Original Article
The establishment of an adjacent intervertebral disc egeneration model in New Zealand white rabbits undergoing lumbar fixation and fusion for intervertebral disc degeneration

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Abstract: Objective: To establish an animal model of adjacent intervertebral disc degeneration in New Zealand White rabbits undergoing lumbar fixation and fusion for intervertebral disc degeneration. Methods: Fifteen healthy, 10-month-old New Zealand rabbits were used in this study. The L3-4 intervertebral disc was located with an 18-G puncture needle under fluoroscopic guidance. Magnetic resonance imaging (MRI) of the spine was performed periodically. When L3-4 disc degeneration occurred, the L3-4 disc was excised, and interbody fusion with the transverse process graft and titanium plate fixation were performed. The changes in the adjacent intervertebral disc were observed periodically via MRI after interbody fusion. The animals were sacrificed 12 weeks after surgery, and the histopathology of the adjacent intervertebral disc was studied. Results: The animal response score was not statistically significant before or after intervertebral disc puncture (F = 1.563, P > 0.05), but it was significantly different before and after the intervertebral disc excision and interbody fusion (F = 6.178, P < 0.001). Compared with the response scores in the animals undergoing disc puncture, the response scores in the animals undergoing fusion and fixation were significantly lower at all studied time points (T = 7.952, 5.791 and 3.944, P < 0.001). The survival rate was 100%. Dynamic MRI revealed that the overall T2 signal intensity started to decrease beginning in the 4th week after disc puncture. Varying degrees of degeneration were present in the adjacent disc after fixation and interbody fusion. The adjacent disc degeneration was not significantly different between the puncture group and the fixation and fusion groups (P > 0.05) in the 4th week after intervention but was significantly different (P < 0.05) in the 8th and 12th weeks. The animals were sacrificed 12 weeks after fixation and interbody fusion, and we observed complete fusion of the fixated segments with surrounding callus formation. A gross pathological examination revealed dark-gray nucleus pulposus with partial fat-like changes. The hematoxylin and eosin (HE)-stained sections showed vague borders of the nucleus pulposus. The nucleus pulposus tissue was replaced by cartilage tissue, and degenerative changes were present in the center. Conclusion: The animal model studied in this work is preliminary for studying adjacent intervertebral disc degeneration after intervertebral disc excision, fixation, and fusion.

Keywords: Adjacent intervertebral disc, degeneration, model

Introduction

Disc degeneration disease (DDD) is a common disease in clinical practice and can cause disc herniation, spinal stenosis, segmental instability, and other clinical symptoms, and it is the major cause of neck, shoulder, and waist pain. The causes of DDD are complex; its pathophysiology and pathogenesis still remain unclear [1, 2]. The occurrence and deterioration of DDD depend on multiple interdependent factors, including a decreased nutrition supply to the intervertebral disc [3], genetic factors [4], mechanical load of the intervertebral disc [5], age [6], the body's inflammatory factors [7] and lifestyle (e.g., obesity, occupation, smoking and drinking) [8]. DDD animal models play an important role in the study of the disease's pathologic mechanisms. To date, the "gold standard" for DDD surgical treatment is still to achieve relief of the clinical symptoms and a higher fusion rate via fixation, decompression, and fusion [9]. However, adjacent disc degeneration may occur following surgical treatment and can cause

new-onset neurological symptoms and physical signs, reportedly with an incidence of 30.3% [10]. The mechanism of this degeneration remains unknown. Moreover, related studies have focused on clinical observations and have failed to explain the pathogenesis of the degeneration's occurrence and development. The aim of this study is to establish a simple animal model of adjacent intervertebral disc degeneration following fixation and fusion for DDD to mimic the changes of adjacent intervertebral discs in terms of histology. Furthermore, the model can provide a platform to study the mechanism of and a prevention strategy for adjacent intervertebral disc degeneration after excision, fixation and fusion of a DDD disc.

Materials and methods

New Zealand white rabbits and perioperative management

Fifteen healthy, 10-month-old New Zealand rabbits, both male and female, weighing 2.0 to 2.5 kg, were provided by the Experimental Animal Centre of Ningxia Medical University. The animals were alert and had free limb movement. Over the course of the experiment, the experimental animals were managed in accordance with the Guidelines for the Care of Laboratory Animals published by the Ministry of Science and Technology of the People's Republic of China in 2006. Under standard conditions, the animals were single-housed and fasted for 6 h and water-deprived for 4 h before the experiment. Cefazolin sodium (0.5 g, once daily) was used for 3 consecutive days to prevent postoperative infection after surgery.

Anesthesia

An injection of 3% pentobarbital sodium (30 mg/kg) was administered via the auricular vein for anesthesia. A 0.5% lidocaine solution (5-10 ml) was applied around the surgical incision.

Establishment of the model

The animal model was established according to our previous study [14]. Each rabbit was placed in the prone position. The surgical field was prepared, disinfected and draped. Under C-arm fluoroscopic guidance, the L3-4 space was located. In reference to the methods reported in the literature, an 18-gauge needle, titling 15°-25° toward the ventral side, was sl-

owly advanced into the L3-4 space via the skin site 1.5 finger widths to the right of the spinous process. The needle tip was moved along the longitudinal axis of the spine from superior to inferior until soft tissue could be felt. Fluoroscopy was repeated to confirm the needle's position toward the L3-4 space. Then, the needle was pushed forward. A firm and rough sensation indicated that the needle tip was in the annulus fibrosus, and a sensation of loss of resistance indicated that the needle tip had completely passed through the annulus fibrosus. A 5-ml syringe was used to create negative pressure by withdrawing the plunger to the scale of 3 ml, which was maintained for 15 seconds [11, 12]. After surgery, magnetic resonance imaging (MRI) was periodically performed until signs of intervertebral disc degeneration in the managed disc were detected (12 weeks after surgery). At this time, the rabbit was placed in the right lateral position and anesthetized. The left abdomen was shaved, disinfected and draped. In reference to the methods reported by Geng Guangqi et al. [13], a 6-cm long longitudinal incision was made from the end of the 12th rib down to the iliac crest. The skin, subcutaneous fascia, abdominal external oblique muscle and abdominal internal oblique muscle were incised. Separation was performed in the posterior direction between the abdominal internal oblique muscle and abdominal transverse muscle to open the fascia between these muscles and the erector spinae muscles and to access the spine. The anterior region of the lumbar spine was exposed via deep separation along the transverse process. Fluoroscopy was repeated to ensure the location of the L3-4 space. Next, the L3-4 disc was removed, and the transverse process was excised, trimmed and placed into the L3-4 space. A 2-hole titanium plate was used to fixate the L3 and L4 vertebral bodies. The wound was washed with saline, and two gelatin sponges containing 0.5 g of cefazolin sodium were placed in the wound anterior to the fixated vertebral bodies. Finally, the wound was closed in layers.

General care of the animals after surgery

The New Zealand white rabbits underwent percutaneous needle puncture and excision, and fixation and fusion of the intervertebral disc. The impacts of the two procedures on the animals were assessed on the day before surgery
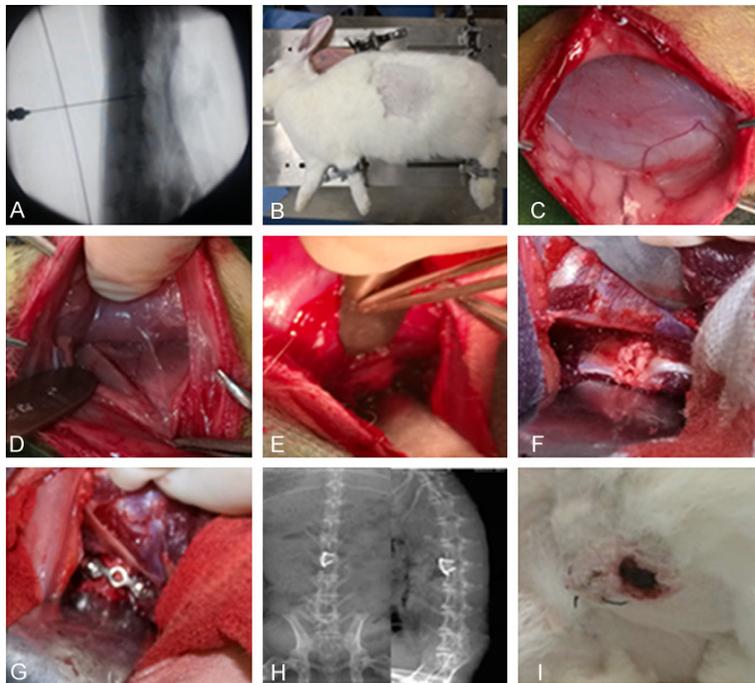
Figure 1. Interbody fixation and fusion procedure. A: Fluoroscopy showing that the puncture needle was located in the L3-4 intervertebral disc; B: Skin preparation in the New Zealand White rabbit before surgery, ranging from the end of the 12th rib down to the iliac crest; C: A 6-cm-long skin incision; D: Exposure of the vertebral body via the space between the muscles; E: Clear exposure of the intervertebral disc; F: Excision of the degenerated disc; G: Implantation of the transverse process graft into the intervertebral space and fixation of the vertebral bodies; H: Postoperative anteroposterior and lateral radiographic views; I: In one rabbit, incision dehiscence occurred 5 days after fixation and fusion. The wound was healed after debridement.

images were obtained using fast recovery fast spin echo sequence (FRFSE sequence) with T1-weighted (TR/TE440/10.7 ms) and T2-weighted (TR/TE2400/103 ms) images in the sagittal plane. The parameters included slice thickness, 2.0 mm; slice distance, 0.2 mm; and field of view, 28 cm × 28 cm. The MRI images were evaluated by a surgeon and a radiologist who were blinded to the study. The modified Thompson classification was used to assess the MRI changes of the intervertebral disc [1], including four grades: grade I, normal; grade II, mildly weak signals with a narrowing high signal area; grade III, moderately weak signals; and grade IV: severely weak signals.

Pathological examination

Twelve weeks after the excision, fixation and fusion of the intervertebral disc, and following a radiographic examination, the rabbits were dissected under anesthesia to expose the fixated L3-4 segments by the titanium plate and its adjacent intervertebral discs. The adjacent intervertebral discs were completely harvested for a gross examination (shape and osteophyte formation) and a pathological examination (hematoxylin and eosin [HE] staining).

Statistical analysis

SPSS 18 statistical software was used for the statistical analysis. The quantitative data are expressed as the means ± standard deviations ($\bar{x} \pm s$). One-way analysis of variance (ANOVA) was used to compare the means of the animal response scores within the group. A T-test was used to analyze the animal response scores between each time point. The MRI scores were compared using the non-parametric Mann-Whitney U test. $P < 0.05$ was considered statistically significant.

and on postoperative days 1, 2, 3, and 6. A comprehensive score was documented according to each rabbit's alertness, food consumption, activity and response to external stimuli [14]. The scale details [14] are as follows. Alertness: alert, 2 points; less alert, 1 point; lack of alertness, 0 points. Food consumption: normal, 2 points; half of the normal amount, 1 point; little or no food consumption, 0 points. Activities: normal, 2 points; moderate, 1 point; significantly less activity, 0 points. Response to external stimuli: sensitivity, 2 points; less sensitivity, 1 point; no response, 0 points. A score of 8 points indicated a good condition. A poorer condition was associated with a lower score.

MRI study

MRI was performed at 4, 8, and 12 weeks after percutaneous needle puncture or excision, fixation and fusion of the intervertebral disc. MRI

Table 1. Response scores of New Zealand White rabbits ($\bar{x} \pm s$)

Group	Pre-op	Post-op day 1	Post-op day 2	Post-op day 3	Post-op day 6
Percutaneous puncture	8.00 ± 0.00	7.23 ± 0.24	7.41 ± 0.49	7.58 ± 0.44	8.00 ± 0.00
Fixation and fusion	8.00 ± 0.00	4.44 ± 0.63	4.79 ± 0.71	6.15 ± 0.63	7.90 ± 0.67
t		7.952	5.791	3.944	0.606
P		0.000	0.000	0.000	0.059

Pre-op: Pre-operative; Post-op: Postoperative.

Table 2. MRI grading of adjacent disc degeneration

Grade	After puncture (n)			After fixation and fusion (n)		
	4 weeks	8 weeks	12 weeks	4 weeks	8 weeks	12 weeks
I	30	30	29	28	25	24
II	0	0	1	2	5	5
III	0	0	0	0	0	1
IV	0	0	0	0	0	0

Results

Survival rates and response scores of the New Zealand white rabbits

The fifteen New Zealand White rabbits underwent the procedures smoothly, and no deaths were reported during the study, for a survival rate of 100%. No lower limb paralysis was observed. In one rabbit, incision dehiscence occurred at 5 days after fixation and fusion, and the wound was healed after debridement (**Figure 1**). Prior to the percutaneous needle puncture or excision, and the fixation and fusion of the intervertebral disc, the animals were assessed according to alertness, food consumption, activity, and response to external stimuli. The animal response score [14] was 8 points in all animals and was not significantly different at any time point before or after the percutaneous needle puncture (F = 1.563, P > 0.05). However, the scores were significantly different on postoperative days 1, 2 and 3 after fixation and fusion compared with the score before the procedures (F = 6.178, P < 0.001). Compared with the response scores after the percutaneous puncture, the scores after excision, fixation, and fusion of the intervertebral disc were significantly lower on postoperative days 1, 2 and 3 (t = 7.952, 5.791 and 3.944, respectively, P < 0.001). On postoperative day 6, the response scores (alertness, food consumption, activity and response to external stimuli) returned to their normal levels (t = 0.606, P > 0.05; **Table 1**).

MRI score

The overall T2 signal intensity of the managed disc began to decrease starting with the 4th week after percutaneous intervertebral disc puncture. The signal intensity of the nucleus pulposus of the intervertebral disc in the 2 groups decreased further beginning with the 8th week, with tendencies for reduced height and degeneration of the intervertebral disc. Of 30 adjacent intervertebral discs, one disc was present with signs of degeneration 12 weeks after percutaneous puncture. Varying degrees of degeneration were present in the adjacent discs after the excision, fixation and interbody fusion of the managed degenerative disc. The degrees of adjacent disc degeneration were not significantly different between the puncture group and the fixation and fusion group in the 4th week after intervention (P > 0.05) but were significantly different in the 8th and 12th weeks (P < 0.05) (**Table 2**; **Figure 2**).
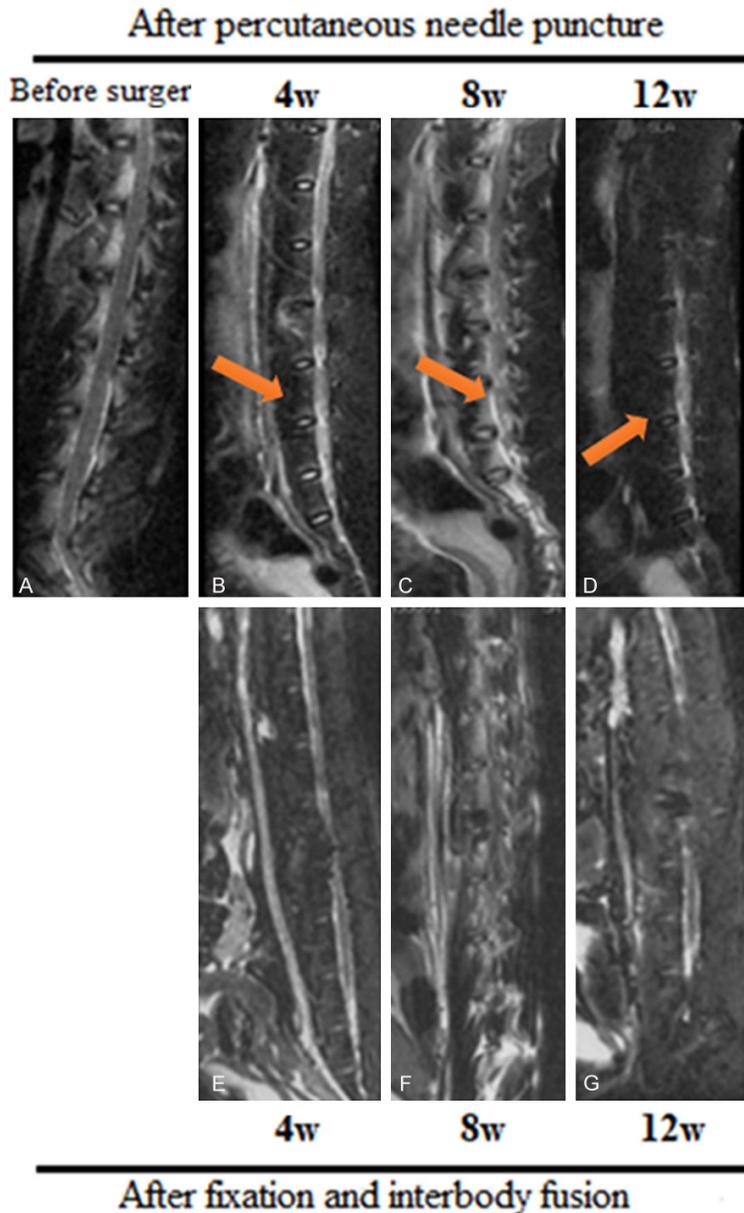
Histopathological observation

At 12 weeks after excision, fixation and fusion of the managed disc, the rabbits were sacrificed, and the intact lumbar spines were harvested. Gross examination revealed that the titanium plate-fixated L3-4 segments were completely covered with the bony callus and were partially black. Osteophyte formation in the adjacent intervertebral discs was not evident. Compared with the normal discs, the gross examination of the adjacent intervertebral discs showed a dark-gray nucleus pulposus with small areas of loose fat-like changes and firm, disoriented annulus fibrosus with hyaline changes in the junction between the annulus fibrosus and nucleus pulposus; pathological examination (HE staining) revealed that the border of the nucleus pulposus was unclear, the nucleus pulposus tissue was replaced by

After percutaneous needle puncture

Figure 2. MRI study after percutaneous needle puncture and fixation and fusion of the vertebral bodies. A: Homogeneous signal intensity of each lumbar intervertebral disc before surgery; B: Image showing slightly decreased signal intensity in the L3-4 intervertebral disc 4 weeks after percutaneous puncture and normal adjacent intervertebral discs; C: Image showing decreased signal intensity in the L3-4 intervertebral disc 8 weeks after percutaneous puncture and normal adjacent intervertebral discs; D: Image showing lower signal intensity (black) in the L3-4 intervertebral disc 12 weeks after percutaneous puncture, decreased height of the intervertebral space and slightly decreased signal intensity in the adjacent intervertebral discs; E-G: Images of the spine 4, 8 and 12 weeks after fusion and fixation showing a slightly decreased signal intensity in the adjacent intervertebral discs.

cartilage tissue and the center of the nucleus pulposus had signs of degeneration (**Figure 3**).

Discussion

Intervertebral disc excision with interbody fixation and fusion has become the mainstream treatment for spinal diseases. However, interbody fixation and fusion is not a perfect regimen for intervertebral disc degeneration. Patients undergoing interbody fixation and fusion for the relief of intervertebral disc pain have to face a series of problems caused by the procedure. Of these problems, degeneration of the adjacent vertebral discs causes the most physical and psychological trouble for patients. To date, studies related to spine degeneration have been focused on imaging diagnosis, but not on the disease's underlying mechanisms. Moreover, there is no suitable animal model to mimic human disc degeneration or adjacent segmental vertebral lesions after interbody fixation and infusion because of the particularity of the human spine.

The currently used animal model of fixation and interbody fusion is created by directly excising the intervertebral disc, followed by fixation and interbody fusion [15]. The disadvantage of this model for investigating adjacent intervertebral degeneration lies in ignoring the self-impact of degeneration of the disc on the adjacent discs. To this end, periodically monitoring the progression of the adjacent intervertebral disc degeneration is key to fully understanding its occurrence and development during the period from onset of the affected intervertebral disc degeneration to the end of surgical treatment with fixation and interbody fusion. In this study, we per-
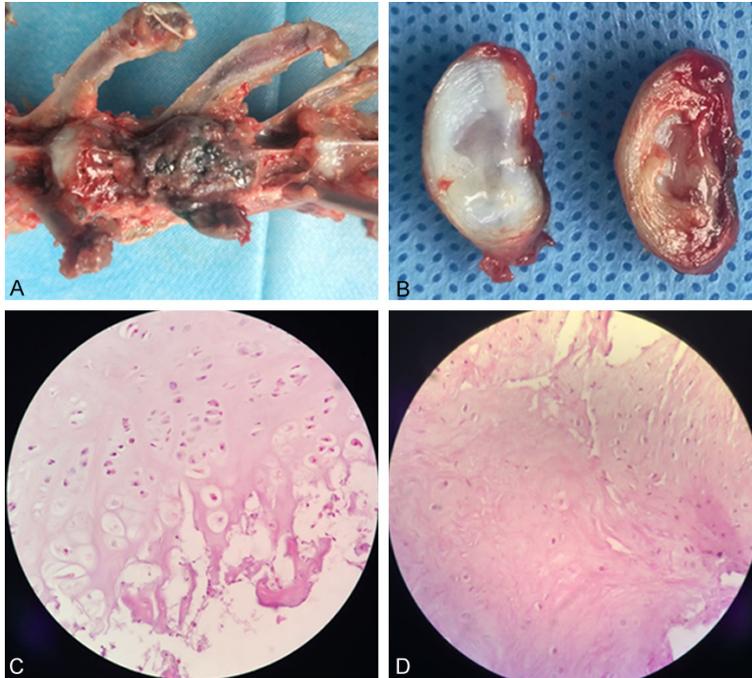
Figure 3. Gross specimen of the lumbar spine of a New Zealand White rabbit after fixation and fusion and HE-stained sections. A: Gross examination showing that the L3-4 segments fixated by the titanium plate were completely covered with bony callus and were partially black, with the screws visualized; B: Compared with a normal disc (left), the gross examination of the adjacent intervertebral discs revealed dark-gray nucleus pulposus with small areas of loose fat-like changes and firm, disoriented annulus fibrosus; C: HE-stained section of a normal nucleus pulposus showing normal cell arrangement; D: HE-stained section (100 ×) of the nucleus pulposus of the adjacent intervertebral disc in a rabbit's lumbar spine after fixation and fusion showing that the border of the nucleus pulposus was unclear. The nucleus pulposus tissue was replaced with cartilage tissue, and the center of the nucleus pulposus had evident signs of degeneration.

formed a percutaneous puncture on the target lumbar intervertebral disc in rabbits to induce its degeneration. When the degeneration was confirmed by MRI, the target disc was excised, and fixation with a titanium plate and interbody fusion with a transverse process graft were performed to create an animal model for investigating adjacent intervertebral disc degeneration after lumbar fixation and interbody fusion.

In this study, the survival rate of the animals was 100% after surgery, and no lower limb paralysis was observed. In one rabbit, incision dehiscence occurred 5 days after fixation and fusion. The wound was healed after debridement. The survival rate is essential for a successful animal model. Our experiences include several considerations. (1) The anesthetic agents must be strictly calculated according to the body weight of the animal. Overdose should be avoided; (2) Aseptic methods are the key to reducing experimental animal deaths caused by infection. (3) A skilled and accurate operation is the key to avoiing damage to the spinal cord and nerve, which can result in postoperative lower limb paralysis. In this study, MRI revealed a time-dependent reduction of the signal intensity of the managed disc 4 weeks after disc puncture. One adjacent intervertebral disc was found to have signal intensity reduction 12 weeks after disc puncture. Moreover, multiple occurrences of intervertebral disc degeneration of the adjacent intervertebral disc were noted on the 4th, 8th and, 12th weeks after excision, fixation, and fusion of the degenerative intervertebral disc. The degeneration in these discs was confirmed in the pathologic examination. Considering disc degeneration is a continuous pathological progression, the results suggest that adjacent disc degeneration may have occurred before fixation and interbody fusion. In other words, a "cause and effect" relationship may exist between two adjacent intervertebral discs: the degenerative disc may trigger the adjacent intervertebral disc degeneration.

The technique of the anterolateral "stab" of lumbar discs was first reported in the rabbit model [16] and was then used in the rodent model due to limitations of operations and costs in the rabbit model [17]. Since then, some researchers have started to create animal models of intervertebral lesions in rats [18], rabbits [1], dogs [19], and goats [20]. Of these animal models, the rat models constitute the majority. However, none of these models can be used as animal models for investigating adjacent disk degeneration after lumbar fixation and interbody infusion. In this study, we

selected rabbits for our animal model, considering their operative difficulty, success rate, and economic cost. This novel animal model can provide a platform for studying lumbar changes subsequent to fixation and interbody fusion in the future.

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Disclosure of conflict of interest

None.

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