HRVATSKO MIKROSKOPIJSKO DRUŠTVO

POZIV NA 261. SASTANAK

Hrvatskog mikroskopijskog društva, koji će se održati u prostorijama **Instituta „Ruđer Bošković“, Bijenička cesta 54,** predavaonica **I. krila**
uz praćenje putem linka: https://mojoblak.irb.hr/apps/bbb/b/7PkPnZWnfxMngXGi
u
utorak, 31. svibnja 2022. u 16:00 sati
u organizaciji Suzane Šegota
uz sljedeći

D n e v n i r e d:

1. **Predavanje stipendista 4. hrvatskog mikroskopijskog kongresa:**
   - **Dominik Hamer:** Thick and cleared – Blood vessels and neurons can be visualized in the cleared mouse brain using inverted fluorescence microscopy
   - **Daniela Petrinec**: *In vivo* imaging to tackle the challenge of visualisation of dynamic processes – example of brain damage evolution after ischemic stroke

2. **Izvješće s 4. hrvatskog mikroskopijskog kongresa povodom 30. godišnjice HMD-a kao samostalnog društva**

3. **Razno**

Tajnica:
Vida Strasser

Predsjednica:
Suzana Šegota

Thick and cleared – Blood vessels and neurons can be visualized in the cleared mouse brain using inverted fluorescence microscopy

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To visualize whole organs or whole body of humans or animals, different imaging techniques can be applied. Novel light sheet fluorescence microscopy (LSFM) allows to visualize large samples, in particular whole organs of the laboratory animals. The variety of tissue clearing procedures are used for achieving sample transparency and the visualisation is based on the labelling of structures of interest by fluorescence. The main goal of this research was to image the structures in the cleared mouse brain with a special task to verify if the clearing procedure can be beneficial even if "classical" easily available fluorescent microscopes were used for sample visualisation. The inverted fluorescence microscope (The EVOS® FL Auto Imaging System, ThermoFisher Scientific) was used as a test instrument for this purpose. Previously naturally transparent parts of mouse embryos were imaged using fluorescence microscopy by our group [1,2]. A fluorescent marker was applied to the brain of live mouse by stereotaxic injection. The fluorescent staining 10 % fluorescein solution (Fluorescite, Alcon) and Isolectin GS-IB4 from *Griffonia simplicifolia*, Alexa Fluor 568 Conjugate (Invitrogen) was injected by help of stereotaxic apparatus (David KOPF Stereotaxic Instrument Small Animal Frame 5001 H7000). Moreover, mouse brains were isolated from two months old animals (Thy1-YFP-16 strain), which naturally expressed yellow fluorescent protein in neurons. As a third approach, blood vessel visualization *Lycopersicon esculentum* Lectin Texas Red (Invitrogen) was injected in the left heart ventricle of living mouse (Fig. 1). In all three cases the mice were perfused by 1× PBS and 4 % formalin solution and subsequently cleared. For whole brain tissue clearing, three methods were used: ECi (optical clearing using ethyl-cinnamate), iDISCO (immunolabeling-enabled threedimensional imaging of solvent-cleared organs) and PEGASOS (polyethylene glycol-associated solvent system). Cleared mouse brain samples were cut on approximately 1 mm thick slices using mold (Alto Acrylic 1 mm Mouse Brain Coronal 40-75gm, CellPoint Scientific), mounted subsequently on the glass slides in the drop of the final clearing solution, covered by coverslips, and imaged using inverted fluorescence microscope. The ECi method was preferred as the protocol for clearing lasted only one day and used chemicals were nontoxic. iDISCO clearing technique made brain slices brittle and difficult to handle. Even without using LSFM, it was possible to visualize fluorescently labelled structures in thick samples. It still remains to be clarified if this type of imaging is suitable not only for qualitative description of the samples, but also for quantitative measurements. In conclusion, the clearing of mouse brain produces thick slices suitable as well for imaging and analysis by fluorescence microscopy.

Keywords: blood vessels, neurons, clearing techniques, mouse brain, fluorescence microscopy

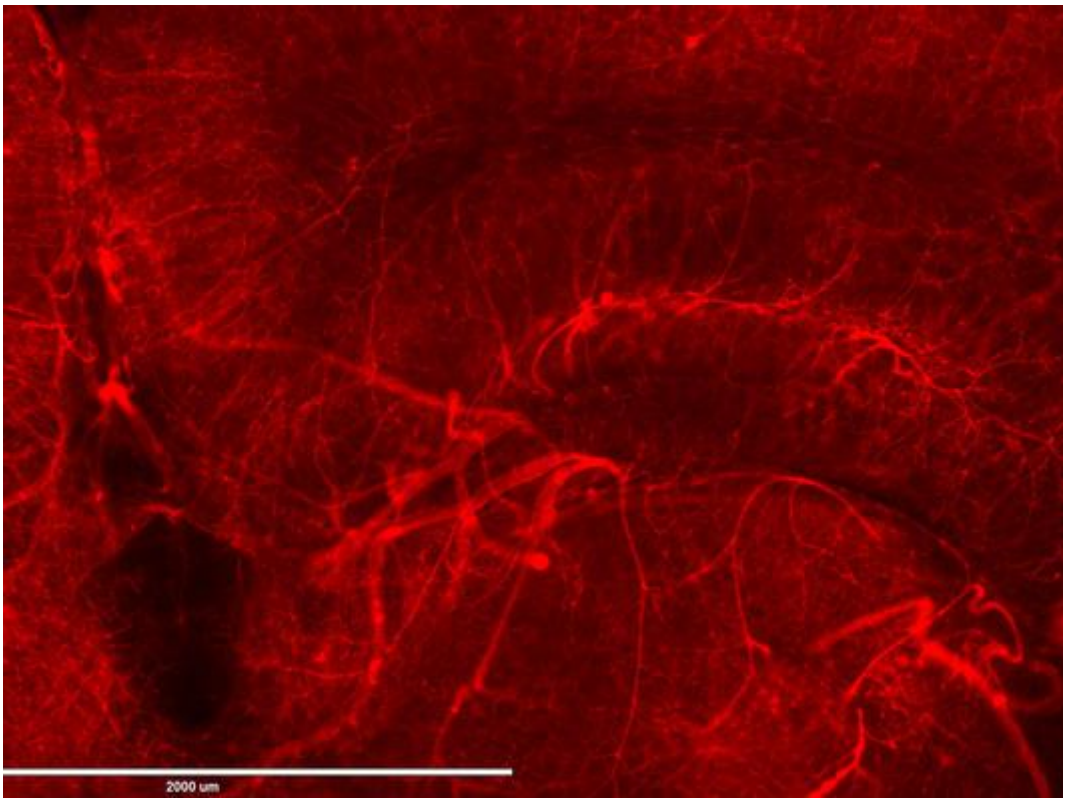

Figure 1. Blood vessels in cleared mouse brain slices (approximately 1 mm sample slice, brain clearing method ECi) labeled with *Lycopersicon esculentum* Lectin Texas Red (Invitrogen) using inverted fluorescence microscope.

References:

1. I. Alić et al., Neurosci. Lett. 634 (2016) 32–41.
2. M. Žižić Mitrečić et al., Cells Tissues Organs 192 (2010) 85–92.

In vivo imaging to tackle the challenge of visualisation of dynamic processes – example of brain damage evolution after ischemic stroke

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The challenge of visualisation of the dynamic processes which change through time remains an important challenge, in particular if combined with methods with different magnification range. Together with light, some other visualisation modalities can be applied, for example magnetic resonance or X-ray-based micro-CT. In animal experiments, the additional issue is the need to reduce the number of animals, refine the animal procedures and replace animals by other experimental approaches. Subsequently, we have organised a platform for *in vivo* imaging of the laboratory animals, which allowed multiple imaging sessions during a single experimental protocol. This was applied to study the animal model of human ischemic stroke, where brain lesion was caused by transient middle cerebral occlusion (tMCAO). Two imaging modalities were used, magnetic resonance imaging (MRI) and optical imaging of bioluminescence (BLI), and the obtained *in vivo* imaging results were combined by histological analysis and immunohistochemistry of the mouse brain and flow cytometry of isolated and separated brain cells. Moreover, the imaging results were related to the functional outcomes of the animals obtained by neurological deficit scoring. The described approach was used to compare the mice deficient for the specific receptor on the microglia, Tlr2, to which particles of necrotic cells bind and subsequently elicit neuroinflammation [1]. The experimental paradigm included the follow up of animals for 28 days after ischemic lesion. The imaging sessions allowed us to compare the brain consequences between Tlr2-deficient to control wild-type animals. Tlr2-deficient animals survived better after ischemic lesion, however, had bigger lesions and neurological scores. After 28 days, Tlr2-deficient animals were comparable to their controls. Bioluminescence imaging showed higher Gap43 expression in Tlr2-deficient animals related to the processes of brain repair. The same was shown by modelling the relation between the ischemic lesion and functional outcome indicating that Tlr2-deficient animals recovered better than the wild-type controls. In conclusion, the *in vivo* imaging modalities combined with other microscopy methods provided a multimodal approach, giving insight into the time-dependent changes of the mouse brain after ischemic lesion. The animals are followed analogously to the human patients which allows testing the preclinical interventions with the aim to design future human therapies of the stroke.

Keywords: magnetic resonance imaging, optical *in vivo* imaging, bioluminescence imaging, mouse brain, ischemic lesion

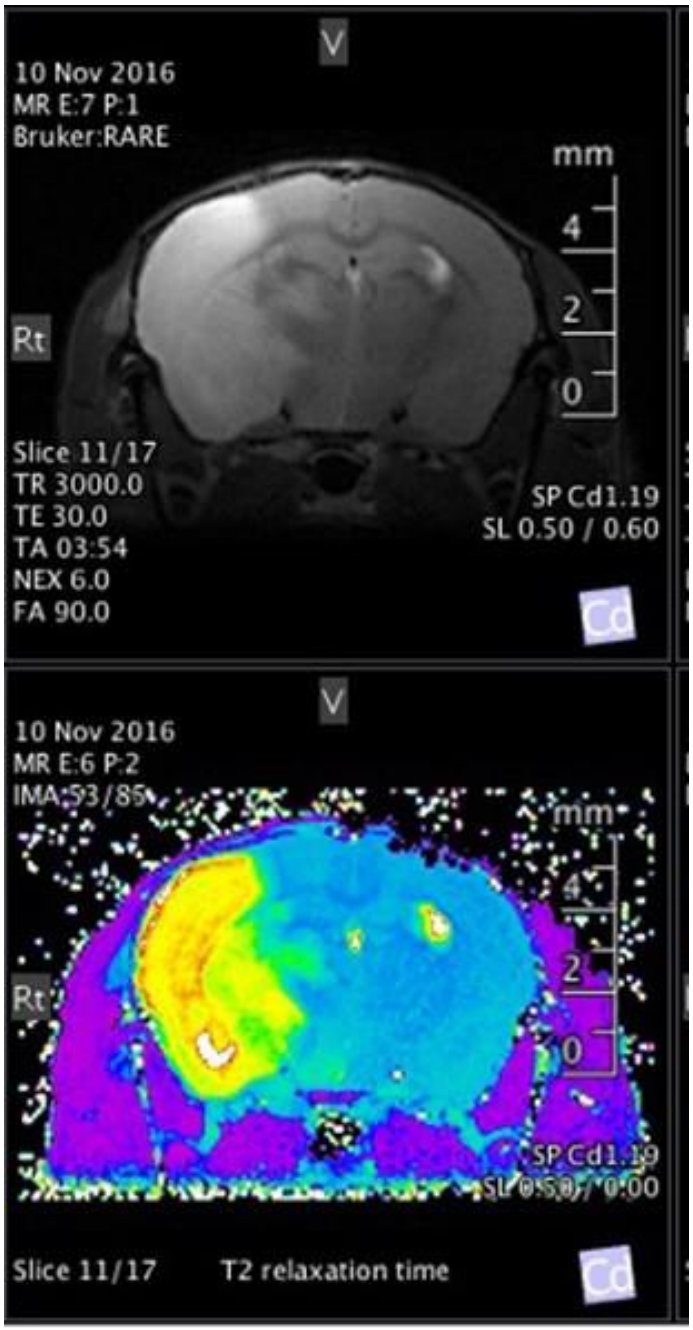

Figure 1. Magnetic resonance imaging of the mouse brain after ischemia shows the size of ischemic lesion by T2 (up) and T2map (down) modalities.

References:

1. I. Bohacek et al., J. Neuroinflammation 9 (2012) 191.