Increasing the repetition frequency of electric pulse delivery reduces unpleasant sensations that occur in electrochemotherapy

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Involuntary muscle contractions and painful sensations during electric pulse delivery are the most unpleasant side effects of electrochemotherapy. The aim of this study was to determine the nature of pain caused by the application of electric pulses and to evaluate patients' tolerance to the standard electric pulses of 1 Hz repetition frequency and the new 5 kHz protocol. A train of eight electric pulses of 1 Hz and 5 kHz repetition frequencies was delivered to the forearms of 40 healthy volunteers. After the conclusion of each protocol the subjects had to complete the short-form McGill Pain Questionnaire with separate visual analog scales for pain intensity and unpleasantness. All subjects selected at least one superficial and one deep pain descriptor; 85 % selected at least two superficial descriptor and 60 % at least two deep description. The application of 5 kHz electric pulses was less unpleasant than the standard 1 Hz pulses; however, the pain intensity did not differ between the protocols. Significantly more subjects chose the new 5 kHz protocol as their choice of treatment (P = 0.017). The frequent use of deep descriptors in our study indicates that muscle contractions contribute to the discomfort felt by the subjects during the delivery of electric pulses. The new 5 kHz protocol considerably shortens the treatment session and is also better tolerated. Therefore, the new 5 kHz electrochemotherapy protocol should eventually replace the 1 Hz pulses as new standard.

Key words: electrochemotherapy, pulse repetition frequency, pain, muscle contractions

Electrochemotherapy is a combination of chemotherapy and the delivery of high-voltage electric pulses (electroporation) that enhance the uptake of nonpermeant chemotherapeutic agents into the tumor by transiently increasing the permeability of tumor cell membranes. This increases the antitumor effect of the chemotherapeutics; therefore lower doses are needed for successful treatment [1]. There have been several reports of successful use of electrochemotherapy with bleomycin or cisplatin in the treatment of head and neck squamous cell carcinomas [2–4], malignant melanomas [5–8], adenocarcinomas [9, 10], basal cell carcinoma [11, 12], Kaposi's sarcomas [13] and breast cancer skin metastases [14].

Despite the success of the therapy, some side effects were reported. Slight oedema, erythema and local burns occurred at the treatment site after the delivery of electric pulses; however, these usually disappeared in less than 24 hours. More unpleasant, according to the patients, were the painful sensations and involuntary muscle contractions during electric pulse delivery [2-14]. Even local anesthesia by lidocaine used in most clinical studies was not successful in alleviating of the pain [5, 6, 11, 14]. Improved pain control was achieved by sedatives (Midazolam, diazepam); however, their use greatly increased the time patients spent in the clinic [3, 9, 12]. A possible explanation for the inadequacy of local anesthesia could be that pain during electric pulse delivery is caused by stimulation of deep nociceptors as well as superficial nociceptors. We believe that although direct stimulation of afferent nerves results in some discomfort associated with electroporation, the high contractile forces evoked by electric pulses can increase the pain and increase the discomfort. If that is the case, alternative ways of alleviating pain during electrochemotherapy (e.g. intravenous regional anesthesia) could prove to be more satisfactory.

A less unpleasant therapy could also be achieved by changing the geometry of the electrodes so that less of the non-target tissue is affected or by changing the electric pulse delivery protocols. Increasing the repetition frequency of the electric pulses from the standard 1 Hz to 5 kHz, which

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was already successfully used in a clinical case [7], lowers the number of painful sensations and muscle contractions, whereas the therapy effectiveness remains at the same level [15]. In addition, the new protocol shortens the duration of the treatment and prevents electrode displacement by muscle contractions during therapy. Unfortunately, the 5 kHz electric pulses evoke higher contractile forces than 1 Hz [15]; therefore it is unclear whether the new protocol would indeed be better tolerated by the patients.

The aim of this prospective study was to ascertain the nature of pain experienced by cancer patients during the electric pulse delivery part of electrochemotherapy and to determine whether the 5 kHz repetition frequency of the electric pulses would be preferred in comparison to the standard 1 Hz frequency. This was investigated by performing electroporation on 40 healthy volunteers who, after receiving the electric pulses, completed a short-form McGill Pain Questionnaire [16] about the quality, intensity and unpleasantness of pain experienced during the procedure.

Methods

Subjects. Experiments were performed on 21 healthy male and 19 healthy female subjects in the age range of 19–41 years (mean value of 25.0 years). Three additional subjects volunteered for the experiments but did not complete them due to temporary discomfort during the delivery of the trial electric pulse. All subjects signed an informed consent form before the start of the experiments. The research was approved by the National Ethics Committee of Slovenia (Doc. no. 112/07/05) and was conducted in accordance with the Declaration of Helsinki.

Study design. The aim of this study was to compare the degree of pain tolerance during delivery of high-voltage electric pulses of 1 Hz and 5 kHz repetition frequency that are used for electroporation in electrochemotherapy. In order to detect a clinically significant difference in pain outcome of 12 mm or more [17, 18] on a 100 mm visual analogue scale (VAS) more than 37 subjects that would undergo both treatment protocols had to be included in the study. The sample size was determined for a paired t-test (SD = 25 mm, power 0.8, $P < 0.05$).

All electric pulses were delivered by the same qualified investigator while the subjects were in a seated position with their right hand placed on a non-conductive surface. The subjects were first exposed to a single trial electric pulse and asked about their experience. If the pulse was deemed acceptable, we proceeded to the electroporation protocols. A train of eight 100 μs electric pulses (voltage to electrode distance ratio 600 V/cm) was delivered to the area over the right *flexor carpi radialis* muscle through two plate stainless steel electrodes with rounded tips and inner distance between them 8 mm (length, 25 mm; width 7 mm; thickness, 1 mm) with the Cliniporator (IGEA, Carpi, Italy). This instrument allowed us also to measure the delivered electric current to assure of the quality of the delivered electric pulses. Good electrical contact between the skin and the electrodes was achieved by an electrocardiograph gel. No anesthesia or analgesia was given to the subjects before the electric pulse delivery. The random order in which the electroporation protocols (1 Hz vs 5 kHz) were performed was determined by a computer generated sequence of random numbers.

After the conclusion of each electroporation protocol the subjects completed the short-form McGill Pain Questionnaire. Part I of the questionnaire served to determine the sensory (descriptors 1-11) and affective (descriptors 12-15) qualities of pain, while part II referred to two separate 100 mm horizontal VAS, which were used to assess pain intensity and pain unpleasantness. The scales were marked "no pain" on one end and "worst possible pain" on the other end. The subjects were allowed to see the values chosen after the first protocol when completing the questionnaire for the second time. After completing both questionnaires the subjects were asked to choose their favorite treatment protocol – 1 Hz, 5 kHz or undecided. Approximately 24 hours after treatment the subjects were examined again to make certain that the electroporation did not cause any longer lasting side effects.

Statistical analysis. Statistical calculations were performed on a personal computer using the statistical software package SigmaStat for Windows 3.01. The differences in the intensity of chosen pain descriptors were tested with the Wilcoxon signed rank test. The difference in the distribution of subjects that chose a particular therapy protocol as their favorite was tested with the binomial formula. The level of significance was set at $P < 0.05$.

Results

Sensorial and affective quality of pain. Fig. 1 summarizes the pain values related to the sensory and affective qualities of pain induced by electroporative pulses of repetition frequencies 1 Hz and 5 kHz. The most frequently selected pain descriptor were *stabbing* (80 %), *cramping* (57.5 %), *throbbing* (60 %) and *shooting* (60 %), *hot-burning* (53.8 %) and *fearful* [50]. More subjects chose superficial descriptor (*throbbing, shooting, hot-burning* and *sharp*) than deep descriptor (*cramping, gnawing, aching* and *heavy*), with 85 % of the subjects choosing at least two superficial and 60 % choosing at least two deep descriptor, respectively. All subjects chose at least one deep and one superficial descriptor. *Fearful* and *punishing-cruel* were the affective sensations selected by almost one half of the subjects; however the intensity values of both were lower than the intensities of superficial and deep sensations. While both protocols of electroporation received similar intensity scores for most descriptors, 5 kHz electric pulses were much less *splitting* and *punishing-cruel* than the 1 Hz pulses.

Intensity and unpleasantness of pain. Fig. 2 shows the intensity and unpleasantness of painful sensations during the delivery of electric pulses. Wide variation in pain experience

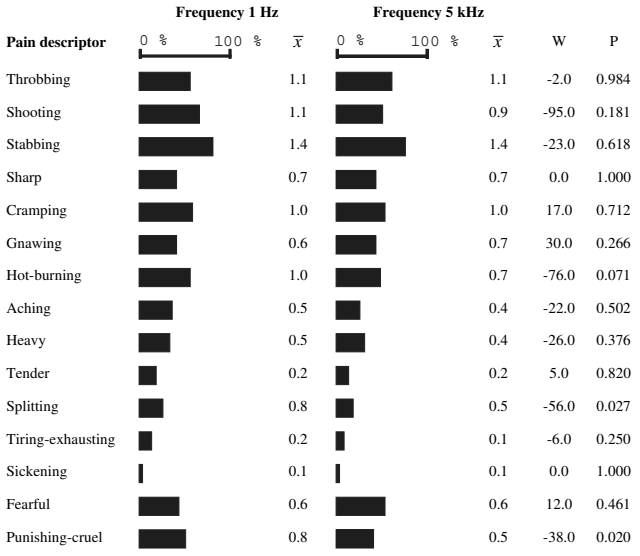

Figure 1 Descriptor chosen by the subjects with their relative intensities. Bar graphs of the percentages of subjects who chose each descriptor and descriptor mean intensity $\bar{\chi}$ (from 0 to 3) attributed to each are presented. The differences in intensity were tested with the Wilcoxon signed rank test (N = 40). The Wilcoxon statistic W and statistical significance of the difference P are presented. Due to multiple comparisons all P values are presented as explanatory values only. Data are shown for electroporation with electric pulses of repetition frequency 1 Hz and 5 kHz.

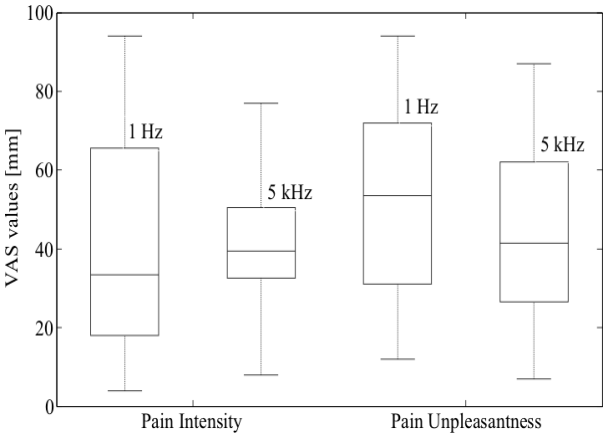

Figure 2 Intensity and unpleasantness of pain evoked by electroporative electric pulses as marked on the VAS. All data are presented as median, 25th and 75th percentiles and 5th and 95th percentiles.

among individuals led to a large variability in VAS pain scores, which ranged from 6 mm to 94 mm. In the subject group 5 kHz electric pulses evoked more intense but less unpleasant sensations than the 1 Hz pulses. However, the change in pain sensation due to different protocols used was bimodal in nature. Some subjects preferred 5 kHz, others 1 Hz and some found no difference between the two. By taking into account only those subjects that found a clinically significant difference in pain sensation between both protocols, we determined that significantly more subjects chose the 5 kHz electric pulses as less unpleasant (Table 1). Most subjects also selected the 5 kHz treatment as their treatment of choice (p = 0.017) and none were undecided.

Treatment tolerance. All subjects reported varying levels of transient discomfort during the electroporation procedure. When 1 Hz electric pulses were applied, each pulse caused a painful sensation and an instantaneous involuntary muscle contraction. When 5 kHz pulses were applied, only one painful sensation and one muscle contraction was reported. A slight redness of the skin was observed in all the subjects immediately after the delivery of electroporative pulses and three subjects complained of muscle fatigue on the day after the experiment; however, both disappeared during the next few days. No other side effects were noticed nor reported during or after the experiment. All subjects, except the three that did not go through with the experiment, tolerated the delivery of electric pulses very well.

Table 1 Pain intensity, unpleasantness and choice of therapy. Numbers of patients that preferred 1 Hz or 5 kHz protocol in regards to pain intensity, pain unpleasantness and choice of therapy are given. The differences in proportion of patients choosing particular protocols were tested by the binomial formula.

	1 Hz pulses	5 kHz pulses	Undecided*	P
Less intense pain	12	8	20	0.503
Less unpleasant pain	6	19	15	0.015
Favorite therapy	12	28	–	0.017

* Two cut-off values were taken into account for pain intensity and pain unpleasantness. An absolute cut-off value of 12 mm, when the pain scores were under 35 mm, and a percentage cut-off value of 25 % when the pain scores were above 35 mm (19).

Discussion

Electrochemotherapy is an efficient treatment of cutaneous and subcutaneous tumors [2-14]. The most unpleasant side effects reported so far are transient painful sensations and muscle contractions that occur when high-voltage electric pulses are applied to the skin. Frequent use of deep and superficial pain descriptor in this study (Fig. 1) confirms our hypothesis that both cutaneous and muscle nociceptors are excited when electroporative pulses are applied. Although superficial descriptor were used more often and with higher intensities than the deep descriptor, all subjects chose at least one of each and more than half chose at least two of each, which indicates that muscle contractions contribute significantly to the discomfort during electrochemotherapy. It also explains why the local anesthesia used in many clinical studies did not succeed in completely alleviating the pain. Local

anesthetics block only the afferent nerve fibers originating in the skin and not those that come from the muscle and tendon tissue. Results, similar to ours, were obtained in studies of tolerance of functional electrical stimulation; these studies determined that involuntary muscle contractions contributed to the discomfort experienced by patients during the delivery of electric pulses [20, 21].

A train of eight 5 kHz electric pulses evoked only a single muscle contraction with a single painful sensation, while the same number of pulses evoked eight painful sensations and muscle contractions during the delivery of 1 Hz pulses. Nevertheless the muscle contractile force was previously found to be higher for the 5 kHz train [16], therefore it was difficult to predict whether higher frequencies would be preferred by the patients. Although many studies of electrical stimulation have suggested that higher frequencies were better accepted by the patients [22], there has been no research in electrochemotherapy that would establish this by using an appropriate study protocol, for instance the McGill Pain Questionnaire or visual analog scales. Our study determined that pain experienced during the application of 5 kHz electric pulses and with it the shorter duration of the therapy was found to be significantly less unpleasant then the delivery of 1 Hz electric pulses and was the preferred choice of the majority of our subjects (Table 1). Even though the subjects were given an option not to choose their favorite protocol, none actually selected this option. A possible explanation for this would be that despite the VAS values being similar, the sensations occurring during both protocols differed sufficiently to enable each subject to prefer one of them. On the other hand, the difference in pain intensity between the 1 Hz and the 5 kHz protocols was not significant. From this we concluded that higher contractile forces caused by the 5 kHz protocol did not significantly alter the perception of involuntary muscle contraction.

The voltage to distance ratio used in our study (600 V/cm) was somewhat lower than the ratios usually used in clinical electrochemotherapy (1300 V/cm). In cancer therapies it is crucial to remove or destroy all cancer cells, otherwise a new tumor grows from the surviving cells of the old one. Higher voltages ensure that all tumor cells are successfully electroporated, thereby enabling the chemotherapeutic agents to enter and destroy them [23]. However, some tumor and even some healthy cells are destroyed by the electric pulses alone. Because we did not want to cause any irreversible damage to the electroporated tissue of our subjects, we decided to employ lower voltages that were just high enough to reversibly electroporate the tissue and cause muscle contractions. We believe that lowering the voltage used did not significantly affect our results and find no reason not to extrapolate them to higher voltages.

The new 5 kHz protocol of electric pulse delivery was preferred by the subjects over the standard 1 Hz protocol. It also shortens the duration of the therapy session and prevents electrode displacement due to muscle contractions during pulse delivery. After the anti-cancer efficiency of the electrochemotherapy with the 5 kHz electric pulses is further established in the clinical environment all its advantages should enable it to replace the 1 Hz pulses as the new standard in electrochemotherapy treatment.

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