様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

令和　３ 年　 ８ 月 ２６ 日現在

機関番号：１２３０１
研究種目：若手研究
研究期間：2019～2020
課題番号：１９Ｋ１７２２４

研究課題名（和文）Potential mechanism of Gadolinium penetration via intact blood-brain barriers and the effect of Gd retention to brain cancer proliferation

研究課題名（英文）Potential mechanism of Gadolinium penetration via intact blood-brain barriers and the effect of Gd retention to brain cancer proliferation

研究代表者

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交付決定額（研究期間全体）：（直接経費）　　2,000,000円

研究成果の概要（和文）：トランスフェリンを培養物中のガドリニウムベースの造影剤（GBCA）と共培養した場合、脈絡叢のGd濃度は類似していることが観察されました。鉄とのインキュベーションは、樹状突起形成に対するガドペンテテートの効果を増加させたが。
腎不全マウスモデルにおける線形GBCAの注射後のNSF様皮膚病変の発症における好中球エラスターゼ（NE）の関与を調査した。血清中のNE活性および皮膚NEの発現は、対照と比較してGBCA処置マウスで有意に高かった。GBCAがC6増殖を有意に増加させることも観察しましたが、TE761細胞ではその効果はそれほど有意ではありませんでした。

研究成果の学術的意義や社会的意義
This project provides the information of:
The effect of Gd to the Purkinje cells, provide a background to reveal the mechanism of Gd retention in the brain, involvement of NE Skin lesion development in the Nephrogenic systemic fibrosis, and the effect of Gd to the proliferation of cancer cell lines.

研究成果の概要（英文）：We found no significant differences in Gd concentration when Gadolinium-based contrast agents (GBCA) were incubated with transferrin. Incubation with Fe(II) did not cause any changes to the Purkinje cell arborization compared the control neurons. Fe(II) increased the effect of gadopentetate to the dendrite arborization, but it did not increase the effect of gadobutrol.
Fibrotic markers were increased in the skin on renal failure mouse model that was injected with gadodiamide, while only collagen 1α and TGF-β mRNA expression were higher in the gadopentetate group. NE activity in the blood serum and the expression of skin NE was significantly higher in the GBCA-treated mice compared to the control. We investigated the GBCA effect on the C6-astroglioma and TE761-medulloblastoma in-vitro. GBCAs significantly increase the C6 proliferation, but the effect was less significant in TE761 cells.

研究分野：Gd造影剤，放射線診断

キーワード：Gadolinium　GBCA　Gadolinium retention　GDD　NSF　choroid plexus

１．研究開始当初の背景

Gadolinium-based contrast agents (GBCAs) are widely used to increase the diagnostic yield of magnetic resonance imaging (MRI) because of their low acute adverse reaction rates and rapid clearance from the body. In the field of neuroradiology, GBCAs are particularly useful for detecting aggressive or metastatic brain tumours and vascular lesions. However, recent studies demonstrated gadolinium (Gd) retention in the brain after multiple GBCA administrations. Although Gd from GBCAs may be retained in the various areas of the brain, the effect of Gd retention in the brain is yet to be fully explained. Gd retention in the brain may pose a threat to the neurons itself. Previous studies showed that Gd3+ may damage the cortical neurons through the oxidative stress pathway. The highest Gd retention in the brain was observed in the area rich of iron, such as the dentate nucleus of the cerebellum.

２．研究の目的

This study aimed to investigate the possible pathway of Gd in distribution via choroid plexus, and to investigate the effect of Gd incubation to the choroid plexus and to the morphological alteration of the cerebellar Purkinje cells in-vitro. Additionally, we investigated the involvement of neutrophil elastase in the development of nephrogenic systemic fibrosis-like skin lesions post-injections of linear gadolinium-based contrast agents in renal failure mouse models.

３．研究の方法

Choroid plexus epithelial cells (CPEC) were obtained from newborn pups were cultured at a density of $1x10^6$ cells per plate, and the medium was changed three times a week until it reaches confluency. Similar experiments were performed in inverted transwell chamber to determine the effect of transferrin to the Gd concentration in the CPEC and the medium. The CPEC were incubated with Gd and Gd+transferrin for 48 hours. Then, the CPEC and the medium were collected to determine the Gd concentration. To determine the effect of Gd to CPEC, anti-transthyretin was used as the primary target.

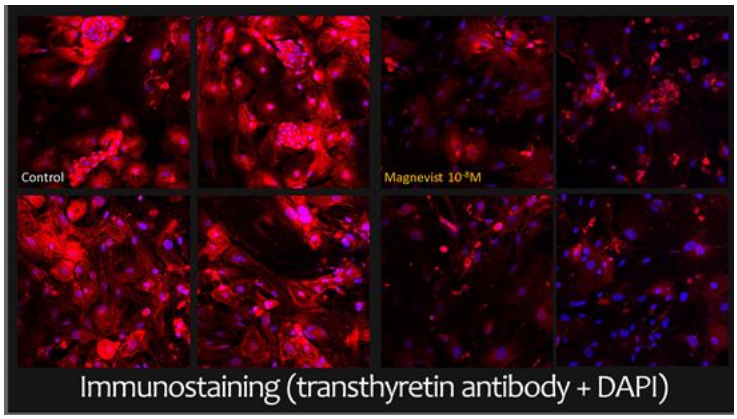
Cerebellar Purkinje cells were cultured at a density of 3×10^5 cells per well. The experiments were performed three times, and each experiment consists of the following group: control group, Fe[II]2+ group (10 nM), GBCA groups (gadopentetate group or gadobutrol group; 1 nM, 10 nM, 100 nM), and GBCA+Fe[II]2+ groups. The experiments were performed to determine the effect of Fe2+ and different GBCAs to the Purkinje cellarborization. The calbindin-28K (anti-CaBP28k) antibody was the primary target and

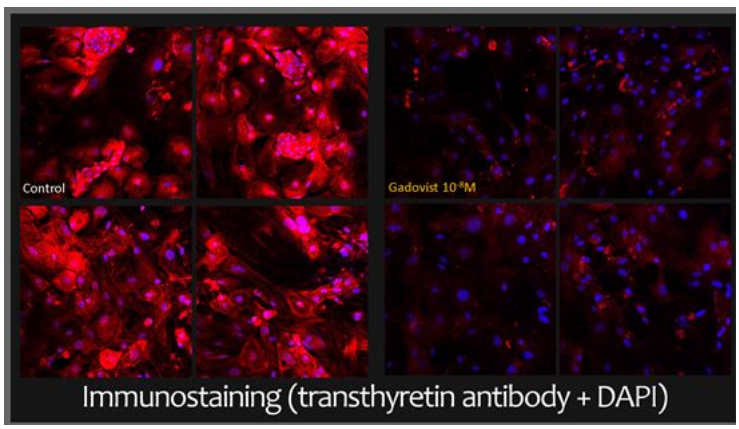
the number of the Purkinje cells and their dendrite arborization were evaluated.

For NE analysis, renal failure mouse models were randomly divided into three groups based on the injection solution: control group, gadodiamide group, and gadopentetate group. Each solution was intravenously administered three times per week for three weeks (total nine injections). Then, the mice were observed daily for skin lesion, and skin samples were obtained at two weeks after the lesion occurred. Quantification of skin lesion, infiltrating inflammatory cells, and the profibrotic cytokines at the lesioned skin were performed by immunostaining and real-time PCR. Blood samples were collected from facial vein to quantify the NE enzymatic activity. The 158Gd concentrations in each sample were quantified using inductively coupled plasma mass spectrometry (ICP-MS).

４．研究成果

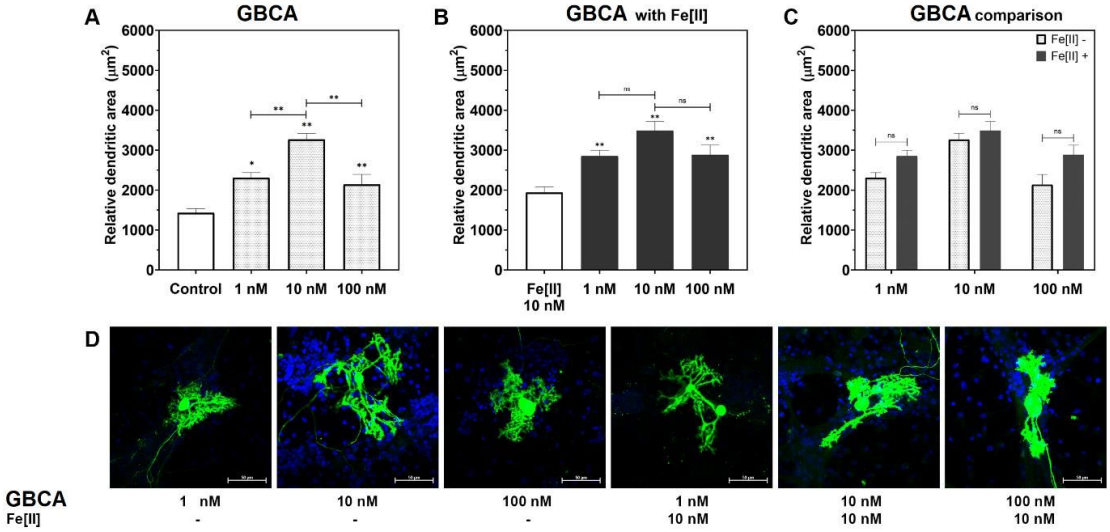
W observed the possibility of transferrin as a transporter of Gd into the brain. We found no significant differences in Gd concentration when Gadolinium-based contrast agents (GBCA) were incubated with transferrin. However, Gd significantly decreased the expression of transthyretin of the CPEC.



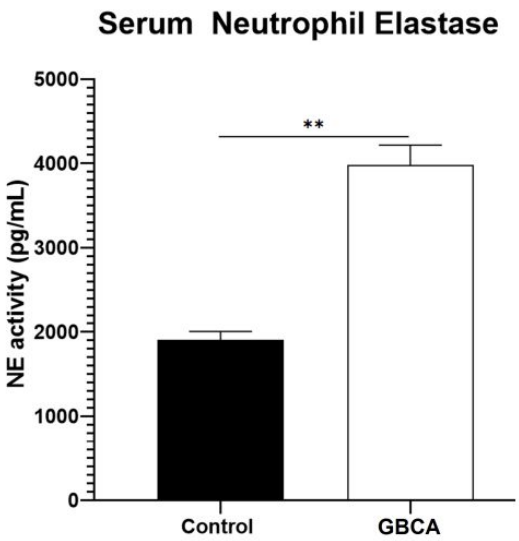


AlthoughFe2+ increased the effect of gadopentetate to the dendrite arborization, it did not increase the effect of gadobutrol, suggesting that the difference in

chelate structures may be important for the toxicity effect of Gd.



Skin lesions were observed in the gadodiamide group and gadopentetate group. In the GBCAs group, the mRNA expression was increased in the skin lesions compared to the control group. The expression of CD3+, CD68+, neutrophil elastase cells and the neutrophil elastase activity in the blood serum were significantly higher in the gadodiamide and gadopentetate groups compared to the control group. Gadolinium concentration in the skin of the gadodiamide group was significantly higher than the gadopentetate group, while almost no traces of gadolinium were found in the control group. Although gadopentetate and gadodiamide affected the fibrotic markers in the skin differently, neutrophil elastase may be involved in the development of fibrosis linked to the GBCAs injections in renal failure mouse models.



５．主な発表論文等

〔雑誌論文〕　計3件（うち査読付論文　3件／うち国際共著　3件／うちオープンアクセス　1件）

１．著者名 Achmad Adhipatria Perayabangsa Kartamihardja, Hirofumi Hanaoka, Putri Andriana, Satomi Kameo, Ayako Takahashi, Hiroshi Koyama and Yoshito Tsushima	４．巻 92
２．論文標題 Quantitative analysis of Gd in the protein content of the brain following single injection of gadolinium-based contrast agents (GBCAs) by size exclusion chromatography	５．発行年 2019年
３．雑誌名 British Journal of Radiology	６．最初と最後の頁 1-8
掲載論文のDOI（デジタルオブジェクト識別子） 10.1259/bjr.20190062	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

１．著者名 Achmad Adhipatria Perayabangsa Kartamihardja and Yoshito Tsushima	４．巻 9
２．論文標題 The extra miles on preventing nephrogenic systemic fibrosis	５．発行年 2019年
３．雑誌名 Quantitative Imaging in Medicine and Surgery	６．最初と最後の頁 1911-1913
掲載論文のDOI（デジタルオブジェクト識別子） 10.21037/qims.2019.09.17	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

１．著者名 Yamaguchi A, Achmad A, Hanaoka H, Heryanto YD, Bhattarai A, Ratianto, Khongorzul E, Shintawati R, Kartamihardja AAP, Kanai A, Sugo Y, S Ishioka N, Tetsuya H, Tsushima Y	４．巻 19
２．論文標題 Immuno-PET imaging for non-invasive assessment of cetuximab accumulation in non-small cell lung cancer	５．発行年 2019年
３．雑誌名 BMC Cancer	６．最初と最後の頁 1-8
掲載論文のDOI（デジタルオブジェクト識別子） 10.1186/s12885-019-6238-4	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

〔学会発表〕　計2件（うち招待講演　0件／うち国際学会　0件）

１．発表者名 Achmad Adhipatria Perayabangsa Kartamihardja
２．発表標題 Changes in the purkinje cell's synaptic density after GBCA exposure: an in-vitro study
３．学会等名 Japan Radiology Conference
４．発表年 2019年～2020年

１．発表者名 Achmad Adhipatria Perayabangsa Kartamihardja
２．発表標題 Quantitative analysis of gadolinium in the protein content of the brain post-administration of gadopentetate
３．学会等名 Japan Radiology Conference
４．発表年 2019年～2020年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関