COL1A1 and COL3A1 elevation and regulation by β2-microglobulin in subsynovial connective tissues of patients undergoing hemodialysis with carpal tunnel syndrome

Kyoko Muneshige
Kitasato University School of Medicine
Kenji Onuma
Kitasato University School of Medicine
Yuji Yokozeki
Kitasato University School of Medicine
Koji Sukegawa
Kitasato University School of Medicine
Yuya Otake
Kitasato University School of Medicine
Yukie Metoki
Kitasato University School of Medicine
Mitsufumi Nakawaki
Kitasato University School of Medicine
Makoto Itakura
Kitasato University School of Medicine
Gen Inoue
Kitasato University School of Medicine
Masashi Takaso
Kitasato University School of Medicine
Kentaro Uchida (kuchida@med.kitasato-u.ac.jp)
Kitasato University School of Medicine

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Abstract

Purpose: Carpal tunnel syndrome (CTS) constitutes a typical complication of patients receiving long-term hemodialysis (HD). However, the mechanisms underlying the manifestation of CTS in patients receiving long-term hemodialysis remain to be studied. We investigated collagen gene expression in the subsynovial connective tissue (SSCT) of patients undergoing hemodialysis and evaluated the effect of β2-microglobulin (B2M) on collagen gene expression in cells derived from the SSCT.

Methods: To study the expression of collagen genes in the SSCT of HD and non-HD patients, SSCT specimens were obtained from 64 patients with CTS (47 non-HD and 17 HD) during carpal tunnel release (CTR). To evaluate the fibrotic condition of SSCTs, COL1A1 and COL3A1 expression were evaluated using qPCR. To evaluate the effect of B2M on collage genes, SSCT cells were stimulated in α-MEM with 0.5% fetal bovine (vehicle) or 1 or 10 μg/ml B2M.

Results: Expressions of both COL1A1 and COL3A1 in the HD group were significantly higher than those in the non-HD group (COL1A1, P=0.021; COL3A1, P=.0.031). Incubating SSCT cells together with B2M for 6 h and 24 h led to increased mRNA expression of COL3A1 compared to that observed in vehicle control cells ($p<0.001$

Conclusions: Our findings suggest that increased expression of COL1A1 and COL3A1 may be one mechanism by which HD patients develop CTS. Further, B2M may be responsible for elevating COL3A1 expression in HD patients with CTS.

Introduction

Carpal tunnel syndrome (CTS) constitutes a typical complication of patients receiving long-term hemodialysis (HD) [1–6]. Its prevalence shows an association with the length of hemodialysis [1, 4], with CTS having been reported in 32–50% of patients over 10 years following the initiation of hemodialysis [4, 7] and in 80% of patients more than 30 years later [7]. However, the mechanisms underlying the manifestation of CTS in patients receiving long-term hemodialysis remain to be studied.

According to recent studies, fibrosis associated with increased collagen synthesis in the subsynovial connective tissue (SSCT) surrounding the median nerve and tendon could be a marker of the development and pathology of idiopathic CTS [8, 9]. However, several types of organ and tissue fibrosis are observed in patients with hemodialysis [10, 11]. One type, known as dialysis-related amyloidosis, involves the laying down of insoluble fibrils of β2-microglobulin (B2M) [12, 13]. According to several reports, B2M may act as an inflammatory cytokine, promoter of aging, and fibrosis-related factor [14–19]. Further, B2M plays a role in the formation of fibrosis in the kidney, heart, and liver [20–23]. Importantly, amyloid deposits are present in the synovial tissue of patients receiving dialysis with CTS [24–26]. However, how B2M affects collagen synthesis in the SSCT remains unclear.

We hypothesized that levels of collagen are increased in the SSCT of patients receiving hemodialysis with CTS and that B2M controls these changes. To test this, we investigated collagen gene expression in the SSCT of patients undergoing hemodialysis and evaluated the effect of B2M on collagen gene expression in cells derived from the SSCT.

Materials And Methods

Patients

The present study was approved by the Ethics Committee at our institute (Clinical Research Review Board of the Kitasato Institute; reference number: KMEO B13-113) and abides by the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. All participants gave written informed consent.

To study the expression of collagen genes in the SSCT of HD and non-HD patients, SSCT specimens were obtained from patients with CTS during carpal tunnel release (CTR). A total of 110 patients underwent CTR. Given that the body mass index (BMI) is a reported risk factor for the formation of CTS [27], we excluded 46 patients who were overweight or obese (BMI ≧ 25 kg/m). All patients underwent diagnostic neurophysiological tests comprising electromyography and nerve conduction studies based on the American Association of Electrodiagnostic Medicine standards and received confirmed diagnosis of CTS [28]. We further excluded patients with a history of thyroid disease, rheumatoid arthritis, osteoarthrosis, degenerative joint disease, flexor tendinitis, sarcoidosis, amyloidosis, peripheral nerve disease, or traumatic injuries to the ipsilateral arm based on their medical charts. Finally, 64 patients with CTS (47 non-HD and 17 HD) were included in this study.

In our study of the pathological role of B2M in patients with CTS, two men and four women with an age range of 52–71 years [mean ± standard deviation (SD), 66.8 ± 7.5 years] were excluded because they had a history of thyroid disease, rheumatoid arthritis, osteoarthrosis, degenerative joint disease, flexor tendinitis, sarcoidosis, amyloidosis, peripheral nerve disease, or traumatic injuries to the ipsilateral arm.

SSCT cell culture

SSCT specimens were treated with 20 ml of 0.1% type I collagenase derived from clostridium histoluticum (Wako Pure Chemicals, Osaka, Japan) for one day at 37°C. SSCT cells were maintained in culture for two weeks in α-MEM with 10% fetal bovine serum (FBS) and 20 mg/ml basic fibroblast growth factor (bFGF). To eliminate any effects of 10% FBS and bFGF on collagen gene expression, cells were washed twice with α-MEM and the medium was replaced α-MEM containing 0.5% FBS. Three hours after replacing the medium, SSCT cells were stimulated in α-MEM with 0.5% fetal bovine (vehicle) or 1 or 10 μg/ml B2M.

Real-time PCR

SSCT cells were homogenized with TRIzol Reagent using a homogenizer. The mixture was centrifuged to obtain the supernatant, from which total RNA was subsequently extracted by Direct-zolTM RNA Micro Prep (Zymo Research, Irvine, CA) based on the manufacturer's protocol. The SuperScriptIII First-Strand Synthesis Super Mix kit (Life Technologies, ThermoFisher, Waltham, MA) was then used to conduct cDNA synthesis, with the purified total RNA as the template. Primers used in the PCR assay are listed in Table 1. To examine *COL1A1* and *COL3A1* mRNA expression, real-time PCR analysis (MiniOpticon, Bio Rad, USA) was conducted using PCR Master Mix (Takara SYBR® PreMix Ex Taq II). mRNA expression levels were normalized to that of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and analyzed using the delta-delta CT method.

Statistical analysis

Shapiro-Wilk's test was used to ascertain whether the data were normally distributed. In the comparison of collagen expression in the SSCT of HD and non-HD patients, variables that were not normally distributed were analyzed using the Mann-Whitney test. In the study of the pathological role of B2M in patients with CTS, data were compared by one-way repeated measures ANOVA and pairwise comparison with Bonferroni adjustment. $P < 0.05$ determined in SSPS software v19.0 (IBM, USA) was used to indicate statistical significant.

Results

Patient demographics

Patients in the HD and non-HD groups showed comparable age and BMI (age, P=0.416; BMI, P=0.135; Table 2). The proportion of male to female patients was likewise similar between the two groups (P=0.0.152; Table 2).

Expression of collagen genes in SSCT of HD and non-HD patients

To evaluate the fibrotic condition of SSCTs, *COL1A1* and *COL3A1* expression were evaluated using qPCR. Expressions of both *COL1A1* and *COL3A1* in the HD group were significantly higher than those in the non-HD group (*COL1A1*, P=0.021; *COL3A1*, P=.0.031; Figure 1A, B).

Impact of B2M on collagen gene expression in SSCT cells

Incubating SSCT cells together with B2M for 6 h and 24 h led to increased mRNA expression of *COL3A1* compared to that observed in vehicle control cells ($p<0.001$, Fig. 2A). Further, according to Bonferroni's multiple comparisons test, stimulating SSCT cells with 10 ng/ml B2M for 24 h had no significant effect on collagen *COL3A1* expression relative to stimulation with 1 ng/ml B2M (p=0.103), but significantly increased expression compared to the control group (p=0.045). In contrast, SSCT cells stimulated with 10 ng/ml B2M for 6 h produced significantly higher levels of *COL3A1* than those stimulated with 1 ng/ml B2M (p=0.014) and the control group (p=0.0.017).

Meanwhile, neither 6-h (p = 0.881) nor 24-h stimulation (p=0.417) with B2M had any significant impact on *COL1A1* mRNA expression (Fig. 2B).

Discussion

The present study demonstrated that *COL1A1* and *COL3A1* levels were elevated in HD patients compared to non-HD patients with CTS. Further, stimulation of SSCT cells with B2M increased *COL3A1* expression but not *COL1A1* expression, suggesting that the presence of B2M in SSCT cells could contribute to the development of CTS in HD patients.

The SSCT of patients with CTS can develop various pathologies, such as proliferation with thickening of the tendon sheath, fibrosis, and vascular lesions, which can include thickening of vessel walls, intimal hyperplasia, vascular proliferation, and thrombosis [8, 29–33]. Such pathological alterations arise when cells interact with extracellular matrix components like collagen, elastin, and proteoglycans [33–35]. Prior research has reported elevated expression levels of fibrotic factors such as type and type collagen in the SSCT of CTS patients [36]. In addition, an immunohistochemical study reported significantly more intense staining of collagen type III fibers in CTS patients relative to a control group. Further, expressions of *COL1A1* and *COL3A1* have also been shown to be higher in patients with idiopathic CTS compared to a control group consisting of fresh cadavers. Moreover, type III collagen-positive cells located in the SSCT of patients with CTS are significantly larger than those in healthy controls [36]. This evidence suggests that COL3A1 may be involved in the development of CTS. In the present study, we showed that *COL3A1* levels were elevated in HD patients compared to non-HD patients, suggesting that increased *COL3A1* may contribute to CTS pathology in HD patients.

B2M, a small membrane protein that binds the heavy chains of class I major histocompatibility complex found on nucleated cells [37], is typically removed by glomerular filtration or later by proximal tubular reabsorption and catabolism [37]. However, patients with end-stage renal disease have significantly reduced catabolism. This leads to increased plasma B2M, which, if allowed to accumulate long-term, deposits into tissues [37]. Prior research conducted using microarray reported that treatment with B2M increased *COL3A1* expression in chondrocytes derived from osteoarthritis patients [38]. Consistent with this, the present study demonstrated that the presence of B2M stimulated *COL3A1* expression in SSCT cells. Thus, the accumulation of B2M in the SSCT cells of HD patients may contribute to the development of CTS by increasing the expression of *COL3A1*.

Conclusion

Our findings suggest that increased expression of *COL1A1* and *COL3A1* may be one mechanism by which HD patients develop CTS. Further, B2M may be responsible for elevating *COL3A1* expression in HD patients with CTS.

Abbreviations

CTS: carpal tunnel syndrome; HD: hemodialysis; SSCT: subsynovial connective tissue; B2M: beta 2-microglobulin; CTR: carpal tunnel release; BMI: body mass index; FBS: fetal bovine serum; SD: standard deviation; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; MHC: major histocompatibility complex

Declarations

Ethics approval and consent to participate

This study was conducted with the approval of the Ethics Committee at our institution (Clinical Research Review Board of the Kitasato Institute; reference number KMEO B13-113) and abides by the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Consent for publication

Not applicable

Availability of data and materials

Datasets supporting the conclusions of this article are included within the article. The raw data can be requested from the corresponding author.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

KM, KO, YY, KS, YO, YM, MN, GI, MT and KU acquired the data. KM, GI, MT, and KU analyzed and interpreted the data. All authors are responsible for the study concept and design, and contributed to writing the manuscript. All authors read and approved the final manuscript.

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Tables

Table 1

Primers used in the study

Gene	Direction	Primer Sequence (5¢–3¢)	Product Size (bp)
COL1A1	F	CCCCGAGGCTCTGAAGGT	145
	R	CACCAGCAATACCAGGAGCA	
COL3A1	F	CCAGGAGCTAACGGTCTCAG	103
	R	CAGGGTTTCCATCTCTTCCA	
GAPDH	F	TGTTGCCATCAATGACCCCTT	202
	R	CTCCACGACGTACTCAGCG	

Table 2

Patients' demographic data

	Hemodialysis	Non-hemodialysis	P value
Age	69.4±6.6	70.3±11.9	P=0.416
Sex (Female/male)	10/7	17/30	P=0.152
BMI	20.4±2.6	21.4±2.3	P=0.135

BMI: body mass index

Figures

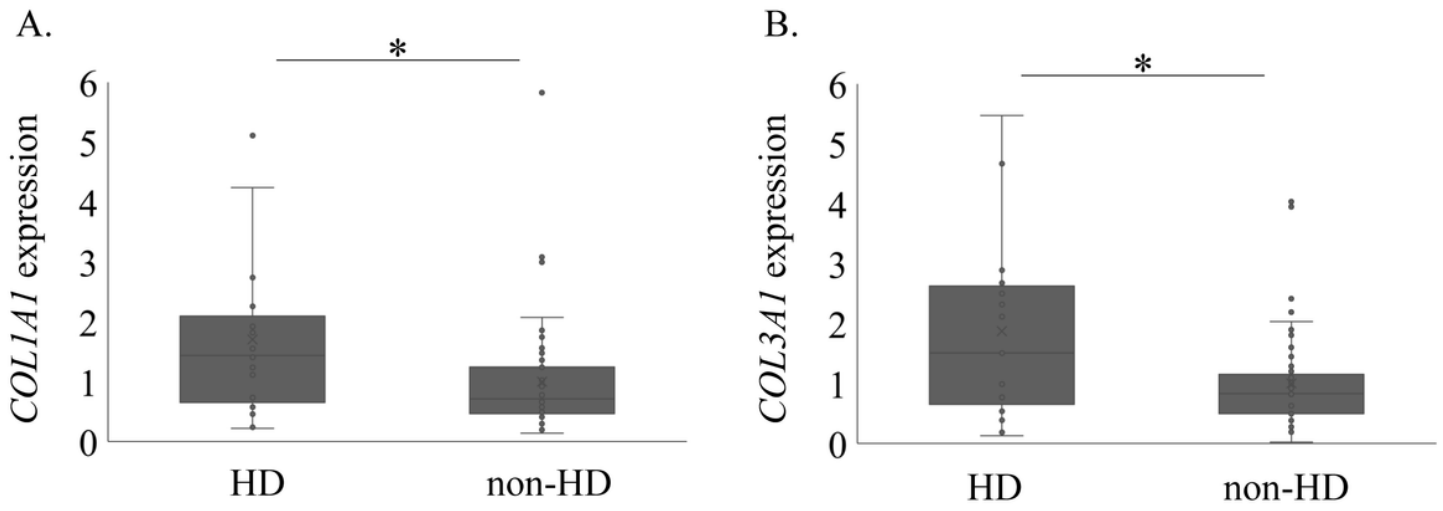

Figure 1

Expression of *COL1A1* and *COL3A1* in the subsynovial connective tissue of hemodialysis (HD) and non-hemodialysis (non-HD) patients with carpal tunnel syndrome

(A) *COL1A1* and (B) *COL3A1*. *p<0.05

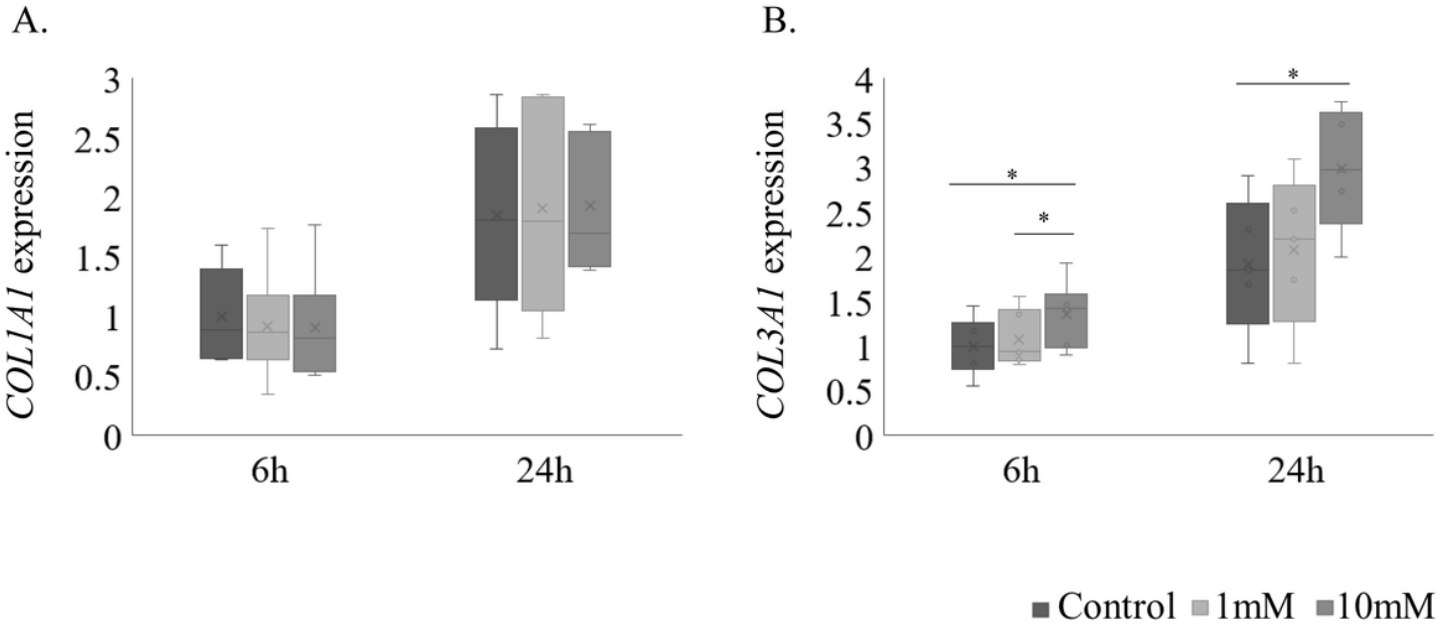

Figure 2

Expression of *COL1A1* and *COL3A1* in subsynovial connective cells following stimulation with B2M

(A) *COL1A1* and (B) *COL3A1* levels in subsynovial connective cells following stimulation with 0 (control), 1 or 10 ng/ml B2M. *p<0.05