Brief Report

Perioperative changes in ganglioside monosialodihexosylganglioside (GM3) molecular species for benign prostatic hyperplasia: a preliminary report

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Abstract: Benign prostatic hyperplasia (BPH) progresses with age and is associated with chronic inflammation. We focused on the relationship between BPH and ganglioside monosialodihexosylganglioside (GM3), a sialic acid-containing glycosphingolipid that is involved in chronic inflammation. GM3 molecular species would have a significant role in regulating inflammatory processes. In this prospective study, preoperative and postoperative serum samples were obtained from patients who underwent holmium laser enucleation of the prostate (HoLEP) for BPH. Preoperative and postoperative measurements of serum GM3 species were performed one month before and three months after HoLEP. Twenty-three patients were included in the study. The average patient age was 75 years, and the average prostate volume was 66 mL. The average weight of the surgically resected prostate tissue was 42 g. At three months after HoLEP, the serum concentration of GM3 species was found to have decreased after HoLEP compared with the preoperative concentration of GM3 species. Six GM3 species such as d18:1-17:0 [C17 acyl chain (-17:0) linked to a C18 sphingosine base with a double bond (d18:1-) by an amide linkage], were significantly reduced. The sample size was small; therefore, this study showed only preliminary results and could not evaluate prostate tissue inflammation. This study showed that the serum concentrations of several GM3 species, which indicate chronic inflammation, may be significantly reduced after BPH surgery.

Keywords: Benign prostatic hyperplasia (BPH); ganglioside monosialodihexosylganglioside (GM3); inflammation; lower urinary tract symptoms

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Introduction

Benign prostatic hyperplasia (BPH) affects over half of all men aged >50 years (1,2). The number of male patients undergoing BPH treatment, including drug therapy and surgery, is increasing (2). BPH progresses with age and is associated with chronic inflammation (3-7). Male patients with chronic inflammation of the prostate had a seven-fold higher risk of BPH than those without inflammation (4). Also, there was a significant correlation between the degree of prostate inflammation, prostate volume, and dysuria after BPH surgery (5,6). It is important to identify the

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relationship between BPH and chronic inflammation to determine the risk of BPH progression and understand its underlying mechanism, in order to achieve an impact on the course and treatment of BPH (7).

Gangliosides constitute one class of glycosphingolipid which contain one or more sialic acid residues (8). Gangliosides can modulate receptor mediated signal transduction within the plasma membrane. Ganglioside monosialodihexosylganglioside (GM3) species act as pro- and anti-inflammatory endogenous toll-like receptor 4 modulators, and potentially serve as diagnostic and therapeutic targets of chronic inflammation diseases (9,10). This study focused on the relationship between BPH and ganglioside GM3, a sialic acid-containing glycosphingolipid involved in chronic inflammation. Patients with chronic inflammatory diseases have increased pro-inflammatory serum GM3 molecular species levels (10,11). We hypothesized that there would be a difference in the levels of GM3, a marker of chronic inflammation, between pre- and post-treatment for BPH. This study aimed to characterize the changes in the levels of GM3 species before and after BPH treatment. We present this article in accordance with the STROBE reporting checklist (available at https://tau.amegroups.com/article/view/10.21037/tau-23-414/rc).

Methods

This study was approved by the Ethics Committee of the Tohoku Medical and Pharmaceutical University Hospital School of Medicine, Japan (No. 2017–2-120), and was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Written informed consent was obtained from all patients.

In this prospective study, the preoperative and postoperative serum samples were obtained from patients who underwent holmium laser enucleation of the prostate (HoLEP) for BPH between September 2018 and March 2020. The HoLEP procedure involves the removal of the line between the outer and inner glands of the prostate tissue and the removal of the inner gland. Therefore, HoLEP is one of the radical resection procedures for BPH. The enucleation procedure was performed following the anteroposterior dissection HoLEP method, as previously reported (12,13). Patients aged 50–90 years with symptoms of BPH for a duration of three months or more were included in the study. Indications for surgery were lower urinary tract symptoms refractory to medical therapy, maximum urinary flow rate ≤15 mL/s, International Prostate Symptom Score (IPSS) ≥8, and prostate volume ≥30 mL. Consecutive patients who met the inclusion criteria and consented to participate in the study were included. Patients who could not be followed up at 3 months after surgery, such as patients referred from distant locations, were excluded. Preoperative and postoperative serum measurements of GM3 species measurements were performed one month before and three months after HoLEP.

Twenty-three patients were included in the study. The median body mass index was 23.3 kg/m^2, 35% of patients had diabetes, and 65% had hypertension.

Total lipids were extracted from 40 µL of plasma, using hexane/methanol (1:4) mixtures containing 20 ng of GM3 (d18:1-(13 C)16:0) as an internal standard, at 40 ℃ for 30 min. After centrifugation at 12,000 ×g for 20 min, the supernatant was collected and combined with equal amounts of the upper phase of the hexane/methanol (2:1) mixture. The mixture was stirred and centrifuged at 1,500 rpm at room temperature for five minutes. The lower phase was collected and evaporated. The samples, which contained 50 ng of deuterium-labeled GM3 (d18:1-[^{2}H]16:0, d18:1-[^{2}H]24:0), were reconstituted with methanol and subjected to liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis, as described in a previous paper, with minor modifications (14). Quantification was conducted by LC-MS/MS analysis of serum GM3 species with differing acyl-chain structures. Each GM3 species were separated using Develosil carbon 30 column (C30-UG, 3 µm, 1×50 mm, Nomura Co. Ltd, Aichi, Japan). The abundance of GM3 molecular species detected by LC-MS was calculated based on the peak area of the internal standard [GM3 (d18:1-(13 C)16:0)], and it was indicated as a relative ratio. A total of 20 different GM3 molecular species were measured in this study (*Figure 1*).

Statistical analyses were performed using the Statcel-the Useful Addin Forms on Excel-4th edition. The paired sample *t*-test was used to evaluate changes in each GM3 species before and after HoLEP. P value of <0.05 was considered statistically significant.

Results

The median patient age was 75 years, and the median prostate volume was 66 mL. The median weight of the resected prostate tissue was 42 g. The median IPSS and quality of life (QOL) scores were 22 and 6, respectively. Based on preoperative uroflowmetry, the median maximal flow rate and postvoid residual urine were 7 mL/s and 100 mL,

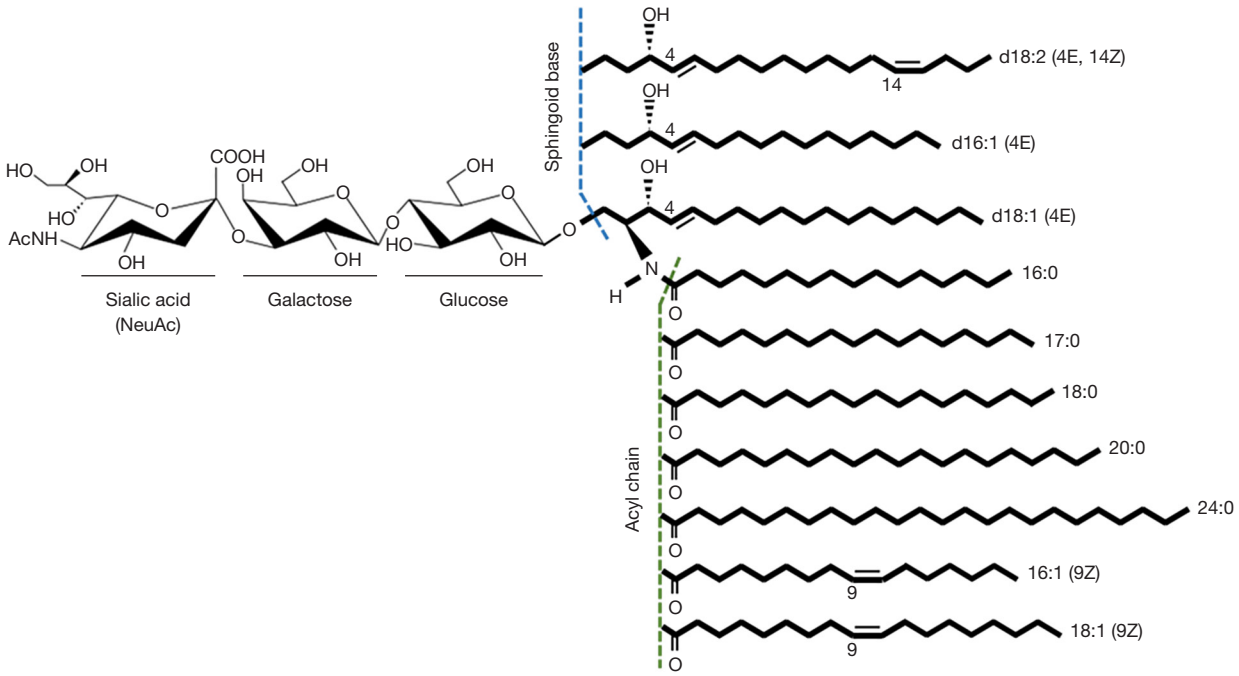

Figure 1 Molecular species of ganglioside GM3 in human serum.

Table 1 Patient background and result

Variable	Median [Q1–Q3]
Age (years)	75 [71–80]
Body mass index (kg/m^2)	23 [22–25]
Prostate volume (mL)	66 [52–83]
IPSS	22 [14–27]
QOL score	6 [6–7]
Maximum flow rate (mL/s)	7 [5–10]
Postvoid residual volume (mL)	100 [64–144]
Resected prostate volume (g)	42 [29–59]

IPSS, International Prostate Symptom Score; QOL, quality of life.

respectively (*Table 1*). At three months after HoLEP, the median IPSS and QOL scores were 5 and 2, respectively, while the median maximal flow rate and postvoid residual urine were 16 mL/s and 20 mL, respectively. Preoperative median white blood cell was 5,580/μL and C-reactive protein (CRP) was 0.2. Preoperative urine culture was performed in all patients. Five patients (22%) had positive cultures and were treated preoperatively with appropriate oral antibiotics.

A lower concentration of the GM3 species was detected after HoLEP. Specifically, the concentration of six GM3 species, notably, d18:1-15:0, d18:1-17:0, d18:1-20:1, d18:2-20:0, d18:2-23:0, and d18:2-24:0 was significantly reduced (*Table 2*). This study showed that the concentration of long-chain GM3 species such as d18:1-17:0 decreased significantly after HoLEP.

Discussion

Among the GM3 species examined in this study, gangliosides, which are glycosphingolipids (such as sialic acid) have at least 1,000 molecular species. Healthy human serum contains approximately 15 μg/mL of gangliosides, and more than 90% of these are GM3 species. Long-chain GM3 variations (16:0 and 18:0) have been used to inhibit inflammatory cytokine production in cases of chronic inflammatory disease (10). GM3 species have a key role in regulating inflammatory processes (15,16). GM3 species are correlated with insulin resistance, hypercholesterolemia, and the mediation of pro- or anti-inflammatory mechanisms by influencing toll-like receptors and consequently the production of pro-inflammatory cytokines (10,16).

The relationship between GM3 and BPH has not been previously reported; furthermore, there are no previous reports on the relationship of GM3 and prostatitis or prostate cancer. In HoLEP, the prostatic adenoma is

Table 2 Changes in molecular species of GM3 in human serum perioperative holmium laser enucleation of the prostate

Species	Preoperative value	Postoperative value	P value
d18:1-15:0	0.175	0.160	0.0270*
d18:1-17:0	0.117	0.106	0.0296*
d18:1-20:1	0.022	0.019	0.0183*
d18:2-20:0	0.270	0.244	0.0266*
d18:2-23:0	0.521	0.472	0.0352*
d18:2-24:0	1.505	1.373	0.0426*
d16:1-22:0	0.657	0.636	0.5949
d16:1-24:0	0.378	0.358	0.4738
d18:1-19:0	0.140	0.130	0.1932
d18:1-20:0	0.992	0.914	0.0998
d18:1-22:0	3.877	3.640	0.1285
d18:1-22:1	0.445	0.421	0.4899
d18:1-23:0	1.847	1.722	0.0771
d18:1-23:1	0.268	0.243	0.0849
d18:1-24:0	5.868	5.460	0.1320
d18:1-24:1	5.510	4.960	0.0826
d18:1-25:0	0.169	0.156	0.1078
d18:2-22:0	1.081	0.994	0.0516
d18:2-24:1	1.420	1.281	0.1536

GM3 was calculated based on the peak area of the internal standard [GM3 (d18:1-(13 C)16:0)] and was indicated as a relative ratio. *, P<0.05, univariate analysis of response. GM3, ganglioside monosialodihexosylganglioside.

completely enucleated, resulting in significant prostate volume reduction. This study examined changes in GM3 species levels, one of the markers of chronic inflammation, between the pre-operative and post-operative period to identify changes in chronic inflammation due to prostate volume reduction and improvement of urinary tract obstruction. LC-MS/MS analysis revealed a significant decrease in the levels of GM3 species, including sphingadienine-GM3 (d18:2-) and odd-chain fatty acid-GM3 (-17:0). These are unique GM3 species, and few studies have documented the changes in their serum concentration during the course of chronic inflammatory disease. Sphingosine-GM3 (d18:1-) and even-chain fatty acid-GM3 (-16:0, 24:0) species positively and negatively regulate inflammatory responses via toll-like receptor 4/myeloid differentiation factor 2 signaling (10). However, the effects of sphingadienine-GM3 (d18:2-) and odd-chain fatty acid-GM3 (-17:0) on inflammatory responses have not been clarified. This warrants further investigation into their bioactivities using chemical biology techniques.

This study had some limitations. First, the sample size was small and the changes in serum GM3 species were not solely influenced by prostate treatment: they were also affected by the patient's general condition. This study had a limited sample size; further research should be conducted using an appropriate sample size. Second, bladder inflammation was not evaluated in this study. HoLEP may help improve bladder inflammation by releasing lower urinary tract obstruction. Third, this study has not been able to evaluate prostate tissue inflammation. Further studies are needed to evaluate the relationship between the prostate tissue and GM3 species.

Conclusions

This study found that the serum concentrations of several GM3 species, which indicate chronic inflammation, may be significantly reduced after surgery for BPH. Further studies are required to establish the pathology underlying the changes in each GM3 species. Future research is needed to identify the GM3 species involved in BPH and examine whether there is a significant correlation with symptoms and course of treatment.

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Footnote

Reporting Checklist: The authors have completed the STROBE reporting checklist. Available at https://tau.amegroups.com/article/view/10.21037/tau-23-414/rc

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the institutional ethics committee of Tohoku Medical and Pharmaceutical University Hospital School of Medicine, Japan (No. 2017–2-120). Written informed consent was obtained from all patients.

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