Antitumor Electrochemotherapy

New Advances in the Clinical Protocol

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BACKGROUND. Electrochemotherapy (ECT) is a new antitumor approach that combines systemic bleomycin (BLM) with electric pulses (EP) delivered locally at the tumor site. These EP permeabilize the cells in the tissue, allow BLM delivery inside the cells, and increase BLM cytotoxicity. As an extension of our initial Phase I trial on patients with head and neck squamous cell carcinoma (HNSCC) permeation nodules, we tested variations of ECT protocol to determine how to improve it.

METHODS. Seven patients with multiple and/or large permeation nodules of HNSCC or of salivary or breast adenocarcinoma were treated in 10 sessions. They received BLM followed by runs of four or eight short (100 ps) and intense (1000 or 1300 V·cm⁻¹) EP delivered at adjacent positions on the nodules to cover all of the tumor surface.

RESULTS. We determined the therapeutic window for EP delivery to be between 8 and 28 minutes after BLM intravenous injection. We showed patient tolerance to a high number of EP, along with ECT feasibility after BLM intraarterial injection or on adenocarcinoma nodules. Clear antitumor effects were obtained, especially in the small nodules. In the largest nodules we observed extended tumor necrosis.

CONCLUSIONS. Relatively efficient ECT can be performed for large and thick nodules, and ECT remains safe even when a large number of EP are delivered. However, in this study, ECT’s effectiveness on large nodules was lower than on the previously treated small nodules, probably due to external electrodes inadequacy. The data reported stimulated us to design a new device for EP delivery. Cancer 1996; 77:956–63. © 1996 American Cancer Society.

KEYWORDS: electropermeabilization, bleomycin, head and neck, squamous cell (epidermoid) carcinoma, Phase I, permeation nodule.

It is now well established that appropriate electric pulses (EP) transitorily and reversibly permeabilize any kind of living cell.1–3 In recent extensive in vitro studies, we have shown that (1) bleomycin (BLM) is a cytotoxic drug that does not freely diffuse through the cell plasma membrane and has a very limited access to the cytosol;4–5, (2) BLM is a very potent cytotoxic molecule when introduced inside the cell;3,5–6; (3) cell as well as tissue electropermeabilization allow BLM to enter the cytosol directly and to exert its cytotoxic potential fully. Preclinical trials have shown that BLM antitumor effects can be greatly increased by the local delivery of permeabilizing EP at the level of the tumors.8–14 We termed electrochemotherapy (ECT)5 this new antitumoral approach combining the administration of a nonpermeant drug such as BLM with local permeabilizing EP. EP bring about an increased drug delivery in the pulsed tumor.7 Moreover, after ECT, local administration of interleukin-215 or of histoincompatible interleukin-2 secreting cells15–16 results in enhanced local16,15 as well as systemic16 antitumor effects.
Small permeation nodes of head and neck squamous cell carcinoma were treated with EP after intravenous BLM injection during a first clinical trial carried out in our institute. Patients received only a preliminary sedation 1 hour before the beginning of theECT session. In one case, the patient was treated under neuroleptanalgesia. ECT was well tolerated, and a majority of objective responses of the treated nodules was obtained in this first trial. However, many questions remained open: What is the appropriate period for EP delivery after BLM injection? How many runs of EP can be tolerated? Is it beneficial to deliver the BLM intraarterially, and at what dose? What results can be obtained in the case of large nodules for which the geometry of the external electrodes apparently is not suitable? Can the ECT be applied to surface tumor nodules of other histological types? These questions were addressed during the 10 ECT sessions for seven patients reported herein. These patients were affected by permeation nodules or lymphangitic skin spreads that are aggressive cutaneous metastases of various tumor origins, e.g., head and neck squamous cell carcinomas and breast carcinomas, often associated with locoregional relapse and/or visceral metastases. They frequently occur close to surgically pretreated and/or irradiated areas, exhibit low chemosensitivity, carry a dismal prognosis, and are difficult to salvage by conventional treatments.

**PATIENTS AND METHODS**

**Inclusion Criteria and Patient Descriptions**

From January, 1992, through August, 1992, five male and two female patients with a mean age of 51 years entered this study. To be eligible, patients had to have recurrent or progressive head and neck or breast carcinoma, be affected by measurable permeation nodules, and be pretreated by radiotherapy and/or surgery and/or chemotherapy. All the patients were excluded from conventional chemotherapeutic protocols and from any other kind of treatment and had to be between 18 and 70 years of age (Table 1). No restrictions were made regarding performance status or life expectancy. All patients signed an informed consent document.

Primary and last treatment before ECT are reported in Table 1. In all cases, tumor progression was clinically evident before the ECT. The treated superficial nodules were located in the neck and/or in the anterior or posterior region of the thorax. One patient (LEN) presented only two large nodules. The other six patients were affected by a large number of nodules that, when possible, were all subjected to the EP. The minimal wash-out interval for other antineoplastic drugs was 22 days (Table 1), and no patient had received BLM previously. For the seven patients, a total surface of approximately 720 cm² was treated with runs of four or eight EP per nodule, delivered step by step at adjacent positions to cover the entire exposed tumor surface (Table 2).

**General Treatment Conditions**

All patients were treated in a surgical area, under continuous monitoring of arterial pressure, cardiac rhythm, and oxymetry. From our initial study, we knew that treatment was painless but disagreeable and that sedation was sufficient when very small surfaces were treated. For the comfort of the patients with very large lesions, such as those included in the present study, as well as for the comfort of the clinicians delivering the treatment, all the patients except CAN, CHO, and MON were treated under neuroleptanalgesia using Midazolam and Alfentanil. Patients CAN, CHO, and MON, with weak respiratory capacities, were treated under general anaesthesia and intubation.

**Bleomycin Administration**

Several modalities for BLM administration were assayed (Table 2). Systemic BLM was delivered by intravenous (i.v.) push (i.e., in 30–45 s) at a dose of 10 or 15 mg/m², irrespective of the number of nodules treated, except in two patients (CHO and MON) in whom the bolus was followed by a slow perfusion of the BLM for 20 minutes (Table 2). In one case (MON), a supplementary dose of BLM was perfused 45 minutes after the initial BLM bolus (Table 2).

In two cases (BAR 3 and LEN 2), BLM was delivered intraarterially (i.a.). In the first case, after femoral puncture, the distal tip of a 5F catheter was placed in the ostium of the left transverse scapular artery. More distal catheterization of this artery was achieved with a 3F coaxial catheter (Tracker, Target Therapeutics, San Jose, CA). BLM was administered at a dose of 10 mg/m² in a 15 min perfusion. In the second case, an arterial branch arising from the lingual artery was catheterized and BLM was administered at a dose of 15 mg/m² in a 17 min perfusion. In both cases, 0.5 ml of Blue Patent (Guerbet, Aulnay, France) was injected i.a. immediately before the treatment. The skin staining after this injection allowed us to determine the area supplied by the catheterized artery.

**Delivery of Electric Pulses**

ECT consisted of the administration of BLM followed by delivery of transcutaneous EP through external electrodes. EP were delivered during the specified time periods (Table 2). The electrodes were two stainless steel strips with round corners (to avoid pointed-corner electrical effects), 10 mm wide, 0.6 mm thick, and 6 mm apart. Skin contact was ensured by electrocardiography paste. For the treatment of small nodules, electrodes were placed at each side of the permeation nodule, and one
run of four or eight EP was delivered. For the treatment of large permeation nodules, several (Table 2; No.) sequential runs of four or eight EP were delivered to cover all the surface of the nodules, our electrodes being placed successively at adjacent positions. The treatment at each position covered by the electrodes consisted of four or eight (Table 2; n) consecutive square-wave EP of 100 μs and 1000 or 1300 V·cm⁻¹ (Table 2; field) delivered at the frequency of 1 Hz by a PS 15 electropulsator (Jouan, France). The last patient (MON) received 1300 V·cm⁻¹ EP on the nodules still covered by a normal or almost normal cutaneous pavement and EP of only 800 V·cm⁻¹ on the ulcerated nodules. The delivered instantaneous amperage was between 0.2 and 13 A. Electrical treatment

### TABLE 1
Patients Description

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Primary</th>
<th>Last treatment</th>
<th>Wash-out</th>
<th>Weight</th>
<th>Size</th>
<th>Creatinine</th>
<th>BLM</th>
</tr>
</thead>
<tbody>
<tr>
<td>TZW</td>
<td>39</td>
<td>Saliv. G.</td>
<td>Surg. + RT</td>
<td>5 mo</td>
<td>40 kg</td>
<td>1.72 m</td>
<td>66 μM</td>
<td>21 mg</td>
</tr>
<tr>
<td>BAR</td>
<td>49</td>
<td>Lung</td>
<td>CT</td>
<td>15 days</td>
<td>62 kg</td>
<td>1.70 m</td>
<td>63 μM</td>
<td>27 mg</td>
</tr>
<tr>
<td>CAN</td>
<td>49</td>
<td>Hypopharynx</td>
<td>Surg. + CT</td>
<td>2.5 mo</td>
<td>56 kg</td>
<td>1.72 m</td>
<td>51 μM</td>
<td>27 mg</td>
</tr>
<tr>
<td>LAZ</td>
<td>67</td>
<td>Tongue</td>
<td>CT</td>
<td>22 days</td>
<td>52 kg</td>
<td>1.56 m</td>
<td>72 μM</td>
<td>16.5 mg</td>
</tr>
<tr>
<td>CHO</td>
<td>50</td>
<td>Tonsil</td>
<td>Surg. + RT</td>
<td>2 yr</td>
<td>70 kg</td>
<td>1.70 m</td>
<td>65 μM</td>
<td>35 mg</td>
</tr>
<tr>
<td>LEN</td>
<td>37</td>
<td>Larynx + Floor of M</td>
<td>Surg. + RT</td>
<td>1 mo</td>
<td>56 kg</td>
<td>1.62 m</td>
<td>57 μM</td>
<td>24 mg</td>
</tr>
<tr>
<td>MUN</td>
<td>67</td>
<td>Breast</td>
<td>CT</td>
<td>2 mo</td>
<td>59 kg</td>
<td>1.61 m</td>
<td>61 μM</td>
<td>15 mg + 7.5 mg</td>
</tr>
</tbody>
</table>

* Primary were squamous cell carcinoma of the various reported localisations except for patients TZW (salivary gland adenocarcinoma) and MUN (breast adenocarcinoma).
BLM: systemic bleomycin; Saliv: G: salivary gland; M: mouth; Surg: surgery; RT: radiotherapy; CT: chemotherapy.

### TABLE 2
Specifications of the Various Treatments Performed

<table>
<thead>
<tr>
<th>Session</th>
<th>BLM (mg/m²)</th>
<th>Route of BLM administration</th>
<th>ELF. (V·cm⁻¹)</th>
<th>n</th>
<th>Time (min)</th>
<th>Nodules (no. and min - max size; in cm)</th>
<th>Nodules surface (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TZW</td>
<td>15</td>
<td>IVb</td>
<td>1000</td>
<td>4</td>
<td>10' 33' 23'</td>
<td>17 20.3 - 2.2 × 1.2</td>
<td>8</td>
</tr>
<tr>
<td>BAR1</td>
<td>15</td>
<td>IVb</td>
<td>1000</td>
<td>4</td>
<td>10' 42' 32'</td>
<td>44 4 + SI (1.5 × 1.5 - 5.5 × 2)</td>
<td>19</td>
</tr>
<tr>
<td>BAR2</td>
<td>15</td>
<td>IVb</td>
<td>1300</td>
<td>4</td>
<td>7' 47' 40' 84</td>
<td>CI (6.5 × 8 - 11 × 8)</td>
<td>97</td>
</tr>
<tr>
<td>BAR3</td>
<td>10</td>
<td>IAV15'</td>
<td>1300</td>
<td>4 (IA)</td>
<td>6' 9' 3' 31</td>
<td>3CI (3.0 × 2.5 - 13 × 12)</td>
<td>200</td>
</tr>
<tr>
<td>CAN</td>
<td>15</td>
<td>IVb</td>
<td>1300</td>
<td>8</td>
<td>9' 29'30 20'30'</td>
<td>51 + SI (5 × 5 - 6.5 × 4.0)</td>
<td>23</td>
</tr>
<tr>
<td>LAZ</td>
<td>10 + 5</td>
<td>IVb</td>
<td>1000</td>
<td>4</td>
<td>8' 44'30 36'30'</td>
<td>160 10 + SI (1.0 - 6.0 × 4.5)</td>
<td>40</td>
</tr>
<tr>
<td>CHO</td>
<td>10</td>
<td>IVb</td>
<td>1300</td>
<td>4</td>
<td>8' 15' 7' 38</td>
<td>3 (2.5 × 2.0 - 4.0 × 2.0)</td>
<td>15</td>
</tr>
<tr>
<td>LEN1</td>
<td>15</td>
<td>IVb</td>
<td>1300</td>
<td>4</td>
<td>12'30' 22' 9'30'</td>
<td>18 2 + SI (6.5 - 2.5 - 2.5)</td>
<td>5</td>
</tr>
<tr>
<td>LEN2</td>
<td>10</td>
<td>IAV</td>
<td>1300</td>
<td>4</td>
<td>10' 37' 27' 95</td>
<td>2 (4.3 × 5.4 - 3.4 × 3.8)</td>
<td>28</td>
</tr>
<tr>
<td>MUN</td>
<td>10</td>
<td>IVb</td>
<td>1300</td>
<td>4</td>
<td>8' 16' 8' 48</td>
<td>7 + CI (1.0 × 0.0 + 20 × 8)</td>
<td>105</td>
</tr>
<tr>
<td></td>
<td>+ 10</td>
<td>IVp20'</td>
<td>400</td>
<td>4</td>
<td>47' 72' 25'</td>
<td>33</td>
<td></td>
</tr>
</tbody>
</table>

**Notes:**
- BLM: systemic bleomycin; IVb: intravenous bolus injection in 30'; IVp: intravenous perfusion (the time indicated besides IVp); IAV: intra-arterial perfusion (the time indicated besides IAV); ELF: electric field, i.e., the ratio of the applied voltage to the distance between the electrodes; n: number of electric pulses per run; No.: number of runs delivered; SI: subcutaneous infiltration, i.e., areas of skin invaded by the tumor without permeation; CI: cutaneous infiltration with permeation.

* Second run of 4 pulses delivered on the same nodules treated by 4n a few minutes before, in the IA perfused area (IA) or in the IV perfused area (IV).
Follow-Up

During ECT and for several hours after, patients were carefully examined to record the treatment effects and were questioned to determine the immediate symptomatology. As a precaution, patients remained hospitalized for 24 hours after the ECT. Patients were examined as outpatients 5 days later and then every 15 days. The longest and the largest perpendicular diameters (in centimeters) of each nodule were measured with a caliper and were photographed before and after treatment at regular intervals. The recommended W10 rules for tumor response assessment were applied to describe, independently, the result of the treatment of individual nodules. Partial response (PR) is a reduction more than 50% of the product of the longest initial diameter by the largest perpendicular diameter. Complete response (CR) is the absence of any clinical trace of the nodule, with recovery of skin integrity. NC indicates no change or a minor response of less than 50%.

RESULTS

Tolerance During Treatment

No significant modification of hemodynamic or cardiological parameters was noticed during the treatment, except for that in patient CAN. During his treatment, we observed transient heart frequency acceleration (112 vs. 76) and increase in maximal blood pressure (195 vs. 160), which returned to the pretreatment values in a few seconds. There were no local or general side effects. One contraction affecting the muscles of the neck, and in some cases of the shoulders and the arms, was observed after each EP. The amplitude of the movements after these contractions depended on the distance between the EP delivery site and the nervous plexus; the closer these were, the greater the movements. Contractions were instantaneous, disappearing immediately at the end of each EP (which lasts 1/10,000th of 1 second). Because of the neuroleptanalgesia, none of the treated patients described recollection of a disagreeable sensation resulting from the EP delivery.

Tolerance After Treatment

Patients did not have any disagreeable impression or any pain, either on waking or in the hours or days that followed the treatment. In two cases (TZV and MON), we noticed a slight hyperthermia during several hours after the session, probably due to the BLM bolus injection. One to two hours after the treatment, the only general noticeable effect was the occurrence of erythema and slight edema at the site of the treated areas, both disappearing within a few days. Phlyctenes were observed at the sites of electrode contact with the skin. Superficial leukonecrosis of some nodules was visible within the following 3 days, and scabs were observed a few days later. In all the cases, marks from the electrodes and superficial epidermal erosion were noted 4 or 5 days after the ECT sessions. ECT was followed by skin ulceration when tumors were very thick, with altered skin at the time of the ECT. Patients reported neither delayed local pain nor any other subjective symptom.

We observed a statistically significant rise in white blood cells, from 9800 ± 2900 (means of the counts per cubic millimeter ± standard deviations of eight determinations 1–3 days before the ECT) to 14,200 ± 4500 (after 40 days on the average; P < 0.01 by Student’s t test for paired data). This was due to a statistically significant increase in the neutrophils (from 8000 ± 2600 to 12,200 ± 4500; P < 0.01). This could be a consequence of the massive necrosis of the treated large nodules. However, after session LEN2 that also induced massive necrosis of the treated volume, no hyperneutrophily was detected. No change occurred in lymphocyte, monocyte, or red blood cell counts. We also noted an increase in the blood platelet count (from 370,000 ± 170,000 to 460,000 ± 220,000; P < 0.05).

Clinical Responses

In almost all the cases, progression of the treated nodules was at least stopped, compared with that of the neighboring nodules not subjected to EP but exposed to the same dose of BLM. On the whole, we observed one CR, one PR, five NC, and two cases of disease progression. One case was not evaluable.

For patient TZV, 3 days after the ECT, edema was evident in all the treated areas. The top of the treated nodules showed a leukonecrosis that dried in a few days, resulting in scabs. All the nodules disappeared by day 18. Thus a clinical CR was achieved. However, a large recurrence developed on the left submaxilla, in an area not treated during the ECT session.

For BAR1, after a single i.v. bolus injection, electric treatment was carried out during a long period (Table 2). All nodules treated before 28 minutes after the end of the BLM administration responded, and the global result was a PR. On the other hand, the nodules treated after this time limit did not show any morphological change and ulteriorly went into progression. The most evident effects, including edema, leukonecrosis, and nodule collapse, were detected in the case of the nodules treated at 15 minutes after the BLM injection.

During the BAR2 session, we treated those nodules that received the EP later than 28 minutes during the BAR1 session as well as new nodules growing outside the previously treated areas. All the nodules were subjected to the EP between 7 and 25 minutes after the end of
BLM administration. On the whole, we observed a 30% response of the treated areas in spite of the fact that this patient had an infiltrating and rapidly growing tumor.

For BAR3, we tried to perform EP delivery after i.a. BLM administration. The permeation nodules extended into both the anterior and the posterior parts of the thorax. It was possible to reach intraarterially only one-half of the area occupied by the nodules. Consequently, we decided to compare the effects of the i.a. BLM with those of the i.v. BLM, considering that the areas not colored by the Blue Patent (the i.a. tracer) were areas receiving the BLM after systemic redistribution, as after the i.v. injection of the BLM. Moreover, due to the extension of the lesions, only some nodules were electrically treated in the i.a. or in the i.v. irrigated areas. Thus four situations were compared, i.a. versus i.v., with or without the EP. Each electrically treated tumor surface received two runs of four EP at two different moments, the first during the i.a. infusion and the second a few minutes after the end of the i.a. infusion. BLM alone, either i.v. or i.a., did not show antitumor effects. In the electrically treated areas, large areas of necrosis were seen, and the effects were more intense in the i.a. regions. Due to the large initial thickness of the treated nodules, only a minor response was obtained.

For CAN, the largest nodule showed a superficial necrosis and a reduction in thickness. Small nodules disappeared and did not reappear during the follow-up period. On the whole, 2.5 weeks after the treatment, a minor response was obtained (40% reduction of lesions size). To preserve the patient's quality of life without performing a new ECT session, we began the palliative oral administration of 1500 mg/day of Hydrea 2 months after the ECT. Then, 4.25 months after the ECT session, disease remained in progression in the deepest part of the treated nodules and ulteriorly at their periphery.

For MON, the treatment was incomplete because of the great thickness of the nodules treated with surface electrodes and of the rapid proliferation of the adenocarcinoma that was extending on large surfaces. This patient received a large amount of BLM that was probably the cause of a transient dyspnea 5 days after the ECT. A slight reduction in the thickness of the treated nodules was detectable, but, on the whole, disease was still in progression.

DISCUSSION
Electrochemotherapy is a new antitumor treatment that relies on the permeabilization of the tumor cells by means of EP locally delivered to the tumor. This allows nonpermanent cytotoxic drugs such as BLM to enter the transiently permeabilized cells and to kill them at plasmatic BLM concentrations that are usually devoid of antitumor or secondary effects. A previous first Phase I–II clinical trial of ECT proved the feasibility of this treatment for human patients, and we extended this in the present study. Our purpose was to address several questions and to determine how to improve ECT in the near future.

Tolerance to the Delivery of High Numbers of Electric Pulses
In contrast to the initial study, in this study we treated patients with a large number of nodules and/or extended nodules covering a large surface. Thus, in several cases, treatment required the delivery of about 100 runs (e.g., BAR2, BAR3), or more (LAZ), each with four EP, i.e., a total of several hundred EP. To perform the treatment in a relatively short period (as discussed below), patients were treated under neuroleptanalgesia. No adverse side effects were observed during or after the delivery of these EP.

The present results indicate that ECT remains safe even when large numbers of EP are delivered. The results also show that the disagreeable sensation that accompanies the EP delivery can be totally overcome by neuroleptanalgesia or general anesthesia. Thus ECT is well tolerated even by patients in poor performance status and suffering from advanced malignancies.

The present study also confirms that a patient can be treated several times (e.g., BAR and LEN), even if each treatment requires a high number of EP. However, we have not yet determined the optimal length of time between two consecutive treatments.
Definition of the Time Window Appropriate for Delivery of Electric Pulses after Bleomycin Injection

The ECT principle is to permeabilize the tumor cells when BLM in an appropriate concentration is present in the interstitial fluid bathing the tumor cells. The pharmacokinetics of BLM concentration in tumor interstitial fluid are not well known, even if they can be approached from BLM pharmacokinetics in blood plasma. In mice, the time window during which EP allows obtaining the most intense antitumor effects is from 3 to 7 minutes after the i.v. injection of 10 µg BLM10 (also, unpublished results). The BARI session, in particular, allowed us to evaluate this window in the case of human patients with permeation nodes of head and neck squamous cell carcinoma: Clear antitumor effects were observed from 8 to 28 minutes after the end of the i.v. bolus of 15 mg/m² BLM. The creatinine levels in this patient were in the normal range. For patients with low renal function, the end of the window might be at a longer time after the BLM injection.

Bleomycin Intraarterial Injection

As was indicated above, the essential point is to achieve a sufficient BLM concentration in the interstitial fluid at the time of EP delivery. We assumed that the i.a. injection of the drug could allow us more easily to reach this concentration in the targeted tissue. In the second treatment of patient LEN, we actually observed a large increase in the antitumor effects of BLM. We also observed considerable skin toxicity. As the dose injected i.a. was equivalent to that injected i.v., the observed effects could in fact be due to an increased local concentration of the drug. This observation demonstrates that ECT can be performed after BLM i.a. supply and that the doses used probably can be decreased.

A comparison of i.a. with i.v. supply was performed during the third treatment of patient BAR. In fact, it was not possible to introduce a catheter capable of supplying BLM to all of the extended tumor area. Again, a higher efficacy of the EP was observed in the area drained by the catheterized artery, previously determined by Blue Patent infusion. The window for EP delivery after the beginning of the infusion was not determined in detail. However, BLM threshold concentration for ECT efficacy seems indeed to be more easily attained after i.a. injection. Thus, we can speculate that, according to the infusion protocol followed (one-half of the dose as initial bolus and then one-half of the dose as continuous infusion for 15 minutes), this window remains open at least during all the infusion period and a few minutes after its end. Decreasing the doses of BLM injected i.a. seems possible but requires further trials.

Treatment of Thick Nodules

ECT efficacy depends on obtaining cell permeabilization by EP. We have demonstrated in preclinical studies7 the importance of the geometry of electric field lines of an intensity higher than the threshold value necessary for cell permeabilization in tissues (400-550 V/cm). Because we used surface electrodes delivering the EP through the skin, it was obvious that the depth to which efficient electric field lines could reach was limited. Thus the deepest portions of thick nodules were assumed to escape the permeabilization.

We treated thick nodules and, as expected, large antitumor effects were observed on the superficial parts of the tumor. Moreover, a large portion of the nodule appeared necrosed after a few days, which resulted in a significant reduction of the nodule thickness. However, as expected, we did not obtain complete sterilization of these treated nodules. The interest of these assays lies in the fact that they allowed us to ascertain the constraints that a good EP applicator must take into account, as presented below.

Antitumor and Cutaneous Effects

In spite of the fact that the external electrodes were not well adapted to the treatment of the large nodules encountered in this study, we can conclude that evident antitumor effects were obtained in the case of extended and thick nodules. However, the level of the skin, the results are very different from those previously described.17 Indeed, in this trial, nodules were not large enough to alter the skin beneath them. As a consequence, the cutaneous pavement was able to reconstruct an apparently normal skin in the area subjected to the EP, even if some scars or burns were transiently observed. In the present study, the size of the nodule resulted in an alteration of the skin over the nodule prior to the ECT. Cutaneous pavement was very thin, and even in the absence of ECT it would have become necrotic after several days of tumor evolution. In this situation, it seemed that ECT accelerated the appearance of this cutaneous necrosis. This has to be taken into account in the follow-up of the patient after ECT in order to prevent it insofar as possible. However, the best method appears to be to try to avoid this effect on the skin by delivering the EP not transcutaneously but directly to the nodule, using the electrodes described below.

Extension of the Electrochemotherapy to Nodules of Others Origins

Preclinical studies supported the notion that ECT can be applied to tumors of quite different origins: in animals we treated transplanted melanomas, carcinomas, sarcomas and gliomas.8-11 In fact, once inside the cell, the chemical reaction of BLM on DNA is very rapid8 and obviously independent of the origin of the cell. Because electro-permeabilization is a universal phenomenon, in principle.
any kind of tumor can be treated by ECT, whatever its usual sensitivity to BLM. Indeed, intact cells’ sensitivity to BLM depends mainly on the cell-dependent efficacy of BLM transport across the plasma membrane (G. Pron et al, in preparation).

The treatment of patient MON demonstrated that antitumor effects can also be obtained in the case of breast cancer nodules. Moreover, part of the nodules treated were ulcerated at the time of treatment. It was possible to treat those nodules still covered by the skin using the usual set of electrical parameters. However, the nodules devoid of a cutaneous pavement were considered to be in the same situation as the intracerebral11 and the intraabdominal (L. Ramirez et al, in preparation) tumors treated in preclinical studies,7 for which pulses of only 800 V·cm−1 are applied, following our basic research results.7 As was expected, antitumor effects were also observed in these areas.

**Design of New Electrodes for Antitumor Electrochemotherapy**

To reach the deepest portion of the nodules, EP have to be delivered in depth. To protect the cutaneous pavement as much as possible, the electric field should not be delivered through the skin. Finally, because the number of EP is not a limiting factor, it seemed possible to divide the volume of the large tumors using an array of needles that would be connected in pairs to the electric generator. The needles would be inserted in depth into the tumor, so that the deepest portions of the tumors would be subjected to the EP. These conductive needles would be covered by nonconductive material (for example, Teflon) at their base, that is, at the level of the skin once the needle is inserted in the tumor. Thus, with the same electrical parameters as those used here in the session MON for the nodules not covered by skin (800 V·cm−1), large tumors would be treated with EP delivered through pairs of needles of an array of needles. A prototype has been built, and preclinical evaluation shows that it is suitable for all the purposes mentioned above (Ramirez et al, in preparation; Mir et al, in preparation).

In summary, in spite of the limitations resulting from the use of transcutaneous EP, we have demonstrated here that there is good tolerance to a very high number of EP, that ECT can be repeated without impairment of the general health status, that antitumor effects can be obtained even in the case of large tumors, and that nodules of an origin other than head and neck squamous cell carcinomas can be treated by ECT. We also have defined the therapeutic time window for the EP delivery after an i.v. bolus of BLM. Thus, the conditions for initiating Phase II studies on less dramatic carcinologic situations seem to be fulfilled.

**REFERENCES**
