

Ex vivo measurement of brain tissue viscoelasticity in postischemic brain edema

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Summary

Knowledge of the biomechanical properties of postischemic brain tissue is important for understanding the mechanisms of postischemic secondary brain tissue injury. We describe the method and results of biomechanical property measurement in ex vivo postischemic brain tissue by applying an indentation method.

Mongolian gerbils were subjected to a transient unilateral hemispheric ischemia. At day 1 after ischemia, multi-parametric MRI was performed, the brain was removed under anesthesia, sliced, and kept in a container with silicone oil for the measurement. A compression probe attached to a pressure transducer was inserted to a predetermined depth at the regions of interest and maintained at a constant speed. A pressure relaxation curve was recorded for the calculation of elasticity modulus (E) and viscosity modulus (η) according to Maxwell-Voigt's 3-element model. One day after ischemia, E and η decreased to 78.7% and 73.1% of the control level, respectively. This decrease corresponded to a mild decrease in apparent diffusion coefficient (ADC) and magnetization transfer ratio, and an increase in T2 value. Tissue water content increased to 105.1% of control. Microvacuolation with demyelination and axonal disruption was evident in the postischemic brain tissue.

Keywords: Cerebral infarction; elasticity; viscosity; Mongolian gerbils; indentation method; multi-parametric MRI.

Introduction

Brain tissue injury secondary to ischemic brain edema and swelling is an important factor in determining the prognosis of stroke patients. It is well known that postischemic brain edema and swelling potentially induces fatal brain herniation at the acute phase. Brain herniation signifies brain mass expansion and protrusion into the free residual spaces through a notch or opening in rigid structures such as the cerebellar tentorium and the foramen magnum. The biomechanical properties of the brain tissue are obviously important in this process. Biomechanical data on brain tissue,

both in the normal and pathological state, are also indispensable for the computer simulation of intracranial disease processes.

In previous studies we have quantified the biomechanical properties of brain with vasogenic edema [3–5]. Here, we describe a method for the measurement of biomechanical properties of ex vivo brain tissue by using an indentation method. This technique was used to examine brains subjected to unilateral hemispheric ischemia and the results compared to morphological and magnetic resonance imaging (MRI) tissue changes. Maxwell-Voigt's 3-element model [1, 2] (Fig. 1b) was applied for the calculation of elasticity modulus (E) and the viscosity modulus (η) of the postischemic tissue.

Materials and methods

Adult Mongolian gerbils of both sexes were used in the experiment. For induction of unilateral hemispheric ischemia, the left common carotid artery was occluded for 10 minutes by using a miniature vascular clip under 2% isoflurane anesthesia. After vascular occlusion, the animal was allowed to recover from anesthesia; the stroke index [7] was measured during the initial occlusion. Animals ($n = 4$) with stroke index equal to or more than 10 were selected as symptom-positive animals, and subjected to the second ischemia for 10 minutes after a 5-hour interval. At day 1 after ischemia, multi-parametric MRI (mapping of apparent diffusion coefficient [ADC], T2 value, and magnetization transfer ratio [MTR]) was performed using an experimental MRI (4.7 T, Unity-INOVA, Varian, Inc., Palo Alto, CA), and the animal was then sacrificed with an overdose of anesthesia. The brain was removed, cut coronally with 3 mm thickness at the chiasma level, and kept in a container with silicone oil heated to 37 °C for the viscoelasticity and tissue water content measurement.

Ex vivo viscoelastic response of the brain tissue was measured by applying an indentation method (Fig. 1). For the measurement, a compression probe attached to a pressure transducer was inserted

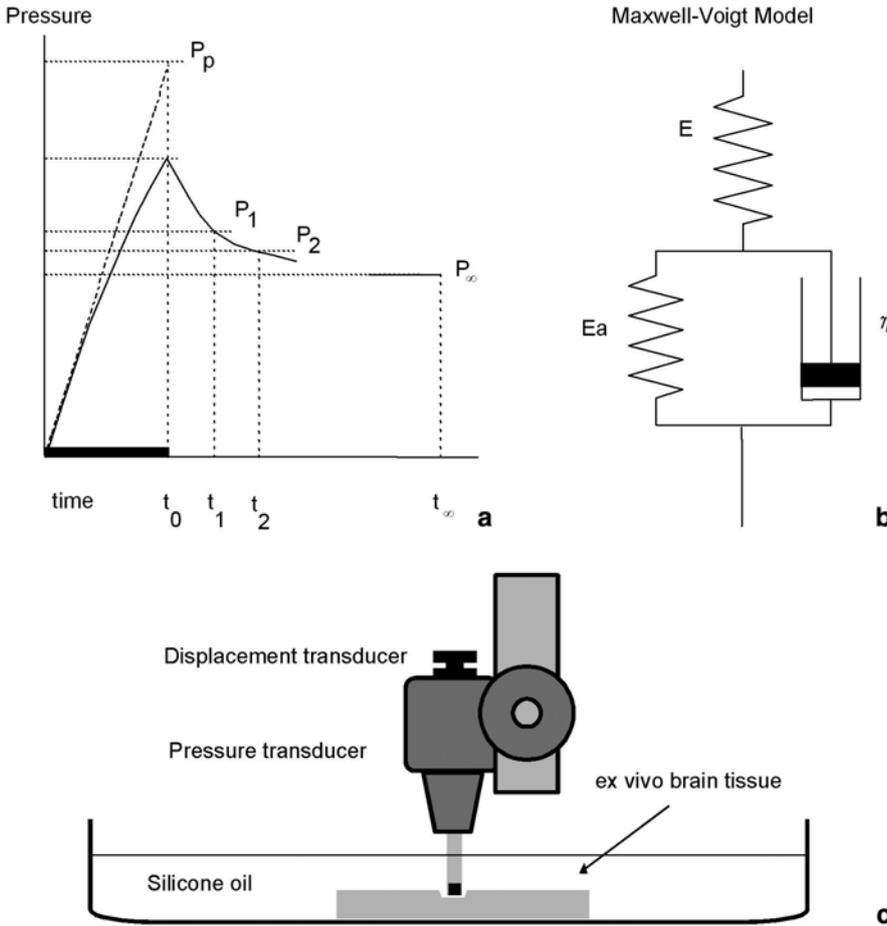


Fig. 1. (a) Schematic drawing of the indentation method using pressure-time relationship; (b) Maxwell-Voigt 3-element model; and (c) a pressure-displacement transducer system

to a predetermined depth (400 μm) at the regions of interest at a constant speed (25 $\mu\text{m/s}$). The time course of pressure response was schematically drawn as shown in Fig. 1a.

Assuming that biomechanical property of the brain tissue is well simulated by the Maxwell-Voigt 3-element model (Fig. 1b), tissue elasticity modulus (E) and viscosity modulus (η) were calculated according the following equations [1].

$$E = \frac{P}{u} \times \frac{\pi(1 - \mu^2)b^2}{2a\{1 - \sqrt{1 - (b/a)^2}\}} \quad (1)$$

$$\eta = E \times \frac{t_1 - t_2}{\log_e \frac{P_1 - P_\infty}{P_2 - P_\infty}} \times \left(1 + \frac{P_\infty/P_p}{1 - P_\infty/P_p}\right) \quad (2)$$

- E : elasticity modulus
- μ : Poisson's ratio
- P_p : Estimated peak pressure at t_0
- P_1 : pressure at t_1
- P_∞ : pressure after tissue relaxation
- a : radius of compression probe
- η : viscosity modulus
- u : insertion depth
- P_2 : pressure at t_2
- b : radius of pressure transducer

After the indentation method, tissue from the region of interest was cut out for specific gravity measurement [6]. The coronal section

with a mirror surface of the above measurement was immersion-fixed in 4% buffered formalin and prepared for light microscopy examination. The results were compared to those obtained from sham operated animals ($n = 4$).

Results

Histological examination revealed postischemic brain edema with neuropil microvacuolation in the postischemic cerebral hemisphere at day 1 postischemia. Demyelination and axonal disruption were also evident. Specific gravity measurement revealed tissue water content increased to 105.1% of the control level. E and η decreased to 78.7% and 73.1% of the control level, respectively. These decreases corresponded to a decrease in ADC and magnetization transfer ratio (70.6% and 85.5% of the control level, respectively), and to an increase in T2 value to 151.9% of the control (Fig. 2).

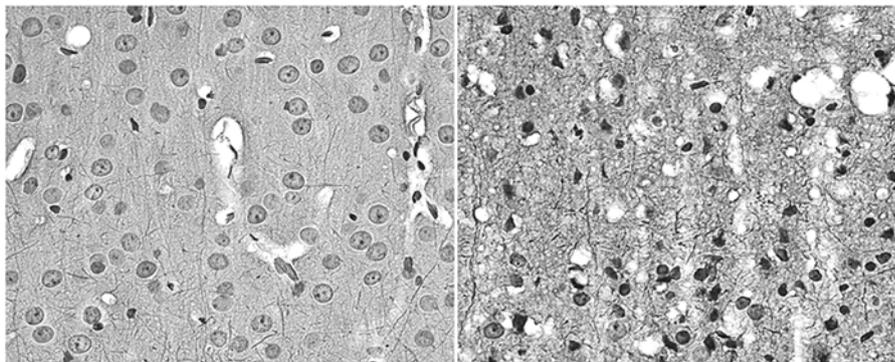
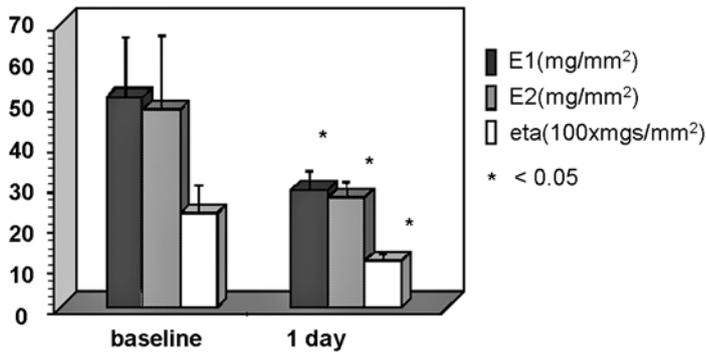


Fig. 2. (a) Change of tissue water content (% water), elasticity modulus (E : mg/mm^2) and viscosity modulus (η : 10^2 mgs/mm^2) of the cortex day 1 after a transient cerebral ischemia (* = $p < 0.05$). (b) Light microscopy of the cortex in sham operated animal (upper), and at day 1 after transient ischemia (lower) (Bodian staining)

Discussion

Biomechanical properties of brain tissue have been examined both in physiological and pathological conditions. Walsh *et al.* [8] measured in vivo brain tissue elastic response under physiological conditions. The measurement was done using a pressure-displacement transducer system attached to the skull of dogs. They observed that the elastic response of the brain was sensitive to systemic factors such as ventilation.

The elastic property of the brain under pathological conditions has also been measured. Aoyagi *et al.* [1, 2] measured brain tissue compliance in dogs subjected to traumatic brain edema, hydration, and dehydration. They found a significant decrease in tissue elasticity in the brain with traumatic brain edema. Thus, in vivo measurement is useful for understanding the overall pressure response of the brain tissue located inside the skull; however, it is not possible to discriminate the influence of systemic factors such as blood pressure and cerebrospinal fluid pressure using this procedure. Meningeal membranes also influence the compressibility of the brain tissue in the in vivo measurement. How-

ever, for understanding the mechanism of secondary brain tissue injury, the biomechanical property of the brain tissue *per se* as a material composing brain structure is important. The influences of cerebrospinal fluid pressure or blood pressure should be excluded in the measurement. This is also true in the biomechanical data for the computer simulation of intracranial disease processes, where biomechanical data of tissue elasticity and viscosity independent from the systemic factors in the pathological as well as normal conditions are essential for simulation procedures such as the finite element method.

We applied an indentation method to ex vivo brain tissue slices. It is well known that a shift of tissue water from the extracellular compartment to the intracellular compartment takes place immediately after energy failure. To minimize this influence and also to prevent tissue from drying, we kept the ex vivo brain in silicone oil during the measurement. By using the indentation method, the size for measurement was approximately 1 mm^2 , which was small enough for regional comparison to tissue water content and histological changes.

Maxwell-Voigt's 3-element model has been used to simulate the biomechanical properties of various materials to a combination of 3 elements, *i.e.*, 1 dash-pot (viscosity) connected in parallel to 1 spring (elasticity), and in series with another spring (elasticity). We calculated the elasticity modulus and the viscosity modulus of the brain tissue under the assumption that the 3-element model simulates biomechanical aspects of brain tissue. We observed that both elasticity and viscosity had significantly decreased already by 1 day postischemia.

Histological examination revealed significant neuropil microvacuolation with ischemic neuronal changes. Bodian staining revealed disruption of neuronal processes in the ischemic tissue. Tissue-specific gravity measurement revealed significant edema. Several factors including sparsely-fibered neuropil, expansion of the extracellular space, loss of cellular integrity, and neural fiber connections in the postischemic brain tissue appear to be responsible for the decrease in tissue viscoelasticity.

Multi-parametric MRI revealed a mild decrease in ADC and MTR and an increase in T2 value. The increase of net tissue water content reflected by T2 increase, and the decrease of tissue water integrated in the macromolecular structure reflected by MTR decrease, appear to coordinate with the decrease in tissue viscoelasticity [9].

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