EXPERIMENTAL STUDY

Characteristics and mechanism of lower limb injury induced by landmine blast: A research in a rabbit model

Sen Zhang, M.D.,[1] Gengfen Han, M.D.,[3] Yan Xiong, M.D.,[1] Ziming Wang, M.D.,[1] Zhong Wang, M.D.,[1] Xinan Lai, M.D.[2]

[1]Department of Orthopedics, Daping Hospital, Army Medical Center, Chongqing-*China*

[2]The 6th Department of Research Institute of Surgery, Daping Hospital, Army Medical Center, Chongqing-*China*

[3]The 969th Hospital of the PLA joint Logistics Support Force-*China*

**Sen Zhang, Geng fen Han and Yan Xiong contributed equally to this work*

ABSTRACT

BACKGROUND: Limb injuries caused by landmine explosions are tricky to treat and difficult to protect. It is necessary to establish an animal model for studying lower limb injury and to investigate the characteristics and mechanisms of lower limb injury induced by landmine blasts.

METHODS: Twenty-six mature white rabbits were randomly divided into sham group (n=10) and injury group (n=16). Landmine blast was simulated by electric detonators under the right lower limb in upright state by a special modified fixation frame. High-speed photography was used to observe the body movements. Vital signs, vascular injury (determining by digital subtraction angiography), pathological characteristics, and ATP concentration of the tibialis anterior muscle and triceps surae of shank were recorded for comparison.

RESULTS: Generally, middle and lower segment of the injured legs of the rabbits was seriously damaged. The limb stump presents a distribution of three areas, tissue free zone, contusion hematoma, and edema contusion. Sneak wound track, myofascial destruction, and periosteum stripping were typical characteristics of landmine blast injury. ATP concentration and pathological analysis showed that the tibialis anterior muscle was the most seriously injured, followed by the gastrocnemius and soleus. ATP concentration of affected muscle of both the contusion and commotio area declined remarkably over time, but the muscle in the avulsion area stayed at a low activity level with no change over the time. Small vascular injury in the contusion area was evident. The site of the sciatic nerve lesion was higher than the muscle. Injured site of sciatic nerve injury was higher than serious contusion muscle. High-speed photography demonstrated that the joints of the injured limb extremely flexed followed by a rapid stretch under the blast shock wave.

CONCLUSION: The established experimental model presents typical effect of lower limbs wounded by the mine blast in war field. Landmine blast can cause typical damage on lower limbs including nerve lesion, knee injury, and microcirculation damage that is progressive over time. The limb stump is divided into three zones based on gross pathology and micropathology, which can provide an important reference for clinical treatments and prognosis.

Keywords: Animal models; blast injury; limb; mine.

INTRODUCTION

Landmine is a kind of explosive firearm that is embedded or laid on the surface of ground. It is cheap prices, convenient gain, and layout, which makes this ideal weapon used widely in the battlefield at wartime for blocking enemies. According to statistics, hundreds of millions of landmines threaten mankind[1] and about 15,000 victims are killed or injured in a landmine blast every year, most of them are civilians.[2] The extensive and long-term harm brought by landmines is be-

Cite this article as: Zhang S, Han G, Xiong Y, Wang Z, Wang Z, Lai X. Characteristics and mechanism of lower limb injury induced by landmine blast: A research in a rabbit model. Ulus Travma Acil Cerrahi Derg 2023;29:1335-1343.

Address for correspondence: Xinan Lai, M.D.
Daping Hospital, Army Medical Center, Chongqing, China
E-mail: 1737305188@qq.com

Ulus Travma Acil Cerrahi Derg 2023;29(12):1335-1343 DOI: 10.14744/tjtes.2023.39560 Submitted: 02.05.2023 Revised: 19.10.2023 Accepted: 23.11.2023

coming more and more prominent because of its significant health-care costs to society[3,4] and the psychological trauma for victims.[5,6] Thus, research related to landmine blast injury is vital for efficient medical care.

The treatment strategy of limb landmine blast injury has historically come mostly from clinical research,[7,8] lacking the corresponding support from basic research. While we still can find that there are many issues worthy of in-depth discussion from many clinical studies, such as the choice of amputation plane and surgery balance between debridement and amputation.[9] Does landmine blast cause limb vascular damage and thrombus formation, and is the level of nerve injury consistent with that of muscle injury? These key clinical problems affecting clinical treatment and rehabilitation have not been concluded. We plan to establish n simple and reliable animal model for the analysis of limb landmine blast injury, studying systematically the blast trauma of limbs treading on a landmine, and answer the above questions, expecting our efforts to provide as much information as possible both for clinicians and later researchers.

MATERIALS AND METHODS

Ethical Approval of the Study Protocol

Procedures were performed strictly in accordance with the guidelines for the use of laboratory animals and approved by the Ethics Committee of the Third Affiliated Hospital of Third Military Medical University.

Animal Grouping and Preparation

Twenty-six male or female New Zealand mature white rabbits, unlimited weight (1.9 kg to 2.4 kg), were provided by the animal center affiliated with Daping Hospital, Research Institute of Field Surgery, the Third Military Medical University, (Chongqing, China). All the animals were randomly divided into two experimental groups: (1) Ten animals in the sham injury group (as normal control) for adenosine triphosphate (ATP) test and pathologic examination (five animals in the 6 h group and 12 h group, respectively) and (2) 16 animals in the injury group were used to study the characteristics of limb injury under high-speed photography.

The animals were anesthetized with intravenous injections of 3% pentobarbital sodium (30 mg/kg; Merck, Darmstadt, Germany) through the marginal vein of the ear at 6 or 12 hours after injury in each experimental group.

Trauma was not induced until the rabbit was in deep anesthesia. Additional doses of 10 mg/kg were administered hourly to maintain anesthesia. Make sure the experiment animals had favorable relief of pain. The fur of the bilateral inguinal regions of right limb was shaved. Local anesthesia was administered with lidocaine hydrochloride before femoral artery cannulation for digital subtraction angiography (DSA).

Experimental Injury Model

We designed a metal frame to hold the anesthetized animals and a metal open-top chamber (L × W × H, 80 cm × 80 cm × 50 cm) for protecting the surroundings (Fig. 1a). The bottom of the chamber was paved with sand at 5–7 cm thickness. To make sure the bilateral hind paws stood on the ground, the height of the frame was adjusted to the length of rabbit. The right lower limb was kept at a 75° slant angle with the ground and detached with the contralateral (the right side behind the left side) to assume a walking posture (Fig. 1b). We applied the RDX paper electric detonators (600 mg, 845 Factory, Chongqing, China) to simulate antipersonnel landmines and placed them under the right hind foot of rabbit. All the wounds of the rabbits were sutured for hemostasis and dressed with sterile gauze immediately after the blast. There were no detonations in the sham group while the other procedures on rabbits in both groups were the same. The rabbits were free of water and food during the observation time after the injury. The house for the experimental animals was kept at 24–27℃ by means of an air conditioner. The movements of a limb caused by the blast were recorded by high-speed photography (Red Lake HG-LE, USA).

Figure 1. Experimental injury model. (a) Injury model (b) Schematic diagram of experimental setup: The angle of injured limb and the ground is about 75°.

High-speed Photography

Reference points were tagged on different parts of the injured hind limb (thigh and calf mainly) in advance for high-speed photography to record movements of the injured limb of the rabbit at the instant after the blast. To image the force of the blast upon the rabbit, we used a high-speed video recording system that consisted of a digital camera (A504kc, Basler, Ahrensburg, Germany) with a 50 mm lens (Canon, Tokyo, Japan) connected to a frame grabber (Karbon-CL, BitFlow, Inc., Woburn, MA) through dual-channel Camera Link protocol. The camera outputs primitive data from the first 512 rows of the sensor (or equivalently, frames at 1280 × 512 resolution at 8 bits per pixel) at 1000 fps and the data were stored by a programmed computer automatically. In post-processing, a demosaicing algorithm was used to gain full-color images. The stored frames recombined as a complete video file and were slowed down 600 times by the multimedia processing software (Corel VideoStudio Pro X4) (Video 1S). The movements of reference points were measured by an Image-Pro Plus software (Version 6.1). Finally, we calculated the acceleration of the different parts of the affected limb at the millisecond level to show the blast event duration.

Imagological Examinations

The animals of the injury group underwent digital radiography and computer tomography (CT) at 6 h after injury. DSA was carried out by professionals with an interventional catheter (4F, 1.35 mm, Cordis Corporation, Miami Lakes, FL, USA) through the left femoral artery in the operation at the intervention center. About 8 ml of iodinated contrast agent (Ultravist, Bayer Vital GmbH, Germany) was injected during the DSA. The process adhered to the standard operating disciplines of examination. The animals were examined under deep anesthesia.

Sample and Reagents Preparation

The rabbits were killed by an overdose of 3% pentobarbital sodium (Merck, Darmstadt, Germany) at 6 h and 12 h post-blast exposure before sampling. Segments of 2–3 cm from the bottom-up of tibialis anterior muscle and triceps surae (the fracture end as a benchmark) were selected. This part is in the contusion area according to our preliminary experiment. At the same time, segments of 1–2 cm and 3–4 cm away from the bottom of the medial head of the gastrocnemius were chosen. The injured nerve was drawn both above and below knee level. Tissue was fixed in 4% paraformaldehyde (Bose, Wuhan, China) at 4℃ for morphologic evaluation. The samples for activity testing were taken under a good anesthetization and stored in liquid nitrogen as soon as possible.

Anatomical and Pathologic Examination

Anatomical examination should be implemented quickly after the death of animals. Length measurement on the tissue and area is performed by a ruler whose smallest scale is 1 mL. The scope of hematoma was measured with both a general view and an anatomical examination. Taking the actual clinic treatments into consideration, we set the tibia fracture end as a benchmark for the measurement of stump uniformly.

Histopathology analysis of the skeletal muscles and limb nerves of experimental animals was processed by routine histology procedures. Five series of sections (5 μm) of each fixed muscle tissue were stained with hematoxylin and eosin and each section was evaluated using a photomicroscope (Olympus Model AX80, Center Valley, PA, Japan). Two non-overlapping pictures of horizons were obtained from each section at random. Image segmentation on necrosis and degeneration of pictures gained from the muscle section was done by Photoshop software (CS-5) for semi-quantitative analysis of the pathology.

Muscle Activity Detection

The content of ATP was used as a marker of activity in the muscle,[10] and it was detected by the means of the fluorescence detection method[11] intensively in our experiment. Frozen tissue was homogenized after weighing. The mixture of homogenate was centrifuged for 5 min at 12000 g. Then, the precipitates were removed. The supernatant was diluted 1000-fold and then was detected using an ATP Assay Kit (Beyotime Institute of Biotechnology, Jiangsu Province, China). Fluorescence intensity was analyzed by a luminometer (Glo-Max 20/20 Luminometer, Promega-GloMax, USA). All the experimental steps were carried out in accordance with the specifications and the operations were implemented on the ice as quickly as possible. Room temperature was maintained at 25±0.5℃.

Statistical Analysis

Data were expressed as mean ± SD and analyzed by SPSS software (IBM Statistics SPSS 20.0). Comparisons measurement data were performed using the one-way analysis of variance (ANOVA) (for three categories) and the Student's t-test (for two categories). The statistical significance was set at $P<0.05$.

RESULTS

Transient Injury Process after Blast

Using a custom high-speed photography system, we recorded the movements of the injured limb accompanying the blast, but the vision was clear only in a transient duration of time (about 0~50 ms) because of smoke and dust under the effect of blast wind. The injured shank rose rapidly around the axis of the knee and the knee flexed extremely, which was followed by a fast lift of the thigh around the axis of the hip; at the same time, the shank also stretched rapidly around the axis of the knee again. The limb joints exposed to the blast underwent a speedy flexion and extension forming like a "whiplash" injury mechanism. The acceleration of the shank and thigh can be calculated through the changing position of reference points marked on the corresponding location (Fig. 2). Based on the analysis of acceleration and its positive peak time point, there are significant differences in the movements

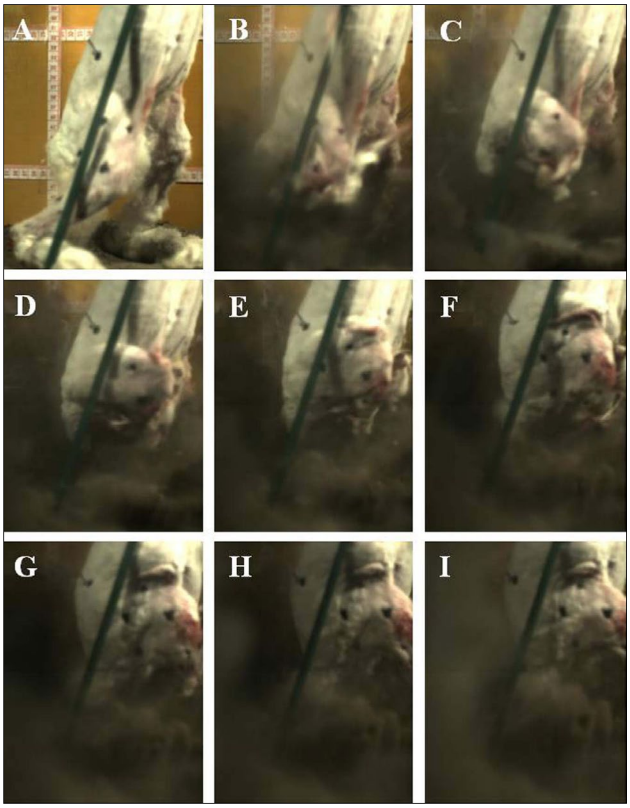

Figure 2. Scenes of high-speed photography experiment. The reference point is directly marked on the skin of the lower extremities.

of the calf and thigh, the peak acceleration of shank is far higher than that of the thigh, and the peak time point of thigh delay obviously. Injury risk factors of the knee were implied by the huge peak acceleration difference between the shank and thigh.

Imageology Examination

Six hours after blast, the injured lower limbs of six rabbits were examined in the clinical imaging department of our hospital. We found distinct multi-segmental fractures existing in one vulnerable tibia of a rabbit and inordinate bone loss from the image of digital radiography (Fig. 3a) and CT (Fig. 3b). Vasospasm can be seen in one injured limbs at 6 h of post-injury through DSA (Fig. 3c). Nevertheless, there was no macroscopic thrombus in the main blood vessels of injured limb, and the blood was still flowing unimpeded.

Gross Anatomy

According to the partition principle on injured lower limbs of victims treading on the landmines,[12] the wounded limbs of experimental rabbits appeared generally in four typical areas (in terms of discrete, avulsion, contusion, concussion area) (Fig. 4a), which was similar to conventional limb injuries induced by anti-personal landmine in spite of our simulative settings. Although the boundaries were not very clear, we were still able to distinguish some characteristics and the approximate range of damage combining with anatomical techniques.

The middle and lower segments of crus were destroyed and the wound was polluted severely by earth and dust. The shanks of the rabbits in the blast-exposed group suffered various degrees of open crushing tibia fracture, periosteum stripping, muscle clearance breaking, and epineurial hemorrhage. Muscle fascia clearance was broken and coagulated blood clots formed, creating the appearance of hematoma (Fig. 4b). The inner scope of hematoma was longer than outer indicating an underlying wound track. There was obvious edema on the stumps of the rabbits, dotted and focal hemorrhage in no constant size, and number distributed in the superior leg irregularly (Fig. 4c). The injured limb nerve was characterized by epineurium bleeding (Fig. 4d). Damage site of the limb nerve was the highest among different types of tissue of the injured leg (Fig. 4e).

Muscles and Nerve Histopathology

The skeletal muscle fiber was exceptionally smooth under the microscope in the control group (Fig. 5a). Muscle fibers necrotized severely in the avulsion area (Fig. 5b).The nibble-like change could be found in the contusion area (Fig. 5c). Focal bleeding showed microcirculation damage in the contusion area while wavy, and edematous myofibers were quite common in the concussion area (Fig. 5d).The limb nerve be-

Figure 3. Imaging characteristics of injured rabbit limbs by simulative landmine blasts. The images were gained from digital radiography (a), computed tomography (b), and digital subtraction angiography (c). The spasmodic blood vessel was pointed out by black arrow.

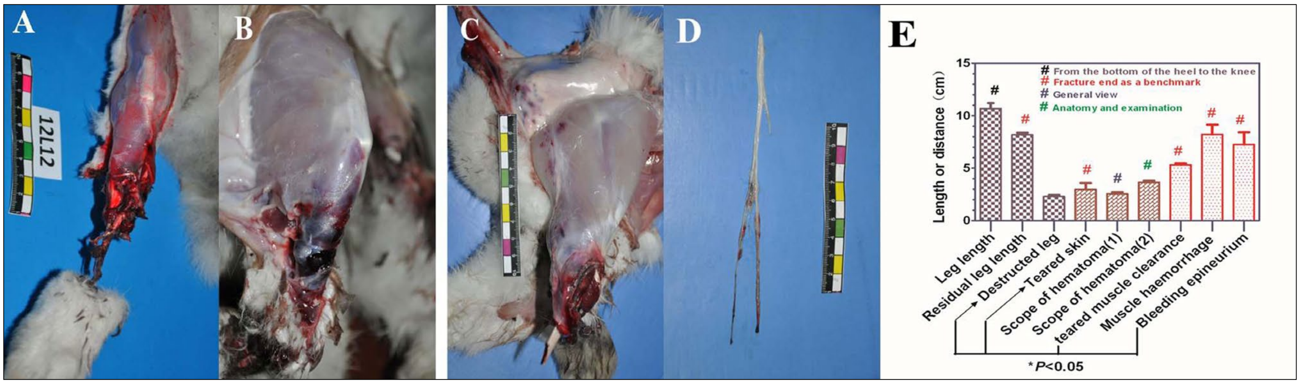

Figure 4. Gross anatomical characteristics of injured rabbit limbs due to simulative landmine blast. **(a)** The wounded rabbit limbs appeared generally four typical areas (in terms of discrete, avulsion, contusion, concussion area) as typical limb injuries induced by anti-personal landmine blast. The boundaries of the subareas were not very clear. The middle and lower segment of shank was destroyed and the wound was polluted severely by earth and dust. The shanks of the rabbits in blast-exposed group suffered various degrees of open crushing tibia fracture, periosteum stripping, and muscle clearance breaking. **(b)** The injured nerve of affected limb was characterized by epineurium bleeding. **(c)** There was obvious edema on the stump of rabbits, dotted, and focal hemorrhage in no constant size and number distributed in the superior leg irregularly. **(d)** Muscle fascia clearance was broken, forming hematoma appearance. The inner scope of hematoma was longer than outer. **(e)** Scope of injured leg of rabbit by simulative landmine. Mostly, the area of the laceration area was within 2 cm, and the muscle contusion/hematoma was about 2–3 cm from the end of the fracture. Damage level of sciatic nerve was the highest among different types of tissue of the injured leg (n = 10, Student's non-paired t-test, P<0.05). Tibia fracture end was taken as a benchmark for the measurement of stump uniformly.

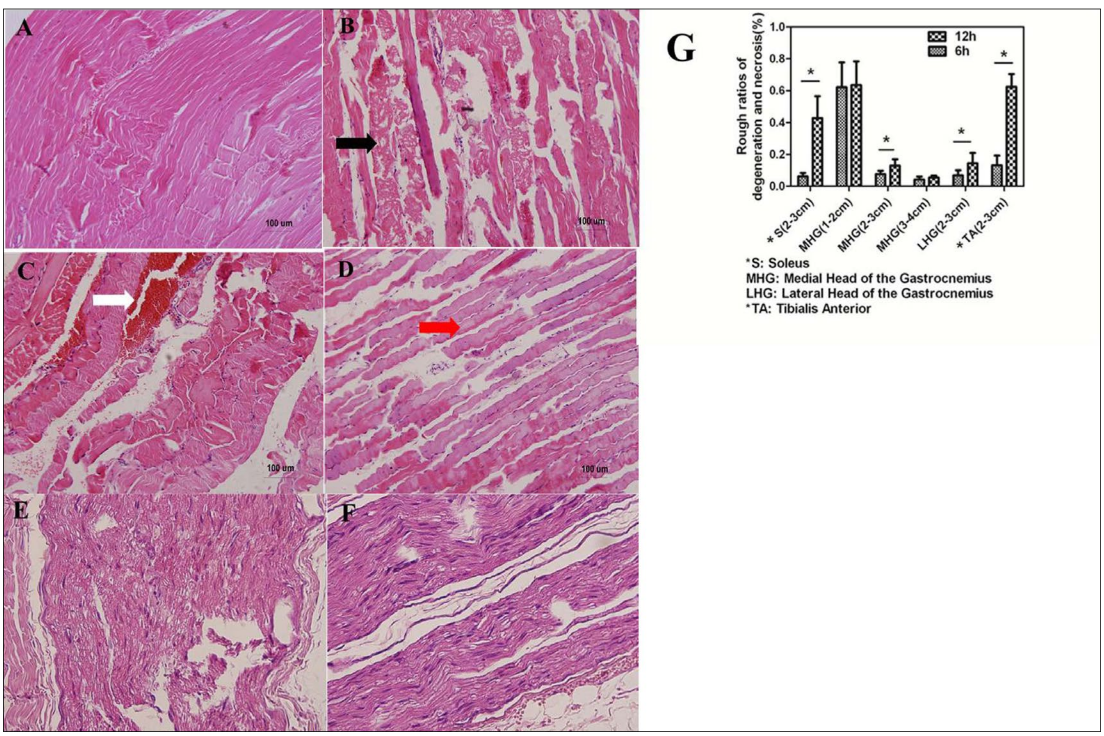

Figure 5. Microscopic-pathology characteristics of the muscles and sciatic nerve of different segments of injured rabbit stump (H&E, ×400). Muscles fibers necrosed severely in the avulsion area, the moth-eaten change was marked by black arrow; **(b)** Contusion area of interior gastrocnemius muscle, there was partial necrosis, the focal bleeding was indicated by white arrow; **(c)** Concussion area of soleus, there is large patchy lightly stained, wavy muscle fibers pointed out by red arrow. H&E: Original magnifications 200× (A, B, C, D and C). **(e)** The sciatic nerve below the knee approaching the detonation performed neurotmesis. **(f)** The sciatic nerve above the knee away from the detonation performed axonotmesis, epineurium bleeding (black arrow). **(g)** Given the result of semi-quantitative pathology analysis (one-way ANOVA, *P<0.05), we found that muscles fibers necrosed more seriously in the avulsion area than those in the contusion and concussion area and that myonecrosis may continue to deteriorate significantly over time if there were no treatments. What is more, damage degree was various between the muscle groups. The tibialis anterior muscle was the most seriously damaged while the soleus was the lightest at the same level. Scale bars: A-100 μm, B-100 μm, C-100 μm, D-100 μm, E-100 μm, F-100 μm.

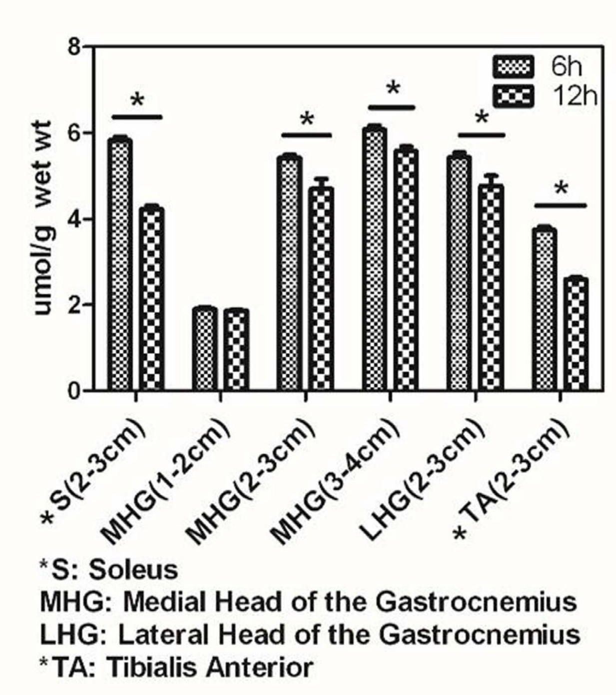

Figure 6. Different injured muscle groups ATP content of the stump.

low the knee approaching the detonation underwent neurotmesis (Fig. 5e) while the limb nerve above the knee away from the detonation underwent axonotmesis (Fig. 5f).Employing semi-quantitative pathology analysis (Fig. 5g), we found that muscle fibers necrotized more seriously in the avulsion area than those in the contusion and the concussion area and that myonecrosis may continue to deteriorate significantly over time if there are no treatments. Moreover, the degree of damage varied between the muscle groups. The tibialis anterior muscle was the most seriously damaged while the soleus was the least damaged.

Changes in Metabolism of Injured Skeletal Muscle

Changes in metabolism were examined through ATP content test (Fig. 6).The activity of affected muscle of both the contusion and commotion area declined remarkably at 12 h post-injury when comparing to either the control group or 6 h group, but the muscle in the avulsion area stayed at a low activity level with no change over the time. The muscle activity difference at the same sampling cross-section was in accordance with the histopathology founding above.

DISCUSSION

The experimental model was designed to investigate the characteristics of limb injury caused by landmine blast, simulating the limb injury of a walker treading on a landmine. We chose the rabbit as our experimental subject not only because its leg has some anatomical similarities with humans but also because its strong hind legs of rabbits provide enough muscle coverage, so it is conducive to research on the pathological characteristics of landmine explosive injury. The shell of the special point blast source is made of paper packaging to exclude the impact of fragmentation and provides a relative single experimental factor. Observation time (6 h and 12 h) of our experiment is mainly based on the evacuation time of most casualties.[13,14] From the pathologic results, its effect is also in line with protruding performance of damaged lower limbs treading on a landmine.

In clinical practice, imaging examinations are essential to trauma diagnosis. As is seen in our animal experiment, for a landmine blast victim, soft-tissue and bone loss are unavoidable.[15] The mechanical mechanism is so complex that multiple types of stress are generated after a landmine blast.[16] It may be the direct cause of the uncommon multi-segmental tibia facture. We failed to acquire some signs in relation to limb angiorrhexis at the upper part of the stump from the frame of DSA. The major blood vessels were not blocked. Vasospasm of the affected limb is a non-specific phenomenon which may happen in an ordinary trauma and its impact still needs further research.

The first principle of partition on typical landmine blast injury was elaborated by Nechaev EA in their research on landmine injury.[12] On the foundation of clinical experience and animal experiments, they divided the injured lower limb treading on mine and triggering blast into four anatomical zones from the distal to the proximal: Dispersion zone, avulsion zone, contusion zone, and commotion zone (Table 1). This anatomical partitioning method has certain instructive significance. Based on their study, we also found some regularity in the gross pathology of our experiment and renamed these anatomical areas. The stumps of all injured lower limbs stepping on landmines can be divided into three zones: Dissociation tissue, contusion hematoma, and edema contusion zones. The dissociation tissue zone is mainly characterized by highly contaminated tissue that was shattered and splintered. As a consequence, the corresponding segment of the fracture end lacks effective tissue covering and is exposed mostly. Large tracts of tissue necrosis were seen in microscopic pathology. Tissue viability of this dissociation tissue zone stayed at a relative low level and did not change significantly at 6-h and 12-h post-injury. Tissue retention in this area is impossible and unnecessary. Removing inactive tissues thoroughly

Table 1. The early principle of partition on typical landmine blast injury

Level	Zone	Description
I	Dispersion	Complete anatomical defect
II	Avulsion	Rupture crush and tissue separation
III	Contusion	Tissue contusion of the remaining part of damaged extremity segment
IV	Commotion	Tissue commotion of adjacent extremity segment and ascending circulatory and neurotrophic disturbance

and early also helps to prevent the expansion of the infection from a clinical standpoint, offering positive significance for the latter treatment.[1] Contusion hematoma area has obvious formation of contusion and hematoma in muscle gaps, but its necrotic area is not very large based on the microscopic pathologic findings. Even so, the necrotic area had expanded significantly at 12 h post-injury compared to 6-h post-injury. The vitality tests on muscle tissue of this zone showed a significant decrease over time. Although debridement played an important role in improving the microcirculation of various kinds of wounds,[17,18] some clinical studies found that excessive wound debridement cannot lead to a better stump functionally and cosmetically in landmine victims.[19] So, this area should be treated as a "danger zone" and we should try to save and avoid excessive debridement on this segment in the early stage (about 6 h after injury) of our clinical practice. Among this area, micro-pathology indicates that there is a certain degree of microcirculatory damage, but the necrosis may be reduced as much as possible by improving microcirculation as soon as possible. Edema is an outstanding feature in the edema concussion area. There were still a small number of point-like focal hemorrhages distributed irregularly or along the vascular sheath but demonstrated no more microscopic necrosis. Considering the main concussion effect acting on this area, there may exist residual effects of contusion that brought a bit of contusion performance, such as hemorrhage. Medical personnel should pay attention to the tightness of a tourniquet since the edema, especially the prolonged application. Of course, all of the proposed partitions are just a relative conception. In fact, the boundaries of districts were not very clear between each other, even adjacent areas presented partial crossover phenomenon. In summary, the renamed partitions give a more legible definition from anatomic aspects. Furthermore, we analyzed the prognosis of each partition and possible mechanisms and provided some rewarding clinical proposals for reference (Table 2).

Now that amputation stumps are wrapped mainly by muscles and the muscle group is composed of some unified functional muscles at the same or neighboring regions. We found some injury differences in muscle groups of affected lower limbs under the same experimental conditions, which were evidenced by muscle activity detection and histopathology. In the same cross-section, the tibialis anterior muscle had the most severe injury while soleus had the least injury. As is commonly known, triceps surae is designed as the major muscle flap tissue covering the amputation stumps in victims of anti-personnel mine blasts,[20] and some rough hints may be extracted from this result only when surgical debridement or amputation is implemented at the first aid scene, supporting the clinical experience of treatment to some extent.[21] Whether these differences have larger or more specific clinical significance requires more follow-up experiments for confirmation. Discrepancy of different muscle groups exists not only in anatomical locations but also in other aspects such as physiological diversity.[22] Additionally, complex reflection and diffraction occurred between the interfaces of different tissues of various densities during shock wave conduction after a blast.[23] As a result, shockwaves behave differently in the lower extremities striking a landmine, so the pressure distribution may not be the same between muscle groups. Unfortunately, we failed to reveal the exact mechanism forming the injury differences in muscles further for lack of some very effective methods. So, we have to prudently point out that the percentages of necrosis are just general not absolute indications.

In our experiment, high-speed photography recorded the general trajectory of lower limbs triggered by a simulated landmine after blasts on rabbits and found the special axial role of the knee and hip. Specifically, extreme flexion followed by a quick extension of the knee forms a "whiplash-like" mechanism of injury. Although the mechanism mentioned is

Table 2. The renamed partitions give a more legible definition from anatomic aspects through our experiments

Subarea	Abbreviation	In general description	Physiological change and prognosis (3R)	Treatment	Mechanism
Edema concussion area	E area	Edema, focal hemorrhage	Edema (Reserve)	Protection, avoid binding up too tightly	main in concussion
Contusion hematoma area	C area	Intact skin, muscle, muscle clearance damage partly, hematoma in muscle clearance, muscle contusion	Dangerous zone, partial and progressive tissue necrosis (Rescue)	Early treatment, removal foreign bodies and muscular clearance of hematoma, may stop progressive tissue necrosis and rescue more tissue	main in contusion
Free zone	F area	Tissue polluted and muscular clearance teared severely	Mostly dead or metamorphic mostly (Resect)	The source of infection, resect as soon as possible	avulsion and splitting

a new concept that has not been recognized before, we can safely infer that it may initiate tertiary blast injury in landmine blast injury. Based on the analysis of the motion parameters of reference markers of different parts, we also found a huge disparity between the peak acceleration and corresponding time point of shank and thigh of affected limbs, indicating that there is a big energy consumption during the shock wave spreading in the calf and thigh of the lower limb treading on a landmine. We speculated that the joint of the injured plays an important role in buffering blast pressure, so there is a high-risk factor of injury at joint connection structures such as ligaments.

The mortality rate was 3.8% after landmine blast, and injuries led to amputation in 73.3% of the victims.[24] Rohit Varma classified complex injuries of mine blast into four types based on the extent and severity of involvement, and these types also helped in predicting the possible line of management and its outcome.[25] However, we believe that it is not suitable for assessing standard mine blast damage, such as broom-like damage, nor for scientific research, because it is impossible to have such a large difference in injuries from the same yield mine blast. Landmine explosions can cause severe limb damage, and surgical treatment is very tricky. In a war environment, amputation is the main treatment method. However, amputation can result in loss of function, and more patients need psychotherapy due to major depression.[26] Therefore, it is important to preserve or reconstruct the limb as much as possible. If the dorsalis pedis is intact and midfoot and forefoot are relatively protected, hindfoot reconstruction should be attempted and hindfoot reconstruction is satisfactory with minor complications than amputation.[26] If skin along with the underlying soft tissue and the neurovascular structures on the dorsum of the foot are spared, then an attempt can be made at limb salvage.[27] Fixation and serial surgical wound debridement can be employed for treating mine blast injuries to maximize residual limb function.[25] Furthermore, the negative pressure wound therapy was helpful in preventing proximal amputations due to mine blast injury and was helpful in satisfactory reconstruction of foot defects.[28] It is worthy of people's attention to the neuropathic pain of the stump, the mechanism of neuropathic pain from landmine blast injury is very complicated, and it involves many factors of peripheral and central processes.[29] In our experiment, we used the epineurium bleeding as marker of nerve damage and confirmed a higher cross-section of nerve injury caused by the landmine blast. The nerve injury below the knee was classified into the first category, while the nerve injury above the knee was classified into the second or third category according to the publication of Sunderland.[30] This may be direct evidence of neuropathic pain from a landmine.

Limitations

Limitations of the experiment are as follows: (1) The physiological differences between experimental animals and actual human beings, and the display of experimental results only adopts specific experimental animals and experimental conditions; (2) there is diversity between experimental conditions and various complex conditions in reality, such as sand and stone composition, body posture, environmental temperature, and treatment conditions, so there are differences in real mine explosion injuries, and it is impossible to simulate real mine explosion injuries; (3) the exposure of rabbits to blasts while under anesthesia in the experiment may inhibit the effects of the sympathetic nervous system and the accompanying changes.

CONCLUSION

The established experimental model presents typical effect of lower limbs wounded by the mine blast in war field well. Landmine blast can cause typical damages on lower limbs including microcirculation damage difference of muscle group that was progressive over time, higher level of nerve lesion, and risky injury factors on the knee. The limb stump is divided into three zones based on gross pathology and micropathology, which can provide an important reference for clinical treatments and prognosis.

Ethics Committee Approval: This study was approved by the Army Medical University Ethics Committee (Date: 03.07.2012).

Peer-review: Externally peer-reviewed.

Authorship Contributions: Concept: Y.X., L.X., G.H.; Design: Z.M.W., G.H.; Supervision: Z.W., S.Z.; Resource: Y.X., L.X.; Materials: Z.W., Z.M.W.; Data collection and/or processing: Z.W., Z.M.W.; Analysis and/or interpretation: Y.X., L.X.; Literature search: Z.M.W., S.Z.; Writing: Y.X., G.H., S.Z.; Critical review: Z.W., S.Z.

Conflict of Interest: None declared.

Financial Disclosure: The author declared that this study has received no financial support.

REFERENCES

1. Mannion SJ, Chaloner E. ABC of conflict and disaster: Principles of war surgery. BMJ 2005;330:1498–500. [CrossRef]
2. Bilukha OO, Brennan M, Anderson M. The lasting legacy of war: Epidemiology of injuries from landmines and unexploded ordnance in Afghanistan, 2002–2006. Prehosp Disaster Med 2008;23:493–9. [CrossRef]
3. Moloney A. Caring for Colombia's landmine survivors. Lancet 2009;373:2013–2014. [CrossRef]
4. Durham J, Hoy D. Burden of injury from explosive remnants of conflict in Lao PDR and Cambodia. Asia Pac J Public Health 2013;25:124–33.
5. Wyper RB. An exploratory study of the perceived impact of health problems of landmine/UXO victims versus another disability group. Health Qual Life Outcomes 2012;10:121. [CrossRef]
6. Hamdan TA. Missile injuries of the limbs: An Iraqi perspective. J Am Acad Orthop Surg 2006;14:32–6. [CrossRef]
7. Changzhi C, Donghai Z, Quanyue L, Haiyan Q, Bocheng C, Linzhoudan. The landmine injuries treated 102 cases of analysis. People's Mil Surg 2010;4:239–40.
8. Cristina Restrepo A, Alberto Lopez J. Clinical and microbiological profiles of anti - personnel mine injuries in the Medellin area of northwestern

Colombia. Biomedica 2010;30:338–44.
9. Walsh NE, Walsh WS. Rehabilitation of landmine victims-the ultimate challenge. Bull World Health Organ 2003;81:665–70.
10. van den Broek NM, Ciapaite J, Nicolay K. Comparison of in vivo post-exercise phosphocreatine recovery and resting ATP synthesis flux for the assessment of skeletal muscle mitochondrial function. Am J Physiol Cell Physiol 2010;299:1136–43. [CrossRef]
11. Kimmich GA, Randles J, Brand JS. Assay of picomole amounts of ATP, ADP, and AMP using the luciferase enzyme system. Anal Biochem 1975;69:187–206. [CrossRef]
12. Nechaev EA, Gritsanov AI, Fomin NF. Mine-Blas Trauma. The 7th International Symposium of Weapons Traumatology and Wound Ballistics, St. Petersburg; 1994. p. 15–6.
13. Necmioglu S, Subasi M, Kayikci C, Young DB. Lower limb landmine injuries. Prosthet Orthot Int 2004;28:37–43. [CrossRef]
14. Pillgram-Larsen J, Mellesmo S, Peck R. Injuries from mines. Tidsskr Nor Laegeforen 1992;112:2183–7.
15. Oztürk S, Bayram Y, Möhür H, Deveci M, Sengezer M. Evaluation of late functional results of patients treated with free muscle flaps for heel defects caused by land-mine explosions. Plast Reconstr Surg 2005;116:1926–36. [CrossRef]
16. Hull JB, Cooper GJ. Pattern and mechanism of traumatic amputation by explosive blast. J Trauma 1996;40:198–205. [CrossRef]
17. Sun YH, Yu DN, Chen X, Hu XH, Zhang GA, Yan RY. Preliminary study on the improvement of wound microcirculation and retrospection on several methods of the management of deep partial thickness burn wound. Chin J Burns 2005;21:17–20.
18. Ichioka S, Yokogawa H, Sekiya N, Kouraba, S, Minamimura A, Ohura N. Determinants of wound healing in bone marrow-impregnated collagen matrix treatment: Impact of microcirculatory response to surgical debridement. Wound Repair Regener 2009;17:492–7. [CrossRef]
19. Khan MI, Zafar A, Khan N, Saleem M, Mufti NJ. Outcome of tissue sparing surgical intervention in mine blast limb injuries. Coll Physicians Surg Pak 2006;16:773–6.
20. Trimble K, Adams S, Adams M. Anti-personnel mine injuries. Curr Orthop 2006;20:354–60. [CrossRef]
21. Parker PJ. Initial medical and surgical management. Curr Orthop 2006;20:333-45. [CrossRef]
22. Schiaffino S, Reggiani C. Fiber types in mammalian skeletal muscles. Physiol Rev 2006;91:1447-531. [CrossRef]
23. Wolf SJ, Bebarta VS, Bonnett CJ. Blast injuries. Lancet 2009;374:405-15. [CrossRef]
24. Afshar A, Afshar N, Mirzatoloei F. Injuries due to landmine blast referred to Shahid Motahhary Hospital, Iran. Med J Armed Forces India 2007;63:157–9. [CrossRef]
25. Varma R, Arora NC, Rai S, Chaudhary A, Wani S. Surgical options in the management of landmine blast injuries of lower limb: A randomised prospective study. Acta Orthop Belg 2019;85:21–34.
26. Bahtiyar Demiral P, Ege T, Kose O, Yurttas Y, Basbozkurt M. Amputation versus functional reconstruction in the management of complex hind foot injuries caused by land-mine explosions: A long-term retrospective comparison. Eur J Orthop Surg Traumatol 2014;24:621–6. [CrossRef]
27. Khan MT, Husain FN, Ahmed A. Hindfoot injuries due to landmine blast accidents. Injury 2002;33:167–71. [CrossRef]
28. Maurya S, Srinath N, Bhandari PS. Negative pressure wound therapy in the management of mine blast injuries of lower limbs: Lessons learnt at a tertiary care center. Med J Armed Forces India 2017;73:321–7. [CrossRef]
29. Muller A, Sherman R, Weiss J, Addison R. Neurophysiology of pain from landmine injury. Pain Med 2006;7:P205-6. [CrossRef]
30. Sunderland S. A classification of peripheral nerve injuries producing loss of function. Brain 1951;74:491–516. [CrossRef]

DENEYSEL ÇALIŞMA - *ÖZ*

Kara mayını patlamasının neden olduğu alt ekstremite hasarının özellikleri ve mekanizması: Tavşan modelinde bir araştırma

Dr. Sen Zhang,[1] Dr. Gengfen Han,[3] Dr. Yan Xiong,[1] Dr. Ziming Wang,[1] Dr. Zhong Wang,[1] Dr. Xinan Lai[2]

[1]Ortopedi Bölümü, Daping Hastanesi, Ordu Tıp Merkezi, Chongqing, Çin Halk Cumhuriyeti
[2]Cerrahi Araştırma Enstitüsü 6. Bölümü, Daping Hastanesi, Ordu Tıp Merkezi, Chongqing, Çin Halk Cumhuriyeti
[3]PLA Ortak Lojistik Destek Gücünün 969. Hastanesi, Çin Halk Cumhuriyeti

AMAÇ: Kara mayını patlamalarının neden olduğu uzuv yaralanmalarının tedavisi ve korunması zordur. Alt ekstremite yaralanmalarını incelemek için bir hayvan modeli oluşturmak ve kara mayını patlamalarının neden olduğu alt ekstremite yaralanmalarının özelliklerini ve mekanizmalarını araştırmak gereklidir.

GEREÇ VE YÖNTEM: Yirmi altı olgun beyaz tavşan rastgele sham grubuna (n=10) ve yaralanma grubuna (n=16) ayrıldı. Kara mayını patlaması, özel modifiye edilmiş bir sabitleme çerçevesi ile dik durumdaki sağ alt ekstremitenin altına elektrikli fünyeler yerleştirilerek simüle edildi. Vücut hareketlerini gözlemlemek için yüksek hızlı fotoğraflama tekniği kullanılmıştır. Vital bulgular, vasküler yaralanma (dijital subtraksiyon anjiyografi ile belirlenerek), patolojik özellikler ve tibialis anterior kası ve şaft triseps surae ATP konsantrasyonu karşılaştırma için kaydedildi.

BULGULAR: Genel olarak, tavşanların yaralı bacaklarının orta ve alt segmenti ciddi şekilde hasar gördü. Ekstremite güdükleri, dokusuz bölge, kontüzyon hematomu ve ödem kontüzyonu olmak üzere üç alanda dağılım gösterdi. Sinsi yara izi, miyofasiyal yıkım ve periost sıyrılması kara mayını patlama yaralanmasının tipik özellikleriydi. ATP konsantrasyonu ve patolojik analiz, tibialis anterior kasının en ciddi şekilde yaralandığını, bunu gastroknemius ve soleusun takip ettiğini gösterdi. Hem kontüzyon hem de komotio alanındaki etkilenen kasın ATP konsantrasyonu zaman içinde önemli ölçüde azaldı, ancak avülsiyon alanındaki kas zaman içinde hiçbir değişiklik göstermeden düşük aktivite seviyesinde kaldı. Kontüzyon alanında küçük vasküler yaralanma belirgindi. Siyatik sinir lezyonu bölgesi kastan daha yüksekti. Siyatik sinir yaralanması bölgesi ciddi kontüzyon kasından daha yüksekti. Yüksek hızlı fotoğraflama tekniği, yaralı uzvun eklemlerinin aşırı derecede büküldüğünü ve ardından patlama şok dalgası altında hızlı bir gerilme olduğunu gösterdi.

SONUÇ: Oluşturulan deneysel model, savaş alanında mayın patlamasıyla yaralanan alt uzuvların tipik etkisini ortaya koymaktadır. Kara mayını patlaması alt uzuvlarda sinir lezyonu, diz yaralanması ve zamanla ilerleyen mikrosirkülasyon hasarı gibi tipik hasara neden olabilir. Uzuv kütüğü, klinik tedaviler ve prognoz için önemli bir referans sağlayabilecek gros patoloji ve mikropatolojiye dayalı olarak üç bölgeye ayrılmıştır.

Anahtar sözcükler: Hayvan modelleri; patlama yaralanması; uzuv; mayın.

Ulus Travma Acil Cerrahi Derg 2023;29(12):1335-1343 DOI: 10.14744/tjtes.2023.39560