Investigation of novel safety biomarker for arteritis using *in vivo* MRI

Graduate School of Frontier Biosciences,

Osaka University

Yuta Fujii

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Summary

Since drug-induced arteritis is difficult to monitor in clinical trials, the occurrence of arteritis in nonclinical toxicological studies of a candidate drug makes development of the drug very difficult. Although arteritis is a severe toxicity, the lesion is completely recovered if the offending drug is discontinued or treatment is initiated at an early phase. If arteritis can be detected in an early phase with a biomarker, clinical trials can be conducted safely. Therefore, biomarker for identifying drug-induced arteritis is highly desirable. Since evaluation in humans is difficult, firstly I conducted the research with rats. On magnetic resonance imaging (MRI) in rodents, evaluation of the organs requires higher resolution due to their small size, and research had not progressed as same as in human. However, *in vivo* imaging techniques, including MRI in rodents, have made remarkable advances in recent years.

I made hypothesis that MRI could be used to find a biomarker candidate for drug-induced arteritis. However, there are no reports on the evaluation of drug-induced arteritis by MRI. Therefore, I conducted this study to clarify whether the finding by MRI can be a biomarker as follows.

First study was conducted to clarify which dosing regimen was appropriate for MRI assessment. Based on the obtained results, subcutaneously administered once daily 100 mg/kg/day in FM and 40 mg/kg/day in MH for 2 days is considered an optimal dosing regimen for MRI assessment.

The second study was conducted to clarify whether fenoldopam mesylate (FM)-induced arteritis in

rats can be detected by MRI. FM causes arteritis due to its vasodilatory effect. Mesenteric arteries were examined with *ex vivo* high-resolution MRI, postmortem MRI and *in vivo* MRI on the day after final dosing or 3 days after administration of the final dose. The *ex vivo* MRI showed low-intensity areas and a high signal intensity region around the artery, and these findings were considered to be erythrocytes infiltrating the arterial wall and perivascular edema, respectively. In the *in vivo* study, the MRI of the FM-administered group showed a high signal intensity region around the artery.

The third study was conducted to clarify whether arteritis induced by vasoconstrictor effect could be detected by MRI. The mesenteric arteries of midodrine hydrochloride (MH)-administered animals were examined using *in vivo* MRI at 1 day or 7 days after administration of the final dose. High signal intensity region around the artery was observed in animals with minimal perivascular lesions and not observed in an animal without histological changes on the day after the final dose. On the 7th day after the final dose, no abnormality was observed in histopathological examinations and no high signal intensity regions were observed by MRI in any animal.

In conclusion, my results indicated that regardless of pathogenic mechanism and degree of changes, high signal intensity region in MRI could be a versatile biomarker for detecting the arteritis with high specificity and high sensitivity. In addition, it is suggested it could be possible to judge the discontinuation of administration of a drug in the phase of minimal lesion, which can be completely resolved. This is extremely useful for conducting clinical trials of drugs that may cause arteritis.

Key words:

Arteritis, biomarker, drug, fenoldopam mesylate, midodrine hydrochloride, magnetic resonance imaging, perivascular edema

Abbreviations:

FA, flip angle; FDA, US Food and Drug Administration; FLASH, fast low-angle shot; FM, fenoldopam mesylate; FOV, field of view; HE, hematoxylin and eosin; MH, midodrine hydrochloride; MRA, magnetic resonance angiography; MRI, magnetic resonance imaging; RARE, rapid acquisition with relaxation enhancement; TE, echo time; TR, repetition time; 0.5% CMC, 0.5 w/v% carboxymethyl cellulose solution

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General introduction

Drugs are useful for treatment of diseases; however, they often have undesired side effects. During drug development, even if a candidate drug is expected to be effective, development may have to be terminated if serious side effects occur. Toxicological findings in nonclinical studies of candidate drugs were the main cause of discontinuation of drug development, accounting for 40% of drug development failures (Waring et al. 2015). The occurrence of drug-induced arteritis in nonclinical toxicity studies is one of the causes for the pre-clinical attrition of candidate drugs (Mikaelian et al. 2014).

In humans, vasculitis including arteritis is known to be induced by several drug classes including antimicrobials and antithyroid medications (Doyle and Cuellar 2003). The predominant site of drug-induced vasculitis is the skin, and lesions are sometimes found in the kidneys and lungs (Doyle and Cuellar 2003). In humans, drug-induced vasculitis is complex and several mechanisms may be involved (Gao and Zhao 2009). In nonclinical toxicological studies, arteritis has been observed in several drugs (Lauden et al. 2019), and there are few cases of phlebitis (Nihon Dokusei Byori, 2017). Therefore, arteritis is important in drug development. The mechanisms of arteritis have not been elucidated and they are not necessarily mutually exclusive; however, it is considered that three major mechanisms may be involved. One of the mechanisms is shear and/or hoop stress, another is the direct pharmacological effect of chemicals and the third is related to immunological and inflammatory response (Kerns et al. 2005).

Drug-induced arteritis is difficult to monitor in clinical trials of candidate drugs because the

underlying mechanism has not been elucidated and no specific and sensitive biomarkers have yet been defined in humans (Louden et al. 2006; Mikaelian et al. 2014). The US Food and Drug Administration (FDA) has been working on various biomarkers of drug-induced arteritis in collaboration with several pharmaceutical companies and other research institutes and the search for biomarkers of arteritis has also been undertaken by the Vascular Injury Working Group (Kerns et al. 2005; Mikaelian et al. 2014). However, it is difficult to narrow these down to specific biomarkers that could become the gold standard (Kerns et al. 2005; Mikaelian et al. 2014). In the absence of noninvasive methods to monitor the onset of drug-induced arteritis, its occurrence in nonclinical toxicity studies can become an obstacle to the development of candidate drugs, even if a drug is predicted to be safe and efficacious in humans (Mikaelian et al. 2014). On the other hand, arteritis is a severe toxicity because the blood shortage due to the artery's decreased ability to carry blood can result in systemic damage of organ and tissue; however, the lesion is completely recovered if the offending drug is discontinued or treatment is initiated at an early phase (Radić et al. 2012). If arteritis can be detected in an early phase by using appropriate monitoring with a specific and sensitive biomarker, clinical trials of candidate drugs can be conducted safely, even if the drugs are associated with drug-induced arteritis in nonclinical toxicological studies. Therefore, a noninvasive method capable of identifying drug-induced arteritis is highly desirable. Since evaluation in humans is difficult, firstly I conducted the research with rats, which are most frequently used rodent in toxicological study for a candidate drug.

On magnetic resonance imaging (MRI) in rodents, evaluation of the organs requires higher resolution due to their small size. Therefore, while MRI has been used for over 30 years in clinical practice, research into MRI in rodents had not progressed to the same degree as that in humans. However, *in vivo* imaging techniques, including MRI in rodents, have made remarkable advances in recent years, and their application to pharmacological evaluation of the central nervous system is progressing (Ni 2021). In the safety assessment of candidate drugs, methods using *in vivo* imaging technology have also attracted a great deal of attention, and demand for these methods is increasing.

I made hypothesis that MRI could be used to find a biomarker candidate for drug-induced arteritis. However, there are no reports on the evaluation of drug-induced arteritis by MRI. Therefore, to clarify whether the finding observed in MRI can be a biomarker, the following three studies were conducted.

(1) Deciding an appropriate dosing regimen of animal models of arteritis for MRI assessment

In order to clarify whether MRI can be used to detect arteritis, it was necessary to use an optimal model for MRI assessment. There is not enough data of animal model of arteritis. The first study was conducted to clarify which dosing regimen was appropriate for MRI assessment. Fenoldopam mesylate (FM) is a drug which is an antihypertensive agent. FM is a dopamine (D1 receptor) agonist, and causes arteritis in rats due to its vasodilatory effect (Dalmas et al. 2011; FDA approval package of CORLOPAM). Midodrine hydrochloride (MH) is a selective peripherally acting alpha-1 adrenergic receptor agonist that is indicated for the treatment of

hypotension (Cruz 2000). MH is known to induce arteritis of the mesenteric artery in rats through its vasoconstrictor action (Tobin et al. 2014; Thomas et al. 2012). In the present study, I aimed to confirm the reproducibility of FM-induced arteritis and determine which arteries are affected by FM. As for MH, I aimed to determine which dosing routes, dosing periods, and dose levels cause arteritis and which arteries are affected. In addition, I conducted histopathological examination for understanding the details of histopathological finding in FM- and MH-induced arteritis.

(2) Detection of fenoldopam-induced arteritis in rats using *ex vivo*/*in vivo* MRI

The present study was conducted to clarify whether FM-induced arteritis in rats can be detected by *in vivo* MRI. Before *in vivo* evaluation using live animals, *ex vivo* MRI and postmortem MRI using euthanized animals were performed. First, *ex vivo* MRI was performed to evaluate whether the changes caused by FM can be detected by MRI under ideal condition (no movement with high resolution). Second, postmortem MRI was performed to evaluate whether the changes can be detected in the situation where both respiration and peristalsis were absent but the other conditions are the same as those in *in vivo* MRI. And finally, *in vivo* MRI was performed in the imaging condition determined from the results of *ex vivo* MRI and postmortem MRI.

(3) Evaluation of usefulness of *in vivo* MRI as a biomarker in rats

The third study was conducted to clarify whether detection of perivascular edema by MRI could be useful for detecting arteritis induced by MH. In other words, the purpose of the present

study is to clarify whether arteritis induced by a non-vasodilatory mechanism, which was evaluated in second study, could be detected by MRI.

As described above, this paper presents the results of research conducted with the aim of clarifying whether the signal change obtained by MRI can be a biomarker (The details of these studies are described below).

Chapter 1:

Deciding an appropriate dosing regimen of animal models of arteritis for MRI assessment

Abstract

In order to clarify whether MRI can be used to detect arteritis, it was necessary to use an optimal model for MRI assessment. However, there is not enough data of animal model of arteritis. Therefore, I conducted the present study to clarify which dosing regimen was appropriate for MRI assessment. Fenoldopam mesylate (FM) was administered subcutaneously to each rat for 1 day at dose of 100 mg/kg/day. Midodrine hydrochloride (MH) was administered orally to each rat once daily for 2 days at dose levels of 25 or 50 mg/kg/day or 4 days at a dose level of 50 mg/kg/day or subcutaneously to each rat once daily for 2 days at a dose level of 25 mg/kg/day or 4 days at a dose level of 40 mg/kg/day. Histopathological examination was performed on mesenteric, pancreatic, gastrointestinal, renal, and femoral arteries and arteries in the heart. Histopathological examination revealed that single dose at 100 mg/kg/day of FM induced arteritis and most frequently and strongest in mesenteric artery. MH also induced arteritis most frequently in mesenteric arteries. In addition, subcutaneous administration of MH did not cause the death up to 40 mg/kg/day and arteritis was induced by two days administration even at 25 mg/kg/day in all animals. Based on the results, I considered that subcutaneously administered once daily 100 mg/kg/day in FM and 40 mg/kg/day in MH for 2 days is an optimal dosing regimen and decided to use this regimen and evaluate the mesenteric artery for MRI assessment.

1. Introduction

In order to clarify whether MRI can be used to detect arteritis, it was necessary to use an optimal model for MRI assessment. However, there is not enough data of animal model of arteritis. Therefore, I conducted the present study to clarify which dosing regimen was appropriate for MRI assessment.

Fenoldopam mesylate (FM) is a drug which is an antihypertensive agent. FM is used for hypertension and hypertension crisis. FM is a dopamine (D1 receptor) agonist, and causes arteritis in rats due to its vasodilatory effect (Dalmas et al. 2011; FDA approval package of CORLOPAM). Although FM-induced arteritis is not observed in mice, dogs and humans, it is also observed in a monkey (FDA approval package of CORLOPAM). In FM-induced arteritis of rats, the mesenteric artery, which is a small- to medium-sized vessel, is the main affected artery (Dalmas et al. 2008; Ikegami et al. 2001). In a monkey, arteritis was observed in arterioles of the gastric and submucosa and a renal arterial branch. The lesion was similar between rats and a monkey (FDA approval package of CORLOPAM). It is reported that subcutaneous administration of FM (1 day or 4 days at dose level of 100 mg/kg/day) induced arteritis in mesenteric artery (Dalmas et al. 2008; Ikegami et al. 2001) and intravenous infusion of FM for 24 hours (at dose levels of 1, 5, 25, 50, or 100 μg/kg/min) induced arteritis in mesentery, pancreas, stomach, intestine, ovary, and kidney in rats (Kerns et al. 1989; Yuhas et al. 1985).

Midodrine hydrochloride (MH) is a selective peripherally acting alpha-1 adrenergic receptor agonist that is indicated for the treatment of symptomatic essential hypotension and orthostatic hypotension

(Cruz 2000). Although no relationship between MH and arteritis has been reported in humans, MH is known to induce arteritis of the mesenteric artery in rats through its vasoconstrictor action (Tobin et al. 2014; Thomas et al. 2012). It is reported that oral administration of MH (4 days at dose level of 25 or 50 mg/kg/day) induced arteritis in mesenteric artery (Tobin et al. 2014; Thomas et al. 2012). However, there were no reports other than the mesenteric arteritis by oral administration.

In the present study, I aimed to confirm the reproducibility of FM-induced arteritis and determine which arteries are affected by FM. As for MH, I aimed to determine which dosing routes, dosing periods, and dose levels cause arteritis and which arteries are affected. In addition, I conducted histopathological examination for understanding the details of histopathological finding in FM- and MH-induced arteritis.

2. Materials and methods

2.1 Compounds

FM (the purity is 95% or more) was purchased from Cayman Chemical (MI, USA). FM was dissolved in saline to reach a concentration of 20 mg/ml. MH (the purity was 99.8%) was purchased from Sigma-Aldrich (St. Louis, MO, USA). MH was dissolved in 0.5 w/v% carboxymethyl cellulose solution (0.5% CMC) to reach a concentration of 5, 8, and 10 mg/ml for administration to the 25, 40, and 50 mg/kg/day groups, respectively.

2.2 Animals and husbandry

All animal studies were approved by the Committee for the Ethical Usage of Experimental Animals of Sumitomo Pharma Co., Ltd.

Female Sprague-Dawley (Crl:CD) rats were purchased from Charles River Laboratories Japan, Inc. (currently Charles River Laboratories Japan G.K.; Kanagawa, Japan) and allowed an acclimation period of more than 1 week. These rats were housed individually in a barrier-sustained room with controlled temperature of 24°C ± 2°C, relative humidity of 55% ± 10%, and a 12-h light (8 a.m. to 8 p.m.)/dark cycle. They were fed a commercial pellet diet (CE-2, CLEA Japan, Ltd., Tokyo, Japan) and tap water *ad libitum*.

2.3 Animal model and experimental design

2.3.1 FM-induced animal model

FM was administered subcutaneously to each rat (3 animals; 6 weeks of age at the start of administration) for 1 day at dose of 100 mg/kg/day in saline (5 ml/kg). The dose was selected based on earlier reports (Dalmas et al. 2008; Gonzalez et al. 2017). Euthanasia was conducted on the day after the dose by exsanguination under anesthesia and necropsied. In the euthanized animals, mesenteric, pancreatic, gastrointestinal, renal, and femoral arteries and arteries in the heart were collected from necropsied animals.

2.3.2 MH-induced animal model

MH in 0.5% CMC (5 ml/kg) was administered orally, the most common dosing route, to each rat (6 weeks of age at the start of administration) once daily for 2 days at dose levels of 25 or 50 mg/kg/day or 4 days at a dose level of 50 mg/kg/day.

Regarding the induction of arteritis, the systemic route of exposure is considered to be important. The state of the induced lesion can depend on the method of systemic delivery; therefore, in addition to oral administration, I performed subcutaneous administration, which has different systemic consequences. Therefore, MH in 0.5% CMC (5 ml/kg) was administered subcutaneously to each rat (6 weeks of age at the start of administration) once daily for 2 days at a dose level of 25 mg/kg/day or 4 days at a dose level of 40 mg/kg/day.

Animals were divided into five groups (3 animals per group) as shown in Table 1-1.

Scheduled euthanasia was conducted on the day after the final dose by exsanguination under anesthesia and necropsied. In the scheduled euthanized animals, mesenteric, pancreatic, gastrointestinal, renal, and femoral arteries and arteries in the heart were collected from necropsied animals.

Table 1-1
Group composition in MH-induced animal model

Group	Route of Administration	Dosing period (day)	Dose (mg/kg/day)
Group 1	Oral	2	25
Group 2	Oral	2	50
Group 3	Oral	4	50
Group 4	Subcutaneous	2	25
Group 5	Subcutaneous	4	40

2.4 Histopathology

All of the collected arteries were fixed in 10% neutral-buffered formalin, embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined by light microscopy.

3. Results

3.1 FM-induced animal model

3.1.1 Clinical signs and necropsy

Throughout the dosing period, no animal died or showed clinical signs. At necropsy, no abnormality was observed in any animal.

3.1.2 Histopathology

Segmental degeneration/necrosis of medial smooth muscle cells accompanied by intramural hemorrhage, infiltration of inflammatory cells into the media and/or perivascular infiltration of inflammatory cells, proliferation of fibroblasts and edema were observed in the mesenteric, pancreatic, and gastrointestinal arteries. In the gastrointestinal arteries, these lesions were mainly observed in arteries in serosa and adipose tissue around the gastrointestinal tract. On the other hand, in the renal

and femoral arteries and arteries in the heart including the coronary arteries, no abnormality was observed. Table 1-2 provides a description of the histopathology of the arteries.

Table 1-2
Histopathological findings in animals administered fenoldopam mesylate

Organs and Tissues	Findings	Fenoldopam mesylate-administered group		
		-	±	+
Mesenteric artery	Degeneration/necrosis of medial smooth muscle cells	0	1	2
	Intramural hemorrhage	0	1	2
	Infiltration of inflammatory cells into the media	0	1	2
	Perivascular infiltration of inflammatory cell and proliferation of fibroblasts	0	1	2
	Perivascular edema	0	1	2
Pancreatic artery	Perivascular infiltration of inflammatory cell and proliferation of fibroblasts	2	1	3
	Perivascular edema	2	1	3
Gastrointestinal artery (stomach/jejunum/cecum)	Intramural hemorrhage	0	3	0
	Perivascular infiltration of inflammatory cell and proliferation of fibroblasts	0	3	0
	Perivascular edema	0	3	0
Renal artery	Finding absent	3	0	0
Femoral artery	Finding absent	3	0	0
Artery of heart	Finding absent	3	0	0

-：No abnormality, ±：minimal, ＋：mild

3.2 MH-induced animal model

3.2.1 Clinical signs and necropsy

In the group 2 (50 mg/kg/day orally for 2 days) and the group 3 (50 mg/kg/day orally for 4 days), one of three animals was found dead before administration on the second day of dosing.

No deaths were found in the other three groups: group 1 (the 25 mg/kg/day orally for 2 days), group 4 (25 mg/kg/day subcutaneously for 2 days), and group 5 (40 mg/kg/day subcutaneously for 4 days).

Throughout the dosing period, no animal showed clinical signs except for the deaths mentioned above.

At necropsy, mottled white lesions in the kidneys were observed in all animals in the group 5 (40 mg/kg/day subcutaneously for 4 days). In the other groups, no abnormality was observed in any animal.

3.2.2 Histopathology

In all groups, minimal to mild perivascular infiltration of inflammatory cells, proliferation of fibroblasts, and edema were observed in the mesenteric arteries. These changes were also observed in the pancreatic arteries, gastrointestinal arteries, and renal arteries in the group 4 (25 mg/kg/day subcutaneously for 2 days; minimal to mild). In addition, these changes were observed in the pancreatic arteries of the group 3 (50 mg/kg/day orally for 4 days; minimal), gastrointestinal arteries of the group 2 (50 mg/kg/day orally for 2 days; minimal) and renal arteries of the group 1 (25 mg/kg/day orally for 2 days; minimal). In regard to the gastrointestinal arteries, these lesions were mainly observed in arteries in serosa and adipose tissue around the gastrointestinal tract. On the other hand, no abnormality was observed in the femoral arteries and arteries in the heart including the coronary arteries in all groups.

Perivascular infiltration of inflammatory cell, proliferation of fibroblasts, and edema were observed in the mesenteric arteries of 2 of 3 animals in the group 1 (25 mg/kg/day orally for 2 days; minimal), 2 of 2 animals in the group 2 (50 mg/kg/day orally for 2 days; minimal to mild), 2 of 2 animals in the group 3 (50 mg/kg/day orally for 4 days; minimal), 3 of 3 animals in the group 4 (25 mg/kg/day subcutaneously for 2 days; minimal to mild), and 1 of 3 animals in the group 5 (40 mg/kg/day subcutaneously for 4 days; minimal). Details of the histopathology of samples from the study to find MH dose and evaluate the details of MH-induced arteritis are shown in Table 1-3 and the lesions

observed in these arteries are shown in Fig. 1-1.

Table 1-3

Histopathological findings in animals administered midodrine hydrochloride

Organs and Tissues	Group	Midodrine hydrochloride-administered group														
	Route of Administration	Subcutaneous			Subcutaneous			Oral			Oral			Oral		
	Dosing period	2 days			4 days			2 days			2 days			4 days		
	Dose	25 mg/kg/day			40 mg/kg/day			25 mg/kg/day			50 mg/kg/day			50 mg/kg/day		
		3*			3*			3*			2*			2*		
	Findings	-	±	+	-	±	+	-	±	+	-	±	+	-	±	+
Mesenteric artery	Degeneration/necrosis of medial smooth muscle cells	2	1	0	3	0	0	3	0	0	2	0	0	2	0	0
	Intramural hemorrhage	2	1	0	3	0	0	3	0	0	2	0	0	2	0	0
	Perivascular infiltration of inflammatory cell and proliferation of fibroblasts	0	1	2	0	3	0	1	2	0	0	1	1	1	1	0
	Perivascular edema	0	1	2	2	1	0	1	2	0	0	1	1	0	2	0
Pancreatic artery	Perivascular infiltration of inflammatory cell and proliferation of fibroblasts	1	1	1	3	0	0	3	0	0	2	0	0	1	1	0
	Perivascular edema	1	1	1	3	0	0	3	0	0	2	0	0	1	1	0
Gastrointestinal artery (stomach/jejunum/cecum)	Perivascular infiltration of inflammatory cell and proliferation of fibroblasts	2	1	0	3	0	0	3	0	0	1	1	0	2	0	0
	Perivascular edema	2	1	0	3	0	0	3	0	0	1	1	0	2	0	0
Renal artery	Perivascular infiltration of inflammatory cell and proliferation of fibroblasts	2	1	0	3	0	0	2	1	0	2	0	0	2	0	0
	Perivascular edema	2	1	0	3	0	0	2	1	0	2	0	0	2	0	0
Femoral artery	Finding absent	3	0	0	3	0	0	3	0	0	3	0	0	3	0	0
Artery in heart	Finding absent	3	0	0	3	0	0	3	0	0	3	0	0	3	0	0

*: Number of animals which were examinded histopathologically
-：No abnormality, ±：minimal, ＋：mild

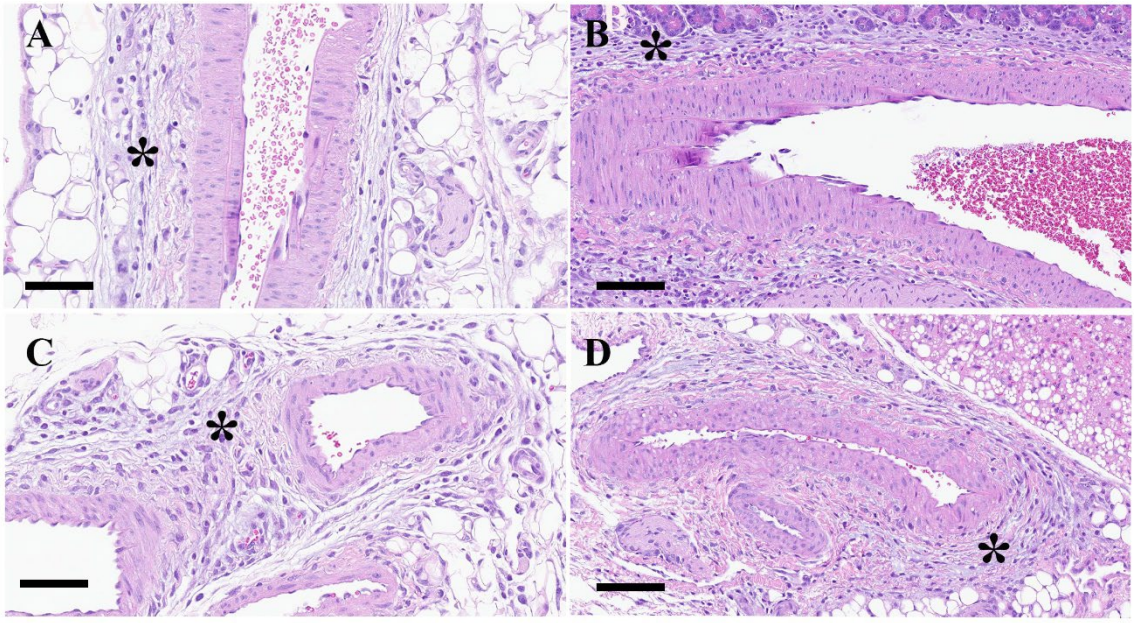

Figure 1-1
Representative histopathological images of affected lesions of the mesenteric, pancreatic, gastrointestinal, and renal arteries stained with hematoxylin and eosin in the MH-administered animals. Edema, infiltration of inflammatory cells, and proliferation of fibroblasts were observed in the perivascular area. (A-D) Histopathological images of the arteries after MH administration. (A) Mesenteric artery; Bar, 70 μm. (B) Pancreatic artery; Bar, 100 μm. (C) Gastrointestinal artery; Bar, 70 μm. (D) Renal artery; Bar, 100 μm. Perivascular edema is indicated with asterisks.

4. Discussion

In the present study, MH-induced arteritis was comprehensively examined. This is the first reported detailed evaluation of MH-induced arteritis for arteries other than the mesenteric artery.

Histopathological examination revealed that single dose at 100 mg/kg of FM induced arteritis in the mesenteric, pancreatic, and gastrointestinal arteries in rats, most frequently in mesenteric and gastrointestinal arteries. The degree of findings is strongest in mesenteric arteries. On the other hand, no abnormality was observed in the renal and femoral arteries and arteries in the heart. As for MH, arteritis was induced in the mesenteric, pancreatic, gastrointestinal, and renal arteries in rats, most

frequently in mesenteric arteries. On the other hand, no abnormality was observed in the femoral arteries and arteries in the heart. Subcutaneous administration of MH did not cause the death up to 40 mg/kg/day and arteritis was induced by two days administration even at 25 mg/kg/day in all animals.

Based on the results, I considered that subcutaneously administered once daily 100 mg/kg/day in FM and 40 mg/kg/day in MH for 2 days is an optimal dosing regimen and decided to use this regimen for MRI assessment, considering dose which death was not observed and frequency of arteritis. Furthermore, because of the high incidence of arteritis in mesenteric artery, I decided to evaluate the mesenteric artery for MRI assessment.

Although arteritis in the renal arteries was not induced by administration of FM at 100 mg/kg/day subcutaneously for 2 days in this study, it was induced by intravenous administration of FM for 24 hours (Kerns et al. 1989; FDA approval package of CORLOPAM). Because D1 receptor is expressed on the renal artery (Amenta et al. 2000), it was suggested that the presence or absence of renal artery lesions may change depending on the duration of vasodilatory effects by FM. In addition, alpha 1 receptor, which is a receptor on which MH acts, is also known to be expressed on the renal artery (Schmitz et al. 1981) and renal artery arteritis induced by MH was observed in some animals in this study.

In this study, arteritis was commonly found in the mesenteric, pancreatic, and gastrointestinal arteries of FM-administered animals and MH-administered animals, suggesting that the mesenteric,

pancreatic, and gastrointestinal arteries may be the predominant site of arteritis in rats. Although D1 receptor is also expressed in coronary and other arteries in the heart, and alpha 1 receptor is also expressed in femoral arteries and coronary arteries (Ozono et al. 1996; Zacharia et al. 2005; Kayki-Mutlu et al. 2020), no lesions were observed in these arteries in this study using FM- and MH-administered animals. Considering that the main lesion in the gastrointestinal arteries was observed in arteries in serosa and adipose tissue around the gastrointestinal tract, a common point of these affected arteries is that the tissues around the arteries, such as adipose tissue and pancreatic tissue, are soft. Therefore, I consider that arteries without hard surrounding tissues are vulnerable to excessive vasodilatory/vasoconstrictor action and therefore becomes predominant sites. No hard tissue around the renal artery, where arteritis was induced, is also consistent with this consideration.

Chapter 2:

Detection of fenoldopam-induced arteritis in rats using *ex vivo*/*in vivo* MRI

Abstract

A method capable of identifying drug-induced arteritis is highly desirable because no specific and sensitive biomarkers have yet been defined. Although magnetic resonance imaging (MRI) may be used to find a biomarker candidate for drug-induced arteritis, there are no reports on the evaluation of drug-induced arteritis by MRI. The present study was conducted to clarify whether Fenoldopam mesylate (FM)-induced arteritis in rats can be detected by MRI. FM, a dopamine (D1 receptor) agonist, is known to induce arteritis in rats. FM was administered subcutaneously to each rat once daily for 2 days at a dose of 100 mg/kg/day. These arteries were examined with *ex vivo* high-resolution MRI or postmortem MRI after euthanasia. These arteries were also examined using *in vivo* MRI on the day after final dosing or 3 days after administration of the final dose. These arteries were examined histopathologically in all experiments. The *ex vivo* MRI showed low-intensity areas and a high signal intensity region around the artery, and these findings were considered to be erythrocytes infiltrating the arterial wall and perivascular edema, respectively. In the *in vivo* study, the MRI of the FM-administered group showed a high signal intensity region around the artery. The perivascular edema observed histopathologically was recognized as a high signal intensity region around the artery on the image of MRI. In conclusion, detection of the high signal intensity region around the artery by MRI is considered to be a useful method for identifying arteritis. Although further investigation is needed to be a reliable biomarker, it is suggested that it could be a biomarker candidate.

1. Introduction

Toxicological findings in nonclinical toxicological studies of candidate drugs were the main cause of discontinuation of drug development (Waring et al. 2015). The occurrence of drug-induced arteritis in nonclinical toxicity studies is one of the causes for the pre-clinical attrition of candidate drugs (Mikaelian et al. 2014).

In humans, vasculitis is known to be induced by several drug classes including antimicrobials and antithyroid medications (Doyle and Cuellar 2003). Drug-induced arteritis is difficult to monitor in clinical trials of candidate drugs because the underlying mechanism has not been elucidated and no specific and sensitive biomarkers have yet been defined in humans (Louden et al. 2006; Mikaelian et al. 2014). Several working groups have been tried to identify the biomarkers of arteritis (Kerns et al. 2005; Mikaelian et al. 2014). However, specific biomarkers that could become the gold standard have not been identified yet (Kerns et al. 2005; Mikaelian et al. 2014). In the absence of noninvasive methods to monitor the onset of drug-induced arteritis, its occurrence in nonclinical toxicity studies can become an obstacle to the development of candidate drugs, even if a drug is predicted to be safe and efficacious in humans (Mikaelian et al. 2014). Therefore, a method capable of identifying drug-induced arteritis is accordingly highly desirable.

In humans, diagnostic imaging techniques such as magnetic resonance angiography (MRA) are used effectively for vasculitis syndrome affecting large- or medium-sized arteries (e.g. Takayasu arteritis, giant cell arteritis, Buerger's disease and polyarteritis nodosa) (JCS Joint Working Group 2011;

Schmidt and Blockmans 2018). Although MRA is the most frequently used method among magnetic resonance imaging (MRI) techniques in the diagnosis of vascular lesion, MRA is mainly employed to detect abnormalities in vascular lumen morphology, such as occlusion and stenosis, by visualizing the vessel lumen, and not to detect abnormalities of the blood vessel itself, because MRA is an angiographic method. On the other hand, it has been reported that contrast-enhanced (late gadolinium enhancement) MRI (1.5-T) could be useful for detecting blood vessel abnormalities (Kato et al. 2015). In this report, the distribution of inflammation in the vessel wall of a large artery was visualized by contrast-enhanced MRI, with the potential to identify pathologic changes in the arterial wall, independent of luminal change. However, there are no reports on the application of MRI to drug-induced arteritis.

In rats, although it has been reported that arteritis in large arteries induced by mechanical stimulation can be detected by MRI (7-T) with gadolinium-labeled perfluorocarbon-exposed sonicated dextrose albumin microbubbles (Anderson et al. 2012), there are no reports of drug-induced arteritis being detected by MRI. Further, in the above report, large arteries were assessed; however, drug-induced arteritis is more likely to develop in medium and small arteries rather than large arteries in rats. Detection of drug-induced arteritis in medium and small arteries by MRI is important, especially in rodents.

Fenoldopam mesylate (FM) is an antihypertensive drug. FM is a dopamine (D1 receptor) agonist,

and causes arteritis in rats due to its vasodilatory effect (Dalmas et al. 2011; FDA approval package of CORLOPAM). The mesenteric artery, which is a small- to medium-sized vessel, is mainly affected (Dalmas et al. 2008; Ikegami et al. 2001). These drug-induced arteritis in medium and small arteries were only evaluated histologically, and there are no reports of *in vivo e*valuation including MRI.

The present study was conducted to clarify whether FM-induced arteritis in rats can be detected by *in vivo* MRI. Before *in vivo* evaluation using live animals, *ex vivo* MRI and postmortem MRI using euthanized animals were performed. First, *ex vivo* MRI was performed to evaluate whether the changes caused by FM can be detected by MRI under ideal condition (no movement with high resolution). Second, postmortem MRI was performed to evaluate whether the changes can be detected in the situation where both respiration and peristalsis were absent but the other conditions are the same as those in *in vivo* MRI. And finally, *in vivo* MRI was performed in the imaging condition determined from the results of *ex vivo* MRI and postmortem MRI.

2. Material and methods

2.1 Compound

FM (the purity is 95% or more) was purchased from Cayman Chemical (MI, USA). FM was dissolved in saline to reach a concentration of 20 mg/ml. Chlorpromazine hydrochloride (the purity is 99% or more), which was used to suppress peristalsis of the intestinal tract, was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Chlorpromazine hydrochloride was dissolved in 0.5

w/v% methyl cellulose solution to reach a concentration of 5 mg/ml.

2.2 Animal husbandry

All animal studies were approved by the Committee for the Ethical Usage of Experimental Animals of Sumitomo Dainippon Pharma Co., Ltd (current name is Sumitomo Pharma Co., Ltd.) and the Animal Welfare Committee of Osaka University.

Female Sprague-Dawley (Crl:CD) rats were purchased from Charles River Laboratories Japan, Inc. (current name is The Jackson Laboratory Japan, Inc.; Kanagawa, Japan) and had an acclimation period of more than 1 week. The animals were housed individually in a barrier-sustained room at a controlled temperature of 24°C ± 2°C, relative humidity of 55% ± 10%, and a 12-h light (8 a.m. to 8 p.m.)/dark cycle.

The rats were fed a commercial pellet diet (CE-2, CLEA Japan, Ltd.; Tokyo, Japan) and tap water *ad libitum*.

2.3 Animal model

FM was administered subcutaneously to each rat (6 weeks of age at the start of administration) once daily for 2 days at dose of 100 mg/kg/day in saline (5 ml/kg). As vehicle control, saline only was administered to animals in the same way described above.

2.4 Experimental design

2.4.1 Experiment 1 (*ex vivo* high-resolution MRI and histopathology)

Animals weighing close to 170 g at the start of FM or saline administration were assigned to the FM group or the saline group (N=3, each group). Mesenteric, pancreatic, gastrointestinal, and renal arteries were collected from animals necropsied on the day after final dosing. The mesenteric arteries were cut into two; one section was evaluated by MRI (*ex vivo*) and the other was evaluated by histopathological examination. In the histopathological examination, pancreatic, gastrointestinal, and renal arteries were also evaluated. In addition, the lumen diameter of mesenteric arteries was measured on both the images of MRI and histopathology.

2.4.2 Experiment 2 (MRI after euthanasia and histopathology)

Animals weighing close to 170 g at the start of FM or saline administration were assigned to the FM group or the saline group (N=4, each group). One animal in the saline group and two animals in the FM group were evaluated by MRI after being euthanized by isoflurane anesthesia or CO_2 inhalation on the day after final dosing. Surviving animals, which were not evaluated by MRI, were euthanized by exsanguination. All animals were necropsied and the mesenteric, pancreatic, gastrointestinal, and renal arteries were collected. These arteries were evaluated by histopathological examination.

2.4.3 Experiment 3 (*in vivo* MRI the day after final dosing and histopathology)

Animals weighing close to 170 g at the start of FM or saline administration were assigned to the FM group (N=5) or the saline group (N=6). These animals were fasted for more than 12 hours and orally administered chlorpromazine hydrochloride (25 mg/kg, 5 ml/kg) to suppress intestinal peristalsis before MRI. All animals of both groups were evaluated by MRI (*in vivo*) and imaging was performed on the day after final dosing. After the completion of MRI, these animals were euthanized on the same day by exsanguination and necropsied. Mesenteric and pancreatic arteries were collected and evaluated by histopathological examination.

2.4.4 Experiment 4 (*in vivo* MRI 3 days after final dosing and histopathology)

Animals weighing close to 170 g at the start of FM dosing were assigned to the FM group (N=8). Without fasting, intestinal peristalsis was strong and it was difficult to obtain a good image. Therefore, these animals were fasted for more than 12 hours. In addition, to solve the problem that images were not clear and arteries were not visible clearly due to peristalsis, I considered agent for suppressing intestinal peristalsis is needed. On the other hand, I had experienced that chlorpromazine hydrochloride delayed the emptying time in the gastrointestinal tract of rats. For this reason, I thought that chlorpromazine hydrochloride would be good, and then I confirmed that peristalsis was suppressed very well by examining peristalsis by ultrasound examination. When MRI was conducted after administration of chlorpromazine hydrochloride, the image became clear and the arteries became

clearly visible. Therefore, chlorpromazine hydrochloride (25 mg/kg, 5 ml/kg) was orally administered to suppress intestinal peristalsis before MRI. All animals were evaluated by MRI (*in vivo*) and imaging was performed 3 days after administration of the final dose. After the completion of MRI, these animals were euthanized on the same day by exsanguination and necropsied. Mesenteric and pancreatic arteries were collected and evaluated by histopathological examination.

2.5 MRI

All MRI was performed using an 11.7 T vertical-bore Bruker Avance II imaging system (Bruker BioSpin, Ettlingen, Germany) and the diameter of the volume radiofrequency coil for transmission and reception (Bruker BioSpin) was 10 mm or 33 mm for *ex vivo* samples and 33 mm for *in vivo* samples.

For *ex vivo* MRI, a mesenteric artery sample was immersed in gadolinium (5 mM)-containing phosphate buffered saline for 2 days or longer. Images were acquired using 3D fast low-angle shot (FLASH) with the following scanning parameters: repetition time/echo time (TR/TE) 40 msec/6 msec, flip angle (FA) 90°, 16 × 16 × 8 mm^3 field of view (FOV), matrix of 400 × 400 pixels, 200 slices with thickness = 0.04 mm, and number of excitations (NEX) = 1 or TR/TE 40/6 msec, FA 70°, 20 × 20 × 12.8 mm^3 FOV, matrix of 512 × 512 pixels, 256 slices with thickness = 0.05 mm, and NEX = 12. Fat suppression was used to reduce the signal intensity of fat on the images.

For euthanized animals and live animals (*in vivo* imaging), anatomical images acquired with

IntraGate FLASH (Bruker BioSpin) were used for slice positioning with the following scanning parameters: TR/TE 62 msec/1.5 msec, FA 15°, 60 mm FOV, matrix of 256 × 256 pixels, and 5 slices with thickness = 1.1 mm. Based on the histopathological image and imaging principle of MRI, I considered that perivascular edema or intramural hemorrhage, which means erythrocytes in the arterial wall, would be useful for detecting arteritis. Considering perivascular edema was observed widely, whereas intramural hemorrhage was observed localized area, perivascular edema is considered to be more useful as a biomarker. Therefore, we decided to use rapid acquisition with relaxation enhancement (RARE) as a sequence which could detect perivascular edema and images for evaluation of arteritis were acquired using RARE with the following scanning parameters: Rare Factor 8, TR/TE 5000 msec/24.8 msec, 35 × 35 mm^2 FOV, matrix of 256 × 256 pixels, 20 slices with thickness = 0.5 mm, and NEX = 1. Fat suppression was used to reduce the signal intensity on the images. During *in vivo* MRI, rats were given a general anesthetic using 0.5%–3% isoflurane (Wako) administered via a mask covering the nose and mouth. Respiratory signals were monitored using a physiological monitoring system (SA Instruments, Stony Brook, NY), and body temperature was continuously maintained at 36.0 ± 0.5°C by circulating water through heating pads throughout all experiments. In addition, in *in vivo* MRI using live animals, a respiratory gating technique was used to minimize the effects of respiration on the images. In addition, to make the image clearer, the gap between the coil and the rat was stuffed with tissue paper, but a difference in the clarity of the image was observed

depending on the site where the tissue paper was stuffed and found that the best images were obtained when the rat's dorsal area was evenly stuffed with tissue paper. Therefore, imaging was performed with tissue paper evenly stuffed on the rat's dorsal area.

2.6 Histopathology

Collected arteries were fixed in 10% neutral-buffered formalin, embedded in paraffin, sectioned, stained with hematoxylin and eosin (HE), and examined by light microscopy.

2.7 Image analysis

2.7.1 Evaluation of lumen diameter of mesenteric artery in Experiment 1

In the samples of Experiment 1, lumen diameter was measured on the image of MRI using ImageJ (NIH, Bethesda, MD, USA) and on the histopathological image using Imagescope (Leica Biosystems, Nussloch, Germany) to assess the vasodilatory effects of FM. For each animal, 10 artery sites were analyzed on the images of MRI and 20 artery sites were analyzed on histopathological images.

2.7.2 Quantitative evaluation of signal intensity around the arteries on the images of MRI in Experiment 3

In the samples of Experiment 3, signal intensity around the arteries on the images of MRI was measured using ImageJ. Signal intensity around the arteries was defined as a value derived from the formula shown in parentheses (signal intensity = intensity around the arteries / intensity of the skeletal muscle around the spinal cord). For each animal, 5 arteries were analyzed and the mean value for 5

arteries was defined as the signal intensity of each animal.

2.8 Statistics

All data are expressed as the mean ± standard deviation. The numerical data were analyzed with two-tailed non-paired t-test to compare the difference between two groups. $P < 0.05$ was considered statistically significant.

3. Results

3.1 Clinical signs and necropsy

Throughout the dosing period, no animal died or showed clinical signs. At necropsy, no abnormality was observed in any animal.

3.2 Histopathology

3.2.1 Experiment 1 (*ex vivo* high-resolution MRI and histopathology) and Experiment 2 (MRI after euthanasia and histopathology)

In the FM-administered group, segmental degeneration/necrosis of medial smooth muscle cells accompanied by intramural hemorrhage, infiltration of inflammatory cells into the media and/or perivascular infiltration of inflammatory cells, proliferation of fibroblasts and edema were observed in the mesenteric, pancreatic, and gastrointestinal arteries. In the pancreas, an increase in single cell necrosis was observed in acinar cells around the artery in which arteritis was induced. On the other hand, in the renal arteries, no abnormality was observed. In the vehicle control group, no abnormality

was observed in any animal. Table 2-1 provides a description of the histopathology of the arteries in Experiments 1 to 4.

Table 2-1
Histopathological findings of the arteries in Experiments 1－4

Organs and Tissues	Findings	Fenoldopam mesylate-administered group															
		Exp. 1 (3*)				Exp. 2 (4*)				Exp. 3 (5*)				Exp. 4 (8*)			
		-	±	+	2+	-	±	+	2+	-	±	+	2+	-	±	+	2+
Mesenteric artery	Degeneration/necrosis of medial smooth muscle cells	0	0	3	0	0	4	0	0	0	4	1	0	0	2	6	0
	Intramural hemorrhage	0	0	0	3	0	1	3	0	0	1	3	1	0	2	5	1
	Infiltration of inflammatory cells into the media	0	0	3	0	0	4	0	0	0	4	1	0	0	2	6	0
	Perivascular infiltration of inflammatory cell and proliferation of fibroblasts	0	0	3	0	0	0	4	0	0	1	4	0	0	0	8	0
	Perivascular edema	0	0	3	0	0	0	4	0	0	1	4	0	3	3	2	0
Pancreatic artery	Degeneration/necrosis of medial smooth muscle cells	0	1	2	0	0	3	1	0	1	4	0	0	0	7	1	0
	Intramural hemorrhage	0	1	1	1	0	1	2	1	0	3	2	0	0	6	2	0
	Infiltration of inflammatory cells into the media	0	2	1	0	0	4	0	0	1	4	0	0	0	7	1	0
	Perivascular infiltration of inflammatory cell and proliferation of fibroblasts	0	0	3	0	0	0	4	0	0	0	5	0	0	1	6	1
	Perivascular edema	0	0	3	0	0	0	4	0	0	0	5	0	4	2	1	1
Gastrointestinal artery (stomach/jejunum/cecum)	Degeneration/necrosis of medial smooth muscle cells	1	0	2	0	2	0	2	0								
	Intramural hemorrhage	1	1	1	0	2	1	1	0								
	Infiltration of inflammatory cells into the media	2	1	0	0	3	1	0	0								
	Perivascular infiltration of inflammatory cell and proliferation of fibroblasts	1	1	1	0	1	2	1	0								
	Perivascular edema	1	0	2	0	1	1	2	0								
Renal artery	Finding absent	3	0	0	0	4	0	0	0								

*: Number of animals in each experiment
-：No abnormality, ±：minimal, ＋：mild, 2＋ ：moderate
No abnormality was observed in saline group

3.2.2 Experiment 3 (*in vivo* MRI the day after final dosing and histopathology)

In the FM-administered group, segmental degeneration/necrosis of medial smooth muscle cells accompanied by intramural hemorrhage, infiltration of inflammatory cells into the media and/or perivascular infiltration of inflammatory cells, proliferation of fibroblasts and edema were observed in the mesenteric and pancreatic arteries. Perivascular edema was observed in all animals. In the vehicle control group, no abnormality was observed in any animal. The lesions observed in the mesenteric arteries are shown in Fig. 2-1.

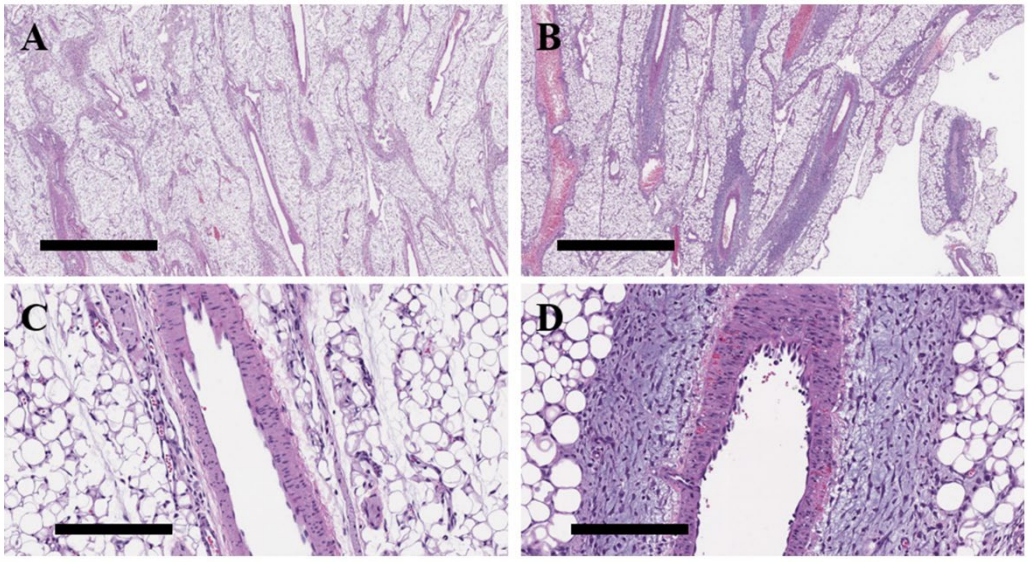

Figure 2-1
Representative histopathological images of the mesenteric arteries with hematoxylin and eosin staining in Experiment 3 (*in vivo* MRI on the day after final dosing and histopathology). (A and C) Histopathological image of the mesenteric artery in the vehicle control group. Bar, 2 mm in A and 200 μm in C. (B and D) Histopathological image of the mesenteric artery in the FM group. Bar, 2 mm in B and 200 μm in D. (B) In the perivascular area, infiltration of inflammatory cells and proliferation of fibroblasts and edema can be seen. (D) Degeneration/necrosis of medial smooth muscle cells accompanied by intramural hemorrhage and infiltration of inflammatory cells into the media were observed.

3.2.3 Experiment 4 (*in vivo* MRI 3 days after final dosing and histopathology)

In the FM-administered group, segmental degeneration/necrosis of medial smooth muscle cells accompanied by intramural hemorrhage, infiltration of inflammatory cells into the media and/or perivascular infiltration of inflammatory cells, proliferation of fibroblasts and edema were observed in the mesenteric and pancreatic arteries. Perivascular edema was observed in 4 of 8 animals necropsied 3 days after administration of the final dose and no perivascular edema was observed in the other 4 animals. In the vehicle control group, no abnormality was observed in any animal.

3.3 MRI

3.3.1 Experiment 1 (*ex vivo* high-resolution MRI and histopathology)

In the vehicle control group, the arterial wall of the mesenteric artery showed uniform and moderate signal intensity, with minor regions of lowered signal intensity observed on the luminal side (Fig. 2-2, A and C). This low signal was attributed to adhesion of erythrocytes to the vascular endothelium. Conversely, in the FM-administered group, low-intensity areas were observed in the arterial walls (Fig. 2-2, B and D). These were attributed to infiltration of erythrocytes into the arterial walls, which was observed in histopathological examination (Fig. 2-2, E and F).

In addition, in the vehicle control group, signal intensity around the artery was low and this was attributed to the presence of fat tissue, which is observed as a low signal in the situation of fat suppression. On the other hand, in the FM-administered group, the signal intensity around the artery was higher than that in the control group (Fig. 2-2, B and D). The high intensity area was attributed to perivascular edema observed in histopathological examination.

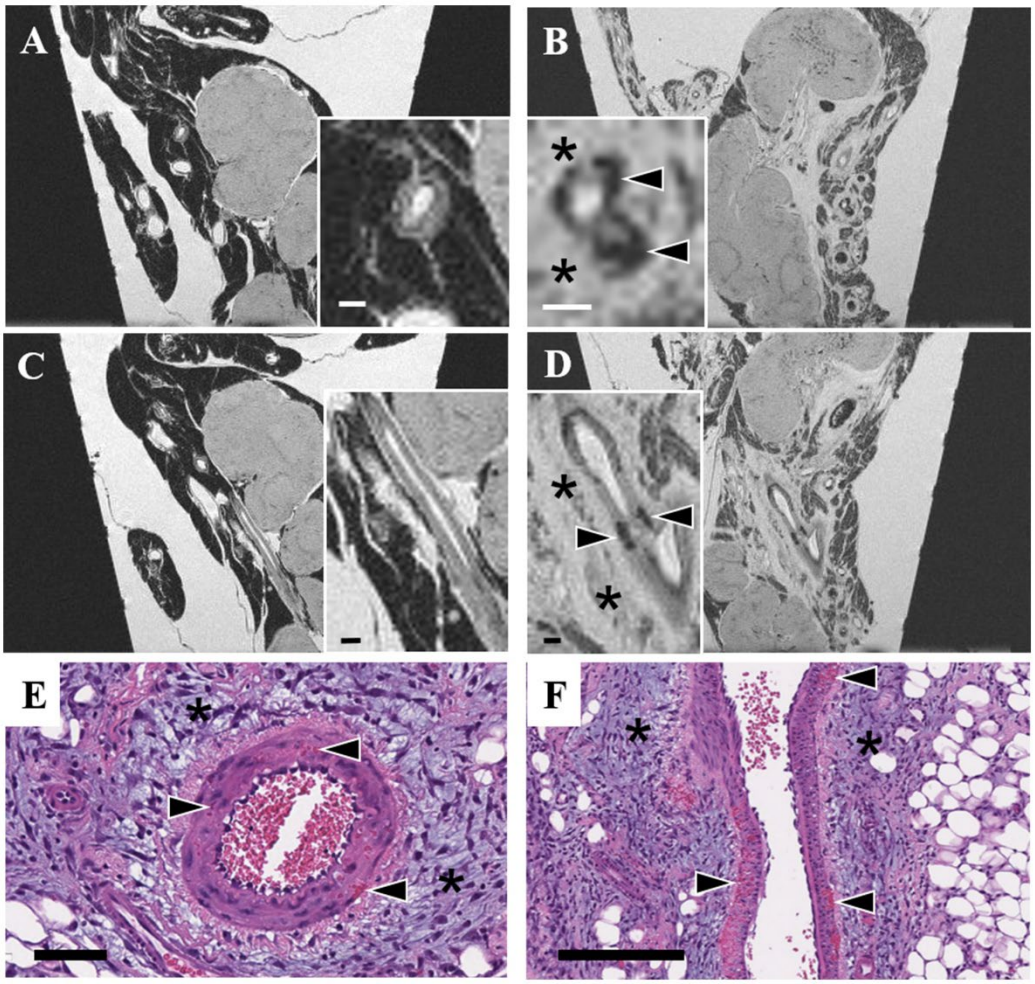

Figure 2-2
Typical images of MRI of mesenteric arteries and histopathological images correspond to the image of MRI in Experiment 1 (*ex vivo* high-resolution MRI and histopathology). (A and C) Images of MRI of the mesenteric artery in the vehicle control group. Inserts show higher magnification of a cross section of aorta in A and a longitudinal section in C. Bar in insert, 200 μm. (B and D) Image of MRI of the mesenteric artery in the FM group. Inserts show higher magnification of a cross section of aorta in B and a longitudinal section in D. Bar in insert, 200 μm. Low-intensity areas in the arterial wall are indicated by arrowheads. The high signal intensity regions around the artery are indicated with asterisks. (E and F) High magnification of histopathological images of the mesenteric artery in the FM group. Bar, 60 μm in E and 200 μm in F. Erythrocytes infiltrating the arterial wall are indicated with arrowheads. Perivascular edema is indicated with asterisks.

3.3.2 Experiment 2 (MRI after euthanasia and histopathology)

In the euthanized animals, some mesenteric arteries with low signal intensity were observed in the vehicle control group. On the other hand, in the FM-administered group, arteries were clearly

visualized and the signal intensity around the artery was higher than that in the control group (Fig. 2-3).

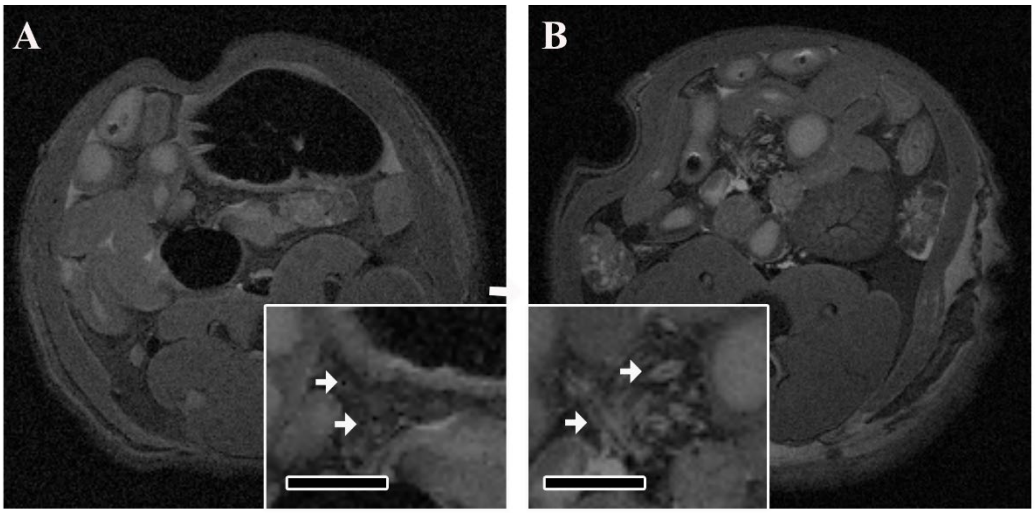

Figure 2-3
Typical images of MRI of mesenteric arteries in euthanized animals in Experiment 2 (MRI after euthanasia and histopathology). (A) Axial image of MRI of the mesenteric artery in the vehicle control group. Inserts show higher magnification of the mesenteric artery. Bar in insert, 3 mm. (B) Axial image of MRI of the mesenteric artery in the FM group. Inserts show higher magnification of the mesenteric artery. Bar in insert, 3 mm. In the FM group, arteries were clearly visualized and the signal intensity around the artery was higher than that in the control group. Arteries are indicated by arrows.

3.3.3 Experiment 3 (*in vivo* MRI on the day after final dosing and histopathology)

In animals administered chlorpromazine hydrochloride as a peristalsis inhibitor, some mesenteric arteries with low signal intensity were observed in the vehicle control group as also seen in the euthanized animals from Experiment 2. In the mesenteric artery of live animals administered chlorpromazine hydrochloride evaluated on the day after final dosing, a higher signal intensity region around the artery was observed in all animals in the FM-administered group (Fig. 2-4). However, during the time when peristalsis was not suppressed, arteries were not observed clearly and no change

in signal intensity was detected.

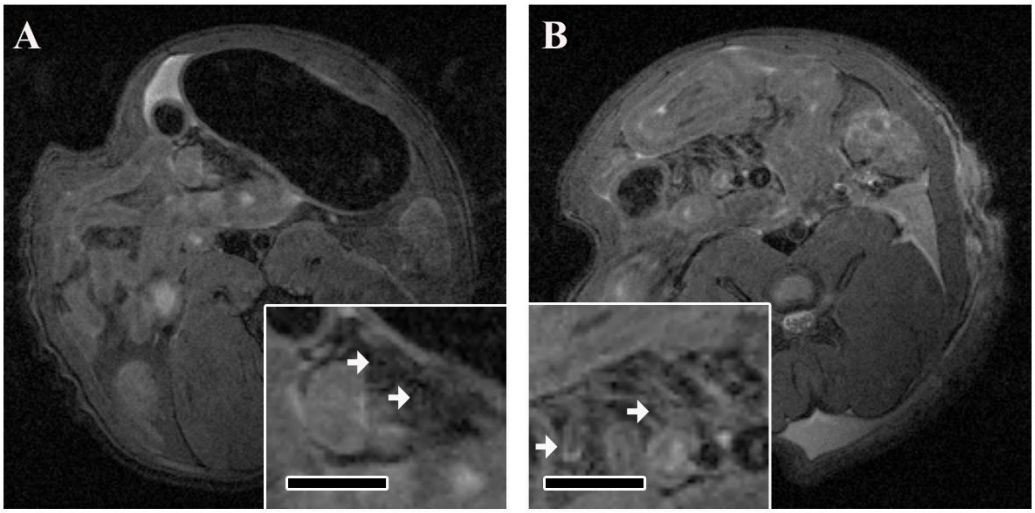

Figure 2-4
Typical images of MRI of mesenteric arteries in live animals administered a peristalsis inhibitor and evaluated on the day after final dosing. (A) Axial image of MRI of the mesenteric artery in the vehicle control group. Inserts show higher magnification of the mesenteric artery. Bar in insert, 3 mm. (B) Axial image of MRI of the mesenteric artery in the FM group. Inserts show higher magnification of the mesenteric artery. Bar in insert, 3 mm. In the FM-administered group, arteries were clearly visualized and the signal intensity around the artery was higher than that in the control group. Arteries are indicated by arrows.

3.3.4 Experiment 4 (*in vivo* MRI 3 days after final dosing and histopathology)

In the mesenteric artery of live animals administered chlorpromazine hydrochloride on the day after final dosing, a high signal intensity region around the artery was observed in 4 of 8 FM-administered animals. Four animals which showed high signal intensity region around the artery were accompanied with perivascular edema confirmed by histopathology. In contrast, in the other 4 animals which did not show the high signal intensity were not accompanied perivascular edema (Fig. 2-5). Table 2-2 provides individual data for comparison of results on histopathological examination and MRI.

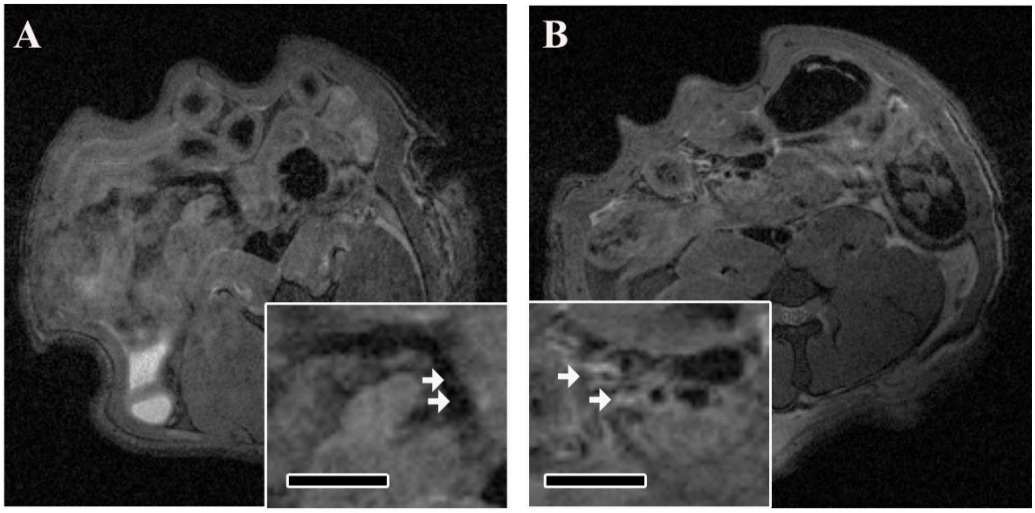

Figure 2-5
Typical images of MRI of mesenteric arteries in live animals administered a peristalsis inhibitor and evaluated 3 days after administration of the final dose. (A) Axial image of MRI of the mesenteric artery in the FM-administered animal without perivascular edema. Inserts show higher magnification of the mesenteric artery. Bar in insert, 3 mm. (B) Axial image of MRI of the mesenteric artery in the FM-administered animal with perivascular edema confirmed by histopathology. Inserts show higher magnification of the mesenteric artery. Bar in insert, 3 mm. A high signal intensity region around the artery was observed only in the animals with perivascular edema. Arteries are indicated by arrows.

Table 2-2
Individual data for comparison of results on histopathological examination and MRI

Histopathological examination		Study								
Organs and Tissues	Findings	Group	FM-administered group							
		Animal No.	3-1	3-2	3-3	3-4	3-5	3-6	3-7	3-8
Mesenteric artery	Degeneration/necrosis of medial smooth muscle cells		+	+	+	±	+	+	±	+
	Intramural hemorrhage		+	+	+	±	+	+	±	2+
	Infiltration of inflammatory cells into the media		+	+	+	±	+	+	±	+
	Perivascular infiltration of inflammatory cell and proliferation of fibroblasts		+	+	+	+	+	+	+	+
	Perivascular edema		-	+	+	-	-	±	±	±

-：No abnormality, ±：minimal, ＋：mild, 2＋ ：moderate

MRI		Study								
Organs and Tissues	Findings	Group	FM-administered group							
		Animal No.	3-1	3-2	3-3	3-4	3-5	3-6	3-7	3-8
Mesenteric artery	High signal intensity around the artery		-	+	+	-	-	+	+	+

-：No abnormality, +: Finding is observed

3.4 Image analysis

3.4.1 Evaluation of lumen diameter of mesenteric artery in Experiment 1

Based on analysis of the histopathological image, the mean diameter of the vascular lumen in the

control group was 66.8 ± 17.7 μm and that of the FM-administered group was 123.6 ± 13.3 μm, revealing that the diameter of the vascular lumen was larger in the FM-administered group than in the control group. In addition, analysis of the image of MRI returned a similar result (the mean diameter of the vascular lumen of the control group was 65.7 ± 12.6 μm and that of the FM-administered group was 111.1 ± 10.7 μm). The bar plots are shown in Fig. 6. The pattern of the bar plot based on analysis of the image of *ex vivo* MRI was similar to that obtained on analysis of the histopathological image.

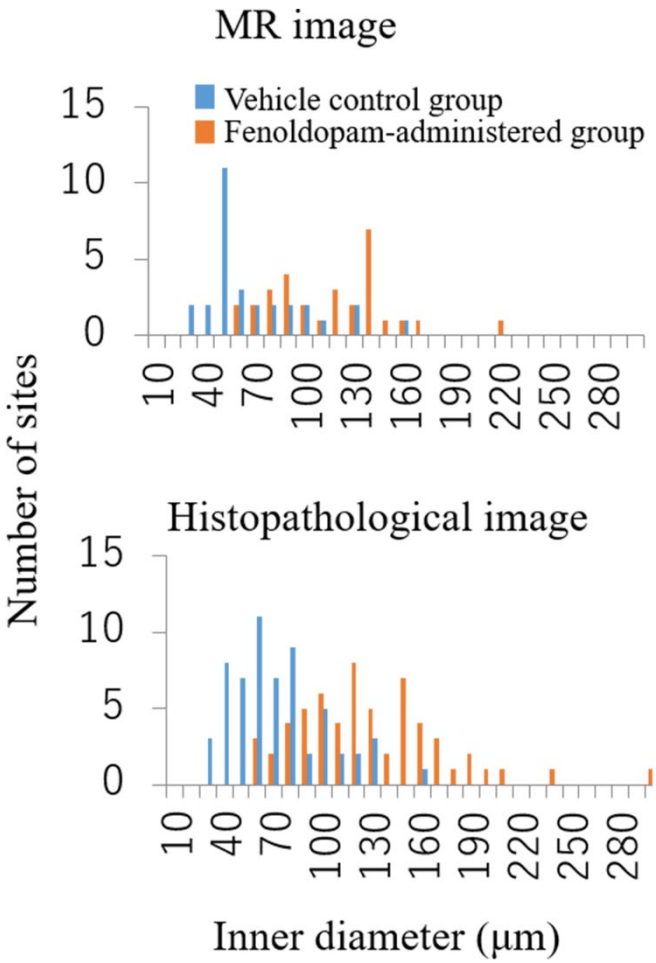

Figure 2-6
Bar plots of inner diameter of vascular lumen measured from the images of MRI and histopathological images. In the FM group, dilation of the blood vessel cavity was observed. The results from images of MRI were consistent with those from histopathological images.

3.4.2 Quantitative evaluation of signal intensity around the arteries on the images of MRI in Experiment 3

The mean value of the control group was 0.91 ± 0.11 and the mean value of the FM-administered group was 1.57 ± 0.20, and there was a statistically significant difference between the values of both groups. The maximum value in the control group was 1.05, whereas the minimum value in the FM-administered group was 1.34, which revealed that the signal intensity was clearly higher in the FM-administered group.

4. Discussion

To the best of my knowledge, this is the first report on the assessment of arteritis in small to medium arteries in rats using MRI. In addition, this is the first time an evaluation of drug-induced arteritis by MRI has been reported.

FM-induced arteritis was detected by histopathological examination following administration of FM at a dose of 100 mg/kg/day for two days. In the mesenteric arterial wall, we observed segmental degeneration/necrosis of medial smooth muscle cells accompanied by intramural hemorrhage and infiltration of inflammatory cells into the media and, in the perivascular area, infiltration of inflammatory cells and proliferation of fibroblasts and edema. These lesions were also observed in the pancreatic and gastrointestinal arteries. The extent and/or frequency of segmental degeneration/necrosis of medial smooth muscle cells accompanied by intramural hemorrhage and

infiltration of inflammatory cells into the media in the mesenteric artery were highest among these arteries as observed in first study. On the other hand, in histopathological examination, no lesions in the renal artery were found. In addition, it has been reported that exudation of fibrin and clear evidence of rupture of the internal elastic lamina could not be observed by special stains in rats with the initial 24-hour infusion and 2-week intravenous administration of FM (Yuhas et al. 1985).

In the *ex vivo* MRI study, the MRI showed low-intensity areas and a high signal intensity region around the artery, and these findings were considered to be the result of infiltrating the arterial wall and perivascular edema, respectively. These findings suggest that intramural hemorrhage and perivascular edema induced by fenoldopam can be clearly detected by *ex vivo* MRI. On the other hand, low-intensity areas observed in the *ex vivo* MRI, attributed to erythrocytes infiltrating the arterial wall, were not observed in the *in vivo* MRI. This is probably due to the fact that the resolution of the *in vivo* MRI is lower than that of the *ex vivo* MRI. The resolution was not high enough to clearly show the arterial wall in the *in vivo* MRI, and we therefore assume that the erythrocytes in the arterial wall could not be detected in the *in vivo* MRI.

From the samples of Experiment 1, an analysis of the histopathological images using Imagescope showed that the diameter of the vascular lumen of the FM-administered group was larger than that of the control group and this is thought to be a change caused by the vasodilatory effect of FM. The results obtained from the images of *ex vivo* MRI were similar to those obtained with the

histopathological images. It can therefore be concluded that, in terms of detecting vasodilatory effects, the accuracy of analysis using images of *ex vivo* MRI is similar to that using histopathological images.

In the *in vivo* study, on the images of MRI, the FM-administered group showed a higher signal intensity region around the artery than the control group. High signal intensity was observed not only in euthanized animals but also in live animals administered the peristalsis inhibitor. In addition, high signal intensity was observed in all animals evaluated on the day after final dosing in the FM-administered group. This suggests that arteritis could also be detected by *in vivo* MRI. Regarding the change in signal intensity around the artery, the signal intensity of the FM-administered group showed a quantitatively significant difference compared to the control group. Therefore, the high signal intensity region around the artery on the image of MRI may serve as a reliable indicator of the presence of arteritis.

In animals examined 3 days after administration of the final dose, a high signal intensity region around the artery was seen in the all of the animals with perivascular edema. On the other hand, high signal intensity was not observed in the animals where perivascular edema was absent. These findings suggest that the perivascular edema observed on histopathology corresponds to the high signal intensity region around the artery on the image of MRI. In addition, we believe that exudation of serous fluid from arteries due to increased vessel wall permeability is identified as perivascular edema on the image of MRI and, as long as the vessel wall remains in a damaged state, arteritis will be accompanied

by perivascular edema. These data and consideration also support that detection of the high signal intensity region around the artery by MRI is a reliable indicator of the presence of arteritis.

In contrast, during the period when peristalsis was not suppressed, the arteries could not be observed clearly and no change in signal intensity was observed. As the mesenteric artery is easily moved by peristalsis, it is essential to suppress peristalsis when evaluating arteritis by MRI.

While the mesenteric artery is the predominant site for drug-induced arteritis in rats (Pereira Bacares 2016), it was difficult to obtain a clear image because the mesenteric artery is free to move within the abdominal cavity and is strongly affected by respiratory and peristaltic movements. However, as shown in this report, by using a respiratory gating technique and suppressing peristalsis, arteritis in the mesenteric artery can be detected by MRI. Since it was possible to detect arteritis in the mesenteric artery, which is a small- to medium-sized artery, evaluation of arteritis in other small- to medium-sized arteries affected by drug-induced arteritis should be possible.

There are no known specific and sensitive biomarkers of arteritis (Louden et al. 2006; Mikaelian et al. 2014); however, the high signal intensity region around the artery seen on the images of MRI can be regarded as a translational biomarker candidate because it can be evaluated in *in vivo*. Peristaltic inhibitors and the respiratory gating technique are also used during intraperitoneal MRI in humans, and humans can consciously pause their own respiration (Lu et al. 2019; Tokoro et al. 2020). Therefore, it is possible to perform intraperitoneal MRI in humans under the same conditions used in this study.

My results show that, in the rat, which is a small animal, it is possible to detect arteritis in the mesenteric artery, which is difficult because this artery is not fixed to the retroperitoneum in the abdominal cavity. In addition, a human study on detecting the distribution of arteritis in a large artery (abdominal aorta) by contrast-enhanced MRI has been conducted (Kato et al. 2015) and delayed contrast-enhanced MRI in Takayasu arteritis can show a high intensity signal in the vessel wall that indicates edema (Jiang et al. 2012). Therefore, arteritis in small- to medium-sized arteries may be diagnosed even in humans by detecting a high signal intensity region around the artery in the image of MRI.

Although the high signal intensity region around the artery seen on the image of MRI could be a biomarker candidate for drug-induced arteritis, further investigation is needed to verify that the high signal intensity is a reliable biomarker (addition of substances, animals etc.).

Chapter 3:

Evaluation of usefulness of *in vivo* MRI

as a biomarker in rats

Abstract

There are no specific and sensitive biomarkers for arteritis, and the occurrence of arteritis in nonclinical toxicological studies of a candidate drug makes development of the drug very difficult. However, I showed in a second study that the high signal intensity region around the artery on magnetic resonance imaging (MRI) could be a candidate biomarker for detection of arteritis. The present study was conducted to clarify whether arteritis induced by a mechanism other than the vasodilatory effect, which was evaluated in a second study, could be detected by MRI. Midodrine hydrochloride (MH), is a selective peripherally acting alpha-1 adrenergic receptor agonist, known to induce arteritis due to its vasoconstrictor action. MH was administered subcutaneously to each rat once daily for 2 days at a dose level of 40 mg/kg/day. The mesenteric arteries were examined using *in vivo* MRI at 1 day or 7 days after administration of the final dose and examined histopathologically. High signal intensity region around the artery was observed in animals with minimal perivascular lesions confirmed by histopathology and not observed in an animal without histological changes on the day after the final dose. On the 7^{th} day after the final dose, no abnormality was observed in histopathological examinations and no high signal intensity regions were observed by MRI in any animal. In conclusion, although further investigation is needed to confirm that high signal intensity is a reliable biomarker for humans, it is suggested that high signal intensity around the artery could be a highly specific and sensitive candidate biomarker for detecting even a minimal change.

1. Introduction

Arteritis is known to occur in nonclinical toxicological studies of several drugs (Louden et al. 2019). Vasculitis including arteritis is also known to be induced by several drug classes including antimicrobials and antithyroid medications in humans (Doyle and Cuellar 2003). Arteritis is a severe toxicity because the blood shortage due to the artery's decreased ability to carry blood can result in systemic damage of organ and tissue; however, the lesion is completely recovered if the offending drug is discontinued or treatment is initiated at an early phase (Radić et al. 2012). If arteritis can be detected in an early phase by using appropriate monitoring with a specific and sensitive biomarker, clinical trials of candidate drugs, which arteritis is observed in nonclinical toxicological studies, can be conducted safely. However, there are no specific and sensitive biomarkers (Louden et al. 2006; Mikaelian et al. 2014), and without these biomarkers the occurrence of arteritis in nonclinical toxicological studies of a candidate drug makes development of the drug very difficult. Therefore, developing a biomarker capable of detecting drug-induced arteritis in an early phase is desirable (Mikaelian et al. 2014). Appropriate monitoring with biomarkers will alleviate the difficulty of conducting clinical trials of candidate drugs associated with drug-induced arteritis.

In a second study, I investigated whether fenoldopam mesylate (FM)-induced arteritis in rats could be detected by magnetic resonance imaging (MRI). FM is a dopamine agonist, and induces arteritis by vasodilatory effect in rats (Dalmas et al. 2011; FDA approval package of CORLOPAM). In that study, the *ex vivo* MRI showed low-intensity areas in the arterial wall and a high signal intensity region

around the artery; these findings were considered to be due to erythrocytes infiltrating the arterial wall and perivascular edema, respectively. In addition, I also showed that FM-induced arteritis could also be detected by the *in vivo* MRI and that perivascular edema observed in histopathology could be recognized as a high signal intensity region around the artery on the images of MRI. Based on the results, I consider the high signal intensity region around the artery to be useful for identifying arteritis. However, no other reports have used MRI to evaluate drug-induced arteritis. Therefore, because further investigation is needed to verify the reliability of high signal intensity as a biomarker for arteritis, I conducted a study using midodrine hydrochloride (MH), which causes arteritis in rats by a different mechanism from the one causing FM-induced arteritis.

MH is a selective peripherally acting alpha-1 adrenergic receptor agonist that is indicated for the treatment of hypotension (Cruz 2000). In addition, it is known that hemorrhage in the arterial wall is rare in rats with MH-induced arteritis (Tobin et al. 2014); this point is different from that in rats with arteritis caused by FM, which has a vasodilatory effect. Although MH-induced arteritis has been reported in the mesenteric artery, the distribution of lesions in other arteries has not been reported.

In the present study, I aimed to clarify whether detection of perivascular edema by MRI could be useful for detecting arteritis induced by MH. In other words, the purpose of the present study is to clarify whether arteritis induced by a non-vasodilatory mechanism, which was evaluated in second study, could be detected by MRI.

2. Materials and methods

2.1 Compounds

MH (the purity was 99.8%) was purchased from Sigma-Aldrich (St. Louis, MO, USA). MH was dissolved in 0.5 w/v% carboxymethyl cellulose solution (0.5% CMC) to reach a concentration of 5 mg/ml. Chlorpromazine hydrochloride (the purity was 99% or more), which was used to suppress intestinal peristalsis, was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) and dissolved in 0.5 w/v% methyl cellulose solution to reach a concentration of 5 mg/ml.

2.2 Animals and husbandry

All animal studies were approved by the Committee for the Ethical Usage of Experimental Animals of Sumitomo Pharma Co., Ltd., and the Animal Welfare Committee of Osaka University.

Female Sprague-Dawley (Crl:CD) rats were purchased from Charles River Laboratories Japan, Inc. (currently Charles River Laboratories Japan G.K.; Kanagawa, Japan) and allowed an acclimation period of more than 1 week. These rats were housed individually in a barrier-sustained room with controlled temperature of 24°C ± 2°C, relative humidity of 55% ± 10%, and a 12-h light (8 a.m. to 8 p.m.)/dark cycle. They were fed a commercial pellet diet (CE-2, CLEA Japan, Ltd., Tokyo, Japan) and tap water *ad libitum*.

2.3 Animal model and experimental design

2.3.1 Experiment 1 (*in vivo* MRI on the day after the final dosing and histopathology)

MH in 0.5% CMC (5 ml/kg) was administered subcutaneously to each rat (6 weeks of age at the

start of administration) once daily for 2 days at a dose level of 40 mg/kg/day. As a vehicle control, 0.5% CMC only was administered to animals as described above.

Rats, whose weight was close to 170 g at the first dose, were assigned to the MH group (N=10) and 0.5% CMC group (vehicle control group; N=5). The MRI examination was conducted on the day after the final dose. On the same day after the MRI examination, these rats were euthanized by exsanguination and necropsied. The mesenteric arteries were collected and evaluated histopathologically.

2.3.2 Experiment 2 (*in vivo* MRI on 7 days after the final dosing and histopathology)

As in Experiment 1, MH was administered subcutaneously to each rat once daily for 2 days at a dose level of 40 mg/kg/day in 0.5% CMC.

Rats, whose weight was close to 170 g at the first dosing, were assigned to the MH group (N=5). MRI examination was conducted on 7 days after the administration of the final dose. As in Experiment 1, mesenteric arteries in all animals were evaluated by *in vivo* MRI. On the same day after the MRI examination, these animals were euthanized by exsanguination and necropsied. The mesenteric arteries were collected and evaluated histopathologically.

2.4 MRI

All MRI examinations were conducted using an 11.7 T vertical-bore Bruker Avance II imaging

system (Bruker BioSpin, Ettlingen, Germany) and 33 mm volume radiofrequency coil for transmission and reception (Bruker BioSpin).

Rats were fasted more than 12 hours and given oral chlorpromazine hydrochloride (25 mg/kg, 5 ml/kg) just before the MRI examination for suppressing intestinal peristalsis. During the *in vivo* MRI, rats were given 0.5%–3% isoflurane for general anesthesia (Wako, Osaka, Japan) administered via a mask covering the nose and mouth. Respiratory signals were monitored using a physiological monitoring system (SA Instruments, Stony Brook, NY, USA), and body temperature was continuously maintained at 36.0 ± 0.5°C by circulating water through heating pads throughout all experiments. Anatomical images used for slice positioning were acquired with IntraGate fast low-angle shot (FLASH; Bruker BioSpin) and the following scanning parameters: repetition time/echo time (TR/TE) 62/1.5 msec, flip angle 15°, 60 mm field of view (FOV), matrix of 256 × 256 pixels, and 5 slices with thickness = 1.1 mm. For evaluation the arteritis, images were acquired using rapid acquisition with relaxation enhancement (RARE) with the following scanning parameters: RARE Factor 8, TR/TE 5000/24.8 msec, FOV 35 × 35 mm, matrix of 256 × 256 pixels, 20 slices with thickness = 0.5 mm, and number of excitations = 1. For reducing the signal intensity of fat and minimizing the effects of respiration on the image of MRI, fat suppression and respiratory gating techniques were used.

2.5 Histopathology

All of the collected arteries were fixed in 10% neutral-buffered formalin, embedded in paraffin,

sectioned, stained with hematoxylin and eosin, and examined by light microscopy.

2.6 Analytical method

2.6.1 Quantitative evaluation of signal intensity around the arteries on the images of Experiment 1

On the images of MRI, signal intensity around the mesenteric arteries was measured using ImageJ (NIH, Bethesda, MD, USA) in samples obtained in Experiment 1. The signal intensity around the arteries was defined as a value derived from the formula: signal intensity = intensity around the arteries / intensity of the skeletal muscle around the spinal cord. The intensities of 5 mesenteric arteries were measured for each animal and the mean value of 5 arteries were calculated as the signal intensity of each animal. Since the signal intensities around the arteries in the MH-administered group were not uniform because the lesions were limited to part of artery, the higher signal intensity regions, which were considered to be affected areas, were selected for analysis of the signal intensity of the area.

2.7 Statistics

The signal intensity data for each group are expressed as the mean ± standard deviation. A two-tailed non-paired *t*-test was used to analyze the difference in data between two groups. I considered p-value less than 0.05 as statistically significant.

3. Results

3.1 Clinical signs and Necropsy

Throughout the dosing period, no animal died or showed clinical signs. At necropsy, mottled white

lesions in the kidneys were observed in 2 of 10 animals necropsied on the day after the final dose and 2 of 5 animals necropsied on 7 days after administration of the final dose.

3.2 Histopathology

3.2.1 Experiment 1 (*in vivo* MRI on the day after the final dosing and histopathology)

In the mesenteric arteries of the MH-administered group, there was degeneration/necrosis of medial smooth muscle cells accompanied by intramural hemorrhage and/or perivascular infiltration of inflammatory cell, proliferation of fibroblasts, and edema. These lesions in the arterial wall and perivascular area were observed in 7 of 10 animals and 9 of 10 animals, respectively. There were no abnormal changes in 1 of 10 animals. Although they were multifocal, these lesions were observed only in a limited area. The extent of lesions was minimal in all cases. In the animals with mottled white renal lesions as gross lesions, acute tubular necrosis was observed. In the vehicle control group, no abnormality was observed in any animal. Table 3-1 describes the histopathology of samples used in the MRI assessment study and the lesions observed in the mesenteric arteries are shown in Fig. 3-1 (A-D).

Table 3-1
Histopathological findings of MH-administered animals

Organs and Tissues	Findings	Midodrine hydrochloride-administered group			
		MRI on the day after the final dosing (10*)		MRI on 7 days after the final dosing (5*)	
		-	±	-	±
Mesenteric artery	Degeneration/necrosis of medial smooth muscle cells	3	7	5	0
	Intramural hemorrhage	3	7	5	0
	Perivascular infiltration of inflammatory cell and proliferation of fibroblasts	1	9	5	0
	Perivascular edema	1	9	5	0

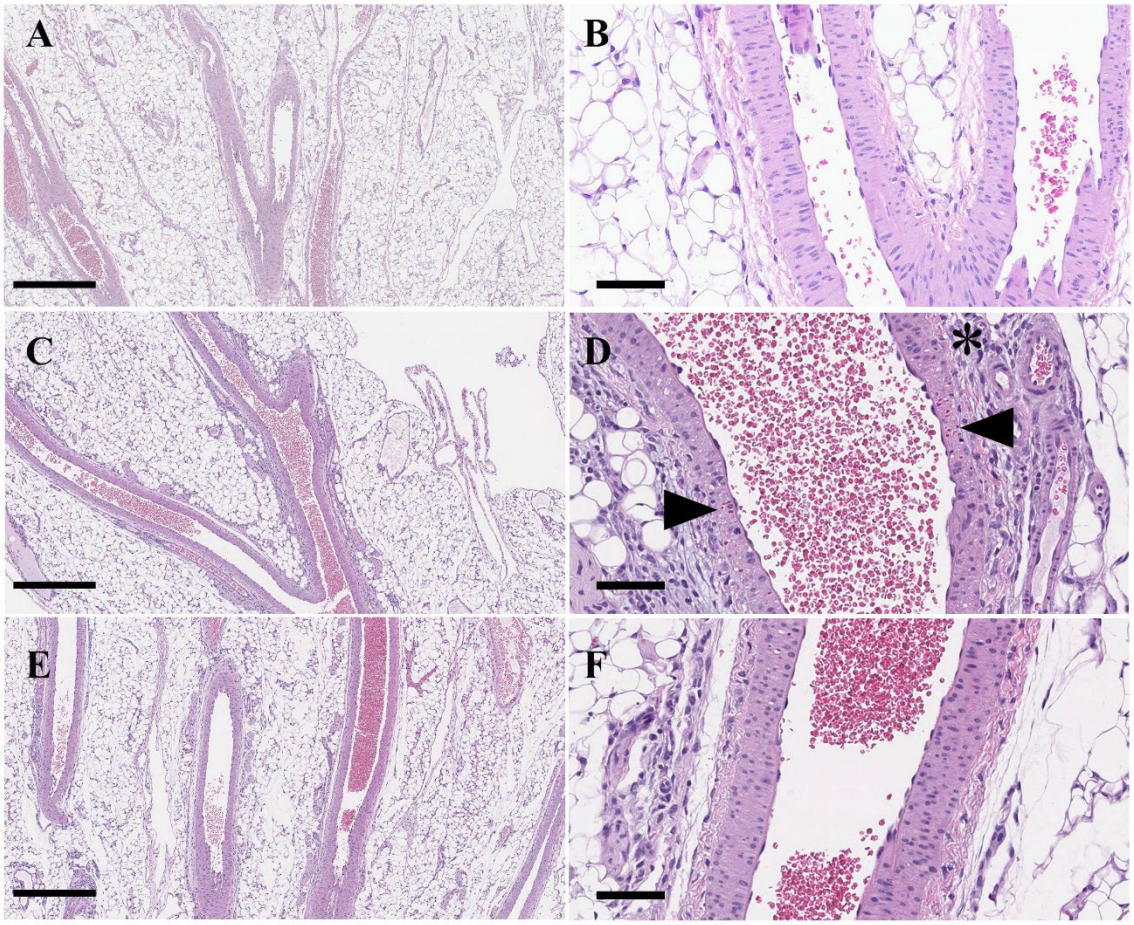

Figure 3-1
Representative histopathological images of the hematoxylin- and eosin-stained mesenteric arteries from MH-administered animals. (A-D) Histopathological images of the mesenteric arteries in Experiment 1 (*in vivo* MRI on the day after the final dosing and histopathology). (A and B) Mesenteric artery in the vehicle control group; Bar, 500 μm in A and 70 μm in B. (C and D) Mesenteric artery in the MH group on the day after the final dose; Bar, 500 μm in C and 70 μm in D. Minimal edema, infiltration of inflammatory cells, and proliferation of fibroblasts can be seen in the perivascular area. In addition, minimal medial smooth muscle cell degeneration and necrosis accompanied by intramural hemorrhage can be seen. Perivascular edema is indicated with asterisks and erythrocytes infiltrating the arterial wall are indicated with arrowheads. (E and F) Histopathological images of the mesenteric arteries in Experiment 2 (*in vivo* MRI on 7 days after the final dosing and histopathology). Mesenteric artery in the MH group on 7 days after the administration of the final dose; Bar, 500 μm in E and 70 μm in F. There were no findings.

3.2.2 Experiment 2 (*in vivo* MRI on 7 days after the final dosing and histopathology)

Although regeneration of renal tubules, hyaline casts, and dilation of renal tubules were observed in the kidneys with grossly visible mottled white lesions, no abnormality in the mesenteric arteries

was observed in any animal on 7 days after the administration of the final dose. Histopathological images of mesenteric arteries are shown in Fig. 3-1 (E, F).

3.3 MRI

3.3.1 Experiment 1 (*in vivo* MRI on the day after the final dosing and histopathology)

In animals in the vehicle control group, a few mesenteric arteries with low signal intensity were observed (Fig. 3-2A). In the MH-administered group, high signal intensity region around the mesenteric artery was observed in animals with perivascular lesions confirmed by histopathology and in limited areas (Fig. 3-2B, 2C). On the other hand, in one animal without histological changes, the image of MRI was similar to that in the vehicle control group (Fig. 3-2D). Table 3-2 provides individual data for comparison of results on histopathological examination and MRI.

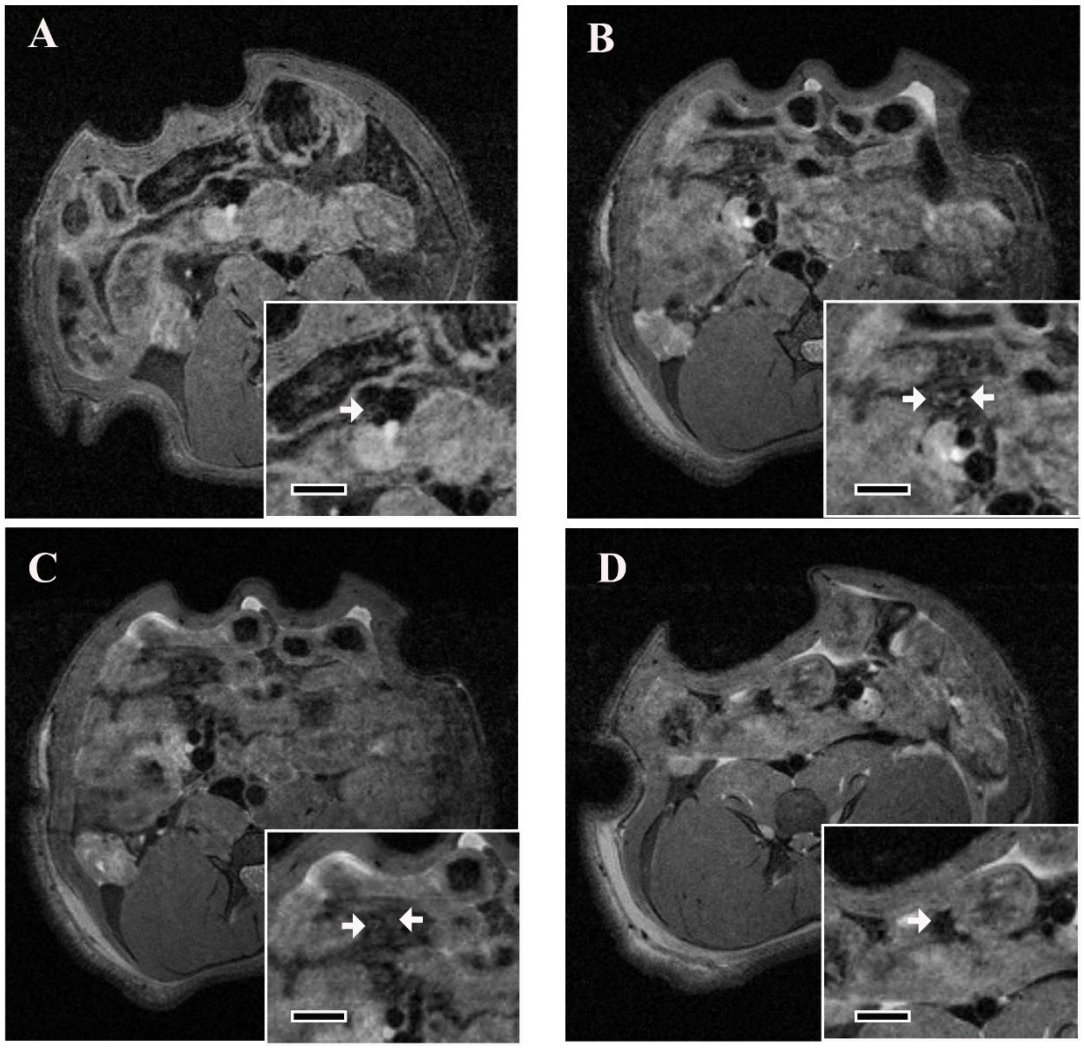

Figure 3-2
Typical images of MRI of mesenteric arteries in Experiment 1 (*in vivo* MRI on the day after the final dosing and histopathology) of MRI assessment study. All images are axial images. (A) Image in the vehicle control group. Inserts show cross section of the mesenteric artery at higher magnification. Bar in insert, 3 mm. (B-D) Images in the MH group. Inserts show cross section of the mesenteric artery at higher magnification. Bar in insert, 3 mm. (B and C) These images are acquired from same animal with perivascular lesions in a limited area confirmed by histopathology. C shows a site that is 1 mm caudal to the site in B. High signal intensity region around the mesenteric artery is seen in the limited area, as observed in the image of B but not in the image of C. (D) The image showing the lack of a high signal intensity region is from an animal without histological change. Arteries are indicated by arrows.

Table 3-2
Individual data for comparison of results on histopathological examination and MRI

Histopathological examination		Study	Experiment 1														
Organs and Tissues	Findings	Group	Vehicle control group					MH-administered group									
		Animal No.	1-1	1-2	1-3	1-4	1-5	2-1	2-2	2-3	2-4	2-5	2-6	2-7	2-8	2-9	2-10
Mesenteric artery	Degeneration/necrosis of medial smooth muscle cells		-	-	-	-	-	±	-	-	±	±	-	±	±	±	±
	Intramural hemorrhage		-	-	-	-	-	±	-	-	±	±	-	±	±	±	±
	Perivascular infiltration of inflammatory cell and proliferation of fibroblasts		-	-	-	-	-	±	±	±	±	±	-	±	±	±	±
	Perivascular edema		-	-	-	-	-	±	±	±	±	±	-	±	±	±	±

-：No abnormality, ±：minimal

MRI		Study	Experiment 1														
Organs and Tissues	Findings	Group	Vehicle control group					MH-administered group									
		Animal No.	1-1	1-2	1-3	1-4	1-5	2-1	2-2	2-3	2-4	2-5	2-6	2-7	2-8	2-9	2-10
Mesenteric artery	High signal intensity around the artery		-	-	-	-	-	+	+	+	+	+	-	+	+	+	+

-：No abnormality, +: Finding is observed

3.3.2 Experiment 2 (*in vivo* MRI on 7 days after the final dosing and histopathology)

No high signal intensity region was observed around the mesenteric artery in any animal (Fig. 3-3).

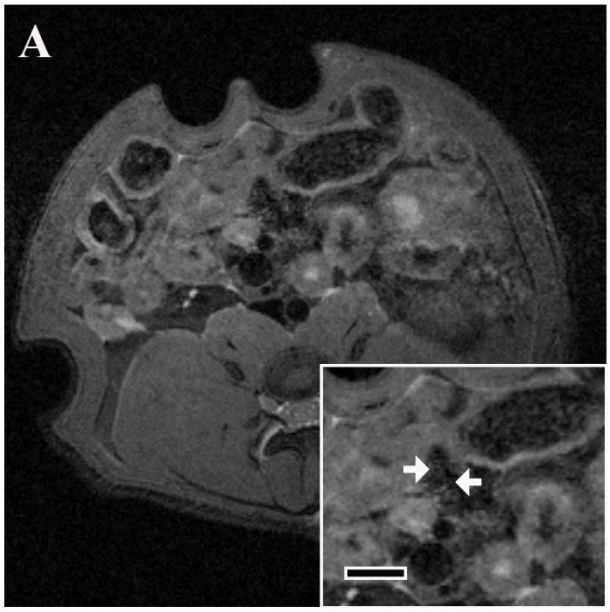

Figure 3-3
Typical images of MRI of mesenteric arteries in Experiment 2 (*in vivo* MRI 7 days after the final dose and histopathology). Axial image in the MH group 7 days after administration of the final dose. Inserts show cross section of the mesenteric artery at higher magnification. Bar in insert, 3 mm. There is no high signal intensity region. Arteries are indicated by arrows.

3.4 Quantitative evaluation of signal intensity around the arteries on MRI images in

Experiment 1

The mean value was 0.62 ± 0.08 and 1.03 ± 0.23 in the vehicle-control and MH-administered groups, respectively, and the difference was statistically significant. The maximum value in the vehicle control group was 0.73, whereas the minimum value in the MH-administered group was 0.85, which revealed that the signal intensity was clearly higher in the MH-administered group.

4. Discussion

To the best of my knowledge, this is the first report of an evaluation of arteritis caused by a vasoconstrictor using MRI.

Histopathological examination revealed that MH induced arteritis in the mesenteric arteries in rats. Degeneration/necrosis of medial smooth muscle cells accompanied by intramural hemorrhage and/or perivascular infiltration of inflammatory cell, proliferation of fibroblasts, and edema were observed.

As a result of MRI assessment, higher signal intensity around the artery was observed in the MH-administered group than the control group. The quantitative analysis of signal intensity around the artery on the images of MRI showed significant differences in signal intensity between the two groups. Although the histopathological lesion induced by MH was minimal, high signal intensity regions around the artery were observed by MRI in all animals with perivascular edema. On the other hand, the one animal without perivascular edema did not show high signal intensity. Therefore, I considered high signal intensity region around the artery using MRI to be a method for detecting the presence of

arteritis with high specificity and high sensitivity. Given that perivascular edema is one of the characteristic lesions of arteritis (Louden et al. 2006), high signal intensity region in MRI could be a versatile biomarker for detecting the arteritis with high specificity and high sensitivity.

In all animals evaluated on 7 days after the administration of the final dose, no lesion was observed in the histopathological examination, suggesting that the lesion had fully resolved during the 6-day recovery period. When the histopathological lesion disappeared, the high signal intensity region around the artery was no longer observed by MRI. This suggests that the presence or absence of a high signal intensity region in MRI can be a useful criterion to confirm the arteritis is recovered or not as an indicator of perivascular edema.

Differences in histopathological changes exist between MH- and FM-administered animals; the extent of the lesions including perivascular edema in MH-administered animals was minimal and that in FM-administered animals ranged from minimal to moderate. As for the perivascular edema, the extent was moderate in most FM-administered animals. Therefore, the lesion induced by MH was considered to be less severe than that induced by FM. Given that the LD_{50} of subcutaneously injected MH in female rats is 51 mg/kg in the interview form of Metligine (trade name of midodrine hydrochloride), 40 mg/kg used for MRI assessment in this study is considered to be close to the maximum tolerated dose. Therefore, the histopathological changes observed in this study were considered to be the strongest of the changes resulting from subcutaneous administration of MH for 2

days. As regard to the mesenteric artery lesions, histopathological changes in the arterial wall such as intramural hemorrhage were observed in all FM-administered animals, but in only 7 out of 10 MH-administered animals, and no lesions including periarterial lesions were observed in 1 of 10 animals. It has also been reported that hemorrhage is rare in MH-administered animals (Tobin et al. 2014). Therefore, I considered that the frequency of arteritis-associated hemorrhage occurrence may be higher with vasodilators. In addition, no renal lesions were observed in FM-administered animals, but renal acute tubular necrosis related to ischemia due to vasoconstriction was observed in some MH-administered animals.

Arteritis caused by the vasoconstrictor action of MH, which is different from that caused by the vasodilatory action of FM, could be detected by MRI by finding a high signal intensity region. Although the severity of MH-induced histopathological changes, including perivascular edema, was less than that of FM-induced histopathological changes and the MH-induced changes were fully resolved, MH-induced arteritis could be detected by MRI. In addition, the high signal intensity on the images of MRI correlated with histopathologic changes, even minimal MH-induced changes. The results of the second study and present studiy suggest that, regardless of pathogenic mechanism and degree of changes, MRI can detect perivascular edema as a sign of arteritis and be used to detecting arteritis without performing histopathological examination.

As shown also in this study, arteritis is known to be fully recovered if the lesion was minimal (Radić

et al. 2012). In this study, even a minimal lesion, which could disappear over time, was detected in rats by MRI by finding a high signal intensity region. Therefore, it is suggested that minimal lesions could be detected in the clinical study. In other words, it could be possible to judge the discontinuation of administration of a drug at the timing when the minimal lesion, which can be completely resolved, is occurred. Being able to determine discontinuation of administration with minimal lesions is extremely useful in conducting clinical trials of drugs that may cause arteritis.

In conclusion, my results indicated that arteritis induced by vasoconstrictor action, in addition to arteritis induced by vasodilatory action as shown in second study, can be detected using in vivo MRI. Furthermore, I also showed that high signal intensity around the artery could be a highly specific and sensitive candidate biomarker.

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Record of research achievements

Publications

Fujii Y, Yoshino Y, Chihara K, Nakae A, Enmi JI, Yoshioka Y, Miyawaki I. Detection of fenoldopam-induced arteritis in rats using *ex vivo* / *in vivo* MRI. Toxicol Rep. 2022 Jul 28;9:1595-1602.

Fujii Y, Yoshino Y, Chihara K, Nakae A, Enmi JI, Yoshioka Y, Miyawaki I. Evaluation of *in vivo* MRI for detecting midodrine-induced arteritis in rats. Toxicol Rep. 2023 Jan 5;10:97-103.

Conference presentations

Title: Detection of drug-induced vasculitis using *in vivo* MRI
Conference: The 47th Annual Meeting of the Japanese Society of Toxicology
Presentation form: Poster

Title: Evaluation of rodent toxicity using MRI: fatty liver and vasculitis
Conference: The 47th Annual Meeting of the Japanese Society of Toxicology
Presentation form: Oral (Work shop)

Title: *in vivo* イメージング技術の毒性評価への応用
Conference: 第 4 回 医薬品毒性機序研究会
Presentation form: Oral (Symposium)

Title: Detection of Fenoldopam-induced arteritis in rats using *ex vivo*/*in vivo* MRI
Conference: The 3rd Annual Scientific Meeting of Asian Society of Magnetic Resonance in Medicine and the 49th Annual Meeting of the Japanese Society for Magnetic Resonance in Medicine
Presentation form: Poster

Title: Detection of drug-induced arteritis in rats using *ex vivo*/*in vivo* MRI
Conference: The 38th Annual Meeting of the Japanese Society of Toxicologic Pathology
Presentation form: Oral (Work shop)

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