KAPOSVÁR UNIVERSITY
FACULTY OF ANIMAL SCIENCE
The Institute of Diagnostic Imaging and Radiation Oncology
KAPOSVÁR

Head of Doctorate School:
PROF. HORN PÉTER, Member of the Hungarian Academy of Science

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Supervisor:
DR. REPA IMRE PHD professor

STUDYING BLOOD BRAIN BARRIER OPENING BY USING NEW IRON BASED MR CONTRAST AGENT IN ANIMAL MODEL AND IN HUMAN CLINICAL TRIALS

Author:
DR. SÁNDOR PÉTER MANNINGER

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Abbreviations

ADEM = ***a***cute ***d***isseminated ***e***ncephalo***m***yelitis • CT = ***c***omputer-***t***omography*)* • MRI = ***m***agnetic ***r***esonance ***i***maging • SPIO= ***S***uper***p***aramagnetic ***I***ron ***O***xid • TOF = ***t***ime ***o***f ***f***light • USPIO = ***U***ltra Small ***S***uper***p***aramagnetic ***I***ron ***O***xid

1. INTRODUCTION

The blood-brain barrier (BBB) is the specialized system of capillary endothelial cells that protects the brain from harmful substances in the blood stream, while supplying the brain with the required nutrients for proper function. Unlike peripheral capillaries that allow relatively free exchange of substance across/between cells, the BBB strictly limits transport into the brain through both physical (tight junctions) and metabolic (enzymes) barriers.

In the United States in the year 2005, it was estimated that there were 43,800 new cases of brain tumors (Central Brain Tumor Registry of the United States, Primary Brain Tumors in the United States, Statistical Report, 2005-2006), which accounted for 1.4 percent of all cancers, 2.4 percent of all cancer deaths, and 20-25 percent of pediatric cancers. These scary numbers only apply to the brain tumors in the US, and lots of other well known CNS diseases (epilepsy, schizophrenia, stroke etc.) are still not mentioned. 98% of all known potential CNS drugs have a molecular weight bigger, than 500Daltons (upper molecular weight limit for crossing BBB), so they can't cross the blood-brain barrier.

To target and successfully treat these CNS lesions, it is obvious that we need to learn about blood-brain barrier as much as we can. This paper consists of three different parts which strongly correlate with each other. The first part is about adaption and introducing osmotic blood-brain barrier disruption in pigs. The second and third part of the work is about new iron-based MR contrast agent, using in central nervous system.

2. OBJECTIVES

1. To adapt osmotic blood-brain barrier opening method to pig.
2. To confirm the reversibility of the blood-brain barrier opening and obtain the reclosing time of the barrier.
3. To study new iron based MR contrast agent (USPIO, ferumoxatran-10) in inflammatory CNS lesions using conventional MR sequences.
4. To study new iron based MR contrast agent (USPIO, ferumoxytol) in CNS using conventional, angiographic and perfusion MR sequences.

3. MATHERIAL AND METHODS

3.1. Animal studies

For adjusting the osmotic blood-brain barrier disruption for pigs we used ten, for define the reclosure time of the open barrier three, and for the MR contrast study two more 25-30kg young pigs. The animal studies were performed in whole anesthesia. Xylazin-ketamin-atropin and isoflurane-O_2 was used for narcosis. Blood-brain barrier opening was performed with a conventional catheter technique, where the catheter was placed at the beginning of the internal carotid artery. 50 ml high osmolality mannitol (40%) was injected into the artery over 30 seconds. The results of the blood-brain barrier opening were proven by in vivo CT and MR examinations and in vitro with vital staining (Evans blue).

3.2. Human studies

Ferumoxtran-10 (Combidex) MR contrast agent study in inflammatory CNS lesions using conventional MR sequences.

Twenty-three patients with different types of intracranial "inflammatory" lesions underwent standard brain MR with and without gadolinium, followed an average of 10 days later by a ferumoxtran-10 scan. Patients were imaged 24 hours after infusion of 2.6mg/kg ferumoxtran-10. All MR images were evaluated subjectively by 4 investigators for a difference in enhancement patterns, which could be useful in differential diagnoses.

Ferumoxytol MR contrast agent study in CNS using conventional, angiographic and perfusion MR sequences.

Twelve patients with malignant brain tumors underwent serial magnetic resonance imaging multiple times up to 72 hours after ferumoxytol injection at both 1.5 and 3T. The enhancement time course was determined for ferumoxytol and compared with a baseline gadolinium scan. Perfusion, time-of-flight and dynamic magnetic resonance angiography and T_1 weighted scans were compared for the two agents.

4. RESULTS AND DISCUSSION

4.1. Results of animal studies

The adaptation of blood-brain barrier disruption method for pigs was successfully, we got a very useable animal model. There were no major side effects. We successfully proved the reversibility of the osmotic opening of the blood-brain barrier and defined the reclosing time of the osmotic opened blood-brain barrier in 30-60 minutes. The outwitting of the blood-brain barrier for the new iron-based contrast agent was also successful.

4.2. Results of human studies

Ferumoxtran-10 (Combidex) MR contrast agent study in inflammatory CNS lesions using conventional MR sequences.

In 5 cases, (one ADEM, 2 strokes, one cavernous venous vascular malformation, and one primary central nervous lymphoma) the ferumoxtran-10 scan showed higher signal intensity, larger area of enhancement, or new enhancing areas compared with gadolinium. Most MS patients showed less enhancement with ferumoxtran-10 than with gadolinium.

Ferumoxytol MR contrast agent study in CNS using conventional, angiographic and perfusion MR sequences.

The lesions were detectable at all field strengths, even with an intraoperative 0.15T magnet. Maximal ferumoxytol enhancement intensity was at 24 to 28 hours after administration, and the enhancing

volume subsequently expanded with time into a non-gadolinium-enhancing, high T_2 weighted signal region of tumor-infiltrated brain. Dynamic studies were assessed with both agents, indicating early vascular leak with gadolinium but not with ferumoxytol.

5. CONCLUSIONS AND SUGGESTIONS

We adapted the osmotic blood-brain barrier disruption for pigs and found this animal model usable for further blood-brain barrier studies. Closing time of the barrier after irreversible osmotic opening was 30-60 minutes. We successfully outwitted the blood-brain barrier for the new iron-based contrast agent, which raises the possibility of its usefulness in central nervous system imaging also in pigs.

Ferumoxtran-10 (Combidex) MR contrast agent study in inflammatory CNS lesions using conventional MR sequences.

Ferumoxtran-10 showed different enhancement patterns in a variety of CNS lesions with inflammatory components in comparison to gadolinium. The impact of timing and therapy need further evaluation to better assess ferumoxtran-10 in addition to gadolinium as contrast agents for use in diagnosis and monitoring therapy in patients with CNS inflammatory lesions.

Ferumoxytol MR contrast agent study in CNS using conventional, angiographic and perfusion MR sequences.

Our most important finding was that gadolinium leaks out of blood vessels early after injection, whereas ferumoxytol stays intravascular in the "early" phase, thereby increasing the accuracy of tumor perfusion assessment. As a magnetic resonance imaging contrast agent, ferumoxytol visualizes brain tumors at all field strengths evaluated, with delayed enhancement peaking at 24 to 28 hours after administration.

6. NEW SCIENTIFIC RESULTS

1. We successfully adapted the osmotic blood-brain barrier disruption for pigs. We found that reclosing time of the barrier after irreversible osmotic opening is 30-60 minutes.
2. We successfully outwitted the blood-brain barrier for the new iron-based contrast agent.
3. We were among the first in the world to use a new iron-based MR contrast agent (ferumoxatran-10) in human study to examine CNS inflammatory lesions. Twenty-three patients were investigated. After dividing inflammatory disorders into three major groups we described the new enhancing patterns observed with the iron-based contrast material compared with conventional gadolinium based contrast agent.
4. We were among the first in the world to use a new iron-based MR contrast agent (ferumoxytol), which can be administered as a bolus, in human CNS study for dynamic MR imaging. Twelve patients with central nervous system tumors were investigated. We described the enhancing properties of the new contrast medium, angiography and perfusion MRI findings observed with the new contrast material compared with conventional gadolinium based contrast agent.

7. PUBLICATIONS AND ORAL PRESENTATIONS FROM THE THESIS STUDIES

7.1. Papers related to the thesis

1. **MANNINGER, S. P.** – MULDOON, L. L. – NESBIT, G. – MURILLO, T. – JACOBS, P. M. – NEUWELT, E. A.: An exploratory study of ferumoxtran-10 nanoparticles as a blood-brain barrier imaging agent targeting phagocytic cells in CNS inflammatory lesions. *AJNR Am. J. Neuroradiol.*, 2005. 26(9): 2290–2300. ***(IF: 2.525)***
2. MULDOON, L. L. – **MANNINGER, S. P.** – PINKSTON, K. E. – NEUWELT, E. A.: Imaging, distribution, and toxicity of superparamagnetic iron oxide magnetic resonance nanoparticles in the rat brain and intracerebral tumor. *Neurosurgery*, 2005. 57(4): 785–796. ***(IF: 2.587)***
3. MURILLO, T. P. – SANDQUIST, C. – JACOBS, P. M. – NESBIT, G. – **MANNINGER, S. P.** – NEUWELT, E. A.: Imaging brain tumors with ferumoxtran-10, a nanoparticle magnetic resonance contrast agent. *Therapy*, 2005. 2(6): 871–882.
4. MULDOON, L. L. – TRATNYEK, P. G. – JACOBS, P. M. – DOOLITTLE, N. D. – CHRISTOFORIDIS, G. A. – FRANK, J. A. – LINDAU, M. – LOCKMAN, P. R. – **MANNINGER, S. P.** – QIANG, Y. – SPENCE, A. M. – STUPP, S. I. – ZHANG, M. – NEUWELT, E. A.: Imaging and nanomedicine for diagnosis and therapy in the central nervous system: report of the eleventh annual Blood-Brain Barrier Disruption Consortium meeting. *AJNR Am. J. Neuroradiol.*, 2006. 27(3): 715–721. ***(IF: 2.279)***
5. NEUWELT, E. A. – VÁRALLYAY, CS. – **MANNINGER, S. P.** – SOLYMOSI, D. – HALUSKA, M. – HUNT, M. A. – NESBIT, G. – STEVENS, A. – JEROSCH-HEROLD, M. – JACOBS, P. M. – HOFFMAN, J. M.: Potential of ferumoxytol nanoparticle magnetic resonance imaging, perfusion, and angiography in central nervous system malignancy: a pilot study. *Neurosurgery*, 2007. 60(4): 601–612. ***(IF: 3.007)***

7.2. Oral presentations

1. **MANNINGER, S. P.** – MULDOON, L. L. – NEUWELT, E. A.: *Rat iron imaging.* AIRC Meeting, Oregon Health & Science University. Portland/Oregon/USA, 10th February 2004.
2. **MANNINGER, S. P.** – NESBIT, G. – MURILLO, T. P. – ORBAY, P. – SOLYMOSI, D. – LACY, N. – TYSON, R. M. – HALUSKA, M. – BENNETT, L. – HEDRICK, N. – DOOLITTLE, N. D. – NEUWELT, E. A.: *Technical strategies in BBBD: Intra-arterial delivery and imaging.* Annual Blood Brain Barrier Meeting. Sunriver/Oregon/USA, 17–20th March 2004.
3. **MANNINGER, S. P.** – NEUWELT, E. A. – PETERS, J. A. – MULDOON, L. L.: *MR Imaging of rat intracerebral tumor Xenograft models: potential for imaging angiolysis by N-Cadherin antagonist (Exherin).* Annual Blood Brain Barrier Meeting. Sunriver/Oregon/USA, 17–20th March 2004.
4. **MANNINGER, S. P.** – MULDOON, L. L. – NESBIT, G. – MURILLO, T. P. – LOVERA, J. – BOURDETTE, D. – JACOBS, P. M. – NEUWELT E. A.: *Ferumoxtran-10 and Gadolinium as imaging agents in PCNSL and other CNS inflammatory lesions.* American Society of Neuroradiology (ASNR) 42nd Annual Meeting. Seattle/WA/USA, 5–11th June 2004.
5. **MANNINGER, S. P.** – MULDOON, L. L. – SOLYMOSI, D. – NEUWELT, E. A.: *Pre clinical and clinical uses of superparamagnetic iron oxide particles as MR imaging agents in the CNS.* OHSU Neurosciences Grand Round Seminars. Portland/Oregon/USA, 10th March 2004.
6. **MANNINGER, S. P.** – MULDOON, L. L. – SOLYMOSI, D. – NEUWELT, E. A.: *Imaging CNS inflammation with USPIO's.* 11th Annual Meeting of the Blood-Brain Barrier Disruption Consortium. Portland/Oregon/USA, 17–19th March 2005.
7. NEUWELT, E. A. – **MANNINGER, S. P.** – SOLYMOSI, D. – NESBIT, G. – JEROSCH-HEROLD, M. – STEVENS, A. – HOFFMAN, J. M.: *Initial timing of MR imaging and assessment of MR Angiography using intravenous superparamagnetic carbohydrate-coated iron oxide particles in primary high-grade brain tumors and/or cerebral metastases.* American Society of Neuroradiology (ASNR) 43rd Annual Meeting. Toronto/Ontario Canada, 21–27th May 2005.
8. **MANNINGER, S. P.** – SOLYMOSI, D. – NESBIT, G. – JEROSCH-HEROLD, M. – STEVENS, A. – HOFFMAN, J. M. – VÁRALLYAY, CS. – NEUWELT, E. A.: *Ferumoxytol for MRA, perfusion, and delayed MR Imaging in Primary High-Grade brain tumors and/or cerebral metastases.* Hungarian Neuroradiological Meeting. Budapest, 23–24th September 2005.