An ultrastructural study on indirect trauma of dental pulp caused by maxillofacial impact injury in dogs

Köpeklerde maksillofasiyal çarpma sonucu diş pulpasında gelişen dolayı yaralanma üzerine ultrastrüktürel bir çalışma

Changqun REN, Ruifeng LIU, Lei TIAN, Peng CHEN, Shuxia ZHOU

BACKGROUND
Indirect injuries of adjacent tissues and organs usually accompany maxillofacial impact injuries. However, studies on indirect dental pulp injury are rare. This study was designed to determine the characteristics of indirect dental pulp injury caused by impact injury of mandible in dogs.

METHODS
Eighteen dogs were divided equally into six groups with random allocation. Right mandible of each dog was impacted but teeth were not injured directly. Then, the animals were killed at appointed time points and ultrastructural changes in dental pulp of assigned teeth of each dog were investigated with transmission electron microscope.

RESULTS
Dental pulp of the fourth premolar of right mandible was injured very severely, but irreversible necrosis did not occur in the end. Dental pulp of the second premolar of right mandible was injured less severely and reversibly. Dental pulp of the second premolar of left mandible was injured mildly and temporarily.

CONCLUSION
In the indirect injury of dental pulp caused by maxillofacial impact injury, the injured area is relatively extensive. The effect of the trauma decreases progressively and sharply as the distance to the impact site increases. Ultrastructural changes in the damaged nerves take place early.

Key Words: Animal experimentation; dental pulp/blood supply/physiopathology; dogs; impact injury; mandibular fractures; maxillary fractures; microscopy, electron; transmission; pressure; ultrastructure.

AMAÇ
Maksillofasiyal çarpma yaralanmaları sonucunda genellikle komşu doku ve organlar da dolayı olarak yaralanırlar. Ancak, diş pulpasinin dolayı yaralanmasi konusunda fazla çalışma yoktur. Çalışmamızda köpeklerin mandibülerinde çarpma sonucu diş pulpasında dolayı olarak gelişen yaralanmanın özelliklerini belirlemeyi amaçladık.

GEREÇ VE YÖNTEM
Onsekiz köpek rastlantalı olarak altı eflit gruba ayrılırdı. Her köpeğin sağ mandibulasında çarpma travması uygulandı. Ancak, dişler doğrudan yaralanmadı. Hayvanlar belirli zamanlarda öldürüldü ve her bir köpeğin diş pulpasındaki ultrastöktürel değişiklikler transmisyon elektron mikroskobu kullanılarak incelendi.

BULGULAR
Sağ mandibuladaki dördüncü premolar dişin pulpasi ağır bir şekilde yaralanmıştı, fakat sonuçta geri dönümsüz bir nekroz oluşmadı. Sağ mandibuladaki ikinci premolar dişin pulpasındaki yaralanma daha az ağırdı ve geri dönümsüzlüydi. Sol mandibuladaki ikinci premolar dişin pulpa ise hafif ve geçici bir şekilde yaralandı.

SONUÇ
Çarpma şeklindeki maksillofasiyal yaralanmalar diş pulpasının dolayı olarak yaralanmasının açısından incelendiğinde, oldukça geniş bir alanın yaralandığı görülür. Travmanın etkisi çarpma sahasından uzaklaşarak giderek ve hızla azalır. Sinir hasarına ait ultraströktürül değişiklikler erken dönemde oluşur.

Anahtar Sözcükler: Hayvan deneyi; diş pulpasi/kan desteğin/physiopathology; köpek; çarpma yaralanması; mandibula kirgii; maksilla kirgii; elektron mikroskobu; basınç; madde, en küçük parça yapısı.
Impact injuries are happening mostly in traffic accidents in daily life or wartime.[1-3] Impact injuries could result from falls from height or being struck by blunt appliance.[1] Maxillofacial region has a high rate of attack and so, maxillofacial impact injury received much attention. In maxillofacial trauma cases, the injured area is quite extensive and many complications are encountered. Indirect injuries of adjacent tissues and organs usually accompany maxillofacial impact injuries. In recent years, a few studies on indirect injuries of adjacent tissues and organs accompanying maxillofacial impact injuries have been reported, such as injuries of brain,[4,5] eyes and optical nerves,[5-7] temporomandibular joint,[8-10] great vessels in the neck,[11,12] and the cervical spine.[13-16] However, studies on indirect injury of the dental pulp were seldom reported. During the treatment of the patient, we should not only save their lives, but also consider the rehabilitation of physiological function after the treatment. As a significant organ for manducation and phonation, teeth should not be ignored in trauma cases of oral and maxillofacial region. Pulp tissues differ from other tissues in morphosis and function. Therefore, it is necessary to study the characteristics of indirect injury to dental pulp accompanying maxillofacial impact injury. In this study, we used transmission electron microscope to determine the characteristics of indirect injury of dental pulp caused by impact injury of mandible in dogs.

MATERIALS AND METHODS

Animals and experimental design

We used eighteen dogs from the animal holding center of our university, whose dentition alternation had been finished. They were 12 to 13 months of age and weighed 12.1 to 13.3 kg. The experiments were done in accordance with the institutional guidelines for the care of laboratory animals. They were maintained in a 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. The groups were noted as Group A, B, C, D, E, F, respectively.

Equipment

We used a new type of level air-pushed multifunctional bio-impactor device (Figure 1) which had been developed by the Department of Mechanics of the Northwest Nuclear Technique Institute (Xi’an, Shaanxi Province, PR China) and our department. By using this equipment, a successive and adjustable (range between 3~40 m/s) impact velocity could be generated. This device could be used for implementing impact injuries with excellent reproducibility. Other characteristics were a satisfactory vulneration effect, relatively simple operability, lower impact noise, optional shape as a ram, and requirement of little space for the experiment.[17]

Vulneration

First, the impactor device had been installed for the experiment. The impact stick we applied was hollow so as to generate higher impact velocity, and the ram capping the tip of the impact stick was a cylindroid iron cap which weighed 0.88 kg. The external diameter was 4.50 cm, internal diameter was 3.10 cm and contact area of impact was 15.90 cm². After approval by an institutional review board the animals were anaesthetized by Sumianxin II (a new type of compound general anesthetic for animal experiments, such as rabbits, dogs or guinea pigs, etc., 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, and their right side of mandible was prepared for experiment. Then a dog was placed face lying on the object stage of the bio-impactor device. Right side of its head was facing
the device. The impact stick was pulled up and the position of the dog was adjusted precisely, so that the impact site would be located at the dog’s right mandible (the area below the fourth premolar) and the centre spot lie on inferior margin of the mandible (Figure 2). Between the tip of the impact stick and the dog’s head (the distance between them was 35 cm), a laser velocimeter device (also developed and produced by the Department of Mechanics of the Northwest Nuclear Technique Institute, Xi’an, Shaanxi Province, PR China) was placed. Subsequently, the impact stick was pushed back to the trigger of emission tube, and Nitrogen gas was injected into the high pressure air chamber with the pressure of 1.0 Mpa. Then the fast-turning-on self-excitation solenoid valve was turned on, vulnerating the dog.

Detection of vulneration parameters

The initial impact velocity of the ram was detected by the laser velocimeter device and mean value was calculated. The impact energy was obtained through the formula $E = \frac{1}{2}mv^2$.

Observation of traumatic conditions

Emergency medical treatment such as packing with sterile absorbent gauze and debridement and suture were performed to each dog immediately after it had been vulnerated. This prevented it from dying of respiratory tract obstruction or excessive loss of blood. In the meantime, traumatic conditions of oral and maxillofacial region was observed and recorded. Penicillin 1 mg/kg was given through intramuscular injection t.i.d. to prevent infection (not more than 4 days). Afterwards, the general condition and wound healing state of each dog were observed when it was killed.

Observation of ultrastructural changes of dental pulp

The dogs of Group A, B, C, D, E, F were killed under anaesthesia 6 hours, 24 hours, 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, with the second premolar of left mandible of each dog were pulled out, and the teeth were split open with caution. The pulp within each of them was unloaded and a one-millimeter-thick segment was derived from the middle part of root pulp. After that, 3% glutaraldehyde compound solution was used to strengthen the tissue fixation for 24 hours. Then the pulp specimens were rinsed, dehydrated with acetone and embedded into Epoxy Resin 618. Subsequently, ultrathin sections were made and stained by osmic acid and observed through JEM-2000 EX transmission electron microscope (Japan Electron Optics Laboratory Co. Ltd., Tokyo, Japan) at an accelerating voltage of 80.0 kV. Meanwhile, representative pictures were taken.

RESULTS

Mean initial impact velocity was $18.09 \pm 0.38$ m/s, and mean impact energy was $143.99 \pm 4.19$ J.

All of the animals suffered the expected wound, i.e., the impact site was located at right mandible (the area below the fourth premolar) and the centre spot lay on inferior margin of the mandible. Lacerations appeared on the soft tissue surfaces at the impact site. The length of the wounds varied from 1.6 cm to 3.0 cm (Figure 3). Mandibular fractures were observed in all of the dogs. All dogs had comminuted fractures at impact region except two dogs with single-line fractures. None of the teeth suffered direct injuries. In the mouth, laceration and hemorrhage were usually observed in gingiva between the third premolar and first molar of right mandible (Figure 4). But neither dislocation nor odontagma could be discovered. In two dogs, belonging to Group C and Group D the loss of blood was rather great as the inferior alveolar vein
was poked by bone fragments; and two other dogs from Group B and Group F had nasal hemorrhage. But all of these dogs survived by haemostasis treatment.

Six hours after the trauma, the dogs were depressed and unwilling to move. They refused to be fed. Twenty-four hours after the trauma, they were less depressed and could feed but they were still unwilling to move. Three days after the trauma, the dogs were clearly in better mood and their movements and food intake increased considerably. Seven days after the trauma, their moods, mobility and food intake were all normal. The soft tissues healed and fracture ends were less mobile. Four weeks after the trauma, fracture mobility nearly vanished.

The ultrastructural changes of dental pulp are summarized in Table 1.

**DISCUSSION**

In this study, we developed an experimental model in dogs to simulate impact injuries. Mean initial impact velocity was 18.09±0.38 m/s (65.12 km/h), which is close to the running speed of motor vehicles in daily life. Moreover, the ram we used to vulnerate the animals was a cylindroid iron cap whose external diameter was 4.50 cm and the contact area of impact was 15.90 cm². This allowed us to simulate impact injuries of traffic accidents through the model and evaluate the characteristics of indirect injury of dental pulp caused by maxillofacial impact injury in dogs.

Damage of impact injuries is produced by high speed collision mainly. It was reported that in maxillofacial impact injuries, transmission effect of the stress waves and impact acceleration effect, resulting from impact and oscillation are primary factors causing indirect injuries of adjacent tissues and organs.¹⁵,¹⁸ Likewise, in the impact injury of the mandible, not only local soft tissues and bones were wounded directly, but also compound stress force aggravated the trauma and made surrounding tissues (for instance, inferior alveolar blood vessels and nerves, dental pulp tissues, periodontium, etc.) being subject to iterative and violent tension and shock. Therefore indirect injuries of dental pulp could happen. In addition, while the mandible body was struck, due to the blast wave and tooth concussion, hard tissues and soft tissues might separate from each other. Therefore, blood vessels passing through apical foramen might break down causing ischemia and the ensuing pathological changes in the dental pulp.

Furthermore, features of endodontic injuries are inseparable from characteristics of anatomy and constitution of dental pulp tissues. Pulp tissue is a type of loose connective tissue which is rich in cells and ground substances. The ground substance (which is abundant in collagen) is the intermediary agent of metabolism of pulp tissues and it has glutinosity,¹⁹ which makes endodontic inflammation difficult to diffuse. In addition, the pulp is wrapped by adamant mineral matter. Although blood supply of pulp tissue is fairly sufficient, all the blood vessels, nerves and lymphatics pass through a narrow

---

Fig. 3. Laceration on surface soft tissues of impact site. (The length of the wound was 2.0 cm)

Fig. 4. Laceration and hemorrhage in gingiva surrounding the third premolar to the first molar of right mandible, but neither dental dislocation nor odontogma could be discovered.
Table 1. Ultrastructural changes of dental pulp of appointed teeth of dogs in each group

<table>
<thead>
<tr>
<th>Group</th>
<th>Dental pulp of the fourth premolar of right mandible</th>
<th>Dental pulp of the second premolar of right mandible</th>
<th>Dental pulp of the second premolar of left mandible</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>(6 hours after wound)</td>
<td>Stratification structure of myelin sheath in part of endodontic nerves has loosened but neuraxes were normal on the whole (Figure 5a); Mild hyperemia occurred within blood vessels but ultrastructure of vessels’ walls was normal.</td>
<td>Stratification structure of myelin sheath in a few endodontic nerves has loosened. Ultrastructural changes of neuraxes or blood vessels were not observed.</td>
</tr>
<tr>
<td>B</td>
<td>(24 hours after wound)</td>
<td>Stratification structure of myelin sheath in more endodontic nerves has loosened; myelin sheath of some nerves was invaginated and neuraxes of which were swelling (Figure 5b). Vacuolization of vascular endothelial cells and swelling of mitochondria of the cells with obvious hyperemia within blood vessels (Figure 5c) could be seen.</td>
<td>Stratification structure of myelin sheath in a few more nerves has loosened, but no ultrastructural change of neuraxes was observed. Mild hyperemia occurred within blood vessels but ultrastructure of vessels’ wall was normal.</td>
</tr>
<tr>
<td>C</td>
<td>(3 days after wound)</td>
<td>Stratification structure of myelin sheath in most of endodontic nerves has loosened, myelin sheath of some nerves was invaginated and neuraxes of which were swelling even demyelinated. Vacuolization of more vascular endothelial cells with aggravated hyperemia showed up. A few endothelial cells shed from the vessels (Figure 5d). Collagenolysis emerged in some regions of ground substance.</td>
<td>The quantity and degree of endodontic nerves involved in resembled those of Group B; mild hyperemia was seen within blood vessels but neither ultrastructural change of vessels’ wall nor collagen rarefaction in ground substance was observed.</td>
</tr>
<tr>
<td>D</td>
<td>(7 days after wound)</td>
<td>Ultrastructural appearance of endodontic nerves was similar to that of Group C, in addition, disintegration happened everywhere in a few neuraxes; breakage of vessels’ wall was aggravated, hemorrhage was discovered and macrophages close to blood vessels contained some lipofuscin (Figure 5e). Collagenolysis occurred everywhere in the ground substance (Figure 5f).</td>
<td>Ultrastructural changes of myelin sheath in more nerves has loosened; myelin sheath of a few nerves was invaginated and neuraxes of which were swelling. Vacuolization of a few vascular endothelial cells together with moderate hyperemia within blood vessels could be seen. Collagenolysis emerged in part of regions of ground substance.</td>
</tr>
<tr>
<td>E</td>
<td>(2 weeks after wound)</td>
<td>Ultrastructural appearance of endodontic nerves was less severe than that of Group D, a few disintegrated nerve fibers and neoformative ones were concomitant (Figure 5g); hyperemia within blood vessels decreased and ultrastructure of vessels’ walls became well. In ground substance, collagen rarefaction occurred in some regions and macrophages containing lipofuscin could be seen occasionally.</td>
<td>The pulp tissue showed normal ultrastructure through TEM.</td>
</tr>
<tr>
<td>F</td>
<td>(4 weeks after wound)</td>
<td>Ultrastructural changes of endodontic nerves was less severe than that of Group D. Hyperemia within blood vessels decreased and ultrastructure of vessels’ walls became well. In ground substance, collagen rarefaction was seldom seen, and a few macrophages containing lipofuscin still existed.</td>
<td>Ultrastructural appearance of pulp tissue was normal roughly (Figure 5h). The pulp tissue showed normal ultrastructure through TEM.</td>
</tr>
</tbody>
</table>

Ultrastructural appearance of pulp tissue was normal roughly (Figure 5h).
Fig. 5. (a) Stratification structure of myelin sheath in an endodontic nerve has loosened but neuraxis was normal on the whole. (TEM, x10k, the bar was equal to 500 nm). (b) Myelin sheath of a nerve was invaginated and neuraxis was swollen. (TEM, x15k, the bar was equal to 500 nm) (c) Vacuolization of vascular endothelial cells and swelling of mitochondria with obvious hyperemia within a blood vessel. (TEM, x7.5k, the bar was equal to 1000 nm) (d) A vascular endothelial cell shed from the vessel wall and collagen covered by it was exposed as well as obvious hyperemia within a blood vessel. (TEM, x4k, the bar was equal to 2000 nm) (e) A macrophage close to a blood vessel contained some lipofuscin within it. (TEM, x7.5k, the bar was equal to 1000 nm) (f) Collagenolysis occurred in ground substance. (TEM, x15k, the bar was equal to 500 nm) (g) Two neoformative nerve fibers. (The myelin sheath of which was thin but the neuraxis was thick relatively). (TEM, x20k, the bar was equal to 200 nm) (h) Ultrastructural appearance of pulp tissue was normal roughly. (TEM, x10k, the bar was equal to 500 nm)
apical foramen and collateral circulation is insufficient in pulp cavity. When there is inflammatory edema in pulp chamber, inflammatory fluids cannot expand outside. This increases the internal pressure inevitably, compressing blood vessels and making blood supply insufficient. Even worse, inflammatory products are difficult to be carried outside, and the pulp tissue is unable to receive potent immunological sustain, generating a vicious circle which results in pulp degeneration and necrosis. On the other hand, pulp tissue is rich in cells including the undifferentiated mesenchymal cells which can differentiate into any type of connective tissue cell, odontoblast or macrophage when stimulated.[20] So, dental pulp has a strong capacity of reparation and regeneration. If it suffers mild or moderate injury, the pathological changes will be usually reversible.

In this study, while the mandible suffered impact injury, dental pulp, not only the tooth, whether it is far from or near the impact site, would show ultrastructural changes to some extent. Dental pulp of the fourth premolar of right mandible, which is located in the impact site, was injured seriously. Six hours after trauma, some early ultrastructural changes such as loosening of stratification structure of myelin sheath of endodontic nerves and vacuolization of mitochondria of Schwann cells could be observed. Later, some other changes, such as invagination of myelin sheath, swelling of neuraxes, vacuolization of vascular endothelial cells, serious hyperemia, collagenolysis, and so on could be seen. Seven days after trauma, disorder of stratification structure of myelin sheath and disintegration of neuraxes were seen in some endodontic nerves. However, neogenetic nerve fibers substituted them quickly. Thus irreversible necrosis did not take place to the dental pulp in the end. Two weeks after trauma, ultrastructural appearance of the dental pulp was better, and 4 weeks after trauma, the dental pulp recovered. Dental pulp of the second premolar of right mandible, which was adjacent to the impact site, was injured less seriously. Disorder of stratification structure of myelin sheath and disintegration of neuraxes did not happen to any of the endodontic nerves. Two weeks after trauma, ultrastructural appearance of pulp tissue was normal. On the opposite side of mandible, dental pulp of the second premolar was damaged slightly and temporarily. Only loosening of stratification structure of myelin sheath in a few nerves and a mild hyperemia could be observed through the electron microscope. Seven days after trauma, the ultrastructural appearance of pulp was normal, and 2 weeks after trauma, the dental pulp recovered fully. All of the above shows that traumatic degree of indirect injury of dental pulp caused by impact injury of the dog’s mandible decreases progressively and sharply as the distance to the impact site increases. Therefore, we think that in patients with oral and maxillofacial impact injury, root canal therapy should not be carried out shortly after the trauma, unless the teeth suffered by impact injury such as dislocation or odontagma. The adjacent teeth should be observed for at least two weeks. Then, vigour of dental pulp is tested, and we decide whether endodontic treatment should be implemented or not.

Also, ultrastructural changes of myelin sheath of endodontic nerves on the wound side occurred 6 hours after the wound. However, there were no marked ultrastructural changes in the endodontic blood vessels or collagen fibers at that time. This indicates that ultrastructural changes of nerve damage may happen early after indirect injury of dental pulp caused by maxillofacial impact injury. This is consistent with the results of Liu et al.[21] on indirect injury of dental pulp caused by a high velocity missile projectile to dog’s mandible.

In conclusion, when a dog’s mandible is subjected to impact injuries, pulp of mandibular teeth will be injured indirectly. The characteristics of this kind of injury are as follows: The injured area is relatively extensive; traumatic degree decreases progressively and sharply as the distance to the impact site increases; ultrastructural changes of nerve damage take place in the early stage after trauma. However, restricted by experimental time and conditions, we did not have the answers to whether pulp of maxillary teeth would be injured indirectly to some extent; how to evaluate the prognosis of teeth that suffered by indirect injuries; whether the injured dental pulp of the teeth which is located in impact site can recover completely and how long it will take for full recovery after trauma; the threshold of initial impact velocity at which dental pulp of the teeth located in the impact site will suffer irreversible necrosis eventually; and
many other related problems. Further research in this field should be carried out in future.

Acknowledgement

This research was supported by the Medical Science Research Foundation of Army for Significant Topics of the Tenth Five-Year Plan of China, No: 01L075.

REFERENCES