

## Gastrointestinal Tolerance and Safety of 3'-Sialyllactose in Subjects Positive with *Helicobacter pylori*: A Pilot Study

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### Abstract

**Background:** This study assessed the gastrointestinal tolerance and safety of the human milk oligosaccharide 3'-sialyllactose sodium salt in subjects positive for *Helicobacter pylori*.

**Methods:** In a randomized, double-blind, placebo-controlled study, 48 adults positive for *H. pylori* were randomized to receive 12g of 3'-sialyllactose sodium salt or placebo (4g after breakfast, lunch, and dinner) for 4 weeks. We used one-way analysis of variance repeated measures analysis and a two-sample t-test to evaluate pre-and post-dose safety and efficacy. Physical examinations and clinical laboratory tests were performed to assess the primary endpoints of gastrointestinal symptoms, safety, and tolerability. The secondary endpoint was <sup>13</sup>C-urea breath test values at week 4 compared with baseline.

**Results:** There were no clinically significant differences between pre- and post-dose gastrointestinal tolerance and clinical chemistry (serum biochemistry, hematology, and urine analysis) outcomes. Pre- and post-dose urea breath test values were not significantly different within or between the treatment and placebo groups. Differences in physical examination and vital signs in the treatment and placebo groups were not statistically significant. Compliance and adverse events were similar between the two groups.

**Conclusions:** 3'-sialyllactose sodium salt was well tolerated, with no major side effects, but it did not effectively attenuate *H. pylori* positive status.

**Keywords:** 3'-Sialyllactose Sodium Salt; Human Milk Oligosaccharides; Gastrointestinal Tolerance; Safety; Adverse Events; *Helicobacter pylori*

### Abbreviations

3'-SL: 3'-Sialyllactose; 6'-SL: 6'-Sialyllactose; AEs: Adverse Events; ANOVA: One-Way Analysis of Variance; ALP: Alkaline Phosphatase; ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; BUN: Blood Urea Nitrogen; bw: Body Weight; CI: Confidence Interval; *H. pylori*: *Helicobacter pylori*; Hb: Hemoglobin; Hct: Hematocrit; HDL-C: High-Density Lipoprotein Cholesterol; HMO: Human Milk Oligosaccharide; GI: Gastrointestinal; GSRS: GI Symptom Rating Scale; KGCP: Korea Good Clinical Practice; LD<sub>50</sub>: Mean Lethal Dose; NOAEL: No Observable Adverse Effect Level; PLT: Platelet; RBC: Red Blood Cells; SL: Sialyllactose; T bil: Total Bilirubin; TC: Total Cholesterol; TG: Triglycerides; UBT: <sup>13</sup>C-Urea Breath Test; WBC: White Blood Cells

## Introduction

3'-Sialyllactose (3'-SL) sodium salt is an oligosaccharide that occurs naturally in human and bovine milk [1]. Sialyllactose (SL) has a combined structure of lactose and *N*-acetylneuraminic acid (also called sialic acid) [2]. The most abundant sialylated oligosaccharides in human milk are 6'-SL, disialyllacto-*N*-tetraose, sialyllacto-*N*-tetraose, and 3'-SL [3]. 6'-SL is the major sialylated oligosaccharide in human milk [3], with 3'-SL the predominant human milk oligosaccharide (HMO) beyond 2-4 months of lactation [4]. Data indicate that SL and sialylated oligosaccharides have significant health benefits for neonates [3,5]. These include the establishment and maintenance of healthy intestinal bacterial microflora that support development of the neonatal immune system [3,6-9]. Due to their negative charge and hydrophilic nature, sialylated HMOs also help modulate cell-cell interactions [5].

The safety of 3'-SL sodium salt has been proven in a series of toxicity studies that include gene mutation, *in vivo* and *in vitro* genotoxicity, and animal toxicity in beagle dogs and rats [10]. The no observable adverse effect level (NOAEL) of 3'-SL sodium salt was determined to be higher than 2,000 mg/kg body weight (bw)/day in an oral subchronic toxicity study in rats. The mean lethal dose (LD<sub>50</sub>) of 3'-SL sodium salt was well above 20 g/kg bw, indicating that the substance is an ordinary carbohydrate with the lowest toxicity rating. Results confirm that the toxicology profile of 3'-SL sodium salt is similar to those of other non-digestible carbohydrates and naturally occurring HMOs and support its safety for human consumption in foods.

Due to its antimicrobial activity, a few studies investigated the effects of 3'-SL in eradicating *H. pylori* [1,7,8]. Previous studies reported that 3'-SL was well tolerated in humans [1,7] and monkeys [8], but was not effective in eradicating *H. pylori*. We conducted this study to investigate the safety and tolerance of 3'-SL sodium salt in *H. pylori* positive adults since these subjects are more sensitive to digestive issues. This study first reports clinical chemistry data in humans consuming 3'-SL or 3'-SL sodium salt.

## Materials and Methods

### Study design

This study was conducted at Chungnam National University Hospital, Daejeon, South Korea. It was approved by the hospital's Institutional Review Board. This double-blinded, controlled, and randomized trial consisted of one 4-week treatment period with 3'-SL sodium salt or placebo powder administered three times a day, right after breakfast, lunch and dinner. The compliance of subjects was determined by evaluating unused investigational products returned to the pharmacy of clinical trials center on Day 28. The study was conducted in accordance with the principles of the Declaration of Helsinki and Korea Good Clinical Practice (KGCP) Guidelines.

### Participants

Males and females (aged 19 - 70 years) were screened by an in-person medical interview, physical examination, and clinical laboratory tests. We used the <sup>13</sup>C-urea breath test to determine *H. pylori* status in subjects; those with  $\geq 2.6$  per mil were considered *H. pylori* positive. No biopsies were conducted to confirm *H. pylori* infection. Exclusion criteria included evidence or history of ulcer or cancer or use of drugs that could affect *H. pylori* infection (e.g. steroids, bismuth compounds, proton pump inhibitors, or antibiotics) within 1 month prior to screening. Individuals were also excluded if they had a history of stomach or duodenum surgery, allergies to drugs or any clinically significant allergies, gastrointestinal disease (e.g. Crohn's disease), drug or alcohol abuse, or surgery (other than appendectomy or hernia repair) that could affect absorption of 3'-SL sodium salt. Females who were pregnant, lactating, or not using reliable contraception were also excluded, as were subjects with current gastrointestinal bleeding, any scheduled stomach or duodenal surgery during the study period, or a history of pyloric diseases, including obstruction, within 1 year prior to screening. All participants provided written informed consent.

Subjects were randomly assigned to one of two groups: 4 g of 3'-SL sodium salt three times per day after breakfast, lunch, and dinner or placebo powder. Baseline data collected prior to treatment included demographics (sex, age, height, weight, medications, and history of

clinical trial participation), vital signs, medical history, and outcomes from a physical examination, electrocardiogram, and clinical laboratory tests. We measured vital signs and performed physical examinations and clinical laboratory tests at screening/Week 0 and the final visit on Week 4. Compliance was assessed at Week 4. Adverse events were monitored throughout the study.

### Measurement of endpoints

The primary endpoint of safety included clinical chemistry and gastrointestinal tolerance of 3'-SL sodium salt. The secondary endpoint was delta  $^{13}\text{C}$ -urea breath test (UBT values) at week 4 compared with baseline.

### Gastrointestinal symptoms

A modified version of the GI symptom rating scale (GSRS) [11] was used to evaluate changes in perceived GI symptoms between baseline and Week 4. The modified GSRS included 5 questions pertaining to abdominal discomfort: stomach grumbling, bloating, belching, and flatulence. These were scored on a scale of 0 to 3, with 0 being 'no symptoms' and 3 being 'extreme symptoms.' One question each related to consistency and frequency of bowel movements. These were scored on a scale of 0 to 4, with 0 and 4 indicating opposite extremes and 2 indicating normal frequency and consistency.

### Clinical chemistry as a safety measure

An automatic analyzer (HITACHI 7080 Chemistry Analyzer, Hitachi, Japan) was used to measure the following serum biochemical parameters: aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT), blood urea nitrogen (BUN), high-density lipoprotein cholesterol (HDL-C), total bilirubin (T bil), total cholesterol (TC), triglycerides (TG), and albumin, creatine, and glucose. Hematological analyses included white blood cells (WBC), red blood cells (RBC), hematocrit (Hct), hemoglobin (Hb), and platelet (PLT). The urine analysis measured protein, glucose, RBC, and WBC.

### Other measurements

The secondary endpoint was delta  $^{13}\text{C}$ -urea breath test (UBT values) at Week 4 compared with baseline. Vital signs included blood pressure, pulse rate, body weight, height, electrocardiogram, and physical examination.

### Statistical analysis

We used repeated measures ANOVA and a two-sample t-test to compare the primary and secondary endpoints at baseline and Week 4.

## Results and Discussion

### Results

Although 121 subjects were screened, 73 were excluded for various reasons. A total of 48 candidates were enrolled in the study and were randomized to receive 12g of 3'-SL sodium salt ( $n = 24$ ) or a placebo powder ( $n = 24$ ) daily, 4g per serving right after breakfast, lunch, and dinner. Eight patients were terminated during the study; 40 completed it. Table 1 depicts demographic data. Both the treatment and placebo groups were similar in age, height, and weight. Those in the 3'-SL sodium salt treatment group were  $50 \pm 12$  years of age versus  $46 \pm 12$  years in the control group. Respective height and weight for the two groups were  $163 \pm 8$  cm versus  $166.3 \pm 7$  cm, and  $62 \pm 12$  kg versus  $65 \pm 12$  kg.

Parameters	Statistics	Treatment group	Placebo group
		N = 24	N = 24
Age (years)	Mean ± SD	50 ± 12	46 ± 12
	Min - Max	32 - 65	27 - 65
Height (cm)	Mean ± SD	163 ± 8	166.3 ± 7
	Min - Max	153 - 181	151 - 179
Weight (kg)	Mean ± SD	62 ± 12	65 ± 12
	Min - Max	42 - 92	46 - 92

**Table 1:** Demographic data of subjects in the 3'-SL sodium salt and placebo groups.

**Gastrointestinal symptoms**

Table 2 shows pre- and post-dose changes in gastrointestinal symptom scores between the treatment and control groups. The respective scores for the 3'-SL sodium salt subjects were 7.6 ± 1.4 (95% CI, 7.0 - 11.0) and 7.6 ± 1.1 (95% CI, 7.0 - 11.0). In the control group, they were 8.1 ± 1.4 (95% CI, 5.0 - 11.0) and 7.2 ± 1.1 (95% CI, 7.0 - 11.0). The pre- and post-dose differences in the treatment and control groups were not statistically significant (P = 0.91), nor was there a statistically significant difference by time (P = 0.16) or interaction effect between treatment group and time (P = 0.11). The difference in maximum decrease in gastrointestinal symptom scores between the treatment and placebo groups was not statistically significant.

	Changes of gastrointestinal symptom scores	
	0 week	4 weeks
Placebo (n = 23)	8.1 ± 1.4 (5.0 - 11.0)	7.2 ± 1.5 (2.0 - 10.0)
3'-SL (n = 17)	7.6 ± 1.4 (7.0 - 11.0)	7.6 ± 1.1 (7.0 - 11.0)

**Table 2:** Pre- and post-dose changes in gastrointestinal symptom score by group (N = 40).

*P* > 0.05 in all comparisons.

**Clinical chemistry tests**

As shown in table 3, there were no significant differences in pre- and post-dose clinical chemistry tests. We found no clinically significant changes between pre- and post-dose hematology tests (Table 3) or urinalyses (data not shown).

Parameter	Normal ranges in adults	Placebo		Treatment Group	
		0 week	4 weeks	0 week	4 weeks
AST	0~37 U/L (M) 0~31U/L (F)	22.7 ± 8.9	18.9 ± 4.7	19.8 ± 4.7	19.9 ± 4.8
ALT	0~41 U/L (M) 0~ 31 U/L (F)	22.5 ± 10.6	18.8 ± 6.6	18.5 ± 8.02	18.9 ± 7.5
ALP	40~130 U/L (M) 35~105 U/L (F)	72.8 ± 18.7	71.9 ± 20.6	72.7 ± 15.7	69.1 ± 16.4
T_bil	0.22~1.2 mg/dL	0.94 ± 0.27	0.88 ± 0.18	0.95 ± 0.45	0.93 ± 0.61
Albumin	4.0-5.0 g/dL	4.60 ± 0.23	4.6 ± 0.2	4.5 ± 0.22	4.5 ± 0.31
TG	45-150 mg/dL	126.6 ± 84.2	140.0 ± 111.3	92.0 ± 42.0	90.8 ± 46.4
TC	125-220 mg/dL	195.1 ± 37.8	198.7 ± 38.6	189.3 ± 34.0	187.9 ± 40.6
HDL-C	> 40 mg/dL	60.4 ± 15.8	60.2 ± 18.0	62.7 ± 13.4	63.1 ± 13.1
BUN	8 - 20 mg/dL	14.1 ± 3.9	12.47 ± 3.6	14.0 ± 4.0	12.5 ± 3.6
Creatine	0.8 - 1.2 mg/dL(M) 0.6 - 0.9 mg/dL(F)	0.76 ± 0.15	0.75 ± 0.14	0.74 ± 0.14	0.74 ± 0.14
Glucose	70-115 mg/dL	98.6 ± 9.9	98.8 ± 11.1	98.6 ± 8.4	96.2 ± 8.0
WBC	3.8~10.0 10 <sup>3</sup> /μL (M) 3.5~10.0 10 <sup>3</sup> /μL (F)	5.83 ± 1.51	5.90 ± 1.44	5.71 ± 1.35	5.97 ± 1.55
RBC	4.2~6.0 10 <sup>6</sup> /μL	4.74 ± 0.47	4.68 ± 0.44	4.50 ± 0.48	4.53 ± 0.47
HGB	13.5~17.5 g/dL (M) 12~16 g/dL (F)	14.6 ± 1.7	14.5 ± 1.5	13.6 ± 1.5	13.7 ± 1.5
HCT	39~53% (M) 36~46% (F)	42.8 ± 4.1	42.1 ± 3.6	40.2 ± 4.1	40.5 ± 4.2
PLT	130~400 10 <sup>3</sup> /μL	221.9 ± 48.1	221.2 ± 41.1	243.4 ± 49.3	247.2 ± 54.6

**Table 3:** Summary of clinical chemistry at Weeks 0 and 4 by group

Placebo vs. Treatment - *P* > 0.05 in all parameters at both week 0 and 4.

M: Male; F: Female; AST: Aspartate Aminotransferase; ALP: Alkaline Phosphatase; ALT: Alanine Aminotransferase; BUN: Blood Urea Nitrogen; HCT: Hematocrit; HDL-C: High Density Lipoprotein-Cholesterol; HGB: Hemoglobin; PLT: Platelet; RBC: Red Blood Cells; T bil: Total Bilirubin; TC: Total Cholesterol; TG: Triglycerides; WBC: White Blood Cells.

### <sup>13</sup>C-urea breath test values (UBT)

Table 4 shows the changes in UBT values between baseline and the Week 4 visit for the treatment and placebo groups. In the former, UBT at baseline was  $24.9 \pm 16.5\%$  (95% confidence interval [CI], 2.6 - 109.1) versus  $29.7 \pm 20.7\%$  (95% CI, 0.2 - 75.8) at the Day 28 post-dose visit. Baseline UBT in the placebo group was  $25.6 \pm 21.2\%$  (95% CI, 7.0 - 59.6), with a post-dose value of  $21.5 \pm 16.9\%$  (95% CI, 3.5 - 68.4). The changes were not statistically significant by treatment group ( $P = 0.47$ ), time ( $P = 0.92$ ), or interaction effect between treatment group and time ( $P = 0.16$ ).

	Changes in <sup>13</sup> C-urea breath test value	
	0 week	4 weeks
Placebo (n = 23)	$25.6 \pm 21.2$ (7.0 - 59.6)	$21.5 \pm 16.9$ (3.5 - 68.4)
3'-SL (n = 17)	$24.9 \pm 16.5$ (2.6 - 109.1)	$29.7 \pm 20.7$ (0.2 - 75.8)

**Table 4:** Pre- and post-dose changes in <sup>13</sup>C-urea breath test values ( $N = 40$ ).

$P > 0.05$  in all comparisons.

### Vital signs and physical examinations

There were no clinically significant pre- and post-dose vital sign changes over time in the treatment or placebo groups (data not shown). We found no clinically significant abnormalities or differences in the vital signs of any subjects, nor were there any clinically significant pre- and post-dose changes in physical examination findings for either group.

### Compliance

At week 4, post-dose compliance in the 3'-SL sodium salt group was  $91.3 \pm 6.1\%$ . It was  $94.2 \pm 5.5\%$  in the placebo group. The difference was not statistically significant.

### Adverse events

The number of adverse events (AEs) and the number of subjects who experienced them did not differ significantly between the treatment and control groups (data not shown). AEs included nausea, loose stools, diarrhea, abdominal pain, and epigastric pain. All were classified as mild, with no moderate or severe AEs reported in either group.

### Discussion

Oligosaccharides are the third most abundant component of human milk [12], with over 200 reported structures [5,13-15]. Our outcomes confirmed prior findings that 3'-SL sodium salt has high tolerability and a good safety profile. Compared with controls, all outcomes were statistically or clinically insignificant. Other human clinical studies have also found that 3'-SL sodium salt is well tolerated and causes no side effects at daily doses up to 20g [1,16,17]. Like other HMOs and dietary fiber ingredients [18], 3'-SL sodium salt has no adverse effects on clinical chemistry parameters or gastrointestinal tolerability.

Our data also support findings from 28- and 90-day animal toxicity studies in which 3'-SL sodium salt caused no treatment-related abnormalities in physical, physiological, biochemical, hematological, or histopathological parameters at daily doses of up to 2,000 mg/kg bw [10]. Evidence from bacterial reverse mutation, *in vitro* chromosomal aberration assay, and *in vivo* mouse micronucleus tests indicate that 3'-SL sodium salt is not mutagenic or genotoxic. The mean lethal dose was higher than 20 g/kg bw in rats. The toxicity profiles of 3'-SL sodium salt were comparable to other carbohydrates and HMOs.

Milk oligosaccharides promote health in a variety of ways, for example, through direct bacterial interactions, immunomodulatory activities, promotion of gut barrier function, and induction of protective transcriptional responses [19]. They enhance adhesion of commensal bacteria to host receptors [20] and alter mucin expression in ways that improve the protection offered by secretory proteins [21]. They also modulate host-cell surface glycosylation, reduce adhesion of enteropathogenic *Escherichia coli* to host cells *in vitro* [22], and upregulate many genes potentially involved in the adhesion process [19,20].

To date, outcomes from several studies support the potential of 3'-SL as a treatment or cure for *H. pylori* colonization. Antiadhesive therapy using 3'-SL has prevented the binding of *H. pylori* to various human gastrointestinal cells *in vitro* [23], and decreased *H. pylori* colonization in rhesus monkeys without side effects [8]. Hester, *et al.* [9] reported that both acidic and neutral HMOs inhibit rotavirus infectivity *in vitro* and in acutely infected piglets, with 3'-SL and 6'-SL inhibiting <sup>125</sup>I-radiolabelled rotavirus cellular binding and infectivity/replication *in vitro*.

However, the findings of this study are consistent with those of Parente, *et al.* [1] and Opekun, *et al.* [7], which showed no clinical benefit in the use of 3'-SL to decrease *H. pylori* infection in humans. Additionally, the study by Mysore, *et al.* [8] demonstrated that the use of 3'-SL was not effective in curing all monkeys infected with *H. pylori*. This lack of treatment efficacy could be due to many reasons, including insufficient dosing delivered at the gastric level or the expression of multiple specificities by bacteria. In the case of *H. pylori*, treatment failure is generally due to poorly designed regimens in terms of doses, frequency of administration, or duration of therapy [24]. Further analysis is needed to investigate the gap between data from *in vitro* or animal studies and human clinical trials.

Outcomes of this study must be interpreted with caution due to the small number of subjects, self-administration of treatment, short duration with no follow-up, and the potential effects of different diets on the absorption and efficacy of 3'-SL sodium salt in the amelioration or eradication of *H. pylori* infection.

## Conclusion

Oral administration of 4 g of 3'-SL sodium salt each time right after breakfast, lunch, and dinner for 28 days in adults positive for *H. pylori* was well tolerated and safe, with no significant changes in clinical chemistry compared with placebo groups. But 3'-SL did not effectively suppress or eradicate *H. pylori* infection or improve gastrointestinal symptoms. The study adds to the limited literature on the potential efficacy and safety of 3'-SL sodium salt as an antibacterial agent in infected adults.

## Trial Registration

This study was registered at Clinical Research Information Service (CRIS), South Korea (CRIS Registration Number KCT0002430). This trial was registered retrospectively (study start date, November 01, 2010; date of registration, August 25, 2017).

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## Conflict of Interest

RBG, JW, and DHK are employees of GeneChem Inc., the sponsor of the study. Other authors (SSC and JHH) declared that they have no conflict of interests.

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