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EXPERIMENTAL HEMORRHAGIC STROKE: SEARCH FOR A BETTER MODEL

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Hemorrhagic stroke is one of the least studied problems in the modern neurology. Development of the treatment leads to examination of the pathophysiology of the process, which mainly can be performed in animal models. Recent years few animal model of the hemorrhagic stroke have been proposed. In this review we discuss the differences and benefits of the existing models in the compliance with the process that take place in human patients with hemorrhagic stroke.

KEY WORDS: hemorrhagic stroke, experimental study, biological model, laboratory animal

ЭКСПЕРИМЕНТАЛЬНЫЙ ГЕМОРРАГИЧЕСКИЙ ИНСУЛЬТ: ПОИСК ЛУЧШЕЙ МОДЕЛИ

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Геморрагический инсульт является одной из наименее изученных проблем в современной неврологии. Развитие новых подходов к его лечению невозможно без установления тонких механизмов развития, которые возможно установить только на адекватных моделях у экспериментальных животных. В последние годы было предложено несколько моделей геморрагического инсульта у животных. В обзоре обсуждаются различия среди существующих моделей в соответствии с процессами, протекающими у пациентов с геморрагическим инсультом, и обосновывается выбор наиболее адекватных среди них.

КЛЮЧЕВЫЕ СЛОВА: геморрагический инсульт, экспериментальное исследование, биологическая модель, лабораторное животное

ЕКСПЕРИМЕНТАЛЬНИЙ ГЕМОРАГІЧНИЙ ІНСУЛЬТ: ПОШУК КРАЩОЇ МОДЕЛІ

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Геморагічний інсульт є однією з найменш вивчених проблем в сучасній неврології. Розвиток нових підходів до його лікування неможливий без встановлення тонких механізмів розвитку, які можливо встановити тільки на адекватних моделях у експериментальних тварин. В останні роки було запропоновано кілька моделей геморагічного інсульту у тварин. В огляді обговорюються відмінності серед існуючих моделей відповідно до процесами, що протікають у пацієнтів з геморагічним інсультом, і обґрунтовується вибір найбільш адекватних серед них.

КЛЮЧОВІ СЛОВА: геморагічний інсульт, експериментальне дослідження, біологічна модель, лабораторна тварина

INTRODUCTION

Hemorrhagic stroke is one of the most severe stroke types and accounts for 20% of stroke patients [1, 2]. 30% of stroke patients die within the first month after stroke incidence, 23% die within a year and only 65% of the survivors can function independently [3-5]. Despite such a high incidence, stroke remains poorly explored, highlighting the necessity of understanding its processes. One of the ways to investigate the processes in hemorrhagic stroke and to find a possible treatment is an experimental animal model. The model should possess certain characteristics such as be standardised, reproducible and represent the main mechanisms of the process as it is in humans [6-9].

This review will discuss and compare current main experimental hemorrhagic stroke models nowadays.

RATS AS A PREFERENTIAL EXPERIMENTING MODEL

Modelling of human neurological disorders in animals is not an easy task.

Rodents, rats in particular, are probably the only laboratory animals that have been studied in detail, providing numerous behavioural studies, which have shown the similarity in human and rats' motor components which are responsible for upper extremity movements in humans and forelimbs in rats during reaching behaviour [9].

There are a few major disadvantages that can be found in the rats' model. The organization of the rat brain is obviously not identical to that of the human brain. Therefore, the clinical relevance of neuroanatomical information obtained in the rat in principle should be confirmed in human material, which often may not be possible for ethical reasons; and conversely, certain important problems like the neurobiology of language in the rat [10].

On the other hand the principle structural organization, functional, behavioral (survival, search for solutions), as well as compensatory mechanisms of lost functions, repair mechanisms, which are realized through the inflammation, as well as fundamental biochemical, immune, functional and other processes are common for all the mammal world, and thus any treatment found for animals could also be applicable in humans [11-14].

A lot of the factors are determined by physiology and thus the reaction to the pathological conditions, which are determined by age, gender, genetical heredity (e.g. vessel pathology). For example in the case of gender it was shown that female hormones, such as estrogens, preserve females rats' brain and narrows the clinical picture in conditions when free blood is present, such as hemorrhagic stroke [15]. The same tendency can be seen in women patients with hemorrhagic stroke, as it was shown that women aged 25-44 have less hemorrhagic stroke death when compared to men at the same age [16]. However other studies suggest that women had higher hemorrhagic stroke associated deaths rate than men [17, 18]. Study performed by Wassertheil-Smolleret al. reported estrogen plus progestin to increase the risk of ischemic stroke in generally healthy postmenopausal women [18]. However the role of female hormones is clearly present in the conditions of hemorrhagic stroke, and neuroprotective effects of estrogen were shown to be of benefit in female rats when give prior the ICH [15]. These findings suggest to use male rats in the hemorrhagic stroke experiments. Age, as an individual evolution – stable period, involution and conditions that are associated with involution such as atherosclerosis (including atherosclerosis of cerebral arteries); genetical problems – aneurysms, stenosis, etc. All these factors influence the research and thus standardization is really important, which limits the research by the gender, age, genetical line according the aims (e.g. hypertensive rats) and etc.

NEOSTRIATUM AS A SITE FOR EXPERIMENTAL HEMORRHAGIC STROKE

Similarity in organization, functional and behavioural mechanisms in neostriatum between human and rats made it a site for modelling hemorrhagic stroke [19, 20] [9, 21-23]. This information has led to the development of a battery of sensorimotor tests that can measure various aspects of both motor impairment and recovery after ischemic and hemorrhagic insult. Further, investigation of the anatomical and neurophysiological organization of the rodent motor system facilitates the identification of the neural mechanisms underlying motor recovery, information that in turn allows for the development of novel adjuvant therapies that may enhance recovery or limit impairment [13, 22, 24, 25]. In addition, rat models enable the use of more complex experimental designs to examine issues such as time course of recovery and dose-response relationships that are not feasible in nonhuman primate experiments [26-29].

HEMORRHAGIC STROKE MODELS

Under normal conditions the blood does not have direct access to the neurons. They are separated by the blood-brain barrier, which is mainly formed by the astrocytes, endothelial cells and extracellular matrix. When an artery in the brain ruptures, this causes a blood leakage

into the brain tissues thus causing a non-physiological condition with corresponding outcome. This condition is called hemorrhagic stroke [30-32].

The volume of the blood leaked out, that forms hematoma, directly correlates to the clinical signs. The blood that has leaked out from the blood vessels in the brain presses the surrounding tissues against the skull thus forming an ischemic zone and hemorrhagic injury [33, 34].

An ideal experimental hemorrhagic stroke model should have the following characteristics characteristics:

1) blood deposition in a distribution consistent with the type of haemorrhage desired;
2) uniform degree of hemorrhage;
3) a mechanism of hemorrhage which closely simulates the human conditions;
4) easily performed;
5) reasonable cost.

The most common experimental intracerebral hemorrhage stroke models are:

1) balloon inflation model;
2) blood injection model;
3) bacterial collagenase model [7,8, 35-37].

BALLOON INFLATION MODEL

This model was introduced by Sinar et.al in 1987. The balloon inflation model is a pure mechanical model of ICH which mimics the space-occupying effect of a hematoma and its removal. The model studies the pathophysiology of these events. A microballoon mounted on a 25-gauge blunted needle is inserted into caudate to a depth of 5.5 mm and inflated to a 50 µl volume over a period of 20 sec with a radiopaque contrast medium. Inflation is confirmed by X-ray fluoroscopy and the balloon is deflated 10 minutes later. Since then the model has been modified for in terms of both the duration and the volume [38, 39].

As the model is performed mechanically the lesion generated is reproducible. Moreover, as it was reported, reproducibility of results and the extent of CBF fall and ischemia in this model can be controlled by the volume and the duration of balloon inflation [10].

Still, cerebral blood flow falls for the next 4 hours after balloon inflation regardless of the time of balloon deflation. Ischemia develops 4 hrs after balloon inflation. Also it has been shown in two separate studies by *Mendelow and Valdes et al*. that ischemic brain injury level can be limited by early deflation of the balloon and that the damage in the region is mainly represented by ischemic processes and that cell death in the lesion is apoptotic [40].

The absence of both blood vessel rupture and the subsequent leakage of blood highlights the physiological differences between this model and hemorrhagic stroke.

BLOOD INJECTION MODEL

A model that used an injection of autologous blood into the brain was firstly proposed by *Bullock et al, 1984*. The blood is collected from the rat's tail vein and stereotaxically injected into the region of interest. The site for injection is based on the common human ICH blood distribution. The basal ganglion is still considered to be the favourite site of blood injection. The volume of the blood injected may vary depending on how big the hemorrhage is designed, but many researches agree that the volume of blood should not exceed 100 µl [23, 41, 42].

One of the major disadvantages in the use of blood injection model is that the hematoma size is hardly reproducible in a series of experiments [10, 43, 44]. The reasons for the lack of reproducibility vary but are most commonly: rupture of ventricle, backflow of the blood infused along the needle track, excess of blood injected.

To minimize the lack of reproducibility the double-injection model has been introduced [45], when the desired volume of blood is injected in two portions at a slow rate over a certain time period with 7 minutes break. The break allows blood to clot along the needle thus prevents the backflow, resulting in no leakage into the subarachnoid space.

However, having the leak limited, the distribution of the blood injected remains inconsistent and as some researches report about 25% of the hematomas have extensions into the adjacent white matter [35, 41], at the same time other researches observe a different size of damage after autologous blood injection and not consistent location of the hematoma [46]. Irregular morphology and variable location of hematoma are the problems encountered with slow infusion [37].

Recent papers suggest using the curved tip of a metallic microcatheter through the canula with turning it four full times to the left and right, thus creating the space for the blood being further injected [6]. However this model is not perfect either, as the canula used for injection is of a quite a large diameter, as 23 G, which leaves a stab wound and produces trauma preceding blood injection.

The main disadvantage of the model most probably lays in that it doesn't represent all the real mechanisms of the hemorrhagic stroke.

BACTERIAL COLLAGENASE MODEL

Collagen IV is the major protein of the basal lamina of blood vessels and plays an important role in integrity. The main principle of the model is that the collagenase disrupts the pro-

tein bonds in collagen and thus rupturing the vessel wall. Originally the collagenase model was introduced by Rosenberg [36] when 0.1-1 units of bacterial collagenase in 2 μl of saline was injected stereotaxically into the brain, and reported that 0.5 units gave expected results, while 1 unit collagenase limited 24-hrs survival of experimental animals. Since then the model has been modified by different authors and the different types of collagenase have been used [23, 47].

The site of collagenase injection in this model remains the striatum. The only other site used collagenase injection model is the primary somatosensor cortex, performed in swine model of intracerebral hemorrhage [48].

The main disadvantage of the collagenase injection model is thought to be an exaggerated inflammatory response, what was suggested by Xue et al. and later by researchers at National Institute of Neurological Disorders and Stroke with an idea that collagenase damages the tissue in the place of injection and plays a role as an artificial chemoattractant [35].

As was suggested, the collagenase model is clearly more suitable to assessing treatments that affect bleeding and hematoma expansion, such as hemostatic therapies or blood pressure therapies. The collagenase model would suit studies in detecting treatment side affects, such as elevated blood pressure by induced hypothermia [14]. The collagenase model was shown to better represent the physiological conditions of hematoma expansion in clinical patients with ICH, as it was suggested that hematoma expansion continues up to 24 hrs [49] after incident.

Most researches report that this model showed good reproducible results with hemorrhage volumes that correlate the collagenase amount injected [10, 25, 50].

COMPARING TWO ICH MODELS

Choosing the model for investigating intracerebral hemorrhage is really important, that is why it is vital to compare existing models and choose the most appropriate one for the research criteria.

The blood injection model and collagenase injection model are the most widely used for intracerebral hemorrhage modulation [7]. However there are a few major differences between.

Starting from the very beginning, from choosing the substance to be injected, in the blood model for example, it is important to consider that in most of the research done the blood has been collected from rat tail vein, which means that venous blood has been injected. Not many researches use arterial blood to simulate hemorrhage, which differs from the real life situations when in most cases arterial vessels rupture, thus causing blood leakage into the brain tissues [6, 14, 51]. Besides blood injection model simulate more the final stage of the intracerebral hemorrhage – the appearance of the blood in the brain tissue, while the speed and the pressure with which the blood have been injected remains questionable. In the collagenase injection model the enzyme affects the basal lamina of the blood vessel directly causing the blood leak into the brain tissues, and the blood mainly appears to be of the mixed type.

The blood injection model was mainly introduced to represent a single large blood leak into the brain tissue [51, 52] and does not represent spontaneous bleeding. As clinical data in ICH patients suggests that bleeding continues up to 24 hours after the incident, the blood injection model is not appropriate for studying the bleeding or interventions that might affect bleeding and is mainly used for routine pathophysiology examination of ICH [14]. That is compared to the collagenase model where the bleeding can be observed in its “natural way” and continues up to 24 hours as in patients after stroke incidence [50]. For example MacLellan et al. showed an increase in hematoma volume in collagenase model compared to a blood injection model over the first 4 hrs after incident [14, 50].

The injection methodology also varies. As the best results in blood injection model have been achieved using a double-injection model, which mean injection of the whole blood in two portions with a short break in between [6, 10, 45]. However 5-7-minute break during whole blood injection doesn’t represent the natural way of blood leakage and the first portion may affect the second portion, e.g. blocking injection by the clot formation. As compared to the collagenase injection model where there is a completelydifferent situation. The collagenase affecting the vessel does represent bleeding as it would be found in real life [36].

The double-injection model demonstrates higher reproducibility of the hematoma due to preventing the backflow of the blood, which is achieved by the blood clotting along the needle tract during the break. However having the leakage limited, the distribution of the blood injected remains inconsistent and as some researches report about 25% of the hematomas have extensions into the adjacent white matter [35, 41]. At the same time other researchers have observed different scales of damage after autologous blood injection which were not consistent with the position of the hematoma [46]. Irregular morphology and variable location of hematoma are problems encountered with slow infusion [37]. A collagenase injection model produces consistent hematoma size and location, according the collagenase concentration

that has been injected [10, 25, 50].

One more important disadvantage of the blood injection model that should be taken into account is that the disrupted vessel wall metabolites do not appear to get into the bloodstream as would happen naturally, and in the collagenase model [51].

It was proposed by Xue et al. that collagenase itself acts as a chemoattractant and that it plays some role in the inflammatory response [35]. It was also stated in the report from a National Institute of Neurological Disorders and Stroke workshop, 2005, that collagenase produces exaggerated inflammatory response and is directly toxic to the neurons. However, some groups using *in vitro* studies showed that collagenase at concentrations used *in vivo* don't cause neuronal death or inflammation, while large doses of collagenase remain toxic to neurons [53]. MacLellan *et al.* also have proposed that the increased damage due to bacterial collagenase injection can be as a result of higher dosage used and thus the distribution of the active enzyme [14]. Another possible reason for more pronounced inflammatory response could be the more natural way of stroke incidence, which is represented by the blood vessel wall disruption, blood leakage and disrupted vessel wall metabolites entering the blood stream, which triggers natural chemoattractactive mechanisms and thus, probably, a more natural inflammatory reaction.

On the other hand, the study performed by *Xue and Del Bigio* showed that in the acute stage all the models of hemorrhagic stroke (both blood injection and collagenase injection models) were characterized with the same signs, such as presence of the cell debris and hypereosinophilic reaction within the first day within the lesion, it was also shown that neutrophils begin to appear in intact brain tissue around the lesion. Neutrophilic reaction, though it was slightly increased in collagenase injection model (compared to a blood injection model), showed the same tendency as the blood injection model – the peak was reached on day 2 after experiment, then it was significantly reduced on day 3 and did not appear to be present on day 7. At the same time it was shown that active lymphocytic reaction was increased reaching its peak on day 7, compared to the blood injection model where the peak was reached on day 2 and decreased [35]. However the exact numbers and changes of the cell types in the hemorrhage region in both models, and in humans, are absent, what makes hard the comparison in between the models and the models to the humans.

Looking deeper into the main stated disadvantage of the collagenase model, pronounced inflammatory response, we should compare the inflammatory response in the model to the inflammatory response in humans, which is represented by local and systemic responses (alteration, exsudation, proliferation) [54-57]. System reaction is presented by the immune and inflammation system cell kinetics, which ideally goes through the steps of leukocyte migration (neutrophiuls 1-2 days, monocytes 2-3 days), chemotaxis (endogenous signaling molecules – lymphokines, exogenous – toxins), phagocytosis (lyzosomal enzymes, free radicals, oxidative burst) [12, 58, 59]. The next step of normal inflammatory reaction is a resolution (which is mainly represented by chemical substances neutralization, normalization of vascular permeability, apoptosis of inflammatory cells, lymphatic drainage) and healing by scar (tissue destruction, fibrinous inflammtion, purulent inflammation which leads to abscess formation). Taking into account that these processes are regulated by the attractants from the injury area (the metabolities of the vessel rupture, the products of died cells, etc) there are the grounds to believe that the collagenase model processes are more relevant to ones in the humans.

Inflammatory molecules, including cytokines (TNF-α, IL-1b, IL-8 and IL-18) and soluble adhesion molecules (L-selectin, E-selectin, P-selectin, sICAM and sVCAM), are elevated in the blood of stroke patients [12]. Cytokines such as IL-1b, IL-6, IL-8 and IL-10 also can be found elevated in the cerebrospinal fluid from the first day of stroke [60-62]. High levels of TNF-α in plasma were shown to be associated with increased perihematoma [63]. Interestingly, neurological impairment on the third day after stroke is correlated with the mRNA levels of IL-1b, IL-8 and IL-17 in peripheral blood mononuclear cells [64]. Serum IL-18 levels within the first 24 h predict the size of the infarct detected by CT scan and clinical outcome at 2 weeks [65]. Similarly, peak plasma IL-6 concentration within the first week of stroke correlates significantly with CT brain infarct volume, early clinical deterioration and clinical outcome after 3 months [66, 67]. The ESR level at 48 h correlates with the clinical outcome at 6 months, and CRP level at 72 h correlates with the mortality rate at 4 years after onset of stroke [68, 69].

A lot of the investigators demonstrate local and systemic inflammatory responses after stroke incidence. Peripherial white blood cell count (WBC), C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) are increased within 24 hours after the onset of stroke [70, 71]. More recent studies showed an elevated rate of WBC, CRP and ESR even 3 months after incindence [72].

In an experimental collagenase induced intracerebral hemorrhage model performed by

Wasserman et al. it was shown that TNF- α, IL-1b, IL-6, ICAM-1 and VEGF increase within hours of ICH incindence, and play a role in BBB disruption and neutrophil recruitment into the area [73, 74].

Wasserman *et al.* presented the data showing that extravasated neutrophils could be observed within 6 hrs at the periphery of hematoma, and on day 3 it was shown neutrophil reaction around the hematoma with separation from the healthy striatum with the band of activated microglia/macrophages [73, 75, 75]. It was also shown that after ICH stroke incidence the cells around hematoma were positive for TNF-α [76]. Infiltrating neutrophils can damage brain tissue directly by generating ROS and secreting pro-inflammatory proteases [77]. Besides, the contents of dying leukocytes can promote inflammatory tissue injury indirectly by stimulating macrophages to release pro-inflammatory mediators [78].

Activated microglia/macrophages were reported to be observed on day 1 throughout the damaged striatum and weakly present in healthy striatum, with further activation and migration of activated microglia/macrophages toward the hematoma and creation of a dense band around the hematoma on day 3. By day 7 it was shown that the hematoma was completely infiltrated with activated microglia/macrophages. Though the primary role for activated microglia is to clear the hematoma, it also expresses and release a variety of cytokines [79-81], ROS [82-84], nitric oxide [85] and other potentially toxic agents, suggesting that activated microglia/macrophages might contribute to ICH induced early brain injury [86, 87]. In addition, a greater degree of microglial activation has been found in aged rats after ICH than in young rats, suggesting that activated microglia might be a contributing component to enhanced brain injury in aged rats [88]. More recently, studies by Yang et al [89] have shown that complement activation may affect inflammatory responses, including microglial activation and neutrophil infiltration, thereby contributing to ICH-induced brain injury.

In the table below can be found the comparative characteristics of the main differences and advantages of the collagenase model in comparison to the blood injection model and in-patient data. It is easy to see that most appropriate model to represent the 'natural' mechanisms of the hemorrhagic stroke is the collagenase model.

Table

Comparative characteristics of the most used model of ICH and in-patients data

Factors	Blood injection model	Collagenase injection model	In-human-process
Technique	Injection model with 5-7 minutes break giving the total volume up to 100 uL in 2 portions	Collagenase injected slowly, in one portion. The volume of portion is 1-3 uL. Collagenase affects basal lamina of the blood vessel, thus causing blood leak into the brain tissue	Due to the damage of the vessel blood leaks into the surrounding brain tissue
Hematoma formation	Autologous blood injection into the chosen region of the brain without damaging the blood vessel wall and participation of its metabolities	Bacterial collagenase disrupts basal lamina of cerebral blood vessel thus causing the blood leak into brain tissue	Blood vessel disruption and thus blood leak into the brain tissue
Blood type	The blood injected into the brain tissue is represented as venous or arterial	Blood type is characterized by the vessel been involved, mainly present mix type (both arterial and venous)	Blood type is characterized by the vessel been involved, mainly present mix type (both arterial and venous)
Bleeding time	Represents one large leak into the brain tissue, thus doesn't represent bleeding	Continues most commonly up to 24 hrs	Continues most commonly up to 24 hrs
WBC count, CRP	—	—	Increased within 24 hrs and stays elevated over 2 month after incindence
Leukocyte reaction	Hypereosinophilia around hematoma on day 1, reaching peak on day 2 and then decreasing	Hypereosinophilia around hematoma on day 1, reaching peak on day 2 and then decreasing. Neutrophilic reaction within 4 hours post-incidence.	Neutrophilic reaction can be observed within 5-8 hours pos-incidence, cell migration 1-2 days
Microglia activation	Activated microglia can be observed withing 1-4 hours in perihematoma region, reaching the peak at day 7 and decreasing at day 28	Activated microglia can be observed within 1-2 hours in the perihematoma region, reaching peak at day 7 and decreasing at day 21	Microglia/macrophages infiltration starts about 3 days and lasts for several years

Factors	Blood injection model	Collagenase injection model	In-human-process
Cytokines	Increase in inflammatory molecules TNF-α, IL-1b	Increase in inflammatory molecules TNF-α	TNF-a, IL-1b, IL-8, IL-18, L-selectin, E-selectin, P-selectin, sICAM and sVCAM are elevated
Reactive oxygen species (ROS)	ROS are present on day 1-3 in the perihematoma region	ROS are present on day 1-3 in the perihematoma region	ROS can be found in perihematoma region
Metalloproteases	MMPs can be found in activated microglia/macrophages	MMPs can be found in activated microglia/macrophages	—
Technique	Injection model with 5-7 minutes break giving the total volume up to 100 uL in 2 portions	Collagenase injected slowly, in one portion. The volume of portion is 1-3 uL. Collagenase affects basal lamina of the blood vessel, thus causing blood leak into the brain tissue	Due to the damage of the vessel blood leaks into the surrounding brain tissue
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WBC count, CRP	—	—	Increased within 24 hrs and stays elevated over 2 month after incindence
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Cytokines	Increase in inflammatory molecules TNF-α, IL-1b	Increase in inflammatory molecules TNF-α	TNF-a, IL-1b, IL-8, IL-18, L-selectin, E-selectin, P-selectin, sICAM and sVCAM are elevated
Reactive oxygen species (ROS)	ROS are present on day 1-3 in the perihematoma region	ROS are present on day 1-3 in the perihematoma region	ROS can be found in perihematoma region
Metalloproteases	MMPs can be found in activated microglia/macrophages	MMPs can be found in activated microglia/macrophages	—

CONCLUSION

As hemorrhagic stroke remains one of the serious problems, the experimental models have been designed to study the pathophysiology for further investigation of possible interventions into the process with subsequent clinical improvement. Although there are many different experimental models used in a variety of different animals, the favoured species for intracerebral hemorrhage is the rat due to its similarity in characteristics to human brain architecture and the relative cost-effectiveness of the model.

Recent decades brought a variety of the experimental hemorrhagic stroke models in rats, among which the most used ones are blood injection model and collagenase injection model. Despite these models are broadly used, there is still no agreement on which model is better for representing the hemorrhagic stroke process. Hence a lot of comparing in between the models has been done, still none of the publications have compared existing model to the processes that take place in humans.

In correspondence to the review the main aim for the intracerebral hemorrhage model

should be reproducibility for further standardization and further precise conclusions on the therapies investigated. The comparison of the main mechanisms of the models, both mechanical and pathophysiological showed that collagenase injection model remains the most appropriate to the mechanisms present in stroke-patients.

Standardization of the approach in experimental hemorrhagic stroke, beginning with the choice of model, is the primary goal for obtaining comparable data, which obviously would highlight further avenues of investigation.

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