Extracorporeal Shock Wave Treatment in Ischemic Tissues: What is the Appropriate Number of Shock Wave Impulses?

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ABSTRACT

The dose-dependent effect of extracorporeal shock wave technology (ESWT) was evaluated using a murine skin flap model. Thirty-six Sprague-Dawley rats were divided into six groups (ESWT groups 1 through 5 and a control group). After surgery, shock wave impulses doses were administered: 200 (group 1), 500 (group 2), 1500 (group 3), 2500 (group 4), 5000 (group 5), and 0 (control group 6). Flap viability was evaluated on day 7. Overall, significantly smaller percentages of necrotic zones were observed in groups 2, 3, and 4 compared with groups 1, 5, and the control group ($p < 0.05$). ESWT treatment with 200 impulses was found to be ineffective. ESWT treatment of 5000 impulses resulted in a significant increase in the percentage of necrosis compared with other ESWT groups ($p < 0.05$). However, ESWT treatments between 500 and 2500 impulses at 0.11 mJ/mm^2 enhanced epigastric skin flap survival significantly.

KEYWORDS: Extracorporeal shock wave, dose dependant, reconstructive flap, ischemic tissues

Extracorporeal shock wave technology (ESWT) has widely been used in the urologic field for over 25 years.[1] Beside its mechanical effects in lithotripsy, it has been shown that ESWT has a biological effect in orthopedics in the treatment of delayed union and nonunion,[2] tendonitis,[3,4] and tendon-bone healing.[5]

In the past few years, the potential of ESWT in the treatment of skin disorders has been described by different groups.[6–8] Experimental investigations by our group on an epigastric skin flap model in rats showed promising results regarding skin flap survival and growth factor release.[9–11] ESWT thereby improved skin flap survival by reducing ischemia and subsequent necrosis. Further, in an early clinical trial, ESWT was revealed to have a highly positive effect on deep partial-thickness burns in humans.[12]

However, and despite the positive effects of ESWT on compromised skin, little is known about the appropriate number of shock wave impulses and the optimal energy carried by each shock wave. The purpose of this study was to investigate the appropriate number of extracorporeal shock wave impulses at a fixed-energy flux density to be applied on an ischemic compromised epigastric skin flap in rats to decrease necrosis.

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MATERIALS AND METHODS

Animals

The animals used in this study were maintained according to the National Research Council guidelines. The 36 male Sprague-Dawley rats weighing 300 to 500 g were randomly divided into six groups of six animals each.

Anesthesia was performed by intraperitoneal injection of 50 mg/kg ketamine (Ketanest 100 mg/mL; Fort Dodge Laboratories, IA) and 1.3 g/kg body weight xylazine (Rampun 20 mg/mL; Bayer Corp., KS) with periodic supplementation as needed.

The Epigastric Skin Flap Model

The epigastric skin flap model used in this study has been described as a modification in flap design.[13] Based solely on the right inferior epigastric vessel, the contralateral distal corner of the flap represents the random portion that predictably undergoes necrosis, amounting to ∼30% of the total flap area. The flap is designed in such a way that the lateral branch of the right epigastric artery is excluded and the flap is supplied by the medial arterial branch alone.

Surgical Technique

Surgical procedures were performed by three surgeons (K.F., O.M., M.R.). At the time of surgery, the surgeons were blinded to the experimental groups. The rats were anesthetized, an epigastric flap measuring 8 × 8 cm was outlined on the abdominal skin, and the relevant area was shaved with an electric razor and then prepared with Betadine and alcohol. The flap was elevated after incising the distal and lateral borders by sharp dissection. Then the inferior epigastric vessels were located bilaterally. The right inferior epigastric artery and vein were left intact, whereas the left inferior epigastric vessels were ligated and divided. Finally, the proximal border of the flap was incised to create a skin island flap pedicle on the right inferior epigastric vessels. Then, the flap was sutured back to its native configuration using interrupted 4–0 nonabsorbable sutures.

ESWT Treatment, Groups 1 through 5

Immediately after the surgical intervention, the anesthetized animals were placed in a supine position. Ultrasound transmission gel (Pharmaceutical Innovations Inc., Newark, NJ) was used as a contact medium between the ESWT apparatus and the flap skin. ESWT treatment with 200 (group 1), 500 (group 2), 1500 (group 3), 2500 (group 4), or 5000 (group 5) impulses at 0.11 mJ/mm^2 (EvoTron^{TM}, Sanuwave, Alpharetta GA) was applied to the left upper corner of the flap. This area represents the portion of the flap that predictably undergoes necrosis.

Table 1 Mean Percentage and SD of Necrosis in Relation to the Respective Total Epigastric Skin Flap Area

	Observer I		Observer II	
	Mean (%)	**SD**	**Mean (%)**	**SD**
Group 1	17.22	8.75	14.05	8.35
Group 2	6.64	5.35	6.66	5.58
Group 3	12.49	3.28	9.88	3.66
Group 4	10.62	5.94	9.98	5.90
Group 5	30.04	16.52	28.61	15.31
Group 6	26.30	18.18	22.58	18.10

There was no statistically significant difference in interobserver error. SD, standard deviation.

Control Group 6

In the control group, the flap was raised and sutured back into place without any shock wave treatment.

Evaluation

On day 7, all animals were anesthetized, standardized digital pictures of the flaps were taken and transferred to a computer, and the animals were sacrificed with an overdose of intraperitoneal pentobarbital (100 mg/kg). The following flap zones were defined for surface area measurement: necrotic zone and total flap area. Surface area of these defined zones was measured by using Image Pro Plus Software (Version 4.1, Media Cybernetics LP, Silver Spring, MD). Areas were measured independently by two investigators (K.F., O.M.) who were blinded to the different groups. The results were expressed as percentage relative to surface area of the total flap (Table 1).

Statistical Analysis

The Kruskall-Wallis test was used to test the equality of median percent necrotic area between the six groups. The interobserver error was determined by comparing the measurements made independently by the two investigators. Statistical significance was defined with a p value <0.05.

RESULTS

None of the epigastric flaps showed any signs of infection, seroma, or hematoma formation. Group 2 (500 impulses) had the smallest mean area of necrosis compared with the other groups. In general, the percentage of necrosis was found to be decreased after 500, 1500, or 2500 impulses compared with the rats that received treatment with 0, 200, or 5000 shock wave impulses (Fig. 1).

A statistically significant decrease in necrosis was calculated for groups 2, 3, and 4 at 500, 1500, and 2500

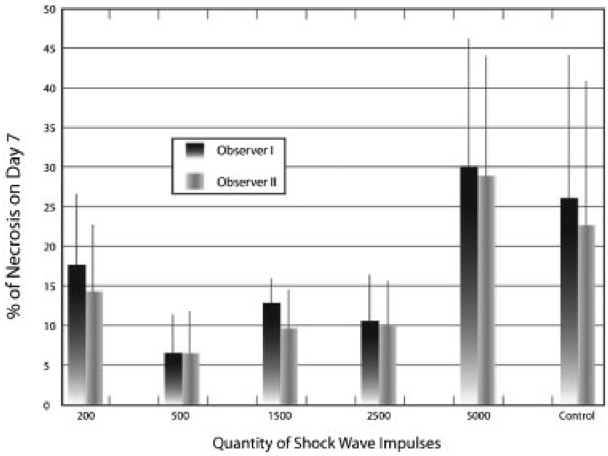

Figure 1 Two observers measured the percentage of necrosis of the respective total epigastric skin flaps independently. Group 2—treatment with 500 extracorporeal shock wave impulses immediately after surgery—resulted in the lowest percentage of necrosis.

impulses, respectively, in comparison to group 5 (5000 impulses) and the control group (Table 2). Although group 1, after the treatment with 200 ESW, showed a larger area of necrosis on day 7 than groups 2 through 4, the results obtained were not statistically significant. Group 5, treated with 5000 ESWT impulses, revealed a larger percentage of necrosis than the control group. However, this was not statistically significant.

Interobserver Error

The correlation coefficient between the two investigators was 0.849. There was no statistically significant difference in interobserver error.

Table 2 Comparison between the Respective Groups

Compared Groups	Observer No.	*p* Value
Group 1 (200) to group 2 (500)	I	0.068
	II	0.100
Group 1 (200) to group 3 (1500)	I	0.361
	II	0.465
Group 1 (200) to group 4 (2500)	I	0.126
	II	0.201
Group 1 (200) to group 5 (5000)	I	0.100
	II	0.045*
Group 1 (200) to group 6 (control)	I	0.228
	II	0.132
Group 2 (500) to group 3 (1500)	I	0.465
	II	0.251
Group 2 (500) to group 4 (2500)	I	0.117
	II	0.465
Group 2 (500) to group 5 (5000)	I	0.016*
	II	0.016*
Group 2 (500) to control group	I	0.008†
	II	0.027*
Group 3 (1500) to group 4 (2500)	I	0.251
	II	0.251
Group 3 (1500) to group 5 (5000)	I	0.047*
	II	0.016*
Group 3 (1500) to control group	I	0.047*
	II	0.036*
Group 4 (2500) to group 5 (5000)	I	0.016*
	II	0.009†
Group 4 (2500) to control group	I	0.006†
	II	0.011*
Group 5 (5000) to control group	I	0.955
	II	0.533

*A p value <0.05 was defined as statistically significant.
†A p value <0.01 as highly significant.

DISCUSSION

According to literature, no attempt has been made to define the appropriate settings for extracorporeal shock wave devices in the treatment of skin disorders. Electrohydraulic shock waves are described as high-energy acoustic waves generated underwater with high voltage explosion and vaporization.[4] ESWT is widely accepted as a technique for treating patients with urinary stones. Recently, ESWT was applied and adapted to different clinical fields.[4] Results of animal experiments and clinical studies have demonstrated that ESWT induced bony union, cell differentiation, and neovascularization.[10,14–16]

However, there had been reports in the past that shock waves could also cause harm to the patient. Zogović reported a case series of 2034 patients with kidney stones admitted to lithotripsy. Systemic side effects such as falls in hemoglobin levels, heart-related problems during or after the treatment, arterial hypertension, and urosepsis were described. Local effects such as transitory hematuria, which occurred in almost all patients, and the creation of a percutaneous nephrostoma in 54 patients were evident. Skin lesions were found in 1004 patients; however, none of them necessitated therapy.[17] Gerdesmeyer et al stated that to remove kidney stones without a surgical procedure, most of the physicians hazard the consequences.[18]

Other studies report impairments that may vary from commonly reported superficial petechial bleedings and formation of haematoma[19] to severe damages in the intima, the medium, and the adventitial layers of arteries and veins as well as injury to the femoral nerve after a direct application of extracorporeal shock waves to the groin region of dogs.[20] However, the settings used in these studies represent the settings that are commonly used in urologic or orthopedic approaches with an energy between 24 kV (humerus, lithotripsy) and 28 kV (femur, tibia) and a high (2000 in lithotripsy, up to 5000 to 6000 in orthopedics) number of shock waves.

Thus, the biological effects of the ESWT seem to be highly dependant on the applied energy flux density, the administered quantity of extracorporeal shock waves, and possibly the mechanism of shock wave generation. Twenty-eight kilovolts is equivalent to 0.62 mJ/mm^2 energy flux density.[21] Thus, the energy flux densities of 0.11 mJ/mm^2 and 0.15 mJ/mm^2 that we used in our investigations regarding epigastric skin flap survival are relatively low for clinical settings.

When we started our studies to investigate skin perfusion after the application of ESWT,[6] the decision was made to use low energy settings for skin disorders. It was, however, an empirical decision, primarily based on our anatomic and histological understanding and the relative weakness of the soft tissue to be treated in comparison to the treated tissues in orthopedics and urology. Second, the decision was also based on the fact that the skin and the subcutaneous tissue in our experimental setting were in direct proximity to the applicator. In comparison, orthopedic and urologic shock wave applications must bridge different tissue layers, which influence the physical behavior of the wave, before reaching their respective therapeutic sites.

However, the results of this study indicate a high influence of the total amount of energy applied to tissue during ESWT treatments. The quantity of applied shock waves directly influences the clinical outcome. Skin flap survival differed significantly in the experimental groups. Two hundred impulse treatments did not meet the clinically relevant threshold needed to trigger the ESWT mechanism of action. Still, the relatively low number of 500 shock wave impulses seemed to be the most suitable for an area of the size of the epigastric skin flap by producing the least amount of necrosis. The results after the application of 1500 or 2500 impulses did not differ significantly from those at 500, but the higher values seem unnecessary given that 500 was clinical significant. Interestingly, the application of 5000 impulses resulted in harm to the tissue as the mean area of necrosis in this group was larger than the area of necrosis in the control group, although not statistically significant. The total amount of energy applied during the 5000 impulses on a small surface area seemed to be inappropriate and negated any clinical benefits of ESWT. We concluded that a high number of shock waves impulses—5000 or more—may impair the blood supply in the skin and its underlying tissues. Further studies could evaluate the possible cellular damage caused by excessive shock wave impulses.

This protocol was performed using an electrohydraulic shock wave device. There are other types of devices that produce shock waves through different methods, and the shock waves produced have different properties including the size of the treatment area and intensity (degree of focus) of the shock wave impulses. It is possible that other types of shock wave devices have different optimum values for clinically relevant impulse dosages and intensities.

From the results obtained, we conclude that the appropriate number of applied shock wave impulses on the epigastric skin flap model is between 500 and 2500 impulses for a electrohydraulic shock wave device at an energy flux density of 0.11 mJ/mm^2. The application of 500 shock waves impulses showed the smallest mean percentage of necrosis after 7 days, making this protocol the most promising for clinical applications treating ischemic conditions.

To get a standardized treatment regimen for compromised soft tissue in animals and humans, further studies are needed to further justify the optimal number of shock wave treatments as well as the optimal time interval between treatments to increase the clinical effects of this technology.

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