 13] The increased effectiveness of these anti-tumor agents is a direct result of electroporation facilitating the uptake of drugs through the cell membrane which has been made transiently more permeable. Electric pulses delivered to the tumor are non-cytotoxic, and cell membrane permeability returns to baseline levels several minutes after the treatment with electric pulses.

Bleomycin has been the drug most often used for ECT for several reasons. Bleomycin is a very potent cytotoxic molecule when introduced inside the cell. The drug works by causing single stranded and double stranded breaks in DNA.[14-17] In addition, only a few hundred molecules are sufficient to be cytotoxic.[10,18] Since bleomycin has an intracellular mechanism of action, the drug must be able to enter the cell to be effective. However, bleomycin is a relatively nonpermeant drug[10] showing minimal intracellular concentration with a systemic dose. Thus, bleomycin cytotoxicity is dependent upon membrane permeability.

Several studies have been performed in both mice and rats and have shown that when bleomycin is administered in combination with electroporation its effectiveness as an anti-tumor agent is greatly enhanced. These studies were done with a variety of tumor types including, melanoma, hepatocellular carcinoma, lung carcinoma, breast carcinoma, fibrosarcoma, glioma and cervical carcinoma.[12,13,19-32] In addition, the combination of electroporation with other chemotherapeutic agents has also been tested.[23,33] One agent that has shown promise is cisplatin. Although cisplatin is a more permeant drug than bleomycin its effectiveness was augmented by electroporation of cells *in vitro* as well as tumors *in vivo*.[33]

Several clinical studies have shown the potential of electrochemotherapy as an antitumor treatment for a variety of cutaneous malignancies.[34-40] Initial trials utilized bleomycin administered intravenously followed by local administration of electric pulses directly to the tumor. Response rates for the treatment of squamous cell carcinoma of the head and neck were 70% with complete responses of 50-60%.[34,35,37] The treatment of melanoma and basal cell carcinoma yielded response rate of 70% with a complete response rate of 33%.[36,39] Subsequent trials for the treatment of melanoma and basal cell carcinoma utilized intratumor administration of bleomycin in conjunction with electric pulses. Response rates in this trial were as high as 99% with a complete response rate of 90%.[39,40]

The results of the animal and human studies have been extremely encouraging. Since electroporation is based on general physical principles and has been shown to work on most mammalian cells, studies have been initiated to examine if ECT could be used to treat other tumor types. The study reported here, examines the use of this antitumor therapy for the treatment of hepatoma and soft tissue sarcoma in rat models. The effects of ECT with bleomycin, cisplatin, taxol, 5-FU and doxorubicin on established hepatomas was investigated first. Sarcomas were then treated with ECT using bleomycin, cisplatin and doxorubicin. Taxol and 5-FU were not used to treat sarcomas because they were

found to be ineffective in the hepatoma model.

Materials and methods

Cell lines and culture methods

Visceral tumor study: N1S1 rat hepatoma cells (ATCC CRL-1604; American Type Culture Collection, Rockville, MD, USA) were grown in Swimms S-77 medium modified to contain, 4mM L-glutamine, 0.01% Pluronic F68, 9% fetal calf serum, and 90μg/ml gentamycin sulfate. Cells were maintained in humidified air that contained 5% CO_2. In addition, the cells used for this study were greater than 95% viable.

Soft tissue sarcoma tumor study: Human A204 rhabdomyosarcoma cells (HTB 82; American Type Culture Collection, Rockville, MD, USA) were used to induce tumors in nude rats. The cell line was grown in McCoy's 5A medium (Mediatech, Washington, DC, USA) supplemented with 10% (v/v) fetal bovine serum (PAA Laboratories, Newport Beach, CA, USA) and 90μg/ml gentamycin sulphate (Gibco, Grand Island, NY, USA). Cells were grown in a humidified atmosphere that contained 5% CO_2. Confluent cultures were prepared for use by detaching with a nonenzymatic cell dissociation solution (Sigma, St. Louis, MO, USA). The trypan blue exclusion dye method was used to determine the viability of all harvested cell batches. Cell viability was greater than 95% for all batches used in this study.

Animals and tumor induction

Tumors were induced in both male Sprague-Dawley rats using N1S1 rat hepatoma cells and nude rats using human A204 sarcoma cells. General anesthesia was administered using isoflurane. Rats were first placed in an induction chamber that was charged with a mixture of 5% isoflurane in oxygen for several minutes. These rats were subsequently fitted with a standard rodent mask and kept under general anesthesia using 3% isoflurane.

Hepatoma study: the right median lobe of the rat was surgically exposed and injected with $1X10^6$ viable N1S1 cells, suspended in 0.5 ml of saline. The animals were closed with surgical staples immediately after injection with tumor cells. The tumors were allowed to grow for 7-10 days. This procedure yielded hepatomas that were approximately 0.75cm in diameter.

Sarcoma study: male athymic rats (Harlan Sprague Dawley, Inc., Indianapolis, IN, USA) that were 3-4 weeks old at the time of tumor induction were used for the sarcoma tumor study. Tumors were induced by injecting $8X10^6$ cells, contained in 70μl of saline, into the biceps femoris muscle of each rear limb of the athymic rats. Tumors were allowed to grow for 7 to 10 days resulting in sarcomas that were 6 to 8mm in diameter for the case of small sarcomas. Large sarcomas were allowed to grow for greater than 35 days which produced tumors that were 18 to 20 mm in diameter.

Tumor treatment

Treatment of hepatoma: After the establishing tumors in the right median lobes, ECT was performed. Bleomycin, cisplatin, taxol, 5-FU, or doxorubicin were injected directly into tumors using different doses in order to determine the effect of each drug separately after the delivery of electric pulses. All doses were administered in a volume of 100 μl. Control animals that did not receive drug therapy received a 100 μl saline injection. Electric pulses were administered ninety seconds after the intratumor injection by inserting a circular array of 6 needle electrodes [41,42] (BTX 878-2a; Genetronics, Inc., San Diego, CA, USA) to a depth of 5 mm around the

peripheral tissue of all tumors so that the entire tumor was contained within the array of needles. The time between drug injection and electric pulse administration was reduced to 90 seconds from the standard of ten minutes, which was used in previous studies,[25] due to the highly vascular nature of hepatoma tumors. Six electric pulses with a field strength of 1000 V/cm were delivered via the inserted needle electrodes in a manner that rotated the applied field around the treatment site.[29,41] A lower field strength was used to treat the hepatoma tumors vs the sarcoma tumors because of the lower impedence of liver tissue compaired to skin tissue. These pulses were administered using a DC generator (BTX T820 generator; Genetronics, Inc., San Diego CA, USA), and the pulses were 99μs in duration with a one second interval between the initiation of each pulse.

Treatment of sarcoma: After injection of human sarcoma cells in the rear limbs of athymic rats, all animals developed firm palpable tumors. All drugs were administered by intratumor injection in a volume that was equal to 25% of the tumor volume. The chemotherapeutic agents were administered at the following concentrations: bleomycin 5 units/ml, cisplatin 1 mg/ml and doxorubicin 20 mg/ml. Control animals that did not receive a chemotherapeutic agent were given an intratumor injection of saline that was equal to 25% of the tumor volume.

The delivery of electric fields to sarcoma tumors was similar to hepatoma electrical treatment except that the electric pulses were administered ten minutes after injection with the chemotherapeutic agent or saline. In addition, the electrode was placed around the perimeter of each tumor to a maximum depth of 1 cm so that the entire tumor was encompassed within the needle array. The ratio of the applied voltage for each pulse to the electrode spacing was 1300 V/cm.[29,41] A larger field strength was used in the treatment sarcoma tumors due to higher tissue impedence. Large sarcomas were too big to fit within the volume delineated by the needle array electrode. These tumors were electrically treated by multiple insertions of the electrode until the entire tumor volume received pulses.

Protocols for the treatment of small and large sarcomas were designed for a single ECT treatment and multiple ECT treatments. Electrochemotherapy was administered once for single treatment experiments and a maximum of three times for multiple treatment scenarios. All single treatment animals received ECT on the same day. Similarly, all multiple treatment animals received their first ECT treatment on the same day. For animals that received multiple treatment, ECT was administered again when palpable tumor within the original treatment site was first detected.

Tumor measurements

Hepatoma study: At days 7 and 14 post ECT treatment, all the animals induced with hepatoma tumors were surgically explored, and the tumors were examined for evidence of response to treatment. Tumor volumes were measured prior to and after treatment using the formula $V=abc\ \pi/6$. Measurements were made using a digital Vernier caliper. Objective responses to ECT treatment was determined based on reduction in tumor volume. A complete response was when no visible tumor was evident. Greater than 50% reduction in tumor volume was considered a partial response, and stable disease was less than 50% reduction in tumor volume. Progressive disease was when the tumor volume continued to increase in size. An objective response was defined as the sum of complete and partial responses. For long term studies, the animals were checked every 14-21 days after day 14.

Sarcoma study: Another study was conducted to confirm the efficacy of ECT in the treatment of highly aggressive human sarco-

ma tumors induced in the rear limbs of male athymic rats. Response to electrochemotherapy treatment was also based on tumor volume. Tumor volume was determined on 3 mutually orthogonal measurements (a,b,c) of the nodule, and the tumor volume was based on the formula V= abc $\pi/6$. Tumors were measured prior to treatment and then at 7 day intervals after treatment. Each tumor was categorized as a complete response, partial response, stable disease, or progressive disease at 28 days post treatment. Animals were considered cured if complete responses were maintained for 100 days.

Histologic analysis

Tissue specimens were fixed overnight in 10% formalin and then processed for routine histopathological examination. Briefly, specimens were dehydrated through a sequence of 50, 70, 95 and 100% ethanol, cleared in xylene and then embedded in paraffin wax. Sections were cut with a microtome (three sections per specimen) and stained with hematoxylin-eosin. The overall condition of the tissue was examined with respect to cellular integrity.

Statistical analysis

The Fisher's test for 2 X 2 contigency tables was used to determine the statistical significance of the complete response rates between the treatment and the control groups. For this test, partial response, stable disease and progressive disease were considered incomplete responses.

Results

A total of 223 established hepatoma tumors were treated in the visceral tumor study and 89 tumors were treated in the sarcoma study. Four different treatment groups were examined. These groups included those with no treatment (D-E-), electrical treatment (D-E+), drug treatment (D+E-), and combined drug and electric pulses (D+E+).

ECT for hepatomas

Treatment with bleomycin: Objective responses were obtained in 84.5% of the tumors treated with both bleomycin (0.5 unit/tumor) and electric pulses (D+ E+ group). This group also had a 69% complete response rate (Table 1). Tumors that received drug only (D+E-) or only electric pulses (D-E+) or no treatment (D-E-), were found to have 100% progressive disease (Table 1). The response was based on tumor measurements taken 14 days after treatment. The complete response rate for the D+E+ group differed significantly ($p< 0.01$) from the other groups. Incomplete responses were considered to be those animals which had progressive disease, stable disease, and partial responses. The number of complete responses for the D+E+ treatment group was significantly greater ($P< 0.01$) than the number of complete responses in each of the control (D-E-, D-E+, and D+E-) groups. In addition, no adverse effects from the treatment were observed in any of the animals.

Treatment with other chemotherapeutic agents: The ability to augment the effectiveness of other chemotherapeutic agents when

Table 1. Treatment of rat hepatomas with bleomycin

Treatment	n	% PD[a]	% SD[b]	% PR[c]	% CR[d]
D-E-	9	100	0	0	0
D-E+	9	100	0	0	0
D+E-	10	90	0	0	10
D+E+	13	15.5	0	15.5	69

a: PD = Progressive disease = tumor increasing in size Day 14 compared to Day 0
b: SD = Stable disease = tumor decreasing less than 50% in size Day 14 compared to Day 0
c: PR = Partial response = tumor decreasing in size more than 50% Day 14 compared to Day 0
d: CR = Complete Response = no tumor present on Day 14

combined with electric fields was tested in the N1S1 rat hepatoma model. The treatment was performed as described above using various concentrations of cisplatin, doxorubicin, taxol and 5-FU. Examination of responses at day 14 showed that hepatomas treated with 0.0357 mg of cisplatin and electric fields resulted in a complete response rate of 67% (Table 2) which is similar to the result obtained with bleomycin. The higher tested cisplatin dose (0.357 mg per tumor) resulted in toxicity related deaths in 40% of the animals in the D+E- and D+E+ groups. The delivery of three different doxorubicin doses, two 5-FU doses, and two taxol doses with electric pulses resulted in low or no complete response rates (Table 2). With all drugs tested, except cisplatin, treatment with drug only or pulses only resulted in little or no response (Table 2). In addition, the toxicity related deaths for doxorubicin of 2 and 3 mg per tumor ranged from 78 - 100%.

Treatment with cisplatin using a dose of 0.0357 mg per tumor was repeated to determine the long term effect of the therapy. A 0.0357 mg cisplatin dose was used because it was what resulted in a high complete response in the initial experiments. The com-

Table 2. Treatment of rat hepatomas with other drugs

Treatment	Drug	Dose[a]	n	% PD[b]	% SD[c]	% PR[d]	% CR[e]
D+E-	5 FU[f]	0.5 mg	4	100	0	0	0
D+E+	5 FU	0.5 mg	5	100	0	0	0
D+E-	5 FU	5.0 mg	10	100	0	0	0
D+E+	5 FU	5.0 mg	10	100	0	0	0
D+E-	DOX[g]	1 mg	9	89	11	0	0
D+E+	DOX	1 mg	8	75	12.5	0	12.5
D+E-	DOX	2 mg	9[h]	11	0	11	0
D+E+	DOX	2 mg	9[h]	11	0	11	0
D+E-	DOX	3 mg	8[i]	-	-	-	-
D+E+	DOX	3 mg	8[i]	-	-	-	-
D+E-	CIS[j]	0.00357 mg	7	100	0	0	0
D+E+	CIS	0.00357 mg	6	100	0	0	0
D+E-	CIS	0.0357 mg	6	83	17	0	0
D+E+	CIS	0.0357 mg	6	33	0	0	67
D+E-	CIS	0.357 mg	10[k]	0	10	10	40
D+E+	CIS	0.357 mg	9[k]	11	0	0	44
D+E+	TAX[l]	0.308	5	100	0	0	0
D+E-	TAX	0.308	5	100	0	0	0
D+E+	TAX	0.0038	5[m]	100	0	0	0
D+E-	TAX	0.0038	5[m]	100	0	0	0

a: dose of drug per tumor
b: PD = Progressive disease = tumor increasing in size Day 14 compared to Day 0
c: SD = Stable disease = tumor decreasing less than 50% in size Day 14 compared to Day 0
d: PR = Partial response = tumor decreasing in size more than 50% Day 14 compared to Day 0
e: CR = Complete Response = no tumor present on Day 14
f: 5 FU = 5 Fluorouracil
g: DOX = Doxorubicin
h: 7 animals died due to toxicity prior to day 14
i: 8 animals died due to toxicity prior to day 14
j: CIS = Cisplatin
k: 4 animals died due to toxicity prior to day 14
l: TAX = Taxol
m: 2 animals died prior to day 14

plete response rate at 35 days for the D+E+ group was 70% (Table 3) with 60% remaining tumor free for greater than 100 days (cure). These results confirmed the initial experiments. The D+E- group had a 35 day complete response rate and cure rate (100 days) of 30% (Table 3). The other control groups (D-E+ and D-E-) showed no response.

ECT for sarcoma

*Treatment with bleomycin:*Examination of the effectiveness of using a single ECT treatment with bleomycin to treat soft tissue sarcoma was performed in an athymic rat model. Tumor response was based on the reduction in tumor volume 28 days after ECT treatment. No objective responses were seen in the D-E-, D-E+, or D+E- control groups (Table 4). All tumors in the control groups showed progressive disease with the exception of one animal which had a tumor that remained stable after treatment with drug only. In contrast, animals in the D+E+ group had a complete response rate of 100% (Table 4). All animals in this group had no palpable tumors 7 days after treatment. Between days 35 and 100, seven of the tumors recurred. Five of the ECT treated tumors (42%) responded completely for 100 days and were considered cured. The complete response rate of the D+E+ group was significantly different from each of the control groups ($p< 0.001$). To confirm the results of this study, random biopsies of the remaining scar from D + E + tumors as well as tumors from control groups

Table 3. Treatment of hepatomas with cisplatin (0.0357 mg/100 µl)

Group	n	Volume Initial	Final	% PD[a]	% SD[b]	% PR[c]	% CR[d]	% Cures[e]
D-E-	8	82.8	12032	100	0	0	0	0
D-E+	10	75.4	7926	100	0	0	0	0
D+E-	10	140.0	16037	70	0	0	30	30
D+E+	10	120.0	480.1	30	0	0	70	60

a: PD = Progressive disease = tumor increasing in size Day 35 compared to Day 0
b: SD = Stable disease = tumor decreasing less than 50% in size Day 35 compared to Day 0
c: PR = Partial response = tumor decreasing in size more than 50% Day 35 compared to Day 0
d: CR = Complete Response = no tumor present on Day 35
e: Cure = No evidence of tumor 100 days after treatment

Table 4. Treatment of rat sarcomas with bleomycin (5 U/ml); injection volume is 25% tumor volume (single treatment)

Group	n	Volume Initial	Day 28	% PD[a]	% SD[b]	% PR[c]	% CR[d]	% Cure[e]
D-E-	12	220.2	3005.9	100	0	0	0	0
D-E+	12	281.2	2998.4	100	0	0	0	0
D+E-	12	140.0	2414.5	91.7	8.3	0	0	0
D+E+	12	278.7	0	0	0	0	100	42
D+E+[f]	8	4621.9	2035.0	12.5	0	37.5	50	12.5

a: PD = Progressive disease = tumor increasing in size Day 28 compared to Day 0
b: SD = Stable disease = tumor decreasing less than 50% in size Day 28 compared to Day 0
c: PR = Partial response = tumor decreasing in size more than 50% Day 28 compared to Day 0
d: CR = Complete Response = no tumor present on Day 28
e: Cure = No evidence of tumor 100 days after treatment
f: Retreatment of D+E- tumors after >35 days (treatment of large tumors)

were taken at day 28. All tumors in the control group showed evidence of malignant sarcoma cells with a high mitotic rate and minimal necrosis of the tumor. However, tumors in the D + E + group showed a few anucleated tumor cell associated with abundant necrotic tissue suggestive of no residual viable tumor.

To examine if the therapy would work on large tumors, tumors of the D+E- group, showing no response 35 days after treatment were treated with ECT. The treatment of these large tumors resulted in a 50% complete response rate and a 37.5% partial response rate (Table 4). In addition, treatment of these large tumors only had a cure rate of 12.5%.

The low cure rate obtained with both small and large tumors was surprising due to the high complete response rate. It is possible that the single treatment was not sufficient to eliminate all tumor cells. Previous work in mouse models with subcutaneous tumors had demonstrated an increased cure rate when multiple treatments were performed.[31] Therefore, an additional experiment was performed to determine if multiple ECT treatments with bleomycin would be beneficial. Small tumors that received multiple ECT treatments showed a cure rate of 83.3% (Table 5) compared to the 42% cure rate obtained with single treatment (Table 4). Treatment of large tumors with multiple treatments resulted in a cure rate of 100% (Table 5).

*Treatment with other chemotherapeutic agents:*The effectiveness of treating sarcoma with cisplatin or doxorubicin in combination with electric pulses was studied. Cisplatin was administered at a dose of 1 mg/ml via intratumor injection. The injection volume was equivalent to 50% of the tumor volume. Two treatment groups were used, drug alone (D+E-) or drug with electric pulses (D+E+). The D+E+ group had a 33% complete response at 28 days and a 33% cure rate (Table 6). The D+E- group had a 17% complete response rate and 17% cure rate (Table 6). Doxorubicin was administered at a dose of 20 mg/ml via intratumor injection. The injection volume was equivalent to 100% of the tumor volume. The D+E+ group had a 17% complete response at 28 days and a 0% cure rate (Table 6). The D+E- group had a 0% complete response rate and 0% cure rate (Table 6). Doxorubicin treatment had a high toxicity as 5 of 6 animals died in each group. All deaths occurred prior to day 14. However, tumor volumes were obtained on day 7 and at time of death.

Discussion

Primary hepatocellular carcinoma is associated with chronic hepatitis B infection and liver cirrhosis. Approximately 80% of patients who develop hepatomas are positive for hepatitis B surface antigen.[43] These patients have

Table 5. Multiple treatment of sarcomas with bleomycin (5 U/ml); injection volume is 25% tumor volume

Group	n	Volume Initial	Final	% PD[a]	% SD[b]	% PR[c]	% CR[d]	% Cure[e]
D+E-	5	213.2	1538.3	80	0	0	20	20
D+E+	6	183.0	25.9	0	16.7	0	83.3	83.3
D+E+	6	3158.2	0	0	0	0	100	100

a: PD = Progressive disease = tumor increasing in size Day 28 compared to Day 0
b: SD = Stable disease = tumor decreasing less than 50% in size Day 28 compared to Day 0
c: PR = Partial response = tumor decreasing in size more than 50% Day 28 compared to Day 0
d: CR = Complete Response = no tumor present on Day 28
e: Cure = No evidence of tumor 100 days after treatment

Table 6. Treatment of rat sarcomas (single treatment)

Treatment	Drug	Dose[a]	n	% PD[b]	% SD[c]	% PR[d]	% CR[e]	% Cure[f]
D+E-	CIS[g]	1.0 mg	6	66	17	0	17	17
D+E+	CIS	1.0 mg	6	50	17	0	33	33
D+E-	DOX[h]	20 mg	6	100	0	0	0	0
D+E+	DOX	20 mg	6	83	0	0	17	0

a: dose of drug per ml
b: PD = Progressive disease = tumor increasing in size Day 28 compared to Day 0
c: SD = Stable disease = tumor decreasing less than 50% in size Day 28 compared to Day 0
d: PR = Partial response = tumor decreasing in size more than 50% Day 28 compared to Day 0
e: CR = Complete Response = no tumor present on Day 28
f: Cure = No evidence of tumor 100 days after treatment response determined 35 days after treatment
g: CIS = Cisplatin
h: DOX = Doxorubicin

chronic subclinical hepatitis infection for many years, and most of them go on to develop advanced liver disease including hepatic cirrhosis. Currently, the treatment of primary hepatocellular carcinoma remains a significant problem not only in the United States, but in third world countries which have a higher incidence of chronic hepatitis B infection.[44] In addition, the diagnosis of hepatocellular carcinoma is made late in the disease process and the chance for curative therapy is relatively low. Since most patients diagnosed with hepatomas have liver cirrhosis and a limited hepatic reserve, they do not tolerate major hepatic resections. Therefore, new and less invasive treatment modalities with higher cure rates are clearly needed to effectively eliminate these tumors.

Hepatic resection of primary hepatocellular carcinoma carries a low 5 year survival rate of 18 to 36%.[44] Furthermore, patients with hepatomas and advanced liver cirrhosis are not candidates for major liver resections. Survival after hepatic resection is dependent on the patients hepatic reserve and whether the liver is able to undergo adequate regeneration after a major resection. A large study of 444 consecutive hepatic resections at John Cochran Veterans Affairs Medical Center demonstrated that hepatic resections in patients with hepatomas is a dangerous procedure with a 21% operative mortality, compared to a 4% operative mortality for major liver resections done for colorectal metastases.[45] Since modalities such as standard chemotherapy, major liver resections, and liver transplantation have been used with very limited success, ECT using cisplatin or bleomycin is an attractive alternative for liver tumors which have been deemed surgically nonresectable due to hepatic failure and advanced liver cirrhosis. ECT can also benefit patients with multiple unresectable tumors and tumors which can not be removed due to their location near vital structures. In addition, animal and human studies demonstrate that ECT is a less invasive and a more effective cytoreductive procedure, which carries a lower morbidity and mortality.

To confirm the effectiveness of ECT, several experiments were conducted using electric pulses and direct intratumor injection of bleomycin to treat measurable liver tumors induced in rats. Hepatomas treated with ECT using bleomycin showed statistically significant responses. These responses are consistent with prior studies and they are readily reproducible. Objective responses were obtained in 84.5% of the tumors treated with both electric pulses and bleomycin. This group also had a 69% complete response rate. Of interest, tumors that received electric pulses only (D-E+), or no treatment (D-E-) were found to have 100% progressive disease.

A minimal 10% response rate resulted in tumors treated with blemycin alone (D+E-).

Several other drugs were also examined for use with ETC. The only drug that showed any effectiveness was cisplatin. The best response using cisplatin was obtained with a dose of 0.0357mg in combination with electric pulses. A 67% complete response rate was obtained. No responses were observed in tumors treated using cisplatin without electric pulses. The study was extended to determine long term responses with cisplatin at this dose. ECT with cisplatin (0.0357 mg) resulted in a 60% cure rate (no evidence of disease for 100 days). Treatment with cisplatin alone resulted in a 30% cure rate. The results presented in this study demonstrate that electric pulses combined with either bleomycin or cisplatin is a safe and effective treatment. ECT can be used to treat liver lesions that are locally extensive, centrally located, or near vital structures. In addition, ECT is a safe alternative in patients with liver cirrhosis and in patients with multiple non resectable lesions.

The soft tissue sarcoma studies also demonstrate the applicability of ECT in the treatment of aggressive human sarcoma tumors. A study was performed using a combination of ECT and intratumor injection of bleomycin to treat human soft tissue sarcomas in athymic rats. No objective responses were seen in the D-E- , D- E+, or D+ E- control groups. All tumors in these control groups showed progressive disease with the exception of one animal which remained stable after treatment with drug only. In contrast, animals in the D+E+ group had a complete response rate of 100%. However, the cure rate of small sarcoma tumors which received a single treatment of ECT was only 42%, and the cure rate of the large tumors was even less (12.5%). Therefore, a trial of multiple ECT treatments was performed to improve the overall cure rates. As a result, small tumors (approximately 250 mm^3) that received multiple ECT treatments showed a cure rate of 83.3% compared to the 42% cure rate obtained with a single treatment. The multiple treatment protocol increased the cure rate of large tumors (4000 mm^3) from 12.5% to 100%. Of interest, the delivery of cisplatin and doxorubicin with electric pulses resulted in minimal antitumor effects.

The current treatment of soft tissue sarcomas have met with limited success, and the survival rates for adults with these tumors remains very poor. In addition, the complete elimination and the successful long term cure rate of highly aggressive sarcoma tumors remains a significant problem today. The use of adjuvant radiation therapy and chemotherapy is of some value in adult patients, but the most effective treatment of aggressive sarcoma tumors is extensive local resection. However, surgical treatment may not be a technically feasible option in tumors which are too extensive for adequate surgical resection or for tumors that are located near vital structures. In contrast, the electrically mediated delivery of bleomycin is a technically feasible and highly effective treatment alternative for sarcoma patients who would otherwise require an amputation because their tumors are located close to joints, bones, and nerves or other vital structures. Since ECT treatment preserves limb function, it can be used in patients as a limb sparing procedure. In conclusion, ECT is a safe procedure with minimal morbidity and functional disability, which may be used in the treatment of aggressive sarcomas that are not amenable to limb sparing surgery.

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