Original Article

Induction of traumatic brain and spinal cord injury models in rat using a modified impactor device

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Abstract

Introduction: The use of standard rodent model, allows for the understanding of neuronal injury physiopathology and helping development of therapeutic strategies. Because of eliminating technical problems, we designed a modified impactor device with ability to induce different degrees according to kilodyne from very mild to very severe of spinal cord injury (SCI) and traumatic brain injury (TBI) models in rat.

Methods: For standardization and determining of optimal performance of the device to induce varying injuries, 47 adult male Wistar rats were used, and 8 different forces were applied in spinal cord and brain tissues.

Results: The hematoxylin and eosin and 2, 3, 5-triphenyltetrazolium chloride (TTC) results demonstrated that by increasing the level of forces, histological changes in the spinal cord and brain were significantly enhanced. Different injuries had significant effect on the Basso-Beattie-Brenham and elevated body swing test outcomes, and there were significant differences between groups in comparison with control group.

Conclusion: Our results showed that the modified device could be valid to produce precise SCI and TBI models, goal to replicate SCI and TBI in humans as much as possible. However, it might be considered that aspects of SCI and TBI models are complicate and more examination is necessary.

Introduction

Traumatic spinal cord and brain injuries are emergency conditions which characterized with sensorimotor, urinary and cognitive impairments. Estimations show that nearly 2.5 million people live with spinal cord injury (SCI) in the world, with annual incidence of more than 130,000 SCI (Thuret et al., 2006). Also, in each year nearly 10 million individuals die and/or hospitalized due to traumatic brain injury (TBI) and estimated 57 million people suffered from brain injuries in the world (Langlois et al., 2006). Several events, including car accident, falls, sports, violence and military injuries can lead to SCI and TBI. Traumatic SCI has two pathological phases, including primary and secondary phases which are similar to TBI. Primary injury, results from external mechanical force to neural tissue and it can lead to direct axonal injury, blood vessels damages, hemorrhage and...
tissue devastation (Gaetz, 2004; Cernak, 2005; Masel and DeWitt, 2010). This primary stage is followed by secondary phase of SCI characterizing with cellular and molecular dysfunctions (Thompson et al., 2005; Marklund et al., 2006; Bramlett and Dietrich, 2007). Among different techniques, contusion method using New York University (NYU), the Ohio State University (OSU) (Stokes, 1992) and the Infinite horizon (IH) devices (Lee et al., 2012) are widely applied for induction of SCI in rodents (Iwanami et al., 2005; Khuyagbaatar et al., 2015). The SCI producing by the NYU device results from dropping of a 10-g rod from different heights on exposed special part of the spinal cord (Gruner, 1992). In the primary version, NYU impactor device couldn’t record digital parameters, but in the ultimate version the device is able to control some mechanical parameters to create different injuries, however, as a disadvantage, duration of impact cannot be exactly controlled (Iwanami et al., 2005). The OSU or electromagnetic spinal cord injury device (ESCID) can produce a range of spinal cord injuries by applying different displacement onto exposed spinal cord (Noyes, 1987; Stokes et al., 1992). The device improved in 2000 to investigate in SCI model in mouse (Jakeman et al., 2000) and cervical spine injury in the rat (Pearse et al., 2005). There is merely a limitation of the OSU impactor that discerning the zero point for the impactor sometimes is difficult (Cheriyan et al., 2014).

Finally, the IH device is made for crating different severity of injuries using a force-controlled impact on the spinal cord tissue and graded contusion injury will generate by entering a predetermined force in specific software which calibrated according to mild (100kdyn), moderate (150kdyn) and severe (200kdyn) forces at different segments of spinal cord in the rat (Scheff et al., 2003). In this device, an apparent limitation revealed by some researchers relating to spinal cord holder which finally caused to create a custom type of clamping instrument to cope with that technical problem (Streijger et al., 2013).

Considering, TBI models divided into four specific models as follow; fluid percussion injury (FPI) (Dixon et al., 1987), weight-drop impact injury (Marmarou et al., 1994), blast injury (Leung et al., 2008) and controlled cortical impactor (Lighthall, 1988; Dixon et al., 1991). The FPI model creates by a fluid rapid injection into the brain space following craniotomy and the different degrees of injury relies on the strength of the pressure (McIntosh et al., 1989). As a beneficial change in next version, FPI model improved by providing a microprocessor-controlled and contemplating adjustable biomechanical parameters (Kabadi et al., 2010). Weight-drop model, a weight releases straight onto the exposed dura matter from a desired height to induce a cortical injury (Feeney et al., 1981). Overtly, one remarkable disadvantage of the weight-drop models relates to high variation in creating different severities of injury; however, because of several reasons, including economic efficiency, easy to accomplish and similar TBI method in human, it should be considered as an applicable and common approach in experimental condition (Xiong et al., 2013). Blast brain injury causes by blast which commonly happens in various military personnel (Long et al., 2009). The controlled cortical impact (CCI), injury model induces by an air or electromagnetic device (Xiong et al., 2013). The advantage of CCI compared to other models is adjustable mechanical parameters, such as velocity, time and depth (Wang and Ma, 2010), and with this way, severity of injury can be appropriate for specific experiments (Goodman et al., 1994).

Therefore, all of these devices were generally employed and developed for traumatic brain and spinal cord injuries, separately, and each one has its specific feature of injury. In this study a modified device is produced inducing either SCI or TBI models in rats. To gain several targets effectively, we have designed a device with usability in the spinal cord and brain trauma models that could create more wide range of injury with slight variation and low heterogeneity in order to help researcher for understanding precise pathophysiological mechanisms.

Materials and methods

Apparatus arrangement of the impactor device
The image of the device and its different components showed in Figures 1 and 2. The device composed of general structure, mechanical and electronic parts and the software.

General structure
The base plate of the device is made from an aluminum platform (referred to animal's platform) and
that was covered with a thickness of 5mm Plexiglas material. Animal's platform is mobile in the X and Y axis which is designed for correct position of animal before creating SCI model. Two clamps are positioned in discrete sides on animal's platform for vertebral column fixing after spinal cord surgery. Height of impactor tip is controllable by hand. The material of impounder tip is stainless steel with chromium coating and it is exchangeable in order to induce SCI in mice and rats. In the animal's platform, a new skull fixative was made in order to create TBI model in the rat which is adaptable with overall structure of the device (Figs. 1A-G).

Mechanical and electronic parts
This device has a gearbox motor (with an external encoder for counting steps) and force sensor system is situated in a rigid box and the contents of this box is interfaced with a minicomputer (ARM: that installed Linux operating system) and a microcontroller. When the gearbox motor turns, causes vertical movement of a bar (impactor tip). By rotating the motor and move the rod tip into neural tissue, force applied by motor to the tissue will be measured directly by force sensor, precisely. When force level reached a threshold level of the desired force, the motor movement will reverse by microcontroller command and impactor tip will withdraw from injured tissue, rapidly (Figs. 2A and B).

The software
The impactor software is composed of two discreet frames. The left frame is for physical parameters setting, comments, video recording, camera and starting experiment. Also in this frame, models of SCI and TBI are selectable before inducing injury in the experimental animals. The right frame is related to experimental information, including force and displacement data graphs to time (Fig. 2C). For creating an injury model, user could select one of the trauma models (SCI or TBI) in the software and then insert a predetermined force, desired force to be applied to the exposed neural tissue.

Study design
In this study, 47 adult male Wistar rats weighing 250-280 g were used randomly. All the rats were kept at 12/12 h dark/night cycle with 25±2°C controlled-
temperature. Experimental animals had ad libitum access to food and water. All intervention procedures were approved by the Committee for Ethics in Animal Research at Tabriz University of Medical Sciences, Tabriz, Iran (approval number: 5.4.11472). Animal’s maintenance was performed according to the National Institutes of Health Guide (NIH Publications No. 8023, revised 1978). SCI and TBI models were induced by the impactor device (Fig. 1). In model of SCI, animals were randomly divided into six groups; laminectomy group (n=7) and five different injury groups as very mild SCI (n=6), mild SCI (n=6), moderate SCI (n=5), severe SCI (n=4) and very severe SCI (n=4). In model of TBI, rats were divided into three different injury groups (5 rats were used in each mild, moderate and severe TBI groups). Applied forces were performed at the T10 level of spinal cord; 50 (very mild SCI), 100 (mild SCI), 150 (moderate SCI), 200 (severe SCI) and 250 (very severe SCI) kdyn and 50, 80 and 110 kdyn forces for induction of TBI models as mild, moderate and severe.

**Equations**

All forces applied based on dyne in both brain and spinal cord tissues. The dyne is a unit of force determined in the centimeter–gram–second system of units (CGS) that considered as standard unit for induction of the trauma models. The dyne unit defined for impactor device in order to set and induce animal trauma models. One dyne is equal to 10 micronewtons: $1 \text{ dyn} = 1 \text{ g-cm/s}^2 = 10^{-5} \text{ kg-m/s}^2 = 10^{-5} \text{ N}$.

**Surgical procedure**

All rats were anesthetized with ketamine (80mg/kg) and xylazine (10mg/kg). At the spinal cord injury group, animals consisted of remove spinal vertebra at the T10 level under laminectomy surgery. For correct

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*Fig. 2.* (A) Schematic view of the new impactor device illustrated from the front of view. (B) Motor drives the bar into exposed neural tissues (brain and spinal cord), and a sensor calculates and shows the force level in the device software. (C) The frame of the software has showed. This part includes experiment mode which user could select trauma models (trauma brain and spinal cord injury models), and two data graphs including, force and displacement data graphs to time. Below of the software, relates to three different force graphs that randomly selected, mild SCI (actual force was 102 kdyn), moderate SCI (actual force was 153 kdyn) and very severe SCI (actual force was 250 kdyn).
inserts of impactor tip, laminectomy region was more than the 2.5mm. The exposed spinal cord was fixed by clamping the caudal T9 and rostral T11 transvers process of spine with special forceps. Before creating contusion injury, the forceps must be tightened carefully with the locking thumb screw in a horizontal level, exactly. It is very important that vertebra column must be fixed to horizontal situation in order to induce bilateral injury. The impactor tip is supplied with a diameter of 2.5mm which was designed for rats. In this group, five different forces (50, 100, 150, 200 and 250kdyn) were inflicted at the T10 level of intact spinal cord. In the traumatic brain injury group, animals were anesthetized by previous procedures. Surgery regions cleaned by an antiseptic solution and shaved. By a midline scalp incision and fascia removed carefully, skull surface was exposed. Desired head trauma site was determined by stereotaxic apparatus on motor cortex=-3mm from bregma and with medial lateral= -2mm. After determining the mentioned area in a stereotaxic apparatus on the skulls, craniotomy was performed using a dental drill and animals head were stabilized in the special skull fixation frame of the device. Three different forces (50, 80 and 110kdyn) inserted to the intact area of motor cortex. Also, craniectomy was performed in left side as control. For postoperative cares, all animals were received 2ml sterile saline subcutaneously (SC) before were placed in a single cage. For analgesics effect after surgery, buprenorphine hydrochloride (0.1mg/kg/day, SC) was used for the first 48h. Also in the spinal cord injury group, bladder was monitored twice a day until bladder normal function returned.

**Tissue preparation**

Animals were euthanized four weeks after injury by ketamine (100mg/kg) and (5mg/kg) xylazine overdose and were perfused intracardially with 0.1M phosphate buffered saline (PBS) and a mixture of 4% paraformaldehyde and 0.1M PBS. Spinal cord tissue was dissected out, carefully. After that, the spinal cord tissue was embedded in fixing solutions and processed in paraffin overnight at the 4°C temperature. In order to determine the degree of injured tissues following trauma, the tissues were sliced in cross sections with a thickness of 4µm by a microtome (DS-8402). Sectioned tissues were stained with hematoxylin and eosin (H&E) staining method (Gonzalez et al., 2003). After tissue staining, images were taken using a microscope (Nikon, eclipse E100) with camera (KECAM, UCMS1000KPA). In order to assess different forces on spinal cord sections, in the present study the histopathological criteria introduced, and based on that criteria scoring was done for each spinal cord sections. Using the histopathological criteria each severities of injury in all experimental groups were determined (Fig. 2) by this way; 0= no damage, 1= lymphocyte infiltration (Li) in posterior funiculus (PF), 2= Li + Vacuolation (V) in PF, 3= Li + V + cystic degeneration (CD) in PF, 4= Li + V + CD + axonal degeneration (AD) in PF, lateral funiculus (LF) and posterior horn (PH), 5= Li + V + CD + AD + hemorrhage (H) in PF, PH, LF, anterior horn (AH) and anterior funiculus (AF). Volume of the brain lesion was measured by staining brain sections with 2,3,5-triphenyltetrazolium chloride (TTC) method (Yu et al., 2004). After motor asymmetry testing at three days post-TBI, the brain was removed and serial sections of brain was immersed in cold saline for 10min and sliced with a thickness of 2mm. The serial sections of the brain tissues were incubated in 2% TTC solution in PBS for 20min at 37°C which this following procedure was explained (Yu et al., 2009). The mean lesion volume was revealed by a lack of TTC staining that represents dehydrogenase deficiency in the brain damaged site. After detecting and quantifying the lesion volume by image analysis system, brain damage volume showed according to the percentage (Yu et al., 2004).

**Basso-Beattie-Brenham (BBB) locomotor test**

Traumatized rats were monitored for locomotion test starting 2 days after surgery procedure, once a week for 4 weeks. Locomotor activity of animals with SCI at T10 segment, evaluated with the BBB locomotor test. This behavioral test is an improved open-field test which is based on scoring hind limb locomotion from 0 to 21 (Basso et al., 1995). Briefly, rats were placed in the open field region and monitored for 4min. The BBB locomotor test in animals with SCI was evaluated by a trained researcher. Before creating the SCI model, normality of locomotor activities of rats was performed.

**Elevated body swing test (EBST)**

Brain traumatized rats were evaluated by EBST for
analysis of motor asymmetry (Yu et al., 2009). Training of rats for this test was not required before injury model. Three different TBI injuries were confirmed with a range of locomotor impairment. The animals were examined for swing movement and their body rotation was recorded. Briefly, the rats were habituated in a Plexiglass box for 10 min in order to define getting a neutral position and after that, the rats were lifted 2 cm from the Plexiglass surface by handling their tail in vertical axis across 20 trials. Swing movement was recorded whenever the animals biased to either side (ipsilateral and contralateral) and biased swing activity expressed as a percentage in TBI groups, and calculated as follows: \([\text{number of left-biased swings/ the total number of swings}} \times 100\%\)] (Wang et al., 2016).

**Statistical analysis**
The results were expressed as mean±SEM. Statistical analysis were done using one-way analysis of variance (ANOVA) and Tukey post-hoc, and differences between experimental groups considered at \(P<0.05\) statistically significant.

**Results**

**Histological and behavioral assessments**
Here, we demonstrated the effect of different injuries (50 to 250 kdyn) on the brain and spinal cord tissues. To determine the severities of injury, the lesion epicenter of spinal cord section compared with intact tissue, and the effect of mechanical force on the spinal cord tissue was analyzed based on histopathological criteria 4 weeks after contusion injury model at T10 segment of spinal cord. Analysis of spinal cord tissues demonstrated that by increasing the amount of applied force, the histopathological score (0 to 5 scores) was enhanced (Figs. 3A and B).

H&E staining of injured sections have shown that in laminectomy group, the white and grey matters are uninjured (Figs. 4A and a); very mild SCI group (50 kdyn), the black arrows indicate the lymphocyte infiltration in posterior funiculus of the spinal cord (Figs. 4B and b); mild SCI (100 kdyn), cystic degeneration is revealed, obviously at injury site (Figs. 4C and c); moderate group (150 kdyn), cystic
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degeneration and lymphocyte infiltration are seen in posterior part of white matter as well as some part of grey matter (Figs. 4D and d); severe SCI (200kdyn), the histopathology changes were showed as mentioned above and also, extensive degeneration is revealed in the majority part of grey and white matters (Figs. 4E and e) and very severe SCI group (250kdyn), the hemorrhage, cystic degeneration and lymphocyte infiltration are seen which is extended at grey matter of spinal cord, completely (Figs. 4F and f). We showed histopathological alteration with signs and quantification has been done based on defined damage levels such as (lymphocyte infiltration and cystic degeneration).

Also, histological staining on brain traumatized sections have shown that the percent of lesion in the

Fig. 4. Contusion-type SCI study on an animal model has been produced by the impactor device for control and experimental groups. Histopathological evaluation of H&E stained sections (5 micrometer). The different degrees of applied forces (kdyn) at lesion site were demonstrated. By increasing the amount of force, overall area of grey and white matters were decreased. (a) Intact spinal cord tissue in laminectomy group. (b) Arrow showed the area of lymphocyte infiltration that produces inflammatory condition in 50kdyn group. (c) Black arrow indicate cavity (empty lesion) with the cavity in posterior funiculus of the spinal cord in mild SCI group. (d) Black arrows show cystic degeneration and lymphocyte infiltration in the majority posterior part of white matter, and small part of grey matter in moderate SCI group. (e) Three arrows in different parts of slice show extensive degeneration and histopathological changes in the majority area of section in severe SCI group. (f) Black arrows indicate extensive hemorrhage, cystic degeneration and lymphocyte infiltration all part of the section determined as very severe SCI group. A higher magnification (×10) photomicrograph from some damaged part of the section in all groups inserted to show the histomorphological alteration of the spinal cord tissue in different groups. Spinal cord dislocation and defined kilo dyne induction are essential items to consequence after SCI. Scale bar, 1mm for panels A to F, under 4X magnification. Apparent of lymphocyte infiltration, cystic degeneration, hemorrhage in the anterior funiculus, and distortion of tissue organization in the spinal cord. Laminectomy group; as sham (n=7 rats) and five different injury groups as very mild SCI; (n=6 rats), mild SCI (n=6 rats), moderate SCI (n=5 rats), severe SCI (n=4 rats) and very severe SCI (n=4 rats) which animals received 50, 100, 150, 200 and 250kdyn forces, respectively. H&E: hematoxylin and eosin.
right hemisphere of the rat brain in comparison with left hemisphere was 7.87% in rats that received mild (50kdyn) force. This lesion percent significantly increased to 14.45 and 20.92% in the moderate (80kdyn) and severe (110kdyn) injury groups. Extensive lesion was seen in the ipsilateral ventricle which was extended to the hippocampus. In the severe TBI group, a wide range of destruction in the subcortical areas was found. There was no lesion in the contralateral cortex in the TBI groups. Rats with three different TBI were also tested by EBST at 3 days post TBI. All behavioral outcomes showing range of locomotor impairments following applied different forces on the brain were confirmed by TTC method. EBST showed that by increasing the amount of force and brain damage, asymmetry activity in mild, moderate and severe injury groups were significantly enhanced, respectively. Behavioral impairments between the 80 and 110kdyn injury groups were enhanced significantly compared with animals that received 50kdyn forces (P<0.001, P<0.05; Fig. 5E). In regards to baseline assessment before TBI, there was no behavioral deficit in all TBI groups. Animals with SCI were assessed randomly by locomotor activity test. Scores of test in the 200 and
250kdyn injured groups as severe and very severe were significantly decreased in comparison with 150kdyn. Also, 100 and 150kdyn groups compared to 50kdyn group, had significant decrease in locomotor score at 4 weeks post contusion injury model. There was significant difference between injury groups in each day ($P<0.05$) and also, different days have been showed significant difference in BBB score in different injury groups ($P<0.05$). Therefore, rising up the level of force, locomotor activity of the rats was significantly decreased which have been showed (Fig. 6).

**Discussion**

In the present study, we described SCI and TBI models using a modified design device which uses a new mechanical design, HD camera, brain and spinal cord fixative as well as force sensor to induce controlled injury in both brain and spinal cord. Applied injury on the exposed brain and spinal cord tissues with our modified device confirmed by histological and behavioral assessments following impact injury. The purposes of designed device were to measure effective parameters of trauma like displacement, time and force which was adjustable in the brain and spinal cord, completely. Trigger of standard SCI and TBI injury models in experimental animals can lead to better assessment of therapeutic interventions in the field of regenerative medicine (Yu et al., 2009; Cheriyan et al., 2014) and these laboratory findings can be translated to the clinic, for SCI and TBI treatments. Approximately, half of human SCI cases are contusion injuries that are why the contusion model is most widely used in the experimental animals. In studies for understanding the pathophysiological mechanisms of secondary injury involved in the SCI, graded spinal cord contusion models is an essential part of a research goal and can be selected (Basso et al., 1995). Therefore, same injury in animals in a study, the variation will be limited between animals and leads to better evaluation of laboratory results aiming to address pathology and/or treatment. In additionally, according to complexity and several factors involved in TBI mechanisms such as tissue lesions, neuronal degeneration, apoptosis, necrotic cell death as well as technical problems, a feasible model that accurately encompass all dimensions of human TBI does not exist in the experiment. However, each experimental model tries to reveal the injury identity of SCI and TBI, but attempts to simulate these models for understanding these injuries mechanism are ongoing. Hence, because of the pivotal role of
biomechanical properties in SCI and TBI models, controlling the primary injury mechanisms by precise devices will be very useful in order to clarify secondary injury cascades for SCI and TBI treatment. Induction of very mild to very severe injury types of SCI and TBI were assessed with the BBB locomotor activity and EBST tests, respectively. Five different applied forces to the spinal cord were evaluated by BBB test for locomotor activity monitoring. In the first week after creation of SCI model, a modest recovery in curves of locomotor activity test was seen and then it curves reached a plateau in each group which these findings are similar to the previous published data.

In keeping with our investigation, morphological evaluation of spinal cord sections at epicenter of injured region demonstrates the significant differences between the amount of force and severities of injury that histopathological score significantly increased when the force level was raised. The histopathological scores as morphologic feature for determining of severities of injury was employed after SCI. Studies showed that when the delivered forces at the epicenter injured site is increasing, the total injury volume was increased and sparing tissue percent was decline that these results previous observed (Basso et al., 1996; Scheff et al., 2003; Dunham et al., 2010; van Gorp et al., 2014). However, in this study histological criteria considered for qualifying of injury degrees for spinal cord sections.

After TBI, significant structural alternations in lesion site may be revealed, such as cerebral structure deformation, ventricular dilation and considerable atrophy in the neocortical and subcortical regions. On the other hand, molecular changes, including, stress oxidative, neuronal loss, altered cellular adhesion and apoptotic cell death can be followed as the secondary phase of TBI.

Our histological assessments in TBI-injured rats showed that by increasing from the mild, moderate and severe injury types, histological changes at lesion area were progressed that have been examined in previous investigations (Brody et al., 2007; Yu et al., 2009; Lin et al., 2015). The behavioral alternations in the TBI groups were monitored by EBST in this experiment. In animals with three levels of TBI injury, asymmetrical motor function was observed and contralateral swing ratio was significantly elevated compared to baseline level of prior to TBI. Also, the worst behavioral impairment was revealed in the severe group of TBI which is comparable with previous study (Yu et al., 2009).

Also, the range of BBB locomotor activity scores are strongly correlated to delivered amount of forces so in the 50kdyn group as lowest injury achieved the highest behavioral score and also, in the 250kdyn group attained lowest score. Thus, the relationship between behavioral deficit and graded SCI in each severity is significant. In agreement with our study, this behavioral evaluation protocol showed that different forces applied using the NYU and IH impactor devices leads to significant behavioral changes (Basso et al., 1996; Rabchevsky et al., 2003; Scheff et al., 2003; van Gorp et al., 2014).

The histological and behavioral findings in the presented study together demonstrated that this device can be used for establish wide range of SCI and TBI injury models. The ability to apply desired forces, precisely to the exposed brain and spinal cord will be useful in the better evaluation of interventions. With new instruments added in the device, laboratory errors are limited as much as possible.

**Conclusion**

This device has some of advantages compared with previous devices made, that contains: 1-One of the most important points in using of this device is adjustable capability in order to induce both SCI and TBI models in the rat with low heterogeneity. The device has more economic efficiency and occupies less physical space in the lab; 2-Also in this device a high-quality camera has used that is capable to capture photo for the standard positioning of animals before the moment of injury which is controllable in the software by user. The ability to record video during injury models of TBI and SCI is another advantage. After recording the moment of tissue damage, researchers will be assured the correct of induced injury; 3-Manipulated of mini-computer in this device created minimal latency and error. There is no latency and error between the first section (A to D section) and last sections (data acquisition and data processing) that improved performance of the device.

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Conflict of interest
The authors declare that they have no conflict of interest.

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