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An ultrastructural study on indirect injury of dental pulp caused by high-speed missile projectile to mandible in dogs

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Abstract – The aim of this study was to evaluate the characteristics of indirect injury of dental pulp caused by high-speed missile projectile to mandible in dogs. Eighteen dogs aged 12–13 months were divided equally into six groups ($n = 3$ in each group) with random allocation, then a high-speed missile projectile (a ball bearing of stainless steel, φ6.0 mm, 0.88 g) was shot at right mandible body (the wound tract was below the fourth premolar, 1 cm or so to the root tips) of each dog, but the teeth were not wounded directly. The dogs were killed 6 h ($n = 3$), 24 h ($n = 3$), 3 days ($n = 3$), 7 days ($n = 3$), 2 weeks ($n = 3$) and 4 weeks ($n = 3$) after the wound, respectively; then ultrastructural change of dental pulp of the fourth premolar and the second premolar of right mandible, and the second premolar of left mandible was observed through transmission electron microscope. The results showed that mean initial velocity of projectiles was 778.0 ± 33.2 m s^{-1} and mean projection energy was 266.1 ± 19.1 J, which were in conformity with parameters of gunshot wound. On the wound side, dental pulp of the fourth mandibular premolar was injured seriously and irreversible necrosis happened in the end; yet, dental pulp of the second mandibular premolar was injured less seriously, reversibly; on the opposite side, dental pulp of the second mandibular premolar was injured slightly and temporarily. It may be concluded that there are several characteristics in indirect injury of dental pulp caused by high-speed missile projectile to dogs' mandible: the injured area is relatively extensive; traumatic degree decreases progressively and sharply with the distance to the wound tract increasing; ultrastructural change of nerval damage takes place in early stage after wound, etc.

Firearm injuries in oral and maxillofacial region have the characteristics of high incidence rate, extensive traumatic area, complicated traumatic condition, etc. (1). In recent years, quite a few studies on injuries of adjacent organ accompanying battle wound in oral and maxillofacial region have been reported, such as injury of brain (2, 3), eyes and optical nerve (4–6), great vessels in cervical part (7), facial nerve (8, 9), etc., but studies on indirect injury of dental pulp were seldom seen. With the transition of medical model, as we rescue the wounded, we should not only save their lives but also think highly of the rehabilitation of physiological function after wound. Teeth are a significant organ of mankind for manducation and phonation; they should not be ignored in trauma care of oral and maxillofacial region. Furthermore, the pulp tissue differs from other tissues of organism in morphosis and function. Therefore, it is necessary for us to study the indirect injury of dental pulp accompanying firearm injury in oral and maxillofacial region. In this study, we try to learn the characteristics of indirect injury of dental pulp caused by high-speed missile projectile to dogs' mandible through transmission electron microscope.

Materials and methods

Animals and experimental design

Eighteen dogs aged 12–13 months and weighing 12.3–13.5 kg (from the animal holding center at Fourth Military Medical University of China) were used in this study, whose dentition alternation had been finished. They were maintained in temperature-controlled room and given unrestricted access to food and water during the experimental period. The animals were divided equally into six groups ($n = 3$ in each group) with random allocation; these groups were noted as Group A, B, C, D, E, F, respectively.

Equipment

A new type of level air-pushed multifunctional bio-impactor device, which had been union-developed by the Department of Mechanics of the Northwest Nuclear Technique Institute (Xi'an, Shaanxi Province, PR China) and Department of Oral and Maxillofacial Surgery of Fourth Military Medical University, was applied in this study. Through the equipment, a successive and adjustable projection speed between 300 and 1500 m s^{-1} could be implemented for experiment. This device could be used for simulating the wound of high-speed missile projectile; likewise, it could be applied together with spherical explosives or detonators for modeling the explosive injury (10).

Vulnerating

The animals were anaesthetized by Sumianxin II (a new type of compound general anaesthetic for animal experiment, developed and produced by Military Veterinary Medicine Institute of Military Medical Science Academy, Changchun, Jilin Province, PR China) through intramuscular injection 0.1 ml kg^{-1}, and right side of mandible was prepared for experiment. Then made a dog face lying on the object stage of the bio-impactor device, right side of the its head facing the device, and a plastic cylinder (φ3.0 cm) was placed between its upper anterior teeth and inferior anterior teeth in order to keep it gaping. The position of its head was adjusted precisely, in order that the orifice of emission tube of the device could aim at its right mandible body (the point below the fourth premolar, 1 cm or so to the root tips), the distance between the orifice and right mandible body was 35 cm; moreover, the dog's head was made right-leaning, so that the outlet of missile projectile would locate its mouth floor, avoiding haemorrhea caused by penetration of body of tongue, or teeth of the opposite side being involved in. Between the orifice of emission tube and the dog's head, a laser velocimeter device (also from the Department of Mechanics of the Northwest Nuclear Technique Institute) was placed (Fig. 1). Subsequently, nitrogen gas with the pressure of 1.5 MPa was injected into the high-pressure air chamber; then, the fast-turning-on self-excitation solenoid valve was turned on, vulnerating the dog. In this study, the missile projectiles we used were ball bearings of stainless steel, φ6.0 mm, weighing 0.88 g.

Fig. 1. Main body of the level air-pushed multifunctional bio-impactor device and the body position of an experimental animal. The brightened dot at dog's surface of right mandible region demonstrated the location we planned to aim at, between the animal and the emission tube was the laser velocimeter device.

Detection of vulnerant parameter

The initial velocity of the missile projectiles was detected by the laser velocimeter device, and mean value was calculated, then the projection energy was obtained through the formula $E = 1/2\ mv^2$.

Observation of traumatic condition

Emergency medical treatment such as packing with sterile absorbent gauze and débridement and suture was performed to each dog right away after it being vulnerated, preventing it from dying of respiratory tract obstruction or excessive loss of blood. Meantime, traumatic condition of oral and maxillofacial region was overviewed and recorded. Penicillin 1 mg kg^{-1} was given through intramuscular injection ter in die to prevent infection (not more than 4 days). Afterwards, the general condition and wound healing state of each dog was observed while it was being killed.

Fig. 2. The inlet of the missile projectile penetrating the dog's right mandible body.

Fig. 3. Comminuted fractures close to the wound tract.

Observation of ultrastructural change of dental pulp

The dogs of Group A, B, C, D, E, F were killed 6 h, 24 h, 3 days, 7 days, 2 weeks, 4 weeks after being vulnerated, respectively; then their heads were perfused by 4% polyoxymethylene solution for tissue fixation. After perfusion, the fourth premolar and the second premolar of right mandible, together with the second premolar of left mandible of each dog were pulled out, then they were split open with caution, the pulp within each of them was unloaded and cut into a 1-mm-thick segment which was derived from the middle part of root pulp, and 3% glutaraldehyde compound solution was used to strengthen the tissue fixation of dental pulp for 24 h. Then the pulp specimens were rinsed, dehydrated with gradient by acetone and embedded into Epoxy Resin 618; subsequently, ultrathin sections were made and stained by osmic acid and observed through JEM-2000 transmission electron microscope (Japanese Electron Mi-croscope Co. Ltd., Osaka, Japan) and representative pictures were taken.

Results

Based on data of this study, mean initial velocity of the missile projectiles was 778.0 ± 33.2 m s^{-1}, and mean projection energy was 266.1 ± 19.1 J.

In this study, all the missile projectiles penetrated the dog's right mandible body, the inlet was below the fourth premolar, 0.9–1.1 cm to the root tips (Fig. 2), and the outlet was located in the submental region, the size of outlet is almost equal to that of inlet. Close to

Table 1. Ultrastructural change of dental pulp of appointed teeth of every group

Group	Dental pulp of right mandibular fourth premolar	Dental pulp of right mandibular second premolar	Dental pulp of left mandibular second premolar
A (6 h after wound)	Invagination of myelin sheath in some endodontic nerves (Fig. 4) and vacuolization of mitochondria in a few Schwann cells. Ultrastructure of Neuraxes and blood vessels were normal roughly.	Loosening of stratification structure of myelin sheath in part of endodontic nerves (Fig. 9). Ultrastructural change of neuraxes or blood vessels was not observed.	Loosening of stratification structure of myelin sheath happened to a few endodontic nerves, ultrastructural change of neuraxes or blood vessels was not observed.
B (24 h after wound)	Invagination of myelin sheath in more endodontic nerves, swelling in part of neuraxes. Vacuolization of vascular endothelial cells (in higher magnification, also swelling of mitochondria) together with obvious hyperaemia within blood vessels (Fig. 5) could be seen.	Loosening of stratification structure of myelin sheath in more nerves, and invagination happened to some of them, but not ultrastructural change of neuraxis was observed. Slight hyperaemia within blood vessels, but ultrastructure of vessels was normal roughly.	Loosening of stratification structure of myelin sheath happened to a few more endodontic nerves than Group A, ultrastructural change of neuraxis or vessels was still not observed.
C (3 days after wound)	Disorder of stratification structure of myelin sheath in many nerves and disintegration of a few neuraxes (Fig. 6). Increased lipofuscin within pulp cells and collagen rarefaction near the cells, more obvious hyperaemia, and vacuolization in more vascular endothelial cells, a few of which shed from vessel wall and collagen covered by them was exposed (Fig. 7).	Loosening of stratification structure and invagination of myelin sheath were seen fairly frequently in endodontic nerves, a few auxiliary fibers were swelling; hyperaemia was aggravated; a bit of endothelial cells had fallen off and collagen covered by them was exposed, collagen rarefaction showed in some region.	The quantity and degree of endodontic nerves involved in resembled those of Group B, slight hyperaemia was seen within blood vessels but neither ultrastructural change of vessels nor collagen rarefaction was observed.
D (7 days after wound)	Ultrastructural change of myelin sheath was aggravated and disintegration happened to a lot of neuraxes, many collagen fibers dissolved; stagnant blood emerged everywhere and breakage of vessel walls was aggravated, haemorrhage was observed; macrophages close to blood vessels contained much lipofuscin (Fig. 8).	A few disintegrated endodontic nerve fibers and neoformative ones were concomitant (Fig. 10), hyperaemia within blood vessels lightened, collagen rarefaction still showed in some region and macrophages containing lipofuscin could be observed occasionally.	The quantity and degree of endodontic nerves involved in decreased significantly compared with Group B and C, slight hyperaemia was still seen but neither obvious ultrastructural change of vessels nor collagen rarefaction was observed.
E (2 weeks after wound)	Most of endodontic nerves (no matter myelin sheath or neuraxis) disintegrated, neoformative nerve fibers were not observed, collagen fibers dissolved completely; angiorrhexis, flowing over of haemocytes and macrophages containing lipofuscin were seen frequently.	Ultrastructure of blood vessels and most of endodontic nerves recovered on the whole, a few disintegrated nerve fibers and neoformative ones were concomitant, hyperaemia subsided, collagen rarefaction were seldom seen, a few macrophages containing lipofuscin still exsisted.	Ultrastructural appearance of pulp tissue recovered on the whole, except that few macrophages containing a little lipofuscin could be observed.
F (4 weeks after wound)	The pulp showed completely necrotic appearance, and no specific structure could be discerned.	Ultrastructure of pulp tissue was normal roughly, except that few macrophages containing lipofuscin could be observed.	The pulp tissue showed normal ultrastructure through TEM.

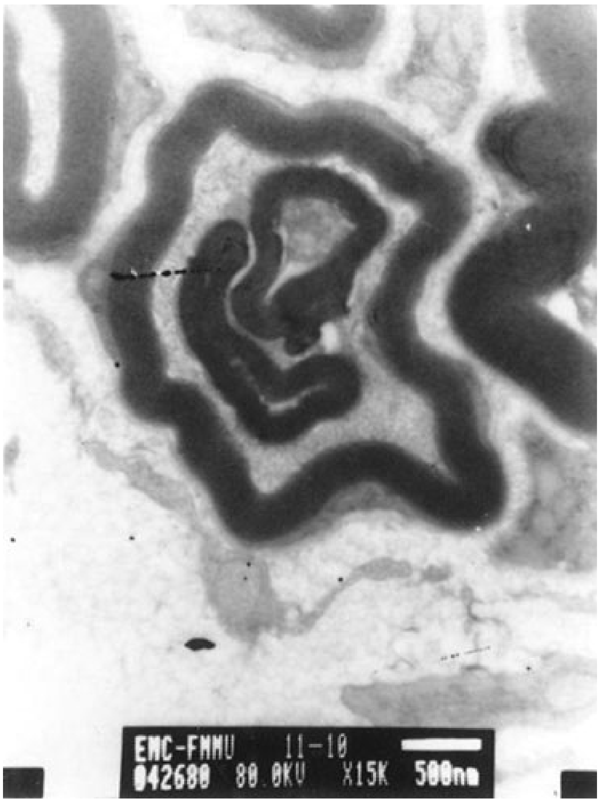

Fig. 4. Invagination of myelin sheath of an endodontic nerve. (TEM, ×15 k, the bar was equal to 500 nm).

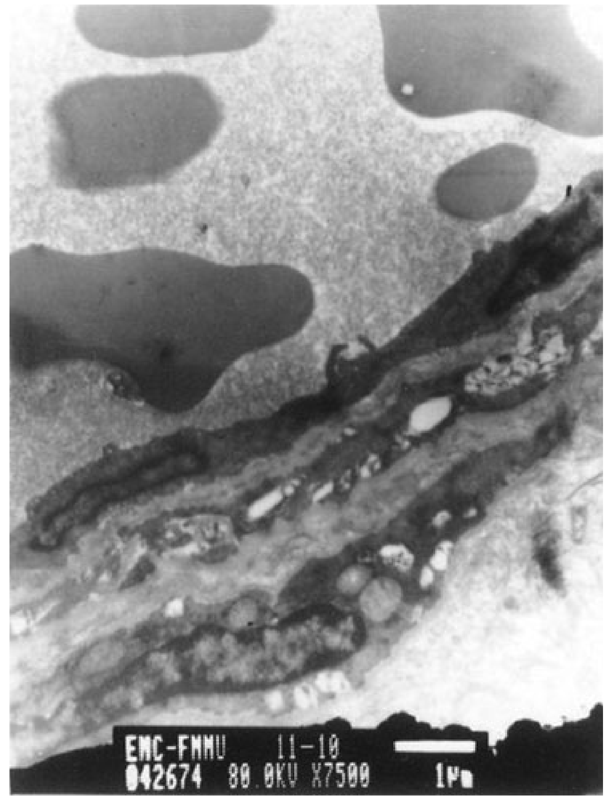

Fig. 5. Vacuolization of vascular endothelial cells and swelling of mitochondria together with obvious hyperemia within a blood vessel. (TEM, ×7.5 k, the bar was equal to 1000 nm).

the wound tract, comminuted fractures could be seen (Fig. 3), but none of teeth suffered direct injuries. In the mouth, laceration and haemorrhage were usually observed in gingiva surrounding the fourth premolar of right mandible, as well, in some dogs, they were seen in gingiva circling the first molar and/or the third premolar of right mandible, but neither dental dislocation nor fracture of teeth could be discovered in any dogs. Of three dogs belonging to Group C, Group D and Group F, respectively, the volume of bleeding was rather great as the missile projectile had penetrated the root of tongue, and two dogs from Group B and Group C, respectively, were stricken by nasal haemorrhage; but all of the above dogs survived by our haemostasis treatment.

Six hours after the wound, the dogs were downcast and unwilling to movement, and they refused to take food; 24 h after the wound, they were less downcast and could take a little food but they were still unwilling to movement; 3 days after the wound, the dogs' spirit was aroused obviously and their quantity of movement and food intake increased significantly; 7 days after the wound, the dogs' spirit, movement and food intake were all normal, and the wound of soft tissue healed, mobility of broken ends of fractured bone diminished a little; 2 weeks after the wound, mobility of broken ends of fractured bone was little; 4 weeks after the wound, mobility nearly vanished and the fracture almost healed completely.

The ultrastructural change of dental pulp of appointed teeth of every group is summarized in Table 1.

Discussion

In our study, we developed an experimental model in dogs to simulate firearm injuries. According to the data, mean initial velocity of projectiles was 778.0 ± 33.2 m s^{-1} and mean projection energy was 266.1 ± 19.1 J, these parameters are in conformity with those of gunshot wound (11, 12). This allowed us to evaluate the characteristics of indirect injury of dental pulp caused by high-speed missile projectile to dogs' mandible.

The basic features of modern firearm injury are complexity of wound tract, catholicity of impaired area and multiplicity of parts involved in (1, 12, 13). When someone is vulnerated by high-speed missile projectile, the endothecium of wound tract would suffer coup injury, besides, tissue in more extensive surrounding area of wound tract will be involved in therefore indirect injury would be resulted in. Numerous studies have shown that main vulnerant modalities of high-speed missile projectile consist of pressurization and laceration, buffeting and blast wave, as well as temporary cavity effect (1–3, 12–14).

Likewise, while the mandible was wounded by high-speed missile projectile, a temporary cavity would emerge and internal pressure of the cavity changes

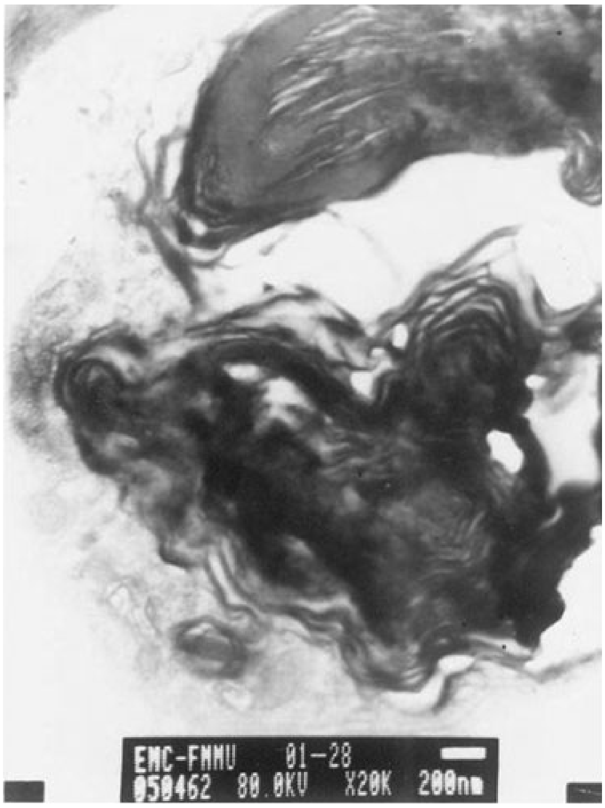

Fig. 6. Disorder of stratification structure of myelin sheath of a nerve and disintegration of the neuraxis. (TEM, ×20 k, the bar was equal to 200 nm).

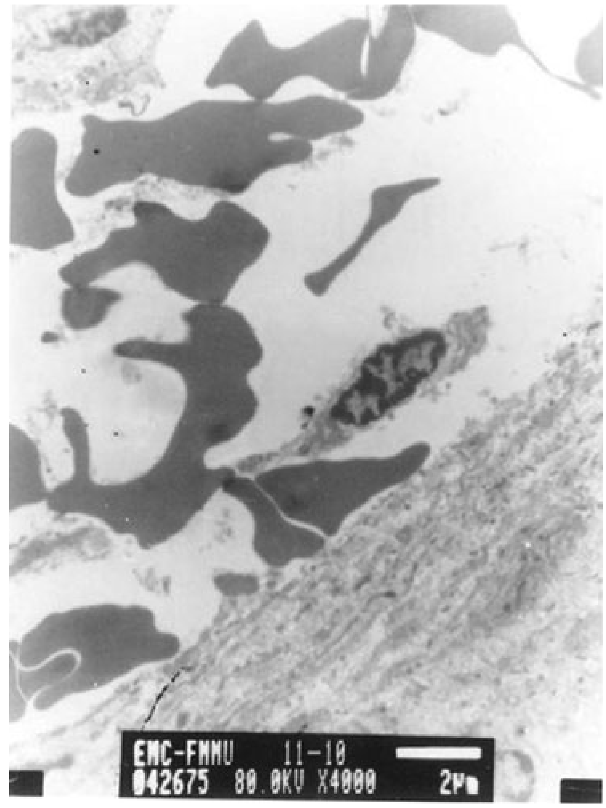

Fig. 7. A vascular endothelial cell shed from the vessel wall and collagen covered by it was exposed as well obvious hyperaemia within a blood vessel. (TEM, ×4 k, the bar was equal to 2000 nm).

sharply; therefore, the surrounding tissues (for instance, inferior alveolar blood vessels and nerve, dental pulp tissues, etc.) would suffer iterative and violent tension and shock, thus indirect injury of dental pulp would happen. In addition, as the high-speed missile projectile permeated through the mandible body, because of the blast wave and tooth concussion, hard tissues and soft tissues might separate from each other and blood vessels passing through apical foramen might break down, so that dysaemia happened to the dental pulp and pathological change ensued. Besides, thermometric change can harm the dental pulp (15, 16), when the projectile permeated the mandible body, temperature in the area nearby the wound tract would step up in a moment, which is also a probable factor of indirect injury of dental pulp. As for teeth of the opposite side, because temporary cavity effect is comparatively feeble, pathological changes of dental pulp may relate to conduction of blast waves. Owing to the two sides of mandible is an integer, while a projectile is cutting through the mandible in one side, the blast waves could convey to the opposite side, so the dental pulp might be damaged.

Moreover, endodontic injury is inseparable from the characteristics of anatomy and constitution of dental pulp. Pulp tissue is a type of loose connective tissue rich in cell constituents and ground substance. The ground substance (which is abundant in collagen) is the intermediary agent of metabolism of pulp tissue and it has glutinosity (17), which makes endodontic inflammation not easy to diffuse. Furthermore, the pulp is wrapped by adamant mineral matter, although the blood supply of pulp tissue is fairly sufficient, all the blood vessels, nerves and lymphatic vessels communicate with surroundings through a narrow apical foramen, in addition, collateral circulation is insufficient in pulp cavity, when inflammatory oedema emerges in pulp chamber, inflammatory fluid could not expand to the outside, thus the internal pressure increases inevitably, compressing blood vessels, which will cause the blood supply insufficient. Even worse, pathological production is difficult to be conveyed to the outside, and the pulp tissue is unable to receive potent immunological sustain, thus infernal circles will be generated with the result of pulp degeneration, even necrosis. But on the contrary, as the pulp tissue is rich of cell components, and the undifferentiated mesenchymal cells can differentiate into every type of cells of connective tissue or odontoblast or macrophage while being stimulated (18), dental pulp has strong reparation and regeneration capacity, if it suffers slight or moderate injury, the pathological change will be reversible in general.

In the present study, while the mandible suffered wound of high-speed missile projectile, dental pulp, not only the tooth was far or near to the wound tract, would generate ultrastructural change of some extent. The dental pulp of right mandibular fourth premolar, which was adjacent to the wound tract, was injured

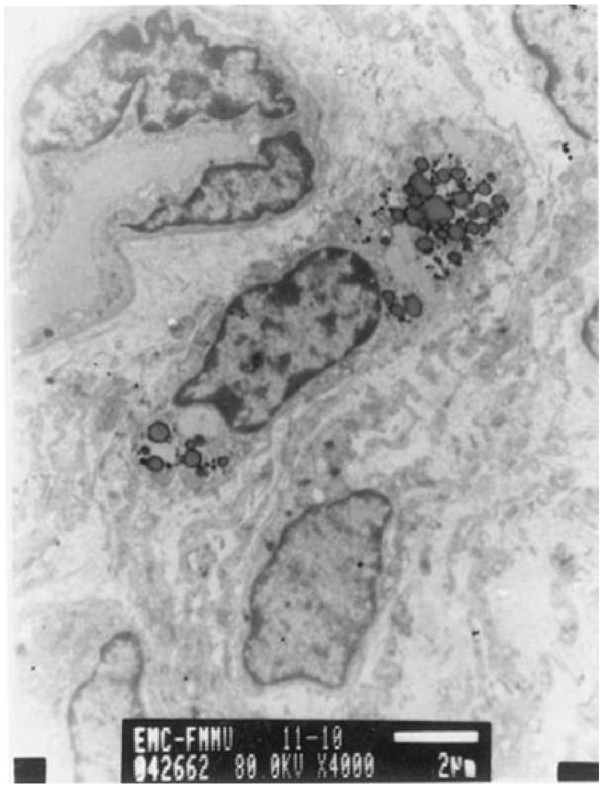

Fig. 8. A macrophages with a deal of lipofuscin within it close to a blood vessel. (TEM, ×4 k, the bar was equal to 2000 nm).

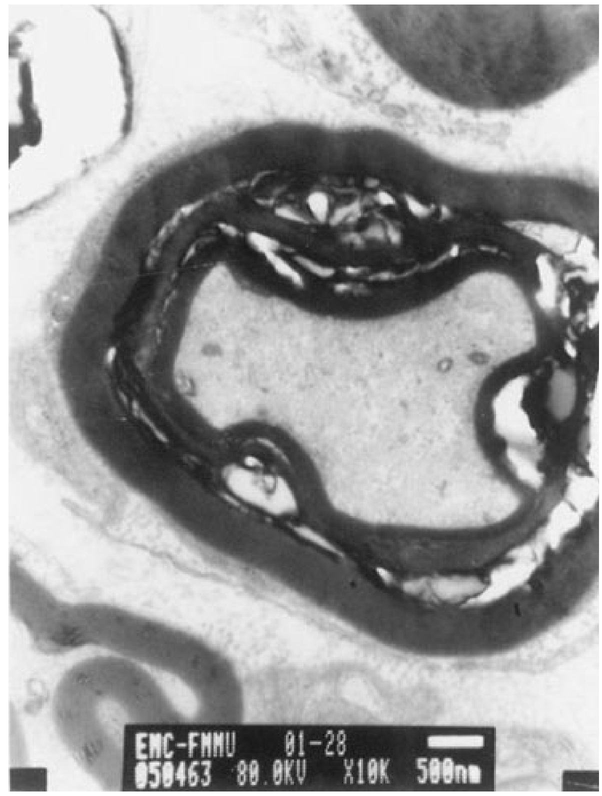

Fig. 9. Loosening of stratification structure of myelin sheath of an endodontic nerve. (TEM, ×10 k, the bar was equal to 200 nm).

swiftly and seriously: 6 h after wound, some early ultrastructural change such as invagination of myelin sheath of endodontic nerves and vacuolization of mitochondria of Schwann cells could be observed; as the time ran on, some other change, for instance, swelling of cylindraxile, vacuolization of vascular endothelial cells, hyperaemia, collagenolysis, and so on, could be seen one after another; 7 days after wound, the dental pulp began to degenerate; and became irreversible necrosis in the end. The dental pulp of right mandibular second premolar, which was not far away from the wound tract, was injured less seriously, necrosis only happened to a few nerves, but neogenetic nerve fibers substituted them in no time; 4 weeks after wound, ultrastructural appearance of pulp tissue was normal on the whole. On the opposite side of mandible, the dental pulp of the second premolar was injured slightly and temporarily, only loosening of stratification structure of myelin sheath in a few nerves and slight hyperaemia could be observed through electron microscope; 2 weeks after wound, the ultrastructural appearance of pulp was normal on the whole, and 4 weeks after wound, the dental pulp obtained full recovery. All of the above showed that traumatic degree of indirect injury of dental pulp caused by high-speed missile projectile to the mandible decreased progressively and sharply with the distance to the wound tract increasing.

From this study, we also saw that ultrastructural change of myelin sheath of endodontic nerves (no matter of the injured side or the opposite side) emerged 6 h after the wound, yet, no marked ultrastructural change happened to endodontic blood vessels or collagen fibers at that time. That indicated ultrastructural change of nerval damage of indirect injury of dental pulp caused by high-speed missile projectile to the mandible should take place earlier.

Based on our observed results, dental pulp of right mandibular fourth premolar, which was adjacent to the wound tract, was injured seriously, and became irreversible necrosis eventually, although the tooth had not suffered direct injury of missile projectile. It suggests that, when treating a patient of oral and maxillofacial injury, besides doing with teeth impaired directly, root canal therapy should be carried out to teeth which are adjacent to the wound tract in spite of not having suffered direct injury.

In conclusion, when the dog's mandible is wounded by high-speed missile projectile, the pulp of mandibular teeth will suffer indirect injury. The characteristics of this kind of injury may include: the injured area is relatively extensive; traumatic degree decreases progressively and sharply with the distance to the wound tract increasing; ultrastructural change of nerval damage takes place in early stage after wound, etc. However, restricted by experimental time and condition, we have not got the message of whether the dental pulp of maxillary teeth was injured indirectly of some extent, nor how to evaluate the prognosis of teeth suffered indirect injuries,

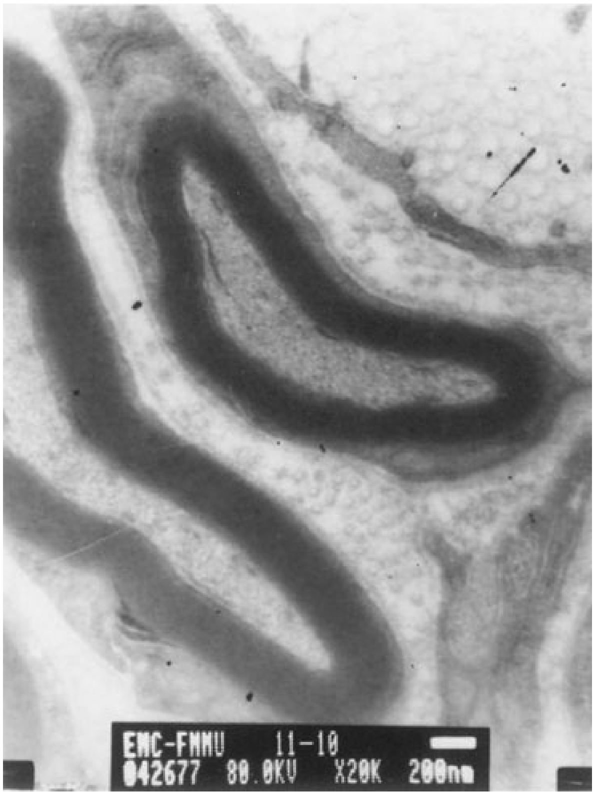

Fig. 10. Two neoformative nerve fibers. (The myelin sheath of which was thin but the neuraxis was thick relatively). (TEM, ×20 k, the bar was equal to 200 nm).

nor many other problems. Further research work in this field should be carried out in future.

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