Hemorrhage Control by Short Electrical Pulses
In Vivo Experiments

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Abstract: An internal hemorrhagic shock is one of the leading causes of death in the battlefield and other trauma events. However the application of direct pressure, as in the treatment of an external hemorrhage, is not possible. Most common techniques to achieve vasoconstriction are through heat; yet heating causes irreversible destruction of organ tissues. Therefore, there is a need for a non-thermal based technology for hemorrhage control. The current research describes, for the first, an attempt to reduce the amount of bleeding in animal model liver injuries by using electrical pulses treatment (EPT). In the experiments, which were performed on 28 rats and 14 rabbits, a short ($25\mu s$ and $50\mu s$) EPT was applied to the treatment groups and the amount of bleeding was compared to the non-treatment (NT) groups. A reduction of 60%, 36% and 44% in blood volume, was found in the $25\mu s$-rats, $50\mu s$-rats and $25\mu s$-rabbits EPT groups, respectively ($P<0.001$). Also, it was found that the hemorrhage control was not caused by the mechanical pressure applied by the electrodes, and there was no evidence for thermal coagulation. Further research is needed to fully expose the potential of this treatment and the modality for hemorrhage control in civilian and military settings.

1 INTRODUCTION

Hemorrhage shock is one of the leading causes of death in the battlefield and other trauma events. Most battlefield hemorrhages are compressible, e.g. they can be controlled by a tourniquet or other means of direct pressure application. A recent survey made by the US army demonstrated the effect of early tourniquet application in increasing survival rates while causing minimal damage (Kragh et al., 2011).

However, bleeding occurring in internal cavities (such as the chest or the retroperitoneal space) or in solid organs (e.g. liver, spleen and kidneys) is considered non-compressible and the application of direct external pressure is not possible. Hemorrhage control from solid organs is challenging even in the setting of an operation theatre, because of their rich vasculature and lack of supportive connective tissues.

In order to cope with this important need, there are several techniques that being researched for hemorrhage control in solid organs, of which the main ones are by mechanical pressure and thermal coagulation. However, each of these technologies has its drawbacks and currently none of them have evolved into clinical devices. For example, High Intensity Focused Ultrasound (HIFU) (Burgess et al., 2007); (Vaezy et al., 1997), induces a rapid temperature increase in tissue, and cavitation formation, both leading to thrombosis and platelet activation. This technique has some adverse reactions such as an irreversible destruction of the liver and blood vessels, and overheating. Nevertheless, these techniques, as well as others, are still under evaluation and were not proved to give full answer the clinical need in the battlefield or in the surgical theater. Thus, there is a need for a non-thermal based technology which will cause vasoconstriction, thrombosis and hemorrhage control.

Thrombosis of a clamped blood vessel was demonstrated by several authors by using a direct current application device (Guarini, 1971); (Hladovec, 1975). However, applying this technique for clinical use in hemorrhage control is not practical because of the expected injury to the tissue. The effect of short electrical pulses on blood vessel constriction and thrombosis was reported in several papers (Gehl et al., 2002); (Matsushima et al., 1994);

(Sersa et al., 1999); (Yu-ling et al., 1997), which studied electrochemical therapy for tumors. These papers demonstrated a significant temporary reduction in blood flow after pulsing.

The specific characterization of various pulse parameters on blood vessels constriction and thrombosis was recently described by Palanker et al., (2008), which reported that the vasoconstriction effect appeared shortly after 10 seconds of electrical stimulation while thrombosis was achieved at 3 minutes. A pulse rate of 0.1Hz was sufficient for maintaining vasoconstriction, yet there were evidence of inflammation and necrosis. The authors reported that the effect is probably non-thermal as the temperature rise during the treatment did not exceed 0.01°C. Nevertheless, the exact mechanism for vessel constriction and thrombosis is still unclear, thus a comprehensive study of these mechanisms is necessary.

The potential application of the electrical pulsing effect on blood vessels is studied by our group. Our long term goal is to develop a portable device to control internal non-compressible hemorrhage from solid organs. The current research describes, for the first time *in vivo* experiments, an attempt to reduce the amount of bleeding caused in rat and rabbit liver injury.

2 METHODS

In vivo experiments were performed on livers of 28 adult Sprague-Dawley rats and 14 New Zealand rabbits. The experiment protocol was approved by the Animal Rights Council of the Israel Ministry of Health and conformed to guidelines for the humane care of animals. Animals were supplied by Harlan Laboratories Ltd., Jerusalem, at the age of 3 months. Average animal weight is depicted in Table 1.

2.1 RAT Experiments

The animals were anesthetized with an IM injection of Ketamine HCl (0.19ml/100 gram) and Xylazine (0.03ml/100 gram) solution. The surgical operation started 20 minutes after anesthetization to get an initially uniform point for all animals, and until that time the animal was weighed and placed on a heating blanket to maintain its body core temperature. Additional anesthetics were given approximately every 20 minutes via titration. A midline abdominal incision was performed and the liver was gently exposed. The median lobe of the liver was resected 13 mm from the lobe edge and the

removed part was weighed using portable scales (Ohaus Company, model N2B110).

Following the liver injury, the animals were divided into four groups. In the control group no treatment (NT) was given after the liver injury. Electrical pulses treatment (EPT) was performed to 2 groups, using a protocol which includes 100 electrical pulses of 500V at a pulse repetition of 1Hz, and pulse duration of 25μs and 50μs, for the EPT25 and EPT50 groups, respectively. In addition, in order to inspect the possibility of bleeding decrease due to mechanical pressure exerted on the liver lobe, a mechanical treatment (MT) group had been defined, so that the electrodes were placed on the median lobe for 200 seconds, as similar to the EPT groups, however, no pulses were delivered.

For all the groups, except the NT, the rat's injured medial liver lobe was placed between two customized copper electrodes, which were attached to a commercial caliper (Figure 1a). The distance between the 2 parallel slabs was adjustable, and determined for each animal by its liver thickness (mean electrode distance was 3.98±0.56 mm). A series of electrical pulses were generated by a square wave electroporation system (ECM 830, Harvard Apparatus), which was operated in the mono-phasic mode.

Following these interventions, the abdomen was closed using continuous sutures and the rats were maintained on heating blanket for 1 hour without any further treatment. Total blood loss was measured 60 minutes after liver injury, by soaking a cotton wool in the peritoneal cavity; the same method reported by previous authors (Hildreth et al., 1996); (Holcomb et al., 1999); (Matsuoka and Wisner, 1996). Blood loss for each animal was normalized by its body weight. All surgical interventions and measurements were performed by the same investigators (GM, YM), to avoid variance in the procedure, which could affect the results.

The liver was removed immediately after euthanasia and fixed into formaldehyde 10%. Histological slices were processed for H&E staining in paraffin sections and then cut perpendicular to liver edge in order to demonstrate the transition between treated and untreated zones.

2.2 Rabbit Experiments

Animals were anesthetized with an intra-muscular injection of Ketamine HCl (50 mg/kg) and Xylazine (3.5 mg/kg) solution followed by maintenance dosage, and 20 minutes following the administration of anesthetic drugs, a midline abdominal incision

Table 1: Animal weight and excised liver weight (normalized to animal weight) in all groups.

	Rat				Rabbit	
Group	NT	MT	EPT50	EPT25	EPT25	NT
N	11	4	8	5	7	7
Animal weight [gr]	398.66±20.14	408.3±49.5	398±32.9	392.3±33.6	3196±448	3106±293
Normalized Excised Liver Weight [%]	0.26±0.07	0.25±0.06	0.22±0.05	0.21±0.05	-	-

was performed and the liver was gently exposed. In this experiment set, different liver injury was performed: 2 cuts of 5mm deep and 3cm long in each front liver lobe (total 6 cuts in 3 lobes).

Following liver injury, rabbits were divided into two groups. EPT25 group received 200 pulses (500V, 1Hz) that were given with pulse duration of 25μs and NT group received no treatment. In the EPT25 group, the rabbit liver was treated with customized electrodes comprised of two copper plates of 4mm wide and 29mm long, and spaced apart by 7.2mm (Fig 1b). Following each liver cut, the electrodes were positioned on the liver surface while the cut is in equal distances between the electrodes and a series of electrical pulses were given, similarly to the treatment described for rat liver injury. In addition, thermal images have been taken using a thermal camera (model A40, FLIR) before and immediately after the treatment, in order to distinguish if there is temperature increase during the electrical treatment.

Following these interventions, rabbit's abdomen was closed using continuous sutures and the animals were maintained on heating blanket for 1 hour without any further treatment. Total blood loss was measured 60 minutes after liver injury in the same method as described for rats. All surgical intervention and measurements for all experiments were performed by the same investigator (GM).

3 RESULTS

Average animal weight and excised liver weight in rats (normalized to the animal weight) are reported in table 1. Both parameters were not significantly different between all animal groups (p>0.1). These results indicate that the injury protocol was pretty much the same, and therefore could not affect the blood loss results.

Blood loss weight can be observed in Figure 2, as a box plot chart of normalized bleeding weight for all animal groups. On each box, the black circle is the mean value, the central line is the median, the edges of the box are the 25th and 75th percentiles, and the lines outside the box are the most extreme data

points (minimum and maximum).

In contrast to the normalized excised liver weight, the blood loss amount in the EPT50 and EPT25 rat groups was significantly reduced by 36% and 60%, respectively, as compared to the NT group (p<0.001 for both groups). Blood loss in the EPT25 group was significantly lower than in the EPT50 group (p=0.025), suggesting that the pulse duration can affect the molecular processes of the electrical treatment (which are not known yet) in becoming faster and/or more efficient. Blood loss in the MT group did not differ significantly from the NT group (p=0.43), and this finding is probably indicates that the mechanical pressure is not the reason for the reduced bleeding from the injured liver.

Similar results were found for the rabbits (Fig 2), where blood loss in the electric pulse treatment group was smaller by more than 44% as compared to the NT group (p=0.004).

4 DISCUSSION

The results, which were achieved by *in-vivo* animal experiments, demonstrated that short electrical pulses of 25μsec and 50μsec decreased the amount of hemorrhage from a rat liver injury by 60% and 36%, respectively, and a rabbit liver injury by 44%.

Apparently, the effect was not caused by the mechanical pressure applied by the electrodes per-se, but by the electrical field applied on the tissue, because there was no significant different in the measured bleeding amount between the NT group and the MT group.

The distinction between the treatment effective between the rats and the rabbits can be explained by the difference in the electrode configuration. In the rabbits' case the treatment was on the surface area, so the electric field was not enough deep. On the other hand in the rats' case the electrodes were in the two sides of the wound, so the electric field was stronger in the injury site, and increase the treatment effective.

Another potential cause for hemorrhage reduction could be a thermal coagulation caused by the increased temperature rise in response to pulse
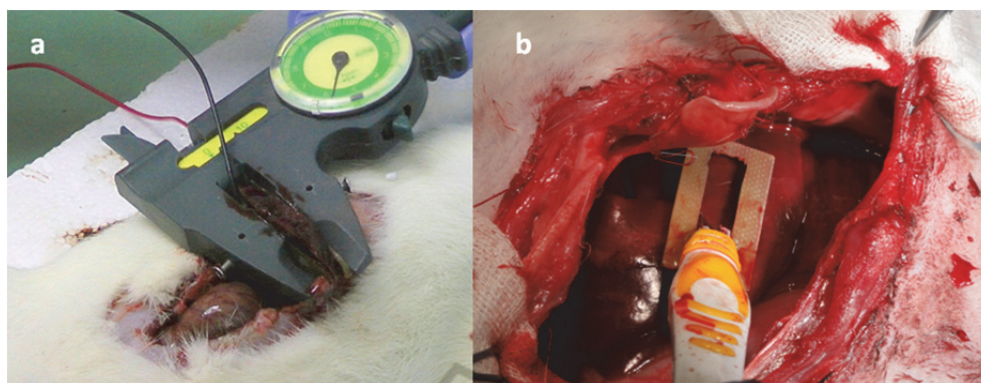
Figure 1: Experimental setup of rat (a) and rabbit (b) liver. Rat liver was treated with two parallel plate electrodes mounted on a hand caliper adjusting for liver thickness. Rabbit liver injury was treated by fix parallel plates electrodes positioned at two sides of the wound.
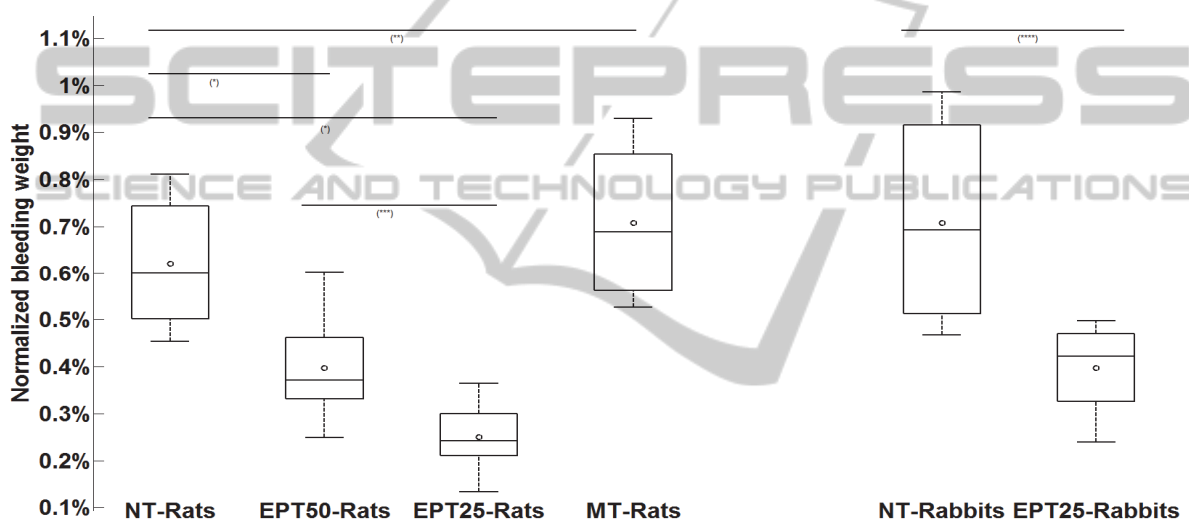

Figure 2: Box plot of normalized bleeding weight in all animal groups (rats and rabbits). Control groups were not treated, EPT50 and EPT 25 were treated by 200 pulses of 50 and 25 µs, respectively, in a repetition rate of 1 Hz. Unpaired t-test results for various comparisons are as follows: (*) $p < 0.001$, (**) $p = 0.43$, (***) $p = 0.025$, (****) $p = 0.004$.

treatment. However, thermo-coagulation is usually expected at temperatures of above 60-70°C (Graham et al., 1998); (Matsuoka et al., 2004), and were probably not achieved in these experiments, even at the longer pulse duration [According to a parallel theoretical study in our group, which investigated the shape of the electric field and the heating that accompanying to the electrical treatment in different configurations of electrodes, through mathematical models and computer simulations in COMSOL and MATLAB].

Further, there was no evidence for thermo-coagulation and temperature rise in the histological sections and the thermal images, respectively.

Interestingly, we found that 25µsec pulses were significantly more effective in reducing hemorrhage volume compared to 50µsec pulses. One possible

explanation may be related to a local increase in liver perfusion in response to the relative temperature rise in the case of 50µsec pulses, as reported in previous studies (Precup et al., 2010). It could be hypothesized that such a local increase in perfusion could cause a relative increase in blood loss, partially reducing the effect of treatment.

We hypothesize that the hemorrhage control observed in this study is associated to endothelial layer damage, leading to irreversible vessel constriction and forming a thrombus. A similar effect was reported in other electrochemical therapy studies (Gehl et al., 2002). Ramirez et al., (1998) reported that electrical pulses of 850 V/cm and 100 microseconds long, caused a decrease in blood perfusion to the spleen and mesenteric arteries, and also reported that electrical pulsing of the liver

caused a decrease in perfusion, as was demonstrated by a color test. Sersa et al., (2008) found that 3 minutes following tumor electric pulsing, blood flow decreased by about 80 percent, and histological evaluation of the endothelial cells showed that they were rounded and swollen causing narrowing of the blood vessels lumen.

Our study has several limitations to be considered. First, blood pressure and pulse were not measured or controlled during the experiments. This could theoretically increase variance in the amount of bleeding. Second, in this preliminary study we did not address the effect of treatment in the case of traumatic coagulopathy, which is expected in cases of severe liver trauma. This issue calls for future research. Other issues to be studied in larger animals are the design of the electrodes in order to optimize electric field geometry, optimize pulse parameters to achieve finer results, better control of tissue temperature, and the possible use of changes in the electrical properties of the tissue for measuring treatment effect.

In conclusion, in this preliminary research we demonstrate that short electric pulses can significantly reduce the amount of bleeding from injured liver in a rat model. The effect is probably non thermal and possibly related to the effect on blood vessels' endothelial layer. Further research is needed in order to fully expose the potential if this treatment modality for hemorrhage control in civilian and military settings.

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