Bipolar Radiofrequency Induced Thermotherapeutic Volumetric Reduction of VX2 Metastases in an Animal Model

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Abstract. Background: Radiofrequency-induced thermotherapy has shown promising results in the palliative treatment of various tumor entities. The purpose of this study was to investigate the effectiveness of bipolar radiofrequency volumetric tissue reduction (VTR) on lymph node (LN) metastases in the VX2 SCC model. Materials and Methods: Six male New Zealand white rabbits, with palpable metastatic disease within the parotid lymph nodes, were treated using the Celon-ProSurge® probe, needle length 110 mm, diameter of 2.3 mm. The animals were sacrificed on the 4th, 8th, 11th, 14th, 18th and 22nd postoperative days respectively. Results and Conclusion: Bipolar radiofrequency VTR could prevent progression of local metastatic disease in one-third of the animals compared to the control group of untreated VX2 carcinoma rabbits. These results encourage further studies, directed at whether this treatment modality could play a role in the palliative therapy of metastatic LN. Future studies should concentrate on the refinement of the treatment parameters and optimization of the treatment duration.

The prognosis of squamous cell carcinomas of the head and neck is predominantly limited by the progress of lymphatic metastatic spread (1, 2). The survival time of patients with an initially advanced tumor stage, e.g. multiple lymph node metastases, can frequently not be elongated despite intensive oncological follow-up (3, 4). According to the results obtained by von Wolfensberger (5), a curative secondary treatment can only be performed in patients with a low T category without recurrent lymph node disease. Considering the full course of the disease, one has to weigh up the risks and side-effects of an extensive surgical intervention, which usually requires extensive reconstructive procedures with surgical flaps. Tumors are occasionally untreatable by the standard means of salvage (6). On the other hand, there is frequently a remarkable desire for ongoing treatment by the patients and a willingness to continue therapy by the physicians. Palliative treatment should prevent devastating complications like a perforation of the metastases through the skin and/or compression, infiltration and erosion of blood vessels by unhindered metastatic growth and spread (7-9). In such palliative situations, there is an increasing need for and interest in new methods of therapy, which at best would be able to stop the tumoral progress and could be realized in an out-patient setting.

Radiofrequency-induced thermotherapy has shown promising results with minimal complications in the palliative treatment of hepatic cell carcinoma, hepatic metastases and advanced lung cancer (10-15). Bipolar radiofrequency interstitial thermotherapy is a technique by which a probe is inserted into the tissue and heated to temperatures ranging between 80°C and 100°C. This allows a targeted interstitial treatment by delivering radiofrequency energy and consecutive heat in a well-circumscribed area around the needle electrode. The main practical advantages of the probes introduced by Celon® is the unification of a bipolar electrode in a single needle, making a neutral electrode unnecessary and avoiding the flow of current through adjacent tissue or organs. Since it uses a low power level (<25W), it generates relatively low tissue temperatures (max. 100°C), causing thermal damage to the tissue, thus resulting in tissue ablation by volume reduction due to secondary scarring.

While reported studies have targeted the impact of this treatment modality on pulmonary and hepatic metastases or primary tumors of these organs, the aim of this experiment was to evaluate the efficacy of bipolar radiofrequency therapy to lymph node (LN) metastases of squamous cell carcinoma (SCC). For the study the VX2 SCC model in New Zealand white rabbits was chosen. This animal model features metastatic spread along a constant cascade similar
to the metastatic spread of human head and neck squamous cell carcinoma (HNSCC) (16-18).

Special attention was paid to the question of whether any volume reduction of the tumor could be observed or downgrading of the status of the LN treated with Celon®’s interstitial thermotherapy could be noted. The treatment consisted of a single therapy session, following the instructions recommended by the manufacturer.

Materials and Methods

The animal use protocol was approved by the Institutional Animal Care Use Committee (IACUC) of the government of Giessen, Germany. The experiments were performed in accordance with the guidelines of the Declaration of Helsinki and in accordance with the Public Health Service Policy of Humane Care and Use of Laboratory Animals (19).

Adult male Iffa Credo New Zealand white (ICO:NZW) rabbits, weighing 1.5 – 2 kg, were used for the study. All the animals were healthy and free of any pathogens. In 6 male New Zealand White rabbits, a VX2 squamous cell carcinoma was induced on the right auricle by injecting 0.3 ml of freshly-prepared VX2 cell suspension subcutaneously, as previously described (16). On the 18th day, a R0 resection of the tumor was performed. The animals were anesthetized with 5mg/kg xylazine hydrochloride (Rompun 2%, Bayer Vital GmbH, Leverkusen, Germany) and 100mg/kg ketamin hydrochloride (Ketavet, Pharmacia GmbH, Erlangen, Germany) (20). The resection line was placed 2 to 2.5 cm distally to the inferior margin of the tumor. The wound was sutured with a 4/0 Prolene®, curved PS-2 needle (Ethicon GmbH, Nordersted, Germany). The neck status was clinically estimated by palpation on a daily basis. The findings were recorded in the following way: 0-no palpable or suspicious neck line was placed 2 to 2.5 cm distally to the inferior margin of the tumor. The wound was sutured with a 4/0 Prolene®, curved PS-2 needle (Ethicon GmbH, Nordersted, Germany). The neck status was clinically estimated by palpation on a daily basis. The findings were recorded in the following way: 0-no palpable or suspicious neck masses; 1-suspicious structure that could not well be differentiated from the normal surrounding structures; 2-clearly palpable mass. For larger masses, dimensions in centimetres were recorded.

The treatment application was performed on anesthetized animals using the Celon-ProSurge® probe (Olympus, Hamburg, Germany) as the applicator of the thermocoagulation. The needle has a length of 110 mm and diameter of 2.3 mm, consisting of a trocar-shaped tip to facilitate its insertion into the tissue and also of marks for measuring the insertion depth. The power was supplied by the Celon Lab PRECISION® power control unit (Olympus) with a power output of 1-25W. The Celon Aquaflow™ Pump system (Olympus) with a power output of 1-25W. The Celon Aquaflow™ Pump system (Olympus) served as the internal cooling device for the applicator, which was filled with distilled water at room temperature as the only cooling fluid. The cooling pump was adjusted to levels 2-3, producing a flow of 20-30 ml/min of distilled water. Each animal was treated once in a single setting. The applied power was 20W, the duration of the application varied between 15 to 30 seconds (15, 15, 20, 25 and 30 seconds, respectively) and was limited by the acoustic feedback signal generated by the power unit, alerting an increase of impedance within the treated tissue and thus preventing adhesions of the probes to the tissue or damage to the adjacent tissue. The cooling pump was adjusted to levels 2-3, producing a flow of 20-30 ml/min of distilled water.

The animals were sacrificed on the 4th, 8th, 11th, 14th, 18th and 22nd postoperative day respectively, with intravenous application of 1 ml T-61 (Intervet Deutschland GmbH, Unterschleißheim, Germany), after being anesthetized with 100mg/kg ketaminhydrochlorid (Ketavet, Pharmacia GmbH, Erlangen, Germany).

In all animals, the sentinel parotid LN was removed and placed in 4% neutral-buffered formalin. After fixation in formalin for 48 hours, the specimens were further processed. The slides were cut on a sliding microtome at 1000 µm. The slides were examined by conventional light microscopy. Special attention was paid to the aspect of the tumoral cells in the LN metastases, the necrosis of the tumor and the fibrosis. To document these items in a more quantitative manner, a rating scale was devised as follows:

For the tumoral aspect (T):
Grade 0 (TG0): No tumoral cells.
Grade 1 (TG1): Viable tumoral cells are detected under the capsule of the tumor exclusively.
Grade 2 (TG2): Viable tumoral cells are detected under the capsule of the tumor with strains to the centre of the LN.
Grade 3 (TG3): Viable tumoral cells are detected disseminated within the LN.

For the induced necrosis (N):
Grade 0 (NG0): No necrosis at all.
Grade 1 (NG1): Small necrotic areas, less than 50% of the tissue.
Grade 2 (NG2): More than 50% of the metastasis is necrotic.
Grade 3 (NG3): Subtotal necrosis of the tumor with few if any viable areas.
Grade 4 (NG4): Total necrosis. No viable tumoral areas. Only LN capsule filled with detritus.

For the induced fibrosis (F):
Grade 0 (FG0): No fibrotic changes in the LN.
Grade 1 (FG1): Subcapsular fibrotic areas.
Grade 2 (FG2): Fibrotic strains stretching from the periphery (capsule) to the centre.
Grade 3 (FG3): Areas of fibrosis spread throughout the LN.

Each single slide was graded according to this scale. On this basis, the mean score for every single LN was calculated.

Results

After the resection of the primary tumor, no local recurrences developed. The histological examination of the resection border after sacrifice revealed no tumoral cells. In all animals, the resection was R0. In this way, the further development of the LN metastases was not influenced by migration of new tumoral cells from the primary location, but rather represents autonomous tumoral development of the metastases.

The metastatic LNs presented with a thick fibrotic capsule. They were usually firmly connected to the underlying neck structures. The LN metastases in the parotid region could be found in close proximity to the large collector vein of the auricle – the caudal auricular vein. A common adjacent surface between the LN and this vessel could be noted. Nevertheless, there were no macroscopic signs of bleeding from vessel erosion.

The dimensions of the metastases upon primary tumor resection, bipolar radiofrequency therapy and on the day of...
sacrifice are presented in Table Ia and Ib. Table Ic summarizes the observation, if and when any volume reduction of the LN or downgrading of their status occurred. Further, the changes of volume during the observation period were analyzed (Figure 1).

Histological findings. The LN metastases at the point of time of the histological examination presented advanced disease with almost complete replacement of the typical LN structures by tumoral cells. The general aspect of the tumor was of a SCC with a very low differentiation and anaplastic appearance. The cells were highly pleomorphic. They were polygonal, contained large cytoplasm and enlarged nuclei. The cell borders were usually well-delineated and sharp and the nucleus was centrally located. Nuclear enlargement and pleomorphism was pronounced. The nuclear chromatin was markedly hyperchromatic, with a coarsely granular pattern and marked chromatin clumping. Mitotic figures were commonly observed. The prominent nucleoli and the mitotic figures, if evident, proved the low differentiation of the tumor. All metastatic LN showed different degrees of necrosis and fibrosis (Table II). The mean tumor score averaged from 1.25 to 2.8, while the mean necrosis score varied from 0.75 to 2.75. Necrosis could also be observed in the LN, which was without evidence of tumoral cells. Only one LN was without evidence of any fibrosis. The fibrosis score averaged from 0 to 2.75, thus showing a great variation without correlation to the period of observation.

Discussion

The prognosis of HNSCC is mainly limited by the progress of lymphatic metastatic spread. As lymphatic metastasis occurs, the treatment of choice consists of surgical removal of metastatic LN by neck dissection and / or radio-chemotherapy. Despite intensive efforts to optimize the treatment of carcinomas of the upper aero-digestive tract, the survival time of patients with an initially advanced tumor stage, e.g. multiple LN metastases, can frequently not be prolonged despite intensive oncological follow-up. Considering the full course of the disease, the risks and side-effects of an extensive surgical intervention have to be weighed up with the expected survival time. This especially addresses cases with significant comorbidities, with extended tumors infiltrating vital structures and / or contraindications for radiation therapy. In palliative situations, the treatment modality of choice should keep hospital stays as short as possible, to improve or maintain the quality of life, and to keep side-effects or the risk of iatrogenic complications as low as possible. It is necessary to comply with the expectations of the patients to be treated effectively with the
adequate treatment option. In addition to this expectation, physicians desire to treat these cases: to avoid lethal complications like metastatic break-through, infiltration of vital organs and vessel erosions or compression and to alleviate disabling, disfiguring and painful symptoms.

Radiofrequency-induced thermotherapy has shown promising results in the palliative treatment of liver tumors, hepatic metastases and advanced lung cancer (10-15). Success has also been reported from the treatment of other tumor entities like renal cell carcinoma (21), and also of benign lesions like prostate hyperplasia (22), meningeoma (23), osteoid osteoma (24), lymphangiomatous macroglossia (25), hypertrophic turbinates (26) and hyperplastic tongue base (27). In the head and neck region, it has been utilized for the treatment of recurrent tongue carcinoma (28) and advanced carcinomas of the oropharynx, oral cavity, maxillary sinus and medullar thyroid carcinoma, using power levels of 90 – 150 W and treatment durations of 5 to 15 minutes (29).

The aim of this investigation was to examine the impact of radiofrequency treatment on apparent LN metastases. Therefore, an animal model was chosen, which is representative for the pattern of lymphatic metastatic spread of SCC. The VX2 auricle model has been used for the evaluation of chemotherapy protocols (30), optimization of intraarterial tumor embolisation (31-33), immunomodulation (34, 35), study of tumor metabolism (36) and studies on piecemeal resection of tumors with CO2-laser (37). In the presented animal model, the resection of the primary tumor was performed before the radiofrequency therapy for the following reasons: to avoid bleeding caused by vessel erosion and to ensure the efficacy of the treatment, not being manipulated by migration of new tumoral cells from the primary tumor into the examined LN.

In hepatic tumors, the maximum size of tissue ablation which could be achieved by radiofrequency therapy ranged from 3 cm to 5.2 cm in diameter (38). Frieser and coworkers (38) found correlation of the coagulation volume with the applied energy, the duration of power application and the perfusion rate in an ex vivo bovine liver model. Kim and coworkers (39) report on a mean coagulation diameter of

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**Figure 1. Assessed diameters of treated lymph nodes.**

**Table II. Semi-quantitative estimation of the tumoral appearance in the metastatic LNs (TG-score), the degree of induced tumoral necrosis (NG-score) and the degree of fibrosis (FG-score).**

<table>
<thead>
<tr>
<th>Animal</th>
<th>LN size at end-point</th>
<th>Mean TG score</th>
<th>Mean NG score</th>
<th>Mean FG score</th>
</tr>
</thead>
<tbody>
<tr>
<td>437</td>
<td>0.8</td>
<td>1.5±1.7</td>
<td>0.75±0.5</td>
<td>2.25±1.5</td>
</tr>
<tr>
<td>436</td>
<td>2.0</td>
<td>1.4±1.34</td>
<td>0.83±0.75</td>
<td>1.66±1.37</td>
</tr>
<tr>
<td>434</td>
<td>2.5</td>
<td>2.08±0.67</td>
<td>1.75±0.75</td>
<td>1.08±1.08</td>
</tr>
<tr>
<td>435</td>
<td>1.5</td>
<td>1.25±0.5</td>
<td>2.75±0.5</td>
<td>0±0</td>
</tr>
<tr>
<td>438</td>
<td>1.0</td>
<td>2.8±0.45</td>
<td>1.2±0.45</td>
<td>1.6±1.14</td>
</tr>
<tr>
<td>433</td>
<td>0.8</td>
<td>0±0</td>
<td>1.1±0.55</td>
<td>1.4±1.01</td>
</tr>
</tbody>
</table>
1.46 cm with radiofrequency therapy alone and an increase of the coagulation volume through the addition of hot saline irrigation in a rabbit liver model. The extent of coagulation necrosis has been reported to be dependent on several factors, such as applied energy, duration of heat exposure, organ perfusion, fluid content of the tissue, probe geometry and other factors (40). Giorgio and coworkers (10) showed, in a study on 84 patients, that radiofrequency ablation was an effective and safe method of treatment for hepatocellular carcinoma, especially for those tumors measuring up to 3 cm in diameter. Vogl and coworkers (14) achieved a local tumor control rate of 85% in a 6-month follow-up of 20 patients with pulmonary metastases treated with CT-guided radiofrequency ablation. The above-mentioned results encourage interest in whether this treatment modality could be utilized for the therapy of metastatic LN in palliative situations.

Two of the 6 animals showed progress of growth over a time period of 4 days or 22 days, respectively. The third animal showed a volume reduction and then a steady condition after an initial increase of size. This could be observed over a total observation period of 18 days. Furthermore, it was noticed that a regression occurred after an initial post-treatment progression within 11 days and also an initial progression, regression and a renewed increase of volume could be noted within 14 days. However, the final volume did not exceed the size of the initial metastasis.

Summarizing, bipolar radiofrequency prevented size progression in one-third of the treated animals compared to the observations made in untreated VX2 carcinoma animals (16). The untreated VX2 carcinoma animal revealed large LN metastases combined with a more accelerated volume increase in an identical observation period.

The observations made under these conditions encourage the setting up of protocols in which multiple applications with optimized power levels and treatment durations could offer an additional treatment option for recurrent LN metastases, where other treatment modalities have failed. The therapy itself is minimally-invasive and causes only minimal discomfort.

Another prospect for the use of radiofrequency energy is the potential option to intensify the effect of radio- and chemotherapy. Some trials have proved the efficacy of a combination of hyperthermia (40-42°C) and radiation therapy in reference to local tumor control (41). Heywang-Köbrunner and coworkers (42) examined a breast tumor specimen, which was treated with radiofrequency therapy before being biopsied. They stated that it might be possible to inactivate tumor cells without significantly impairing the morphological architecture of the tumor, provided the applied temperature did not exceed 70°C. They concluded that this might facilitate the recovery of histopathologically representative biopsies from suspected organs without the risk of tumoral cell spread.

The intra-interventional monitoring of produced temperatures in the depth of the tissue would be a very beneficial tool to correlate the histological findings and volumetric changes induced by thermotherapy with the applied energy and other contributing factors. This will eventually open up new perspectives for the evaluation of the significance of radiofrequency-induced thermotherapy in the treatment of head and neck cancer. A first step would be the initiation of a trial on limited applications of radiofrequency energy with temperatures below the coagulative-ablative effect in an animal model. It would be of special interest to compare the impact of a sole thermoablative technique with a combined thermo-sensitizing radio- or chemotherapy, to evaluate the effectiveness of radiofrequency therapy on the chemosensitivity of lymphatic metastases and to consider if any additional thermosensitive impact besides the coagulation-induced tumor ablation exists.

References