

# In-vivo imaging of hind paws micro vessel damage in the STZ-induced diabetes rat using dynamic contrast enhanced-MRI

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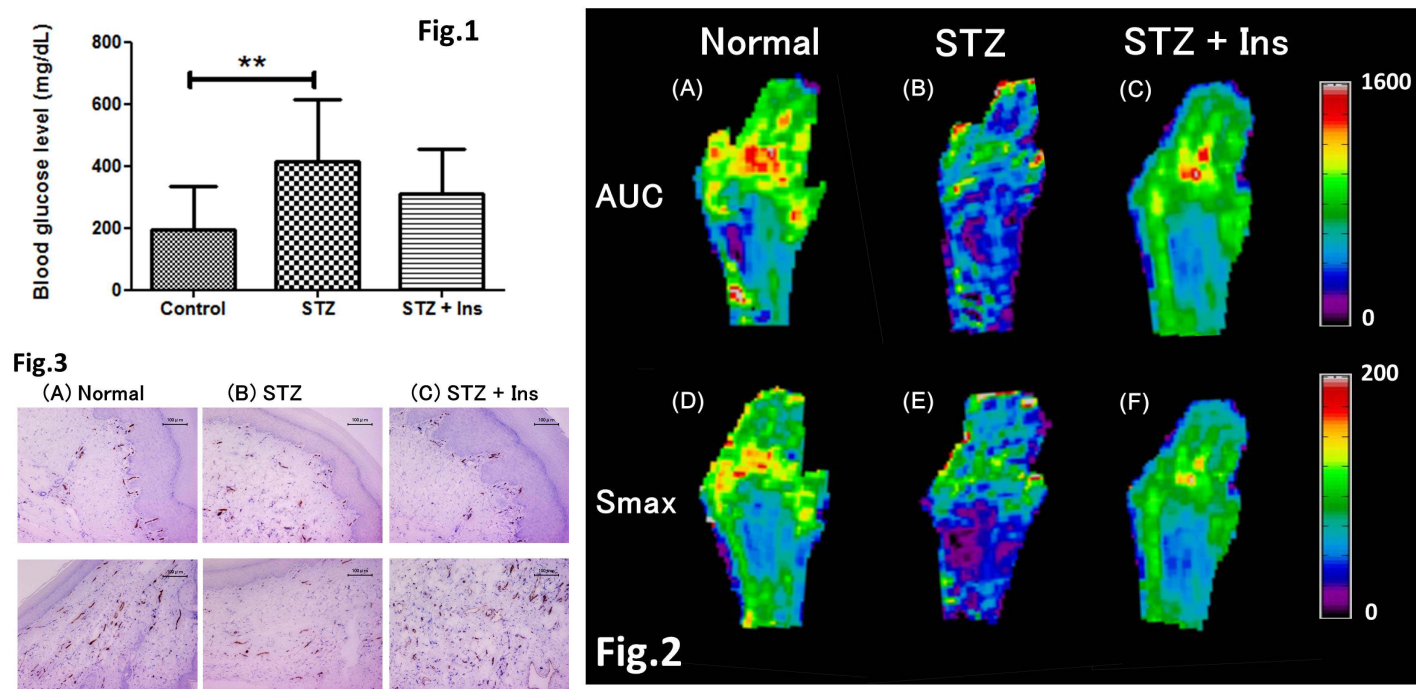
## INTRODUCTION

Hyperglycemia causes damage in the blood vessels and nerves of the body, which in turn develop into the major complications of diabetes [1]. Peripheral neuropathy [2] and peripheral circulatory disorder [3] may induce diabetic foot lesions. Our purpose of this study was to assess peripheral micro-vessel damage in streptozotocin (STZ)-induced diabetic rats by using dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) and histological experiment.

## MATERIALS AND METHODS

A rat diabetes model was produced by intravenous injection of STZ. Diabetes was induced by injecting STZ (65 mg/kg, Sigma Chemical, St. Louis, MO) into the tail vein after overnight fasting. Normal control rats were injected with the same volume of saline (1 mL/kg). Diabetic rats were treated with either saline or insulin during 4 weeks. The DCE-MRI studies were performed with an MRI system for animal experiments equipped with a 1.5-tesla permanent magnet (MRmini, DS Pharma Biomedical Co., Ltd., Osaka, Japan). The DCE-MRI data were acquired using 3D the fast low angle shot (FLASH) sequence with the following parameters: a repetition time (TR) of 50 ms, an echo time (TE) of 6.7 ms, an excitation pulse flip angle (FA) of 36 degrees, a field of view (FOV) of 60.0 × 60.0 mm<sup>2</sup>, a matrix size of 256 × 128, and a number of excitations (NEX) of 1. Gd-DTPA (0.1 mmol/kg, Magnevist, Bayer Schering Pharma, Osaka, Japan) was manually injected into the tail vein via a 26-gauge indwelling needle connected to a 50-cm extension tube and 1.0-mL syringe. Hind paw tissue perfusion was measured by signal intensity (SI) enhancement after Gd-DTPA injection in DCE-MRI study and quantified using the area under the SI-time curve (AUC) and the max values of SI (Smax) at 4 weeks after STZ injection. Peripheral micro-vessel was also assessed CD-31 staining as a marker of tissue damage.

## RESULTS and DISCUSSION



**Fig 1. Blood glucose level:** blood glucose level for three groups at 4 weeks after saline or STZ injection. The blood glucose level of the diabetic rats was significantly elevated compared with that of the control rats at 4 weeks ( $p < 0.01$ ).

**Fig 2. Typical images of AUC and Smax:** (A) – (C) the AUC maps in the whole right hind paw for each experiment groups. (D) – (F) the Smax maps in the whole right hind paw for each experiment groups. The AUC values of the diabetic rats showed a significant reduction (B and E,  $p < 0.001$ ) compared with that of the normal control rats, and significant improvement was observed in the insulin-treated diabetic rats (C and F).

**Fig 3. CD-31 staining:** CD-31 staining demonstrated the endothelial cell structure. The density of CD-31-positive cells for the STZ-induced diabetic rats were significantly smaller than that of the control and insulin treatment animals (B and C).

DCE-MRI perfusion measurement was performed to investigate and characterize peripheral tissue perfusion disorder and to assess the effect of insulin on peripheral tissue perfusion disorder [Fig2]. In the diabetic state, the endothelial tissue of blood vessels is exposed to chronic hyperglycemia, which induces endothelial dysfunction leading to diabetic angiopathy [1]. We can observe the micro vessel damage using the CD-31 staining [Fig.3]. Previous studies have shown that endothelial dysfunction of mesenteric microvessels [4] or reduction of hindlimbs blood flow [5] was observed after 2 weeks of STZ-induced diabetes, which agrees with our results that 4 weeks of a diabetes state leads to deterioration of peripheral tissue perfusion and micro-vessel damage.

## CONCLUSION

In conclusion, this study demonstrated that the DCE-MRI is useful for the assessment of STZ-induced diabetic rat peripheral circulatory disorder.

## REFERENCES

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