• Letter to the Editor •

Increased plasma galectin-1 correlates with advanced glycation end products and interleukin-1β in patients with proliferative diabetic retinopathy

Keitaro Hase, Atsuhiro Kanda, Kousuke Noda, Susumu Ishida

Laboratory of Ocular Cell Biology and Visual Science, Department of Ophthalmology, Faculty of Medicine and Graduate School of Medicine, Hokkaido University, Sapporo, Hokkaido 060-8638, Japan

Correspondence to: Atsuhiro Kanda. Laboratory of Ocular Cell Biology and Visual Science, Department of Ophthalmology, Faculty of Medicine and Graduate School of Medicine, Hokkaido University, N-15, W-7, Kita-ku, Sapporo 060-8638, Japan. kanda@med.hokudai.ac.jp

Received: 2018-06-18 Accepted: 2019-01-25

DOI:10.18240/ijo.2019.04.28

Citation: Hase K, Kanda A, Noda K, Ishida S. Increased plasma galectin-1 correlates with advanced glycation end products and interleukin-1β in patients with proliferative diabetic retinopathy. *Int J Ophthalmol* 2019;12(4):692-694

Dear Editor,

Galectin-1, one of galactoside-binding lectin family proteins, has been shown to regulate cell growth, migration, and proliferation, where it binds many receptors depending on their glycosylation profile, rather than its specific receptors. We and others reported galectin-1 as a newly recognized hypoxia-induced angiogenic factor that binds to the N-glycans on vascular endothelial growth factor receptor (VEGFR) 2 while increasing VEGFR2 activation (*i.e.* phosphorylation), stimulating its downstream signaling pathway in endothelial cells, and consequently promoting angiogenesis[1-2]. Importantly, compared with non-diabetic patients, vitreous galectin-1 protein levels were increased in proliferative diabetic retinopathy (PDR) eyes, showing no correlation with elevated VEGF-A levels[2]. Recent reports including ours revealed a significant increase of galectin-1 levels in aqueous humor samples of diabetic retinopathy (DR) patients with the severity of clinical stages[3-4], but not in eyes with non-diabetic retinal vascular occlusions[3]. In addition to galectin-1 expression regulated by hypoxia[1-2], we newly proposed that diabetes-induced advanced glycation end products (AGEs) accumulation activates interleukin-1β (IL-1β)-linked inflammatory cues in macrophages, resulting in Müller glial galectin-1 upregulation along with the progression of DR[3]. In the present study, we explored correlations between protein levels of galectin-1 and potentially related molecules in the plasma samples of PDR patients.

Plasma samples from 20 PDR cases (aged 62.4±1.9 years old) and 20 non-diabetic, age- and gender-matched cases with idiopathic macular diseases (non-DM, aged 63.4±1.0 years old) were collected, of which detailed characteristics were indicated in our previous report[5]. The levels of proteins [galectin-1, IL-1β, VEGF-A, C-reactive protein (CRP) and IL-6] were measured using the Luminex assay kit (R&D Systems, Minneapolis, MN, USA) and MAGPIX (Millipore, Austin, TX, USA) per the manufacturers' instructions. The protein levels of AGEs and erythropoietin and the activity of lactate dehydrogenase (LDH) in the plasma were determined with OxiSelect AGE competitive (Cell Biolabs, San Diago, CA, USA) and human erythropoietin (Thermo Fisher Scientific, Waltham, MA, USA) enzyme-linked immunosorbent assay kits and LDH assay kit (Serotec, Hokkaido, Japan), according to the manufacturers' protocols. All results are presented as the mean±standard error of the mean (SEM). Student's *t*-test following the analysis of variance and Spearman rank correlation were used for statistical analyses. This study was conducted in accordance with the tenets of the Declaration of Helsinki following approval from the institutional review committee of Hokkaido University Hospital (IRB No.011-0172), and written informed consent was obtained from all patients.

As compared with those in non-diabetic patients, plasma levels of galectin-1, AGEs and IL-1β significantly increased in cases with PDR (Table 1), but those of VEGF-A, CRP, erythropoietin, IL-6, and LDH did not. Importantly, in the PDR group, elevated plasma galectin-1 levels were positively correlated with AGEs and IL-1β levels (Table 2), both of which were also correlated with each other (r=0.495, P=0.026), while there were no correlations between galectin-1 and any of other parameters: VEGF-A, CRP, erythropoietin, IL-6 or LDH. No correlations between galectin-1 and those protein levels were found in the non-DM group (Table 2). In PDR patients, plasma galectin-1 levels exclusively exhibited significant

Table 1 Plasma concentrations of galectin-1 and other molecules in patients with non-DM and PDR

Molecules	Non-DM	PDR	*P*
Galectin-1 (ng/mL)	3.36±0.10	4.55±0.25	<0.001
AGEs (μg/mL)	35.76±2.63	68.48±4.88	<0.001
IL-1β (pg/mL)	0.37±0.02	0.49±0.03	<0.001
VEGF-A (pg/mL)	0.41±0.02	0.44±0.04	0.605
CRP (μg/mL)	0.11±0.04	0.09±0.03	0.690
Erythropoietin (mIU/mL)	5.68±0.32	6.53±0.71	0.282
IL-6 (pg/mL)	0.22±0.03	0.25±0.04	0.455
LDH (U/L)	186.05±8.23	203.40±8.57	0.152

DM: Diabetes mellitus; PDR: Proliferative diabetic retinopathy; AGEs: Advanced glycation end products; IL: Interleukin; VEGF: Vascular endothelial growth factor; CRP: C-reactive protein; LDH: Lactate dehydrogenase.

Table 2 Correlations between plasma galectin-1 and other molecules in patients with non-DM and PDR

Molecules	Non-DM		PDR	
	r	*P*	*r*	*P*
AGEs (μg/mL)	0.082	0.730	0.628	0.003
IL-1β (pg/mL)	0.393	0.086	0.791	<0.001
VEGF-A (pg/mL)	0.144	0.543	0.088	0.713
CRP (μg/mL)	0.211	0.371	-0.093	0.696
Erythropoietin (mIU/mL)	0.296	0.205	0.037	0.876
IL-6 (pg/mL)	0.229	0.332	-0.086	0.719
LDH (U/L)	0.240	0.309	0.403	0.078

DM: Diabetes mellitus; PDR: Proliferative diabetic retinopathy; AGEs: Advanced glycation end products; IL: Interleukin; VEGF: Vascular endothelial growth factor; CRP: C-reactive protein; LDH: Lactate dehydrogenase.

negative and positive correlations with estimated glomerular filtration rate (eGFR; $P<0.001$, $r=-0.820$) and serum creatinine (sCr; $P<0.001$, $r=0.741$), respectively, out of various basal characteristics of participating patients (*e.g.* body mass index, HbA1c, random blood sugar, and duration of diabetes)[5]. These results showed close correlations mutually between galectin-1, AGEs, and IL-1β in the plasma samples of PDR patients, and a possible link of galectin-1 to the kidney changes in diabetes.

The present study demonstrated, for the first time to our knowledge, important findings concerning the significant correlations between galectin-1 and renal dysfunction parameters (*i.e.* eGFR and sCr levels) in PDR patients. Previous reports have revealed a significant association between the prevalence of retinopathy and nephropathy in diabetes, relating low eGFR levels with the onset and severity of DR[6-7]. Galectin-1 expression upregulated in the kidney cortex of diabetic mice, and inhibition of galectin-1 showed a significant decrease in the protein expression of a fibrosis marker fibronectin[8], suggesting the contribution of renal galectin-1 to the pro-fibrotic activity of diabetic nephropathy. Importantly, we have recently revealed that the significant involvement of galectin-1 in choroidal neovascularization and subretinal fibrosis[9]. This may also be true of retinopathy with angiogenic and fibrogenic processes in nature, given a significant increase of galectin-1 in eyes of DR patients along with the severity of clinical stages[3].

Several cross-linking biochemical pathways have been proposed as potential links among hyperglycemia, oxidative stress, and diabetic complications. These pathways cause acceleration of AGEs formation, induction of pro-inflammatory cytokines, and microvascular injury leading to retinal hypoxia[10]. The accumulation of AGEs, a complex and heterogeneous group of products resulting from nonenzymatic glycation and oxidation of proteins, has been reported as one of the key molecules for inflammatory response in diabetes. Under hypoxia, several transcription factors including hypoxia-inducible factor-1 induce the production of pro-inflammatory molecules (CRP, IL-6), angiogenic factors (VEGF-A, erythropoietin) and anaerobic glycolytic enzyme in various organs including the eye[11]. We recently clarified two distinct regulatory mechanisms of retinal galectin-1; hypoxia-mediated and AGE/IL-1β pathways[2-3]. The current data suggest that AGEs upregulate plasma galectin-1 *via* IL-1β, although the cellular components involved in the eye are different. Given that plasma galectin-1 levels were associated with diabetes-induced renal as well as retinal abnormalities, elevated plasma galectin-1 may in part result from AGE-triggered inflammatory stimuli rather than hypoxic induction in the diabetic kidney vulnerable to chronic inflammation.

This study has limitations. We retrospectively analyzed routine blood samples collected from patients waiting for surgery for complicated PDR, the most advanced stage of DR. Since plasma levels of both AGEs[12] and IL-1β[13] have been reported to increase along with the progression of DR, future studies are warranted to clarify a possible correlation between plasma galectin-1 and the severity of DR. Nevertheless, the present findings suggest that plasma galectin-1 potentially represents both a pathogenic biomarker and a therapeutic target in the management of patients with DR.

ACKNOWLEDGEMENTS

Foundation: Supported by Bayer Yakuhin Ltd, the Uehara Memorial Foundation, the Eye Research Foundation for the Aged, and the Japan National Society for the Prevention of Blindness.

Conflicts of Interest: Hase K, None; **Kanda A,** None; **Noda K,** None; **Ishida S,** None.

REFERENCES

1 Croci DO, Cerliani JP, Dalotto-Moreno T, *et al*. Glycosylation-dependent lectin-receptor interactions preserve angiogenesis in anti-VEGF refractory tumors. *Cell* 2014;156:744-758.

2 Kanda A, Noda K, Saito W, Ishida S. Aflibercept traps galectin-1, an angiogenic factor associated with diabetic retinopathy. *Sci Rep* 2015;5:17946.

3 Kanda A, Dong Y, Noda K, Saito W, Ishida S. Advanced glycation endproducts link inflammatory cues to upregulation of galectin-1 in diabetic retinopathy. *Sci Rep* 2017;7(1):16168.

4 Ridano ME, Subirada PV, Paz MC, Lorenc VE, Stupirski JC, Gramajo AL, Luna JD, Croci DO, Rabinovich GA, Sánchez MC. Galectin-1 expression imprints a neurovascular phenotype in proliferative retinopathies and delineates responses to anti-VEGF. *Oncotarget* 2017;8(20):32505-32522.

5 Hase K, Kanda A, Hirose I, Noda K, Ishida S. Systemic factors related to soluble (pro)renin receptor in plasma of patients with proliferative diabetic retinopathy. *PLoS One* 2017;12(12):e0189696.

6 El-Asrar AM, Al-Rubeaan KA, Al-Amro SA, Moharram OA, Kangave D. Retinopathy as a predictor of other diabetic complications. *Int Ophthalmol* 2001;24(1):1-11.

7 Man RE, Sasongko MB, Wang JJ, MacIsaac R, Wong TY, Sabanayagam C, Lamoureux EL. The association of estimated glomerular filtration rate with diabetic retinopathy and macular edema. *Invest Ophthalmol Vis Sci* 2015;56(8):4810-4816.

8 Al-Obaidi N, Mohan S, Liang S, *et al*. Galectin-1 is a new fibrosis protein in type 1 and type 2 diabetes. *FASEB J* 2019;33:373-387.

9 Wu D, Kanda A, Liu Y, Kase S, Noda K, Ishida S. Galectin-1 promotes choroidal neovascularization and subretinal fibrosis mediated via epithelial-mesenchymal transition. *FASEB J* 2019;33:2498-2513.

10 Stitt AW, Curtis TM, Chen M, Medina RJ, McKay GJ, Jenkins A, Gardiner TA, Lyons TJ, Hammes HP, Simó R, Lois N. The progress in understanding and treatment of diabetic retinopathy. *Prog Retin Eye Res* 2016;51:156-186.

11 Semeraro F, Cancarini A, dell'Omo R, Rezzola S, Romano MR, Costagliola C. Diabetic retinopathy: vascular and inflammatory disease. *J Diabetes Res* 2015;2015:582060.

12 Ono Y, Aoki S, Ohnishi K, Yasuda T, Kawano K, Tsukada Y. Increased serum levels of advanced glycation end-products and diabetic complications. *Diabetes Res Clin Pract* 1998;41(2):131-137.

13 Koleva-Georgieva DN, Sivkova NP, Terzieva D. Serum inflammatory cytokines IL-1beta, IL-6, TNF-alpha and VEGF have influence on the development of diabetic retinopathy. *Folia Med (Plovdiv)* 2011;53(2):44-50.