Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi:

Application of ultrasonic inspection in monitoring dynamic healing of mandibular fracture in rabbit model

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ARTICLE INFO

Article history:
Received 15 February 2012
Received in revised form 15 March 2012
Accepted 15 April 2012
Available online 20 May 2012

Keywords:
Fracture
Ultrasonic inspection
Fracture healing
X-ray

ABSTRACT

Objective: To investigate the feasibility of ultrasonic diagnosis for monitoring fracture healing. **Methods:** Thirty rabbit models with fraction of mandible body were established by surgically removing partial lower jawbone. At the 1st, 2nd, 4th, 6th, 8th and 12th week after the operation, they were examined by X-ray and ultrasound, respectively. All detection results were scored according to a generally accepted standard. Spearman rank correlation analysis was conducted to explore the relationship between the results of the two inspection methods. **Results:** In each healing stage, the results of the ultrasonic inspection were basically consistent with those of the X-ray examination, as supported by a Spearman rank correlation coefficient of 0.892 (P<0.001). **Conclusions:** Non-invasive ultrasonic inspection can be used instead of X-ray examination to monitor and diagnose fracture healing.

1. Introduction

In clinic, the X-ray examination is commonly used to detect fracture ends regularly after reduction and fixation of bone so as to observe osteal healing and growth. However, the X-ray radiation damages human body, especially pregnant women, fetuses and children. Presently, the non-invasive ultrasonography has been an alternative of the X-ray examination to monitor dynamic healing process of fracture, which has become a hot research spot. Compared with the X-ray examination, the ultrasonography is simple, rapid, non-invasive and accurate. Furthermore, the ultrasonoscopy is more suitable for the examination of early callus than the X-ray examination. To further investigate the feasibility of ultrasonic inspection for monitoring fracture healing, we used the ultrasonoscopy technique to observe dynamic healing process of fracture in rabbit model with fraction of mandible body.

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Foundation project: Supported by the Research and Development Project of Scientific and Technological Industry of Guangdong Province (2011B080701053).

2. Materials and methods

2.1. Establishment of rabbit fracture model

Two healthy rabbits were selected randomly from 32 experimental rabbits and used as control. The other 30 were used to establish fracture models by the following surgery. After general anesthesia with ketamine (30-50 mg/kg· body weight), the forepart of lower jawbone, about 1 mm-wide bone segment, were amputated, and the bone was reconstructed by internal fixation with titanium plates and screws. A 1-mm long bone gap was produced for the observation of fracture healing.

2.2. X-ray inspection

At the 1st, 2nd, 4th, 6th, 8th and 12th weeks after the operation, the same part of each experimental rabbit was inspected regularly by a designated technical personnel using a digital X-ray imaging system (GE, USA). The same conditions of projection were used. The results of X-ray examination were scored according to the clearness of fracture line, callus number, and formation of continuous callus. The scoring standard described by Moed was used. Four levels were used in the quantitative scoring standard, and they are as follows: Level Ⅰ (1 point) manifested as no callus, clear fracture gap, slightly fuzzy boundaries of fracture ends; Level Ⅱ (2 points) manifested as a few calluses and clear fracture gap; Leve Ⅲ (3 points) manifested as more calluses and obscure but still existent fracture gap; and Level Ⅳ (4 points) manifested as indistinct fracture gap, a large number of calluses and completely connected fracture ends.

2.3. Ultrasonic inspection

At the 1st, 2nd, 4th, 6th, 8th and 12th weeks after the operation, the experimental rabbits were inspected regularly by a designated technical personnel using a Philips iU22 ultrasound system (GE, German). Double-blind method was used for the ultrasonic examination. The testing results were recorded and used to evaluate the extent of fracture healing. The ultrasound images were scored according to the standard also described by Moed, and the aforementioned quantitative scoring standard was also used for the ultrasonic inspection.

2.4. Statistical analysis

The scoring results were analyzed using the Spearman rank correlation test. The postoperative time, results of X-ray examination and results of ultrasonography were defined as variables 1, 2 and 3, respectively.

3. Results

3.1. Dynamic analysis of fracture healing of mandible body by X-ray inspection

As examined by X-ray, progressive calcification indicating new bone formation was observed in the gap of mandibular fracture. With the healing of fracture, the fracture gap gradually narrowed, radiopaque shadow was gradually becoming stronger, calluses appeared and became gradually more, and the fuzzy fracture line disappeared. The radiographs were scored, and the rabbits were assigned at different grades of healing (Table 1).

3.2. Ultrasonography of normal tissues in mandibular part

The rabbit skin with a thickness of 0.75-1.50 mm made hyperechoic or medium echoes. The subcutaneous tissue and muscular layer made hypoechoic or medium echoes, and these tissues had varying thickness due to their positions and nutritional status. Some connective tissues and muscle fibers were linear hyperechoic. The low jawbone made continuously hyperechoic echoes, and its ultrasonic image had a smooth boundary and a posterior acoustic shadow. The surface of the low jawbone was 2.5-5.0 mm far away from skin in the mandibular part.

3.3. Dynamic analysis of fracture healing of mandible body by ultrasonography

After the ultrasonic examination, the images were scored, and the rabbits were assigned at different grades of healing according to the above-mentioned standard (Table 1).

At the 1st week after the operation, the swelling of soft tissue at the fracture site subsided obviously. The number of echoes increased, and the hyperechoic line of cortical bone was interrupted. The boundaries of the fracture ends were slightly fuzzy, and the echoes from the fracture gap were enhanced and became anechoic or hypoechoic. At this time, the fracture gap was about 1 mm wide.

At the 2nd week after the operation, the swelling of soft tissue at the fracture site faded away. The echoes were enhanced, and irregular weak hypoechoic echoes possibly from the original calluses appeared gradually on the fracture ends. The anechoic region decreased in size or disappeared. The number of echoes from periosteum increased. The fracture ends had obscure edges and were connected by periosteum. At this time, the fracture gap was narrowed,

Table 1

Scoring results of X-ray and ultrasonic inspections in different healing stages of mandibular fracture (n = 30).

Postop. time (wk)	Level Ⅰ		Level Ⅱ		Level Ⅲ		Level Ⅳ	
	X-ray	Ultrasound	X-ray	Ultrasound	X-ray	Ultrasound	X-ray	Ultrasound
1	30	26	0	4	0	0	0	0
2	30	12	0	15	0	3	0	0
4	6	0	24	6	0	15	0	9
6	0	0	6	0	20	6	4	24
8	0	0	0	0	8	2	22	28
12	0	0	0	0	0	0	30	30

0.5-0.7 mm in width.

At the 4th week after the operation, the echoes from the calluses were enhanced and became uneven high sound. The fracture ends were connected by the calluses. The fracture gap became vague and disappeared. Arch-shaped lamellar irregular structure was formed at local parts, and the acoustic beam could go across the gap.

At the 6th week after the operation, the echoes at the fracture site were enhanced and almost resembled that from normal lower jaw bone. The uneven echoes became gradually even. The gap had almost disappeared, and the local arch-shaped structure was gradually reduced in size and flattened. An acoustic shadow appeared with posterior acoustic transmission.

At the 8th week after the operation, the echoes were further enhanced to be dense, even and strong ones, which were difficult to be distinguished from the normal mandibular echoes. The hyperechoic line of cortical bone became continuous, and the local arch-shaped structure became flat.

At the 12th week after the operation, the fracture part was connected with the surrounding normal bones and became indistinct.

3.4. Comparison between X-ray inspection and ultrasonography

The Spearman rank correlation coefficient was 0.892 between the results of the X-ray examination and those of the ultrasonography (P=0.000<0.001). The test fails to reject the null hypothesis at the default α = 0.05 significance level. Based on the statistical analysis, we found that the results of the X-ray examination were correlated with those of the ultrasonography.

4. Discussion

After reduction and fixation of fracture, some unexpected operation sequelae such as the failed connection between fracture ends, poor or delayed healing and failure of internal fixation may occur occasionally due to various reasons. Regular testing is thus needed to determine the growth and healing of fracture[1]. The X-ray inspection is a routine clinical method to monitor fracture repair. However, it is always influenced by many factors including conditions of projection and film developing, subjective factors of readers, and radiation injuries[1,2]. In different stages of fracture healing, cellular components and bone matrix vary, and the attenuation coefficients of tissues with different densities also change. These changes result in different ultrasonic echoes, which is the biological basis for ultrasonography to be used to observe fracture healing. The B-ultrasonic examination reflects fracture healing by testing the fracture gap as well as the number and area of callus formation[2-4]. It is meaningful to investigate the feasibility of safe quantifiable non-invasive B-ultrasound in examining fracture healing in clinic. In the rabbit model with fraction of mandible body, the results of the X-ray examination had correlation with those of the ultrasonography. Therefore, we believe that the ultrasonic examination be feasible and credible to monitor the dynamic process of fracture healing. Besides, intensity of echoes from fracture gap can be used to reflect callus calcification, and the sound beam below callus can be used to indicate new bone formation[2-6]. In our study, in the middle stage of healing, the sound beam raising outwards was found in the fracture gap and could also go through the gap. In the late stage of healing, the sound beam disappeared and became an acoustic shadow, indicating the increasing density, good calcification and good mineralization of calluses.

In the routine X-ray examination, the content of bone minerals in callus should reach at least 25% for macroscopical distinguishment[2]. However, the ultrasonic inspection can detect fibrous and cartilaginous calluses that are difficult to be observed by X-ray. Thereby, the ultrasonic monitoring is superior to the X-ray examination for the observation of early porosis. By observing the healing of fracture of tibia body, Mode reported that the ultrasonic inspection can provide earlier information than the X-ray examination to reveal the appearance of callus, forecast the final result of fracture and diagnose fracture recovery[2]. In addition, blood circulation in fracture ends can also be observed by the ultrasonography, but this is an impossible task for the X-ray examination. Blood supply to fracture site is important for the growth, healing and rebuilding of bones, and the colour Doppler ultrasound can be used to evaluate the development of fracture healing by detecting blood volume[2,5,6]. Fracture patients having rich blood signals in or around callus had well-formed calluses, while those having no blood signals also showed poor callus formation[3,7,8]. Bottinelli *et al* also thought the colour Doppler ultrasound could be used to observe fracture healing and surrounding blood supply[7]. Therefore, the ultrasonic inspection can help to monitor callus formation dynamically and to observe tissue injuries and blood supply in fracture part, which is very important for early prognosis and treatment. In addition, the B-ultrasound examination can be used to show the continuity of cortical bone at fracture site, development of fracture ends and dynamic reduction of bone, making the reset operation intuitive and accurate[8-10].

Many clinical experts and scholars aim to find non-invasive examination methods, and the non-invasive ultrasonic examination attracts much attention. Pregnant women, fetuses and children are more sensitive to X-ray radiation, and repeated X-ray detection is unacceptable. The ultrasonic examination is greatly popular in clinic and has an advantage in observing fracture healing of sensitive populations, which is incomparable with the X-ray inspection. In clinical practice, the ultrasonic inspection can be used instead of the X-ray examination to perform reduction and fixation for severed finger reunion, traditional Chinese bone-setting, and fractures in children and pregnant women. Doctors and patients can also be protected from long exposure to X-ray radiation during surgery[11-13]. With the continuous improvement and innovation of ultrasound technology and the accumulation of clinical experience, the ultrasonic inspection will have broad prospects in monitoring fracture healing.

Conflict of interest statement

We declare that we have no conflict of interest.

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