Journal of BUON 9: 47-50, 2004
© 2004 Zerbinis Medical Publications. Printed in Greece

ORIGINAL ARTICLE

Electrochemotherapy of skin tumours: comparison of two electroporation protocols

E. Peycheva[1], I. Daskalov[2]

[1]National Hospital of Oncology, Clinic of Oncodermatology, Sofia; [2]Centre of Biomedical Engineering, Bulgarian Academy of Sciences, Sofia, Bulgaria

Summary

***Purpose**: To study the effect of electric pulses duration and number in a sequence for electrochemotherapy of primary non-melanoma skin tumours.*

***Patients and methods**: Two electroporation sequences were applied: sequence A, consisting of 16 biphasic pulses of 25+25 μs duration, spaced at 0.6 ms, with a duration-number product of 0.8 ms; and sequence B, consisting of 8 50+50 μs biphasic pulses, spaced at 1.0 ms, with the same duration-number product of 0.8 ms.*

The two sequences were used for electrochemotherapy of clinical stage I (T1N0M0) basal cell and spindle-squamous cell carcinoma lesions in two groups of patients. Sequence A was applied to 59 patients (group A; subgroup A1 with 45 patients with basal cell carcinoma, and subgroup A2 with 14 patients with spindle-squamous cell carcinoma) and sequence B to 54 patients (group B; subgroup B1 with 40 patients with basal cell carcinoma, and subgroup B2 with 14 patients with spindle-squamous cell carcinoma). Intratumoral bleomycin was administered to all patients.

***Results:** All group A and B patients achieved initially complete response (CR). The sustained CR rate in subgroup A1 patients was 80%, due to 9 recurrences and for subgroup A2 patients it was 71.4% because of 4 recurrences. No subgroup B1 patient developed recurrence. Three subgroup B2 patients showed disease recurrence, for a sustained CR rate of 78.6%.*

***Conclusion**: The efficiency of electrochemotherapy in cutaneous non-melanoma tumours depends on the duration of electric pulses. For biphasic pulses, duration below 50 μs per phase (or 50+50 μs for the total pulse) is not recommended, in spite of a markedly reduced pain sensation. The rule about equal efficiency of larger number of shorter pulses and longer pulses of lesser number has its limits for external application, especially through the skin.*

Key words: cutaneous tumours, electrical pulse parameters, electrochemotherapy, therapeutic efficacy

Introduction

The high morbidity of malignant non-melanoma skin tumours, involving lymphatic and hematogeneous metastatic spread, outline their importance in the clinical therapeutic practice. Electrochemotherapy for the treatment of these tumours, applied for about a decade, has proven very effective [1,2]. Our experience in the treatment of basal cell and spindle-squamous cell carcinoma (T1N0M0) with series of biphasic electropermeabilization pulses showed very high CR rates [3-5]. We also treated several rare cases of mycosis fungoides (clinical stage I, 3 patients with 8 lesions, CR 100%) and Kaposi's sarcoma (2 patients with 7 lesions, CR in 6 of them) [6,7].

Traditionally pulses of 100 μs have been used with 1s interpulse interval [1,2]. In our previous treatment protocols [3-5] we used 8 biphasic pulses of 50+50 μs duration and 1 ms interpulse interval, resulting in a total sequence duration of 7.1 ms. We

Received 05-01-2004; Accepted 17-02-2004

Author and address for correspondence:

Ivan Daskalov, MSEE, PhD, DrSc
Centre of Biomedical Engineering
Bulgarian Academy of Sciences
Acad. G. Bonchev Str., Block 105
Sofia 1113
Bulgaria
Tel: +359 2 8700326
Fax: +359 2 723 787
E-mail: ikdas@argo.bas.bg

have selected these shorter biphasic pulses and much shorter interpulse interval, as they resulted in a single sensation for the patient, instead of the of 8 "classical" successive pulses. Our motive was that this sequence was much better tolerated by the patients, especially by those with lesions on sensitive locations such as lips, eyelids etc. We also speculated that biphasic pulses might permeate a higher number of cells, as cell orientation and size in a compact tissue vary with respect to the field polarity. In addition, the pore formation has been considered a process, which develops within microseconds [8].

Subsequent theoretical and experimental studies of the Mir group were in agreement with our results, concerning the better efficiency of bipolar pulses [9] with higher pulse repetition frequencies [10].

Meanwhile Orlowski and Mir have reported that the *in vitro* electropermeabilization effect depends on the total electrical charge introduced in a given tissue [11]. In other words, the product of pulse duration and amplitude and the number of pulses per series was the determining factor. It would follow then that increased number of shorter pulses could be used in a sequence.

Having already established that shorter pulses and interpulse intervals, under all equal conditions, are better tolerated by the patients, we decided to try a modified pulse sequence.

The objective of the present study was to investigate whether higher number of shorter pulses would yield the same or similar treatment outcome compared to our previous results [3-7].

Patients and methods

This study benefited from a previous approval of the Ethical Committee and presently electrochemotherapy is a routinely applied therapeutic modality in the National Hospital of Oncology.

According to the object of our study, we prepared two electroporation sequences:

A) 16 biphasic pulses of 25+25 μs duration, spaced at 0.6 ms, with a duration-number product of 0.8 ms and total sequence duration of 9.6 ms

B) Our previous sequence of 8 biphasic pulses of 50+50 μs duration, spaced at 1.0 ms, with a duration-number product of 0.8 ms and a total sequence duration of 7.1 ms.

Two groups of patients were treated by electrochemotherapy, each with one of these two electric pulse sequences.

Group A, treated with pulse sequence A, consisted of 59 patients with 59 lesions of basal cell and spindle-squamous cell carcinoma. Subgroup A1 included 45 patients (37 women and 8 men) with 45 lesions of basal cell carcinoma (T1N0M0, clinical stage I). Subgroup A2 included 14 patients (9 women and 5 men) with 14 lesions of spindle-squamous cell carcinoma (T1N0M0, clinical stage I).

Group B, treated with pulse sequence B, included 54 patients with 54 lesions of basal cell and spindle-squamous cell carcinoma. Subgroup B1 included 40 patients (27 women and 13 men) with 40 lesions of basal cell carcinoma (T1N0M0, clinical stage I). Subgroup B2 included 14 patients (5 women and 9 men) with 14 lesions of spindle-squamous cell carcinoma (T1N0M0, clinical stage I).

Additional details for the subgroups are given in Table 1.

Inclusion criteria were as follows:

1. Confirmed diagnosis of the primary tumour by cytology or histology.
2. Clinical TNM disease stage I (T1N0M0).
3. Absence of lesions in the thoracic cardiac region (for patients with implanted pacemaker or defibrillator).
4. Monthly follow-up for at least 12 months.

All patients gave signed informed consent after been informed about their respective disease and the

Table 1. Patient groups and data

Patient groups	*No of patients*	*Mean age (years)*	*No of lesions*	*Mean lesion size (mm)*	*Mean bleomycin dose (units)*	*Mean electr.field intensity(V.cm^{-1})*
A1	45	66.45±1.23	45	13.2±1.85	0.5	1200
A2	14	59.5±0.96	14	10.2±1.50	0.5	900
B1	40	62.25±1.2	40	10.64±4.55	0.75	1200
B2	14	67.5±0.9	14	9.0±4.21	0.8	900

A1 – basal cell carcinoma patients treated with 25+25 μs pulses; A2 – spindle-squamous cell carcinoma patients treated with 25+25 μs pulses; B1 – basal cell carcinoma patients treated with 50+50 μs pulses; B2 – spindle-squamous cell carcinoma patients treated with 50+50 μs pulses

existing types of therapy (surgery, radiotherapy and chemotherapy) and about electrochemotherapy as an alternative method with all details about the procedure of application, expected outcome and possible side effects.

The previously established electrochemotherapy procedure was applied [4-7]. Briefly, it consisted of injection of lidocaine (1%, 1-3 ml around the lesion), followed by intratumoral injection of bleomycin (dose approximately corresponding to the tumour size, and ranging between 0.5-0.8 units), and application of the electrical pulses after 10 min. The generator (Chemopulse, Centre of Biomedical Engineering, Sofia) was programmed to deliver the above described pulse sequences A and B. The intensities were about 1200 $V \cdot cm^{-1}$ for transcutaneous application and about 900 $V \cdot cm^{-1}$ for mucosal tissue.

Results

All group A and B patients achieved initially CR. However, in 9 A1 subgroup patients with 9 lesions, suspected tumour recurrence was confirmed by histological examination. In 3 of them recurrence occurred after 2 months and in the remaining 6 after 5 months. Therefore, the sustained CR rate for this patients' subgroup was 80%.

The results for subgroup A2 patients with spindle-squamous cell carcinoma were as follows: recurrence was found in 3 of them at the 2nd monthly control and in one more at the 4th month, for a sustained CR rate of 71.4%.

In subgroup B1 patients with basal cell carcinoma the CR rate was 100% with no tumor recurrence for the 12-month follow-up period.

Three patients from subgroup B2 with spindle-squamous cell carcinoma had disease recurrence at the 6th month for a sustained CR rate of 78.6%.

These results are summarized in Table 2, where results from one of our previous studies (using 50+50 μs pulses) [5] are added for comparison. That study included 99 patients with 194 lesions of basal cell carcinoma and 23 patients with 23 lesions of spindle-squamous cell carcinoma. The continuous CR rate was above 95%.

Discussion

According to the objective of our study, it can be concluded that the pulse sequence B, with higher number of shorter (25+25 μs) pulses, is considerably less efficient than the sequence with 50+50 μs pulses (Table 2). CR dropped from 100% to 80% for basal cell carcinoma and from 78.6% to 71.4% for spindle-squamous cell carcinoma.

These results are also inferior compared with our previous study [5], which was performed using the 50+50 μs pulse sequence only, with CR rates of 97.3% and 95.65% for basal cell and spindle-cell carcinoma, respectively.

These data suggest that most probably short pulses are smoothed (or integrated) by their passage through solid tissue, resulting to less sharp rising edge and reduced amplitude. Of course, it is possible to compensate the lowered pulse amplitudes by raising the field intensity, but this way the advantage of pain reduction would be practically lost.

It is possible to consider the cell membrane capacity as an integrating factor too. The shortest pulses used by Orlowski and Mir were 10 μs [11]. This could be adequate for *in vitro* electroporation. However, in case of solid tissue it could be speculated that the short pulses were already affected by their passage through superficial structures before reaching tumour cell membranes.

We conclude that the therapeutic efficacy of electrochemotherapy for non-melanoma cutaneous tumours depends on the duration of electric pulses. For

Table 2. Summary of treatment results

Patient groups	*No of patients*	*Lesions localization and number*	*Sustained CR (%)*
Basal cell with short pulses (A1)	45/45	face 29, chest 8, arm 5, leg 3	80
Spindle-squamous cell with short pulses (A2)	14/14	lover lip 8, abdomen 3, arm 1, leg 2, back 1	71.4
Basal cell with long pulses (B1)	40/40	face 27, arm 4, leg 2, back 5, abdomen 2	100
Spindle-squamous cell with long pulses (B2)	14/14	lower lip 7, abdomen 2, back 1, leg 2, neck 2	78.6
Basal cell with long pulses (previous study, [5])	99/129	face 96, back 10, arm 10, leg 11, abdomen 2	98.3
Spindle-squamous cell with long pulses (previous study, [5])	23/23	lower lip 16, face 3, arm 2, leg 1, abdomen 2	96.6

biphasic pulses, duration below 50 μs per phase (or 50+50 μs for the total pulse) is not recommended. The rule about equal efficacy of larger number of shorter pulses and longer pulses of lesser number has its limits for external application, especially through the skin.

References

1. Mir LM, Glass LF, Sersa G et al. Effective treatment of cutaneous and subcutaneous malignant tumors by electrochemotherapy. Br J Cancer 1998; 77: 2336-2342.
2. Hofmann GA, Dev SB, Nande GS, Rabussay D. Electroporation therapy of solid tumors. Crit Rev Ther Drug Carrier Syst 1999; 16: 523-569.
3. Daskalov I, Mudrov N, Peycheva E. Exploring new instrumentation parameters for electrochemotherapy. Attacking tumors with bursts of biphasic pulses instead of single pulses. IEEE Eng Med Biol 1999; 18: 62-66.
4. Peycheva E, Daskalov I. Electrochemotherapy of skin tumours. J BUON 1999; 4: 185-188.
5. Peycheva E. Electrochemotherapy of cutaneous malignant tumours. CR Bulg Acad Sci 2001; 54: 103-106.
6. Peycheva E, Daskalov I. Electroimmunotherapy of mycosis fungoides: first results. J BUON 2000; 5: 85-87.
7. Peycheva E. Electrochemotherapy of Kaposi's sarcoma. CR Bulg Acad Sci 2001; 54: 101-103.
8. Saulis G, Venslauskas MS. Cell electroporation. Part 1. Theoretical simulation of the process of pore formation in a cell. Bioelectrochem Bioenerg 1993; 32: 221-235.
9. Kotnik T, Pucihar G, Rebersek M, Miklavcic D, Mir LM. Role of pulse shape in cell membrane electropermeabilization. Bioch Biophys Acta 2003; 1614: 193-200.
10. Pucihar G, Mir LM, Miklavcic D. The effect of pulse repetition frequency on the uptake into electropermeabilized cells in vitro with possible applications in electrochemotherapy. Bioelectrochem 2002; 57: 167-172.
11. Orlowski S, Mir LM. Cell electropermeabilization: a new tool for biochemical and pharmacological studies. Biochim Biophys Acta 1993; 1154: 51-63.