BMP-7 PROTEIN EXPRESSION IS DOWNREGULATED IN HUMAN DIABETIC NEPHROPATHY

Renata Ivanac-Janković[1], Marijana Ćorić[2], Vesna Furić-Čunko[3], Vesna Lovčić[1], Nikolina Bašić-Jukić[3] and Petar Kes[3]

[1]Department of Internal Medicine, Bjelovar General Hospital, Bjelovar; [2]Department of Pathology and Cytology, Zagreb University Hospital Center, [3]Department of Nephrology, Arterial Hypertension, Dialysis and Transplantation, Zagreb University Hospital Center, School of Medicine, University of Zagreb, Zagreb, Croatia

SUMMARY – Bone morphogenetic protein-7 (BMP-7) is expressed in all parts of the normal kidney parenchyma, being highest in the epithelium of proximal tubules. It protects kidney against acute and chronic injury, inflammation and fibrosis. Diabetic nephropathy is the leading cause of chronic kidney disease, and is characterized by decreased expression of BMP-7. The aim of our study was to analyze whether the expression of BMP-7 is significantly changed in advanced stages of human diabetic nephropathy. Immunohistochemical analysis of the expression of BMP-7 was performed on archival material of 30 patients that underwent renal biopsy and had confirmed diagnosis of diabetic nephropathy. Results showed that BMP-7 was differently expressed in the cytoplasm of epithelial cells of proximal tubules and podocytes among all stages of diabetic nephropathy. At early stages of diabetic nephropathy, BMP-7 was strongly positive in proximal tubules and podocytes, while low expression was recorded in the majority of samples at advanced stages. In conclusion, increased expression of BMP-7 at initial stages of diabetic nephropathy with subsequent decrease at advanced stage highlights the role of BMP-7 in the protection of kidney structure and function. Further investigations should be focused on disturbances of BMP-7 receptors and signaling pathways in patients with diabetic nephropathy.

Key words: *Kidney; Bone morphogenetic protein-7; Diabetic nephropathy; Kidney tubules, proximal; Podocytes; Immunohistochemistry*

Introduction

Bone morphogenetic proteins (BMPs) are members of the transforming growth factor beta superfamily. They are also known as osteogenic proteins (OPs), and were originally identified by their ability to induce the formation of endochondral bone at extraskeletal sites[1,2]. BMP-7 is synthesized predominantly in the kidney at gestational age of 5-14 weeks[3,4], and has been recognized as a key signaling molecule during kidney development[5]. It is mostly expressed in the epithelial cells of the glomeruli and distal tubules in the kidney[6]. BMP-7 has the ability to protect kidney against acute renal failure in postnatal life[5].

The absence of BMP-7 gene expression in mice causes uremia and death within 24 h after birth, and dysplastic kidneys and hydroureter are found on autopsy[7]. Experimental animal models of acute and chronic renal injury confirmed the important role of BMP-7 in the prevention of inflammation and fibrosis[8].

Several studies confirmed that expression of BMP-7 and the number of podocytes are decreased in diabetic nephropathy[9]. In diabetic nephropathy, tubular cells produce a large amount of extracellular matrix proteins, which leads to interstitial fibrosis. BMP-7

Correspondence to: *Renata Ivanac-Janković, MD*, Department of Internal Medicine, Bjelovar General Hospital, Mihanovićeva 8, HR-43000 Bjelovar, Croatia
E-mail: renata.ivanac@gmail.com

Received December 11, 2014, accepted February 21, 2015

can reduce increased expression of interstitial extracellular matrix proteins[10]. Wang *et al*. have presented a hypothesis that deficiency of BMP-7 signaling may play crucial role in podocyte damage in early diabetes in rodents, and its prevention has an important role in reducing diabetic renal injury. They suggest endogenous glomerular BMP-7 as an autocrine regulator of podocyte integrity *in vivo*[11].

Recent data collected on a diabetic mouse model of diabetic nephropathy which disregard the physiological differences between the rat and mouse models, have reaffirmed the observation that the progression of diabetic nephropathy leads to decreased BMP-7 expression. These data show that BMP-7 has a crucial role in adult kidney function, especially in the protection from renal injury in diabetic condition[12].

Studies on rodents have shown that BMP-7 is an effective therapy for diabetic nephropathy, especially in reversing proteinuria. Diabetic injury resulted in the loss of tubular epithelial and glomerular podocyte phenotype manifested as the loss of BMP-7 expression, and therapy with BMP-7 restored the phenotype of the collecting duct manifested by restoration of BMP-7 expression[13].

In our study, we postulated that expression of BMP-7 is different among stages of diabetic nephropathy on human kidney biopsies, and that it might be associated with the severity of disease. Therefore, the objective of this study was to analyze whether expression of BMP-7 is significantly changed in advanced stages of human diabetic nephropathy.

Materials and Methods

Human tissue

Renal tissue samples for immunohistochemical analysis of the expression of BMP-7 were obtained from the archives of the Department of Pathology and Cytology, Zagreb University Hospital Center, Zagreb, Croatia, from 30 patients with previously histologically confirmed diagnosis of diabetic nephropathy. During the processing, two of the samples were extracted from the analysis due to material insufficiency. Healthy kidney tissue of 12 human kidneys obtained after surgical nephrectomy in patients with localized renal tumors was used as controls. Mean age of the controls was 58.8±4.3 years and 67% were men.

For determination of the stage of diabetic nephropathy we used the Renal Pathology Society Research Committee classification, which divides diabetic nephropathy into four stages of glomerular lesions based on the degree of interstitial and vascular involvement[14].

Immunohistochemistry

Anti-human BMP-7 monoclonal antibodies (R&D Systems, USA) were used for immunohistochemistry. Paraffin sections (4-5 μm) were deparaffinized, antigen BMP-7 masked in the PT-module (DakoCytomation, Denmark) at 97 °C for 20 minutes in a buffer from EnVision FLEX commercial kit (K8010, DakoCytomation, Denmark) and processed by standard automated method in the Autostainer + camera (DakoCytomation, Denmark), then stained with hematoxylin.

Quantification

Globally sclerosed glomeruli were excluded from analysis. Results of immunohistochemistry were assessed using a light microscope, on at least five fields under high magnification (X400). The presence of staining in >5% of cells was considered another positive expression of BMP-7. BMP-7 immunostaining was semiquantitatively evaluated for intensity as previously described[15].

Statistical analysis was carried out using Statistica (version 10) and MedCalc 12.0 computer program.

Results

Patient characteristics

Three patients had diabetes type 1 and 25 patients diabetes type 2. At the time of biopsy, the youngest patient was aged 24 and the oldest 70, average age 53 years. There were 60% of male patients. Biopsies were conducted in the period from 1998 through 2012.

BMP-7 expression in proximal tubules

BMP-7 was differently expressed in the cytoplasm of epithelial cells of proximal tubules and podocytes at all stages of diabetic nephropathy (Fig. 1).

Four patients had diabetic nephropathy stage I or II, and BMP-7 was in all cases strongly positive (3+)

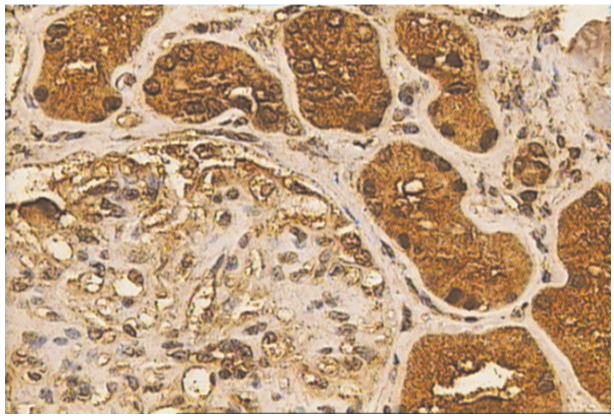

Fig. 1. BMP-7 is highly positive in the cytoplasm of epithelial tubular cortex and in podocytes (X400).

in proximal tubules. Twenty-four patients had stage III or IV diabetic nephropathy, and BMP-7 expression was of medium intensity (2+) in 7 samples, while strong intensity (3+) was found in 17 samples (not significant) (Fig. 2).

BMP-7 expression in control samples was less intensive than at the initial stages of diabetic nephropathy (not significant).

BMP-7 expression in podocytes

Expression of BMP-7 in podocytes was variable. BMP-7 expression at initial stages of diabetic nephropathy was high, either 2+ or 3+. At advanced stages, low expression (1+) was recorded in the majority of samples. Thus, at stage III, 1+ BMP-7 expression was found in 62.5%s, 2+ in 12.5% and 3+ in 25% of samples. At stage IV, 50% of samples had expression 1+, 14% 2+, while the remaining 36% had high (3+) intensity of BMP-7 expression (Fig. 3).

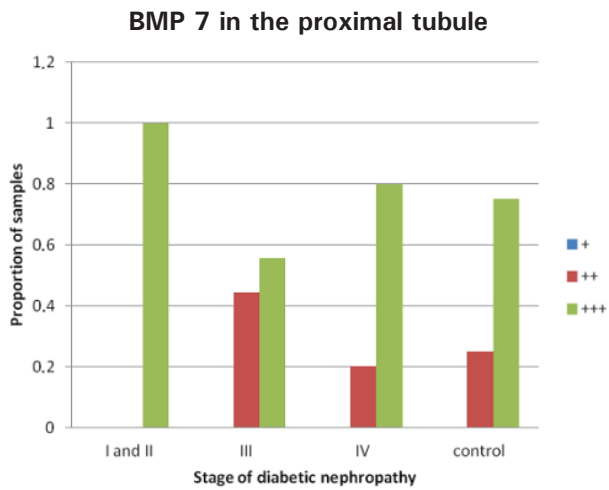

Fig. 2. Expression of BMP-7 in proximal tubules at different stages of diabetic nephropathy.

Discussion

Over the last decade, BMP-7 has emerged as an antifibrogenic agent. Exogenously administered rh-BMP-7 reduced renal fibrosis in experimental models of unilateral obstructive nephropathy or diabetic glomerular sclerosis[16,17]. In the rat model of streptozotocin-induced diabetic nephropathy, BMP-7 was found to partially reverse diabetes-induced kidney hypertrophy, restore glomerular filtration rate, urine albumin excretion, and glomerular histology[13].

BMP-7 exerts antifibrogenic actions *in vitro by* inducing Smad signals that block activation and nuclear translocation of TGF-β-induced Smad 2/3[18]. In cultured cells, BMP-7 acts as a counter-regulator of TGF-β profibrogenic actions[19]. BMP-7 decrease occurred early in kidneys of experimental animals, even before the progression of structural damage to the kidneys[10].

In this study, we examined the expression of BMP-7 at different stages of diabetic nephropathy in humans. Our results revealed that BMP-7 expression increased at the initial stages of diabetic nephropathy, with subsequent decrease at advanced stages of the disease. It is probably a protective mechanism that occurs in order to protect kidney structure or function. However, it is in contrast with the loss of endogenous

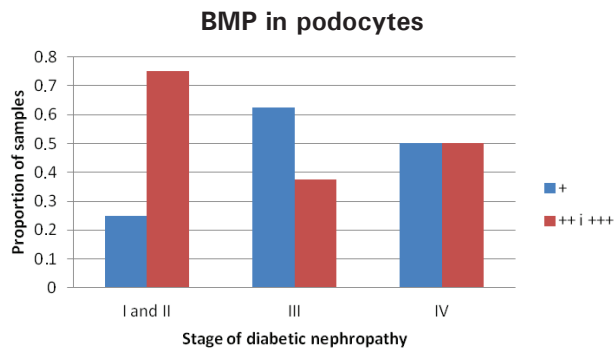

Fig. 3. Expression of BMP-7 in podocytes at different stages of diabetic nephropathy.

renal BMP-7 that has been observed in early diabetes in rodents[10]. BMP-7 expression in proximal tubular cells was strongly positive at early stages and less intensive at advanced stages. Expression of BMP-7 in podocytes was less consistent. At initial stages of diabetic nephropathy it was high, 2+ or 3+, while at advanced stages, the majority of samples exerted low expression (1+). Also, marked expression of BMP-7 at initial stages of diabetic nephropathy compared to control group was found.

BMP-7 was proposed as a survival factor for podocytes in animal models of diabetic nephropathy[11], as it was found for neuronal cells[20]. Maintenance of BMP-7 in podocytes improves podocyte survival, which may account for lesser albuminuria in diabetic mice that express the BMP-7 transgene compared with their WT counterparts. It was not found to affect the diabetes-induced increase in TGF-β but reduces early accumulation of collagens and fibronectin and maintains renal collagenase activity, consistent with the TGF-β activity-opposing action. The beneficial effect of BMP-7 on podocyte survival may also be explained by its antagonism to the effects of TGF-β[21].

Although samples of patients with more advanced stages of diabetic nephropathy had a decreased intensity of BMP-7 staining compared to samples obtained at initial stages of the disease, the difference was not statistically significant.

The major limitation of our study was a small sample size. Further investigations with large samples are needed to determine the significance of such findings with certainty. However, data on human samples are valuable as they partially support data from animal experiments that emphasize the role of BMP-7 in preservation of kidney structure and function. Higher-than-normal expression of BMP-7 at initial stages of diabetic nephropathy may be in line with these findings. In human diabetic nephropathy, Turk *et al*. showed that the loss of podocytes and podocyte differentiation markers is associated with a decreased BMP signaling activity[9]. Our findings of increased BMP-7 expression in early diabetic nephropathy samples could indicate a disorder in BMP receptors or the cell signaling pathway that results in consecutive overstimulation of BMP-7, so future research should be focused in this direction.

Conclusion

Increased expression of BMP-7 at initial stages of diabetic nephropathy with subsequent decrease at advanced stage highlights the role of BMP-7 in the protection of kidney structure and function. Further investigations should be focused on disturbances of BMP-7 receptors and signaling pathways in patients with diabetic nephropathy.

References

1. Urist MR. Bone: formation by autoinduction. Science. 1965;150:893-9.
2. Vukicevic S, Basic V, Rogic D, *et al*. Osteogenic protein-1 (bone morphogenetic protein-7) reduces severity of injury after ischemic acute renal failure in rat. J Clin Invest. 1998;102:202-14.
3. Vukicevic S, Latin V, Chen P, *et al*. Localization of osteogenic protein-1 (bone morphogenetic protein-7) during human embryonic development: high affinity binding to basement membranes. Biochem Biophys Res Commun. 1994;198:693-700.
4. Helder MN, Ozkaynak E, Sampath KT, *et al*. Expression pattern of osteogenic protein-1 (bone morphogenetic protein-7) in human and mouse development. J Histochem Cytochem. 1995;43:1035-44.
5. Bašić N. The role of bone morphogenetic proteins in compensatory renal growth. Lijec Vjesn. 1997;119:53-5.
6. Markić D, Čelić T, Španjol J, *et al*. Expression of bone morphogenetic protein-7, its receptors and Smad1/5/8 in normal human kidney and renal cell cancer. Coll Antropol. 2010;34:S149-S153.
7. Dudley AT, Lyons KM, Robertson EJ. A requirement for bone morphogenetic protein-7 during development of the mammalian kidney and eye. Genes Dev. 1995;9:2795-807.
8. Simic P, Vukicevic S. Bone morphogenetic proteins in development and homeostasis of kidney. Cytokine Growth Factor Rev. 2005;16:299-308.
9. Turk T, Leeuwis JW, Gray J, *et al*. BMP signaling and podocyte markers are decreased in human diabetic nephropathy in association with CTGF overexpression. J Histochem Cytochem. 2009;57:623-31.
10. Wang SN, Lapage J, Hirschberg R. Loss of tubular bone morphogenetic protein-7 in diabetic nephropathy. J Am Soc Nephrol. 2001;12:2392-9.
11. Wang S, de Caestecker M, Kopp J, *et al*. Renal bone morphogenetic protein-7 protects against diabetic nephropathy. J Am Soc Nephrol. 2006;17:2504-12.
12. Qu Y, Du E, Zhang Y, *et al*. Changes in the expression of bone morphogenetic protein 7 and Tamm-Horsfall protein in the early stages of diabetic nephropathy. Nephrourol Mon. 2012;4:466-9.

13. Wang S, Chen Q, Simon TC, *et al*. Bone morphogenetic protein-7 (BMP-7), a novel therapy for diabetic nephropathy. Kidney Int. 2003;63:2037-49.
14. Tervaert TW, Mooyaart AL, Amann K, *et al*.; Renal Pathology Society. Pathologic classification of diabetic nephropathy. J Am Soc Nephrol. 2010;21:556-63.
15. Basic-Jukic N, Hudolin T, Radic-Antolic M, *et al*. Bone morphogenetic protein-7 expression is down-regulated in human clear cell renal carcinoma. J Nephrol. 2011;24:91-7.
16. Morrissey J, Hruska K, Guo G, *et al*. Bone morphogenetic protein-7 improves renal fibrosis and accelerates the return of renal function. J Am Soc Nephrol. 2002;13;S14-S21.
17. Zeisberg M, Hanai J, Sugimoto H, *et al*. BMP-7 counteracts TGF-beta1-induced epithelial-to-mesenchymal transition and reverses chronic renal injury. Nat Med. 2003;9:964-8.
18. Wang S, Hirschberg R. Bone morphogenetic protein-7 signals opposing transforming growth factor beta in mesangial cells. J Biol Chem. 2004;279:23200-6.
19. Wang S, Hirschberg R. BMP7 antagonizes TGF-beta-dependent fibrogenesis in mesangial cells. Am J Physiol Renal Physiol. 2003;284:F1006-F1013.
20. Yabe T, Samuels I, Schwartz JP. Bone morphogenetic proteins BMP-6 and BMP-7 have differential effects on survival and neurite outgrowth of cerebellar granule cell neurons. J Neurosci Res. 2002;68:161-8.
21. Schiffer M, Bitzer M, Roberts IS, *et al*. Apoptosis in podocytes induced by TGF-beta and Smad7. J Clin Invest. 2010;108:807-16.

Sažetak

IZRAŽAJ KOŠTANOG MORFOGENETSKOG PROTEINA-7 JE SNIŽEN U HUMANOJ DIJABETIČKOJ NEFROPATIJI

R. Ivanac-Janković, M. Ćorić, V. Furić-Čunko, V. Lovčić, N. Bašić-Jukić i *P. Kes*

Koštani morfogenetski protein-7 (engl. *bone morphogenetic protein-7*, BMP-7) izražen je u svim dijelovima bubrežnog parenhima zdravih bubrega, najviše u epitelnim stanicama proksimalnih kanalića. Glavna mu je uloga zaštita bubrega od akutne i kronične ozljede, upale i razvoja fibroze. Dijabetička nefropatija je vodeći uzrok kroničnog zatajenja bubrega, a obilježena je smanjenim izražajem BMP-7. Cilj našega rada bio je istražiti mijenja li se izražaj BMP-7 s napredovanjem humane dijabetičke nefropatije ovisno o stadijima bolesti. Imunohistokemijskom metodom analizirali su se arhivski uzorci materijala 30 bolesnika kojima je učinjena biopsija bubrega te potvrđena dijagnoza dijabetičke nefropatije. Rezultati su pokazali da je BMP-7 različito izražen u citoplazmi epitelnih stanica proksimalnih kanalića i podocita između pojedinih stadija dijabetičke nefropatije. U ranijim stadijima, u proksimalnim kanalićima i podocitima uočen je snažno pozitivan izražaj, dok je slab izražaj zabilježen u većini uzoraka kasnijih stadija bolesti. U zaključku, povećan izražaj BMP-7 u početnim stadijima dijabetičke nefropatije s kasnijim smanjenjem izražaja u poodmaklim stadijima naglašava ulogu BMP-7 u zaštiti strukture i funkcije bubrega. Daljnja istraživanja u bolesnika s dijabetičkom nefropatijom potrebno je usmjeriti na otkrivanje poremećaja na razini BMP-7 receptora i signalnih putova.

Ključne riječi: *Bubreg; Koštani morfogenetski protein-7; Dijabetička nefropatija; Bubrežni kanalići, proksimalni; Podociti; Imunohistokemija*