Stroke solutions

Professor Masahiro Nishibori discusses his specialism in ischaemic stroke and the condition's prompting of dangerous inflammatory reflexes, which he aims to combat

Could you explain why brain infarction is such a serious human health problem?

The massive death of brain tissue in brain infarction causes a diverse range of neurological symptoms including motor paralysis, sensory disturbance, speech disturbance, cognitive impairment and mood disorders depending on the brain regions involved. In many cases, patients require constant physical, mental and financial support. The number of patients with brain infarction is high in developed countries. Thus, the burden on society is huge.

How did you come to focus your investigations on preventing the damage caused by neuronal death, particularly that which is ischaemia- and brain trauma-induced?

These investigations were born out of several important hypotheses. One of my primary hypotheses was that damage-associated molecular patterns may be involved in triggering brain inflammation during ischaemic and traumatic injury. The other hypothesis, which came from a 2004 study of a fulminant hepatitis model in mice, was that excessive inflammatory response occurs during the very acute phase after the brain insult.

What is high mobility group box-1 (HMGB1) and why is it a suitable target for the prevention of penumbral inflammation?

HMGB1 is a non-histone chromatin DNA binding protein. Once released into extracellular space, HMGB1 plays a cytokine-like role through the stimulation of plural receptors including a receptor for advanced glycation endoproduct (RAGE) and Toll-like receptor-4/2.

Therefore, HMGB1 is considered a useful ready-made mediator in triggering a cascade of events in inflammatory responses. In the ischaemic and traumatic brain, HMGB1 release is induced in neurons in the acute phase. This response starts very quickly, as early as two hours after the onset of infarction.

Why is it important to target inflammation within the penumbra region of the brain when developing novel drugs to prevent neuronal death?

In contrast to necrotic cells in the ischaemic core, in the penumbra region the brain tissues receive different levels of blood supply from collateral blood vessels. Here, the inflammatory responses that occur include

blood-brain barrier disruption associated with the activation of microglia and upregulation of expression of inflammation-related molecules such as tumour necrosis factor-alpha and inducible nitric oxide synthase.

You use monoclonal antibodies (mAbs) in your studies. Could you explain how they can be employed for therapy?

A specific antibody can be used in animal experiments for validation of specific molecules as the therapeutic target in diseases. Also, we can discern the efficacy of the mAb therapy by using suitable animal models at the same time. Therefore, specific mAbs for extracellular antigens can be applied for the evaluation of target molecules in specific diseases.

Concerning the availability of mAb therapy for brain infarction, we demonstrated that therapeutic mAb is accumulated in the ischaemic areas and can enter, to some extent, into the brain's parenchyma in middle-cerebral artery occlusion models.

Have your investigations involving the implementation of anti-HMGB1 mAb therapy on rodents with neuropathic pain produced promising results?

Using a sciatic ligation model in rats, we demonstrated that anti-HMGB1 mAb therapy reduced the neuropathic pain administered by acute injection even after the hyperalgesic state had been established. We are trying to examine the effects of anti-HMGB1 mAb on spinal cord injury, in addition to brain trauma, in order to ultimately expand its clinical application. The preliminary results are promising.

You identified three rat mAbs against HMGB1, which recognised different amino acid sequences in HMGB1. Why did you choose the first clone – mAb #10-22 – for your experiments?

mAb #10-22 has the highest affinity for HMGB1 among the three mAbs we raised, and recognised the HMGB1-specific sequence at the C-terminal, whereas the other two clones recognised the sequence in B-box common to HMGB2.

Does anti-HMGB1 mAb therapy have the potential to inhibit atherosclerotic plaque formation?

In the atherosclerosis model of ApoE-/- in mice with high fat diets, chronic treatment with anti-HMGB1 mAb reduced the number of macrophages infiltrating into the intimal layer of the atherosclerotic plaque and the expression of inflammation-related molecules. In the plaque, expression of HMGB1 was increased and shown to stimulate chemotactic activity on monocytes/macrophages. Therefore, it is speculated that the inhibition of monocyte/macrophage migration and infiltration may be one of the main mechanisms of anti-HMGB1 mAb on atherosclerotic plaque formation.

What still remains to be achieved before anti-HMGB1 mAb therapy is ready to enter the clinical arena?

It will be necessary to obtain a humanised or complete human mAb against HMGB1. It will also be important to evaluate the side-effects of anti-HMGB1 mAb after high dosages and chronic treatment in experimental animals in order to establish the safety of the treatment.

Expanding antibody therapy

Medicinal solutions for the devastating consequences of stroke fail to meet the needs of patients. Researchers at the **University of Okayama**, Japan are developing an innovative new alternative

ACCORDING TO THE World Health Organization (WHO), stroke is the second leading cause of death worldwide. 6.2 million people died by the rupturing of blood vessels in the brain in 2011 alone, an alarming increase from the 5.6 million lives taken in 2000. As well as its immediately traumatising effects, cerebrovascular disease is also a leading cause of disability in adults, permanently diminishing its survivors' quality of life and placing serious emotional and financial strain on those who must support them.

Ischaemic stroke is the most common variety of stroke, and occurs when blood clots, usually forming in areas where arteries have partially collapsed or have been blocked with fatty plaques, obstruct the passage of blood to the brain. These clots, or thrombi, together with emboli – travelling intravascular masses – are known to cause neuronal death in the core ischaemic zone. Neuronal death can ultimately be traced to an inability to sustain membrane ion gradients, excitotoxicity and, crucially, disintegration of the blood-brain barrier (BBB), which, when functioning, provides a wall between circulating blood and extracellular cerebral fluid. When the BBB is breached in this way, life-threatening consequences include brain oedema – intracranial pressure or swelling.

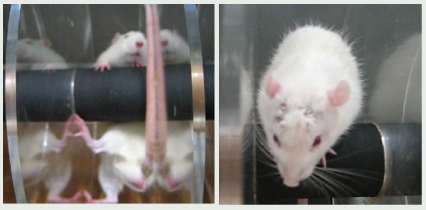
Professor Masahiro Nishibori of the Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences' Department of Pharmacology, succinctly underlines the centrality of BBB compromise to complete cerebral breakdown: "BBB disruption forms a positive feedback loop with the inflammatory events in the brain. In other words, BBB disruption is one of the major processes of brain inflammation, leading to brain infarction". The penumbra, or area surrounding the ischaemic core, is compromised dangerously in the case of ischaemic stroke. Owing to restrictions on the penumbra's usual blood supply, a sequence of inflammatory responses is activated and may damage any surviving neurons. Fortunately however, Nishibori's research offers some hope for curtailing symptoms of ischaemic stroke, most especially when they result from destruction of the penumbral region.

A CHAIN OF EVENTS

Nishibori's considered preclinical studies postulate a highly credible interplay between high mobility group box-1 (HMGB1), a DNA binding protein, and the central nervous system. HMGB1 is released from cells in the stages of death, or from cells undergoing other kinds of intense stress. His team has concluded that the regulation of this protein may have a protective effect on cerebral tissue. The group's innovative new antibody treatment for HMGB1 suggests the possibility of a fuller recovery of the areas of the brain altered by inflammatory reflexes, and is already emerging as far superior to innumerable other candidate drugs which have been subjected to less successful preclinical and clinical studies.

ANIMAL MODELS

In the earliest stages of Nishibori's research, a number of rat monoclonal antibodies



Effects of anti-HMGB1 mAb on brain infarction in rats.

INTELLIGENCE

ANTI-HMGBI MAB THERAPY FOR BRAIN INFARCTION, BRAIN TRAUMA AND NEUROPATHIC PAIN

OBJECTIVES

To develop high mobility group box-1 (HMGB1) monoclonal antibodies (mAbs) as a novel therapy for the treatment of brain infarction, brain trauma, neuropathic pain and atherosclerosis.

KEY COLLABORATORS

Professor Shuji Mori, Department of Pharmacology, School of Pharmacy, Shujitsu University, Okayama, Japan

Professor Hideo K. Takahashi, Department of Pharmacology, Kinki University, Faculty of Medicine, Osaka-Sayama, Japan

Professor Yasuko Tomono, Shigei Medical Research Institute, Okayama, Japan

FUNDING

Japan Society for Promotion of Science (No. 2439006115)

Ministry of Health, Labor and Welfare of Japan (No. WA2F2343)

Japan Science and Technology Agency (No. 7211200174)

CONTACT

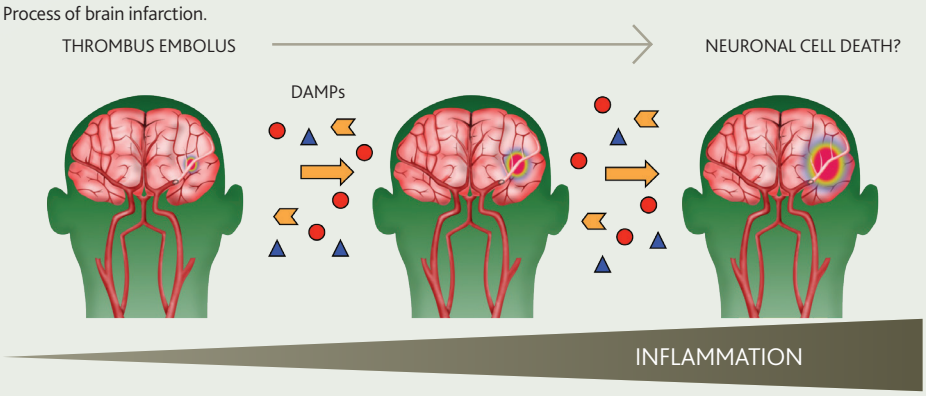
Professor Masahiro Nishibori
Principal Investigator

Graduate School of Medicine, Dentistry and Pharmaceutical Sciences
Okayama University
2-5-1 Shikata-cho
Kita-ku, Okayama
700-8558
Japan

T +81 86 235 7140
E mbori@md.okayama-u.ac.jp
E mn3130162007@yahoo.co.jp

PROFESSOR MASAHIRO NISHIBORI

obtained a PhD in 1985 from Okayama University, where he remained as Research Associate, Assistant Professor, Associate Professor and finally Professor, a position in which he remains at the Graduate School of Medicine, Dentistry and Pharmaceutical Sciences to this day. Among many awards throughout his career, most recently he received the 2011 Saito Award from the Bio Business Japan Award and the 2012 Sanyo Newspaper Award (Academic field).



(mAbs) were pitted against bovine HMGB1. The #10-22 variety soon emerged as most suited for the suppression of HMGB1. Its compatibility with the harmful protein, attributable to surface plasmon resonance, led to inhibited upregulation of intercellular adhesion molecule-1 (ICAM-1) expression of monocytes, a variety of white blood cells, in human mononuclear cells. Given this aptness of the #10-22 mAb, it was then utilised in a series of experiments testing its suitability for the treatment of brain infarction, as well as brain trauma, neuropathic pain and atherosclerosis.

The neutralising impact of this selected mAb upon brain infarction was tested in rats with obstructed middle cerebral arteries. Happily, the mAb acted swiftly even when administered a very short time after reperfusion; Nishibori's research associates witnessed both a 90 per cent reduction in the size of necrotic tissue and fewer locomotive defects in the rats' brains. The anti-HMGB1 mAb treatment provided protection of the BBB; reduction of the activation of microglia and expression of tumour necrosis factor and inducible nitric oxide synthase; and suppression of enzyme matrix metalloproteinase 9.

In order to accurately measure the translocation of HMGB1 in contact with mAb in this selection of rats, the researchers observed the protein acting unhindered in control experiments. They found that its immunoreactives were translocated into the cytosolic compartment in ischaemic stroke, then formed granular structures and were ultimately released into the affected areas; a sequence suggestive of HMGB1 movement into extracellular space.

This busy, unobstructed HMGB1 activity was very much inhibited when coupled with mAbs as a neutralising agent. In this instance, astrocytes displayed a weaker tendency towards end feet swelling, end feet detachment from the basement membrane of capillary vessels was suppressed and the junctions between vascular endothelial cells remained taut. This set of results at once highlighted the instrumentality of HMGB1 in a harmful chain of inflammatory reactions and indicated how appropriate it is as a target for treatment.

BRAIN TRAUMA

Traumatic brain injury (TBI) is another serious cause of death and disability which might benefit from antibody treatment. The cerebral oedema this entails claims thousands of adolescent and young adult lives every year, a fact WHO has been attempting to counter with its WHO Helmets publicity campaign since 2011. Sadly though, only 21 American states and the District of Columbia have made helmet-wearing for bicycle and motorcycle users compulsory, and death tolls are set to increase proportionally with the increasing speeds of vehicles.

Given its similarities in BBB disruption induced by ischaemic stroke and its increasing prevalence worldwide, Nishibori's team made the innovative decision to analyse the effects of anti-HMGB1 mAb on TBI. Remarkably, anti-HMGB1 mAb minimised fluid percussion-induced brain swelling, HMBG1 translocation and helped the BBB to remain intact in rats. As in the case of brain infarction, motor function also suffered less damage, and was even shown in some cases to ameliorate. As such, this second investigation not only proved the effectiveness, but also the versatility of the novel antibody therapy.

UNDERSTANDING CHRONIC PAIN

Not content to conclude his research after successful mAb application to brain infarction and TBI, Nishibori decided to maximise its potential in animal models of chronic pain, including pain stemming from diabetes, cancer and neuropathy.

Pain produced by the compression of the sciatic nerve in rats was impressively alleviated by the mAb treatment, dulling hind paw hypersensitivity at timed intervals of up to 21 days. This sequence of tests also enabled Nishibori to pinpoint neuronal sources of HMGB1 – its movement from the nucleus to the cytosol predominantly occurred in dorsal horn neurons located in sections of grey matter in the spinal cord. This process can be held accountable for symptoms of pain in the nervous system.

Nishibori's steps towards not only solutions for acute pain, but also a richer comprehension of how it is generated during assaults on the brain, are cause for great optimism. Quite deservedly, his contributions to ischaemic medicine have been recognised by his award of the 21st Century Encouragement of Invention Prize by the Japan Institute of Invention and Innovation and the 2011 Saito Prize conferred at the Bio Business Award ceremony in Japan.