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Original Article

Effect of Isosorbide Dinitrate Spray on Esophageal Peristalsis in Patients with Distal Esophageal Spasm

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ABSTRACT

Background: Nitric oxide (NO) has been showed to modulate esophageal peristalsis contractions in healthy humans.

Objective: To study if isosorbide dinitrate (ISDN) which is an exogenous NO donor can restore esophageal peristalsis contractions in symptomatic patients with distal esophageal spasm.

Methods: Ten patients were randomized to undergo high resolution manometry (HRM) with ISDN spray (1.25 mg/puff) or normal saline (NSS) spray, in 2 times at least 48 hours apart, in a crossover randomized controlled trial (assessor blind). For each HRM study, esophageal contractions in response to 12 wet swallows were studied at baseline, after the first 1-puff and the second 1-puff of test agents. Esophageal contraction parameters were analyzed using ManoView analysis software version 2.0.1.

Results: Prevalence of esophageal peristalsis contractions was similar at baseline (p>0.05) and increased by ISDN significantly only after the first dose (65% vs. 50%, p=0.045) but not the second dose compared to NSS (p>0.05). ISDN decreased esophageal distal contractile integral (DCI) after the first dose (1421±839 vs. 2363±1581 mmHg $s^{-1}cm^{-1}$, p=0.050) and significantly decreased DCI after the second dose (1399±739 vs. 2409±1289 mmHg $s^{-1}cm^{-1}$, p=0.006) compared to NSS. ISDN significantly increased residual upper esophageal sphincter (UES) relaxation pressure after the first dose (0.5±3.7 vs. –4.0±6.2 mmHg, p=0.026) and the second dose (1.2±4.5 vs. –3.9±6.5 mmHg, p=0.027) compared to NSS. However, there was no significant difference of pressurization front velocity (PFV), 4-second integrated relaxation pressure (IRP) and UES resting pressure comparing between ISDN and NSS at baseline, after the first dose and the second dose of the test agents (p>0.05).

Conclusions: In patients with distal esophageal spasm, proportion of esophageal peristalsis contraction was increased overtime after HRM catheter insertion. ISDN significantly improved esophageal peristalsis contractions earlier than NSS, decreased DCI or force of contractions, and increased residual UES relaxation pressure. This study suggests the role of central nervous system or esophageal adaptation to local stimulus and exogenous NO on the restoration of esophageal peristalsis contractions in patients with distal esophageal spasm.

Key words : Nitric oxide, esophageal peristalsis, esophageal spasm

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Introduction

Esophageal motility involves co-ordinated contractions in the esophageal body, and relaxation of the upper and lower esophageal sphincters. Normal functioning of the two sphincters and esophageal peristalsis requires fine neuromuscular co-ordination. Motor disorders of the esophagus are characterized by failure of such co-ordination, with abnormal esophageal peristalsis and/or sphincter relaxation[1]. Diffuse esophageal spasm (DES) is characterized by normal peristalsis intermittently interrupted by simultaneous contractions. The first description of esophageal spasm is attributed to Osgood, who, in 1889, described six patients with severe chest pain and dysphagia with meals[2]. The first recorded description of the manometric features of DES was made by Creamer *et al*. in 1958[3] and by Roth *et al*. in 1964[4], in which they found repetitive simultaneous pressure waves with sustained contractions in the distal third of the esophagus. Diffuse esophageal spasm is defined manometrically by the presence of simultaneous contractions after 20% or more of wet swallows in the distal esophageal body with normal peristalsis. Other manometric findings less consistently found include long-duration contractions, repetitive waves (three peaks or more), spontaneous non-swallow-induced contractions, and abnormalities of lower esophageal sphincter (LES) pressure or relaxation[5]. Sperandio M and Tutuian R, *et al*. have been proposed that the term diffuse esophageal spasm is a misnomer and is more appropriately described as "distal" esophageal spasm because most of the simultaneous contractions are concentrated in the distal esophagus[6].

The prevalence of DES is less than 10% in patients with chest pain and/or dysphagia, and is 3-4% in series of unselected patients undergoing esophageal manometry[7,8]. There are no data on the impact of age, gender, or race on the prevalence of DES. Nevertheless, it is seen most commonly in patients older than 50 years. Recurrent chest pain and dysphagia are the most common presenting symptoms. The symptoms of DES, however, are very variable, ranging in severity from mild to severe, lasting from seconds to minutes, occurring in a variety of locations, may be precipitated by solids or liquids, or can occur independently of eating[5,9].

The etiology of DES remains unknown, even though physiologically impaired deglutitive inhibition has been suggested. The dominant concept of the pathophysiology of DES is that it involves an impairment of inhibitory innervations leading to both premature and rapidly propagated or "simultaneous" contractions in the distal esophagus. In the proximal esophagus, peristalsis is directed by excitatory vagal motor neurons that arise in a nucleus ambiguous of the brain stem and that innervate the striated muscle esophagus in a craniocaudal distribution. However, in the distal esophagus, composed of an increasing proportion of smooth muscle, peristalsis is controlled by interactions among the central nervous system (CNS), the peripheral nervous system (PNS), and the smooth muscle of the esophagus. The role of the CNS in modulating peristalsis in the smooth muscle esophagus has yet to be clearly defined. On the contrary, it appears that the progressive nature of peristalsis in the smooth muscle esophagus is programmed peripherally[10,11]. The esophageal smooth muscle was found to exhibit many unique responses due to the intramural nerves, including 1) the off or rebound contraction, and 2) the latency gradient. Christensen and colleagues found that the circular smooth muscle of the esophagus remained quiescent during electrical stimulation but contracted only on cessation of the stimulus. They concluded that esophageal circular muscle contraction was a rebound following stimulation of the inhibitory nerves[12]. The ultimate contraction is mediated via excitatory (cholinergic) and inhibitory (nitric oxide) ganglionic neurons. The degree of cholinergic and noncholinergic inhibitory influence varied at different levels of the esophagus so that cholinergic influence was most marked in the proximal parts of the esophagus and decreased distally along the esophagus. On the other hand, the inhibitory nerve influence was least prominent in the proximal strips and increased distally along the esophagus. Upon stimulation of the inhibitory nerves alone, the latency of contraction increases gradually distally along the esophagus, resulting in peristaltic sequence of contraction that is entirely located locally in the wall of the esophagus. However, cholinergic nerves further reduce the latency, particularly in upper levels of the esophagus because of their greater influence in the upper esophagus[13,14].

Nitric oxide (NO) is the primary inhibitory nonadrenergic non-cholinergic (NANC) neurotransmitters in the gastrointestinal (GI) tract. It is synthesized by the activation of neuronal NO synthase (NOS) in the myenteric plexus. Its release in response to nerve stimu-

lation of the myenteric plexus causes relaxation of the smooth muscle. It regulates esophageal inhibition, LES relaxation, latency gradient and contraction amplitude of esophageal peristalsis[15-17]. Therefore, inhibition of NOS affects the timing of the off-response. The period from the end of stimulus to the beginning of the off-response (latency) is shortened by NOS inhibitors. Shortening becomes progressively more pronounced in the distal esophagus so that the greatest decrease in latency occurs in muscle strips taken from just above the LES. The result is a loss of latency gradient along the length of smooth muscle[11].

Several animal and human experiments showed that inhibition of NO in control subjects induced simultaneous esophageal contraction from reduction in inhibitory function. Administration of the NO scavenger human recombinant hemoglobin in healthy volunteers produced simultaneous contractions and chest pain[18-20]. Additionally, the administration of NOS blocker NG-monomethyl-L-arginine in humans provoked a significant reduction in the latency period between swallows and the onset of contractions, resulting in increased propagation velocity of the peristaltic waves in the distal esophagus[21]. Then they supported the hypothesis that NO played an important role in maintaining esophageal peristalsis. The currently accepted concept attributes distal esophageal spasm to an altered endogenous NO synthesis and/or degradation[22,23].

Nitrates are the potent relaxants of gastrointestinal (GI) smooth muscle through the stimulation of cyclic guanosine monophosphate (cGMP)-dependent pathway. The administration of nitrates which is NO donors has been proposed to treat distal esophageal spasm based on the pathophysiological mechanisms. Several studies revealed that nitrates improved symptoms and esophageal peristalsis from manometric findings[23-29]. However, all studies were open label including a small number of patients and no placebo-controlled trials have been completed. Furthermore, they used conventional manometry as the tool for assessment of esophageal peristalsis.

To date, the pathophysiology of distal esophageal spasm is not completely clear. Thus, the aim of this randomized cross-over study was to investigate the role of depletion in NO as one of the pathogenesis in symptomatic patients with distal esophageal spasm by administration of isosorbide dinitrate (ISDN) which is exogenous NO donor. The primary research question was whether NO played a major role in helping patients with distal esophageal spasm by restoring their normal peristalsis. The secondary research question was to determine the rate of normal esophageal peristalsis in patients with distal esophageal spasm after we increased the amount of NO.

In this study, we used HRM to study the esophageal contractions. HRM is now the method of choice to assess esophageal contractile function. The concept of HRM is to overcome the limitations of conventional manometric systems by using advanced electronic technologies and increasing the number of pressure sensors on the manometric assembly. A sufficient number of pressure sensors which spaced about 1 cm apart so that, by interpolating between adjacent sensors, intraluminal pressure can be viewed as a continuum throughout the length of the esophagus and adjacent sphincters without significant loss of contractile information. Besides, it provides visual assessment of esophageal peristalsis, propagation velocity of the esophagus, amplitude of distal esophageal contraction, and EGJ function among other manometric parameters.

Material and Methods

Study patients

Patients who underwent HRM (ManoScan360TM (Sierra Scientific Instruments Inc., Los Angeles, CA)) and were diagnosed distal esophageal spasm between December 2011 and November 2012 at King Chulalongkorn Memorial Hospital (KCMH) were recruited. The patients who met the following criteria were enrolled in the study. The inclusion criteria were 1) patients were older than 18 years of age, 2) patients who stopped the medication known to potentially interfere with esophageal motor functions and smooth muscle relaxant at least 3 days prior to undergoing HRM (e.g., nitrate, anticholinergic, prokinetic drug and calcium channel blocker), and 3) patients were willing to participate in this study. The exclusion criteria were 1) patients with history of low blood pressure (less than 90/60 mmHg), 2) patients with history of hypersensitivity to ISDN spray or their ingredients, 3) patients with disease that could be worsened by nitrate drugs such as hypertrophic obstructive cardiomyopathy, constrictive pericarditis, etc., and 4) patients who were using phosphodiesterase 5 (PDE5) inhibitor agents such as sildenafil, vardenafil, and tadalafil because concur-

rent using of PDE5 inhibitor agents with nitrate drugs can worsen the low blood pressure condition. This study was approved by the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University and informed consent was obtained from each subject.

Study design

This crossover randomized controlled study (assessor blind) was conducted at King Chulalongkorn Memorial Hospital. Prior to manometry study, the patients were assigned to complete a questionnaire regarding their esophageal dysmotility symptoms and underlying diseases. Manometric studies were done with the patients in the upright position after at least a 6-hour fast. The HRM catheters were solid-state assemblies with 36 circumferential sensors at 1-cm intervals (Given Imaging, Los Angeles, CA). Transducers were calibrated at 0 and 300 mmHg using externally applied pressure. The manometric assemblies were placed transnasally and positioned to record from the hypopharynx to the stomach. All patients were randomized to undergo HRM with ISDN spray (1.25 mg/puff) or normal saline (NSS) spray which was placebo, in 2 occasions at least 48 hours apart, in a randomized cross-over fashion. One actuation (0.05 mL) of ISDN spray (Isomack®) contains 1.25 mg isosorbide dinitrate in form of oromucosal spray. After sprayed into the oral cavity, the active drug, ISDN, is rapidly absorbed by the mucosa. Pharmacological effects can be observed within 1-3 minutes after administration of isomack® spray with maximal plasma levels within 3-6 minutes. Elimination takes place with a half-life of 30-60 minutes. Within a period of 90-120 minutes, plasma concentration drops to baseline values again. ISDN is metabolized to isosorbide-2-mononitrate and isosorbide-5-mononitrate which have a terminal half-life of 1.5 to 2 and 4 to 6 hours, respectively. Both metabolites are pharmacologically active.

For each HRM study, after a 10-minute rest period, the esophageal contractions in response to 12 wet swallows were studied at baseline, 7 minutes after the first 1-puff and the second 1-puff of ISDN or NSS spray. But only the first 10 wet swallows at baseline, 7 minutes after the first 1-puff and the second 1-puff of ISDN or NSS spray were analyzed using ManoView analysis software. Blood pressure and pulse rate were monitored during HRM examination. All adverse events from test agents were recorded.

HRM analysis

In this study, the esophageal contractions were classified according to the Chicago Classification of esophageal motility[30]. Each swallow was characterized as normal peristalsis if pressurization front velocity (PFV) was <8 cm/s, distal contractile integral (DCI) was <5000 mmHg $s^{-1}cm^{-1}$, esophagogastric junction (EGJ) pressure was normal (10-35 mmHg), and deglutitive relaxation was normal (eSleeve 3-s nadir <15 mmHg). Simultaneous contraction (spastic contraction) was characterized on the basis of rapidly propagated pressurization if PFV was >8 cm/s (increased PFV attributable to rapid contractile wavefront). In addition, distal esophagus was divided into 2 sub-segments as segment 2 (S2) and segment 3 (S3). Therefore, segmental esophageal spasm was defined as simultaneous contraction which limited to S2 or S3. Diffuse esophageal spasm was defined as simultaneous contraction which involved both S2 and S3 (Figure 1). Aperistalsis was defined as no continuous pressure domain above an isobaric contour (IBC) of 30 mmHg in the distal esophageal segment in any swallow.

Moreover, we also analyzed other esophageal contraction parameters, i.e. PFV for proximal esophagus (S1), residual upper esophageal sphincter (UES) relaxation pressure, 4-second integrated relaxation pressure (4-second IRP), UES resting pressure, and distal contractile integral (DCI) using ManoView analysis software version 2.0.1 (Sierra Scientific Instruments Inc., Los Angeles, CA).

The HRM analysis was performed by a same researcher. Before analysis, each manometric studies were renamed by other staff to blind the assessor from the patients' names and test agents.

Statistical analysis

This is a pilot study thus no sample-size calculations were performed. Ten patients with diffuse or segmental simultaneous contractions of the esophagus ≥ 20% of wet swallows were randomly assigned to undergo HRM with ISDN spray or NSS spray, in 2 occasions at least 48 hours apart, in a randomized cross-over fashion.

Qualitative data were reported as a percentage or frequency, and compared between groups using Chi-squared or Fisher's exact test. Quantitiative data were reported as mean and standard deviation (SD), and compared between groups using paired *t*-test. All *P*

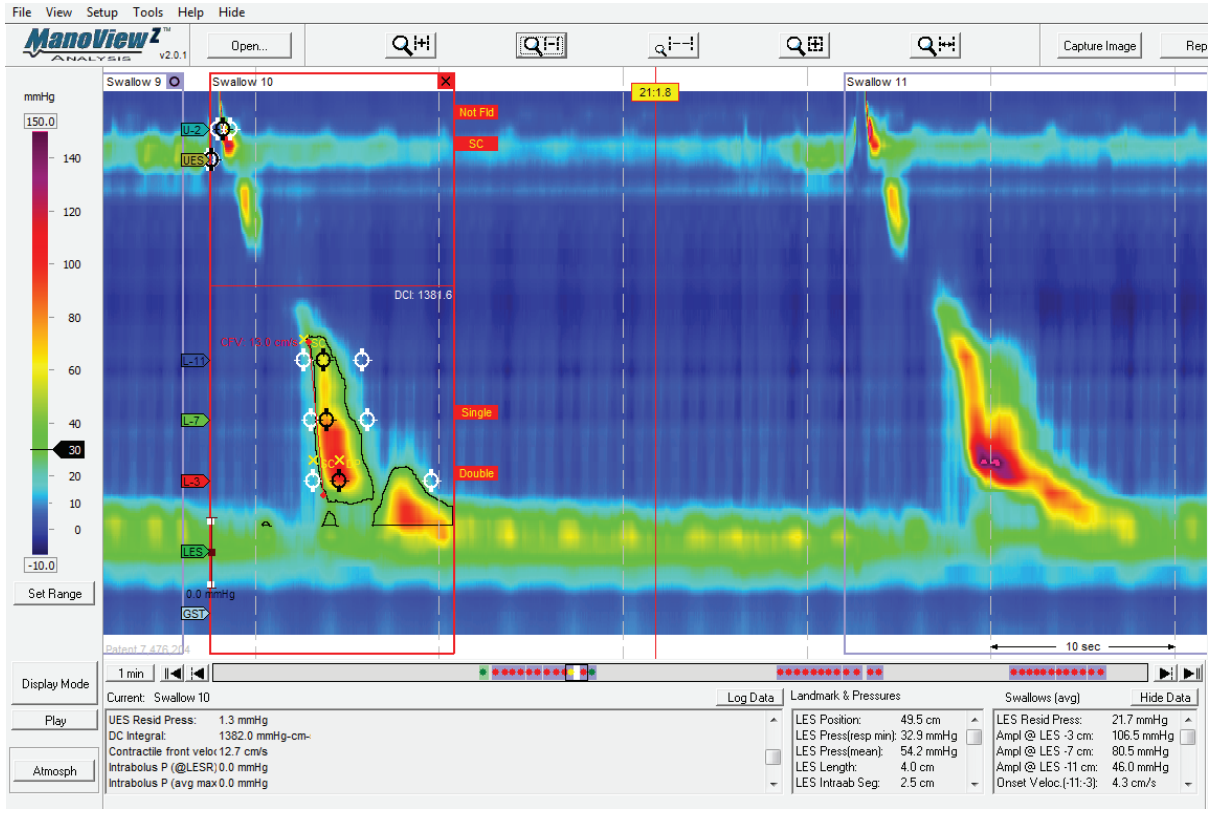

Figure 1. Swallow pressure topography spanning from the pharynx to stomach of a patient with simultaneous contraction. The heavy black line delineates the pressure domain ≥30 mmHg. The PFV of the 30 mmHg isobaric contour domain is 13.0 cm/s and would fit criteria for a rapid contraction.

values were two-tailed with the level of significance defined at less than 0.05. Data analysis was performed using Excel and Statistical Package for the Social Sciences (SPSS) 17.0.

RESULTS

Demographic and patient characteristics

Ten patients with diffuse or segmental simultaneous contractions of the esophagus ≥ 20% of wet swallows were enrolled. All patients completed the studies. The mean age was 49 ± 10 years, and 8 were females (80%). The mean esophageal length was 24.4 ± 1.4 cm. The mean resting pressure of UES was 33.1 ± 10.1 mmHg. The mean resting pressure of LES was 28.8 ± 13.7 mmHg.

Chief complaint of esophageal dysmotility symptoms was globus sensation in 5 (50%), dysphagia in 3 (30%), chest pain in 1 (10%) and food regurgitation in 1 patient (10%). The mean duration of chief complaint was 32.0 ± 37.7 months. Three patients (33.3%) had co-morbidities including diabetes, hypertension and chronic kidney disease in 1, systemic sclerosis in 1 and dyslipidemia in 1 patient.

All patients were symptomatic at the time of diagnosis and presented with globus sensation (n =8), dysphagia (n =7), acid regurgitation (n =5), heartburn (n =5), chest pain (n =5), food regurgitation (n =4), nausea/vomiting (n =3) and anorexia (n =2). Among 7 patients who had dysphagia, the symptom was triggered by swallowing of both solid and liquid food in 6 (85.7%) and solid food in 1 patient (14.3%). Dysphagia occurred intermittently in 6 (85.7%) and persistently in 1 patient (14.3%). The baseline esophageal dysmotility symptoms are summarized in Table 1.

Swallow subtypes according to the Chicago classification

According to the Chicago Classification of HRM, we determined the swallow subtypes as simultaneous contraction, aperistalsis and peristalsis contraction to compare between the ISDN group and the NSS group

Table 1. Summary of the esophageal dysmotility symptoms of 10 patients with distal esophageal spasm.

Symptom	Number of patient (%)	Frequency of symptom (%)		Symptom duration (month) mean ± SD	Severity (%)		
		≤ 1 d/wk	> 1 d/wk		mild	moderate	severe
Dysphagia	7 (70%)	2 (28.6%)	5 (71.4%)	39.5 ± 43.5	0	5 (71.4%)	2 (28.6%)
Globus	8 (80%)	1 (12.5%)	7 (87.5%)	15.9 ± 19.1	0	6 (75%)	2 (25%)
Acid regurgitation	5 (50%)	2 (40%)	3 (60%)	26.7 ± 30.6	1 (20%)	4 (80%)	0
Food regurgitation	4 (40%)	2 (50%)	2 (50%)	19.8 ± 27.0	1 (25%)	3 (75%)	0
Heartburn	5 (50%)	2 (40%)	3 (60%)	39.8 ± 50.6	1 (20%)	4 (80%)	0
Chest pain	5 (50%)	2 (40%)	3 (60%)	38.5 ± 51.6	1 (20%)	3 (60%)	1 (20%)
Nausea/Vomiting	3 (30%)	2 (66.7%)	1 (33.3%)	22.3 ± 32.7	1 (33.3%)	2 (66.7%)	0
Anorexia	2 (20%)	1 (50%)	1 (50%)	35.0 ± 35.4	1 (50%)	1 (50%)	0

Table 2. The distribution of the Chicago Classification swallow subtypes comparing between the ISDN group and the NSS group at baseline, after the first dose and the second dose of test agents.

Agent	Simultaneous contraction (%)	Aperistalsis (%)	Peristalsis contraction (%)	*p*-value
Baseline of ISDN group	49	8	43	0.876
Baseline of NSS group	47	10	43	
After the first dose in ISDN group	25	10	65	0.017
After the first dose in NSS group	44	6	50	
After the second dose in ISDN group	25	6	69	0.542
After the second dose in NSS group	32	5	63	

at baseline, after the first dose and the second dose of test agents (Table 2). There were no significant differences in percentage of swallows in each subtype in the ISDN group and the NSS group at baseline. Interestingly, there were significant differences in percentage of swallows in each subtype in the ISDN group and NSS group only after the first dose ($p = 0.017$) but not after the second dose of test agents ($p = 0.542$).

There were no significant differences in percentage of simultaneous contractions at baseline (ISDN 49% vs. NSS 47%, $p = 0.887$) and significant decrease in ISDN group only after the first dose (ISDN 25% vs. NSS 44%, $p = 0.007$) but not after the second dose (ISDN 25% vs. NSS 32%, $p = 0.347$) compared to NSS group (Figure 2). Likewise, there were no significant differences in percentage of esophageal peristalsis contractions at baseline (ISDN 43% vs. NSS 43%, $p = 1.000$) and significant increase in ISDN group only after the first dose (ISDN 65% vs. NSS 50%, $p = 0.045$) but not after the second dose (ISDN 69% vs. NSS 63%,

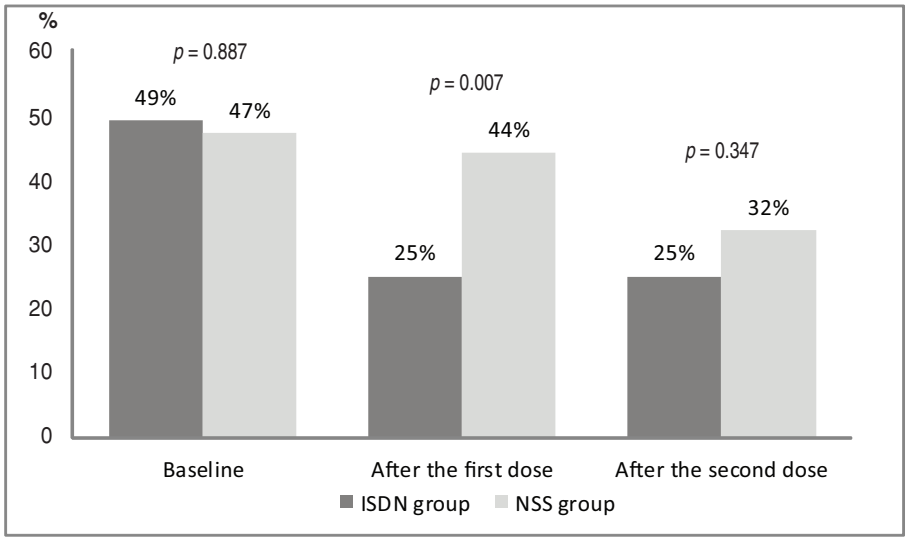

Figure 2. Percentage of simultaneous contractions comparing between the ISDN group and the NSS group at baseline, after the first dose and the second dose of test agents.

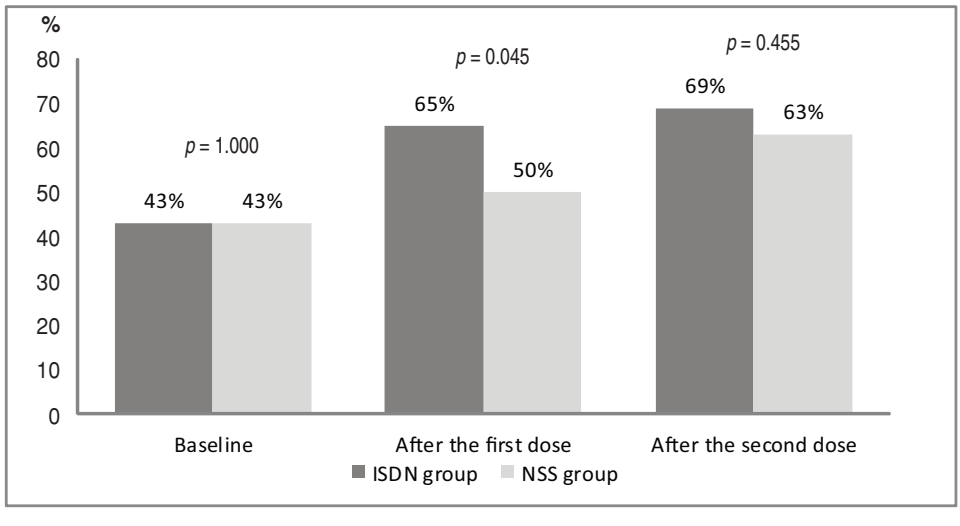

Figure 3. Percentage of peristalsis contractions comparing between the ISDN group and the NSS group at baseline, after the first dose and the second dose of test agents.

$p = 0.455$) compared to NSS group (Figure 3).

Comparison of manometric parameters at baseline between the ISDN group and the NSS group

Of the 100 swallows analyzed in each group (the ISDN group and the NSS group), the esophageal pressure topography (EPT) characteristics at baseline were expressed as mean ± SD and compared using paired *t*-test (Table 3). There were no significant differences in mean manometric parameters between the two groups ($p > 0.05$).

Comparison of manometric parameters after the first dose of test agent between the ISDN group and the NSS group

Of the 100 swallows analyzed in each group (the ISDN group and the NSS group), the esophageal pressure topography (EPT) characteristics after the first dose of test agent were expressed as mean ± SD (Table 4). There were significant differences in mean residual UES relaxation pressure, DCI of S3 in the 30-mmHg

Table 3. Comparison of EPT characteristics at baseline between the ISDN group and the NSS group.

	ISDN group (mean ± SD)	NSS group (mean ± SD)	*p*-value
UES resting pressure (mmHg)	51.9 ± 17.8	46.5 ± 10.1	0.148
Residual UES relaxation pressure (mmHg)	-1.0 ± 3.5	-3.2 ± 4.9	0.101
4-second IRP (mmHg)	10.9 ± 7.5	11.2 ± 8.4	0.932
PFV of S2 on the 30-mmHg IBC (cm/s)	8.6 ± 7.0	5.1 ± 11.9	0.496
PFV of S3 on the 30-mmHg IBC (cm/s)	8.7 ± 6.8	8.0 ± 6.4	0.687
PFV of S2 and S3 on the 30-mmHg IBC (cm/s)	7.1 ± 6.6	6.8 ± 6.5	0.920
DCI of S3 in the 30-mmHg IBC (mmHg $s^{-1}cm^{-1}$)	716 ± 479	962 ± 672	0.126
DCI of S2 and S3 in the 30-mmHg IBC (mmHg $s^{-1}cm^{-1}$)	820 ± 562	1119 ± 786	0.090
DCI of S1, S2 and S3 in the 30-mmHg IBC (mmHg $s^{-1}cm^{-1}$)	1634 ± 714	1765 ± 792	0.521
DCI of S3 in the 10-mmHg IBC (mmHg $s^{-1}cm^{-1}$)	1375 ± 741	1657 ± 979	0.247
DCI of S2 and S3 in the 10-mmHg IBC (mmHg $s^{-1}cm^{-1}$)	1639 ± 875	1986 ± 1118	0.185
DCI of S1, S2 and S3 in the 10-mmHg IBC (mmHg $s^{-1}cm^{-1}$)	3176 ± 1072	3310 ± 1206	0.684

UES, upper esophageal sphincter; IRP, integrated relaxation pressure; IBC, isobaric contour; PFV, pressurization front velocity;
DCI, distal contractile integral; S1, segment 1 (proximal esophagus); S2, segment 2 (distal esophagus); S3, segment 3 (distal esophagus);

Table 4. Comparison of EPT characteristics after the first dose of test agent between ISDN group and NSS group.

	ISDN group (mean ± SD)	NSS group (mean ± SD)	*p*-value
UES resting pressure (mmHg)	47.2 ± 21.3	44.4 ± 9.1	0.621
Residual UES relaxation pressure (mmHg)	0.5 ± 3.7	-4.0 ± 6.2	0.026
4-second IRP (mmHg)	10.3 ± 8.2	9.9 ± 7.8	0.900
PFV of S2 on the 30-mmHg IBC (cm/s)	7.4 ± 4.6	6.9 ± 5.5	0.996
PFV of S3 on the 30-mmHg IBC (cm/s)	7.4 ± 4.4	7.1 ± 2.4	0.839
PFV of S2 and S3 on the 30-mmHg IBC (cm/s)	7.2 ± 4.3	6.4 ± 2.3	0.551
DCI of S3 in the 30-mmHg IBC (mmHg $s^{-1}cm^{-1}$)	570 ± 387	1216 ± 992	0.040
DCI of S2 and S3 in the 30-mmHg IBC (mmHg $s^{-1}cm^{-1}$)	673 ± 507	1376 ± 1178	0.046
DCI of S1, S2 and S3 in the 30-mmHg IBC (mmHg $s^{-1}cm^{-1}$)	1479 ± 751	1989 ± 1118	0.106
DCI of S3 in the 10-mmHg IBC (mmHg $s^{-1}cm^{-1}$)	1195 ± 656	1997 ± 1335	0.053
DCI of S2 and S3 in the 10-mmHg IBC (mmHg $s^{-1}cm^{-1}$)	1421 ± 839	2363 ± 1581	0.050
DCI of S1, S2 and S3 in the 10-mmHg IBC (mmHg $s^{-1}cm^{-1}$)	2970 ± 1047	3676 ± 1491	0.103

UES, upper esophageal sphincter; IRP, integrated relaxation pressure; IBC, isobaric contour; PFV, pressurization front velocity;
DCI, distal contractile integral; S1, segment 1 (proximal esophagus); S2, segment 2 (distal esophagus); S3, segment 3 (distal esophagus);

IBC, and DCI of S2 and S3 in the 30 mmHg IBC between the two groups. The mean residual UES relaxation pressure was significantly higher in the ISDN group (0.5 ± 3.7 vs. -4.0 ± 6.2 mmHg, $p = 0.026$) than the NSS group. The mean DCI of S3 in the 30-mmHg IBC was significantly lower in the ISDN group (570 ± 387 vs. 1216 ± 992 mmHg $s^{-1}cm^{-1}$, $p = 0.040$) than the NSS group. Likewise, the mean DCI of S2 and S3 in the 30 mmHg IBC was significantly lower in the ISDN group (673 ± 507 vs. 1376 ± 1178 mmHg $s^{-1}cm^{-1}$, $p = 0.046$) than the NSS group. Nonetheless, in the 10-mmHg IBC, the mean DCI of S3 and DCI of S2 and S3 had trend to decrease in ISDN group ($p = 0.053$ and $p = 0.050$, respectively). In contrast, no significant difference of mean UES resting pressure, 4-second IRP, PFV, and DCI of S1, S2 and S3 were seen in both groups after the first dose of test agent.

Comparison of manometric parameters after the second dose of test agents between the ISDN

Table 5. Comparison of EPT characteristics after the second dose of test agents between ISDN group and NSS group.

	ISDN group (mean ± SD)	NSS group (mean ± SD)	*p*-value
UES resting pressure (mmHg)	42.4 ± 18.0	46.5 ± 11.0	0.178
Residual UES relaxation pressure (mmHg)	1.2 ± 4.5	-3.9 ± 6.5	0.027
4-second IRP (mmHg)	10.8 ± 9.2	9.9 ± 8.2	0.797
PFV of S2 on the 30-mmHg IBC (cm/s)	12.0 ± 17.2	4.0 ± 1.5	0.225
PFV of S3 on the 30-mmHg IBC (cm/s)	5.2 ± 1.6	6.1 ± 2.5	0.429
PFV of S2 and S3 on the 30-mmHg IBC (cm/s)	5.1 ± 1.6	5.2 ± 1.4	0.961
DCI of S3 in the 30-mmHg IBC (mm Hg $s^{-1}cm^{-1}$)	532 ± 353	1196 ± 799	0.006
DCI of S2 and S3 in the 30-mmHg IBC (mmHg $s^{-1}cm^{-1}$)	621 ± 465	1351 ± 924	0.006
DCI of S1, S2 and S3 in the 30-mmHg IBC (mmHg $s^{-1}cm^{-1}$)	1568 ± 1046	2020 ± 978	0.068
DCI of S3 in the 10-mmHg IBC (mmHg $s^{-1}cm^{-1}$)	1145 ± 569	2053 ± 1119	0.005
DCI of S2 and S3 in the 10-mmHg IBC (mmHg $s^{-1}cm^{-1}$)	1399 ± 739	2409 ± 1289	0.006
DCI of S1, S2 and S3 in the 10-mmHg IBC (mmHg $s^{-1}cm^{-1}$)	2843 ± 794	3757 ± 1337	0.011

UES, upper esophageal sphincter; IRP, integrated relaxation pressure; IBC, isobaric contour; PFV, pressurization front velocity;
DCI, distal contractile integral; S1, segment 1 (proximal esophagus); S2, segment 2 (distal esophagus); S3, segment 3 (distal esophagus);

group and the NSS group

Of the 100 swallows analyzed in each group (the ISDN group and the NSS group), the esophageal pressure topography (EPT) characteristics after the second dose of test agents were expressed as mean ± SD (Table 5).

There were significant differences in mean residual UES relaxation pressure, DCI of S3 in the 30-mm Hg IBC, DCI of S2 and S3 in the 30-mmHg IBC, DCI of S3 in the 10-mmHg IBC, DCI of S2 and S3 in the 10-mmHg IBC, and DCI of S1, S2 and S3 in the 10-mmHg IBC between the two groups. The mean residual UES relaxation pressure was significantly greater in the ISDN group (1.2 ± 4.5 vs. -3.9 ± 6.5 mm Hg, $p = 0.027$) than the NSS group. In the 30-mmHg IBC, swallows analyzed in ISDN group had significantly lower of DCI of S3 (532 ± 353 vs. 1196 ± 799 mmHg $s^{-1}cm^{-1}$, $p = 0.006$), and DCI of S2 and S3 (621 ± 465 vs. 1351 ± 924 mmHg $s^{-1}cm^{-1}$, $p = 0.006$) (Figure 4) than in NSS group after the second dose of test agents. Similarly, in the 10-mmHg IBC, swallows analyzed in ISDN group had significantly lower of DCI of S3 (1145 ± 569 vs. 2053 ± 1119 mmHg $s^{-1}cm^{-1}$, $p = 0.005$), DCI of S2 and S3 (1399 ± 739 vs. 2409 ± 1289 mmHg $s^{-1}cm^{-1}$, $p = 0.006$) (Figure 5), and DCI of S1, S2 and S3 (2843 ± 794 vs. 3757 ± 1337 mmHg $s^{-1}cm^{-1}$, $p = 0.011$) than in NSS group after the second dose of test agents. Although after the second dose of test agents, there were still no significant differences of mean UES resting pressure, 4-second IRP, PFV, and DCI of S1, S2 and S3 in the 30-mmHg IBC in both groups.

Overall, the mean PFV changes comparing between ISDN group and NSS group at baseline, after the first dose and the second dose of test agents were depicted in Figure 6 (PFV of S2, S3 on the 30-mmHg IBC) and Figure 7 (PFV of S2 and S3 on the 30-mm Hg IBC).

Adverse events of the drug

Two out of 10 patients enrolled (20%) reported headache in 1 and palpitation in 1 patient after administration of ISDN spray whereas no patient complaining for adverse events after administration of NSS spray (placebo).

DISCUSSION

NO is a major inhibitory neurotransmitter in GI tract. In the smooth circular muscle of human esophagus, it acts as an inhibitory non-adrenergic, non-cholinergic neurotransmitter regulating the latency period, the latency gradient, and the contraction amplitude of esophageal peristalsis[15,17,31]. Distal esophageal spasm is characterized by normal peristalsis intermittently interrupted by simultaneous contractions of distal esophagus. The etiology of this condition is unclear. The currently accepted concept attributes this simulta-

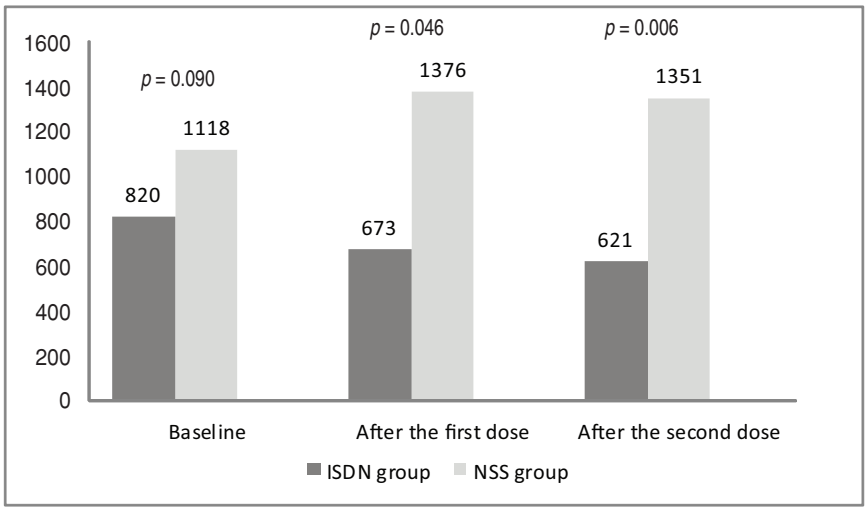

Figure 4. Mean DCI of S2 and S3 in the 30-mm Hg IBC changes comparing between the ISDN group and the NSS group at baseline, after the first dose and the second dose of test agents.

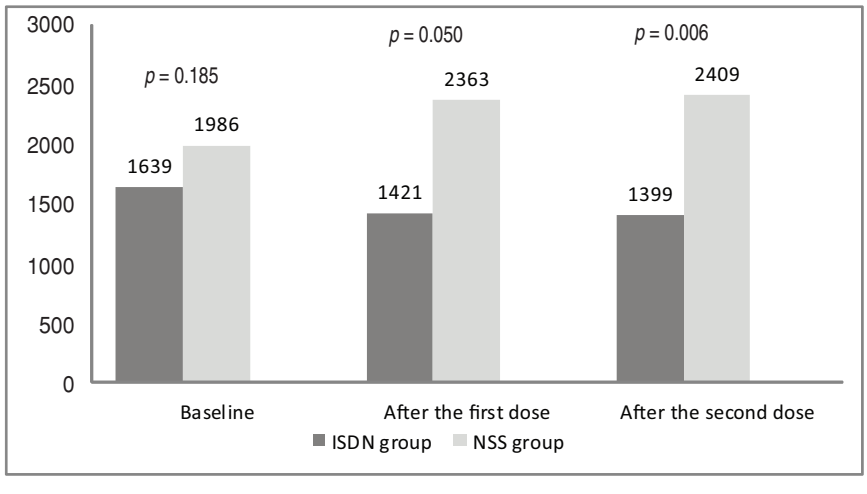

Figure 5. Mean DCI of S2 and S3 in the 10-mm Hg IBC changes comparing between ISDN group and NSS group at baseline, after the first dose and the second dose of test agents.

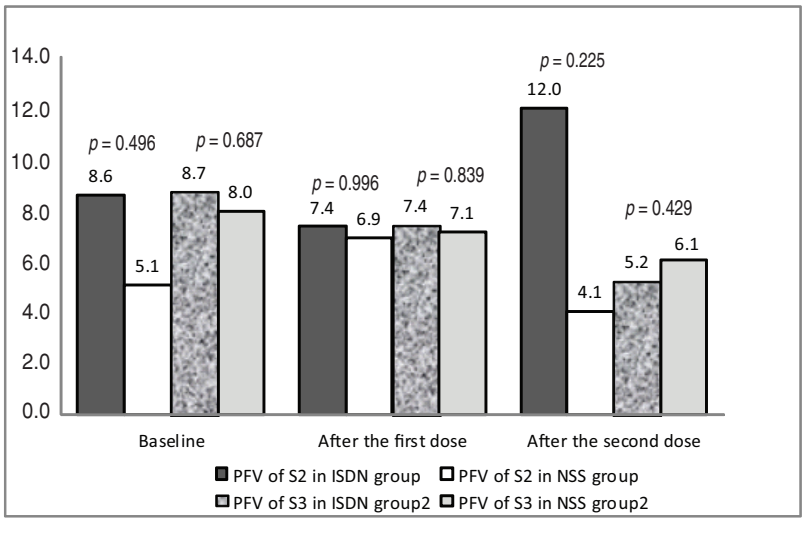

Figure 6. Mean PFV of S2, S3 on the 30-mmHg IBC changes comparing between ISDN group and NSS group at baseline, after the first dose and the second dose of test agents.

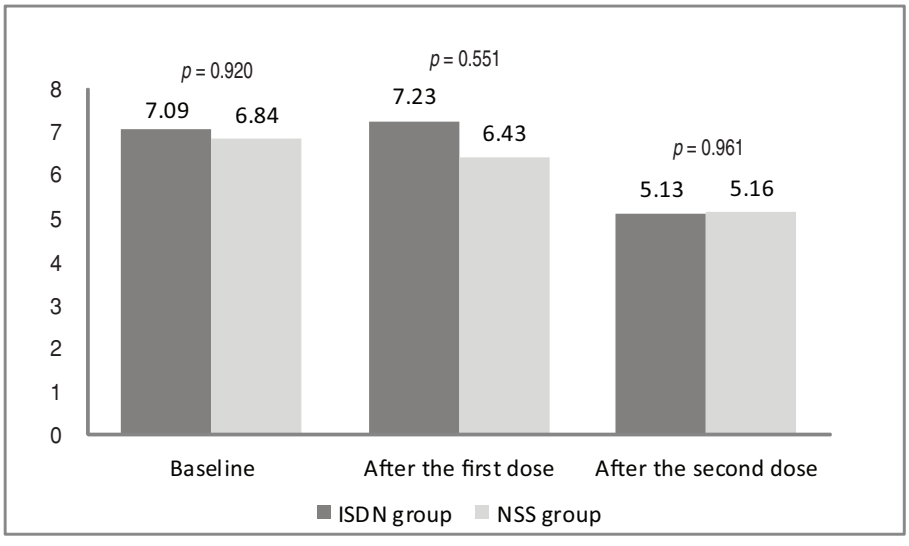

Figure 7. Mean PFV of S2 and S3 on the 30-mmHg IBC changes comparing between ISDN group and NSS group at baseline, after the first dose and the second dose of test agents.

neous contraction of distal esophagus to an altered endogenous NO synthesis and/or degradation[22,32].

Based on the pathophysiological mechanisms of distal esophageal spasm, the agents that result in the augmentation of NO may improve the clinical and manometric patterns of these patients. Even though nitrates have long been used for spastic esophageal motility, on the basis of clinical observation that nitrates improved chest discomfort and manometric findings in conventional manometry, they have never been studied in a placebo-controlled trial[10,16]. Therefore, we investigated whether ISDN, an exogenous NO donor, can restore esophageal peristalsis contractions in symptomatic patients with diffuse or segmental simultaneous contraction of the esophagus in this randomized cross-over study. Moreover, we also studied the effect of ISDN on other esophageal motor functions in these patients.

Besides, we used HRM which offered several basic technical advantages over conventional manometry to analyze esophageal motility. HRM permits the visualization of esophageal contractility with isobaric conditions among sensors indicated by isocoloric regions on the pressure topography plots, thus it can delineate the spatial limits, vigor, and integrity of individual contractile segments along the esophagus.

In our study, the most frequent symptom of distal esophageal spasm was globus sensation (80%) and dysphagia (70%), respectively. Using the Chicago classification of HRM[30], the prevalence of swallows in each subtypes i.e. simultaneous contraction, aperistalsis and peristalsis contraction comparing between the ISDN group and the NSS group were similar at baseline. Interestingly, there were significant differences in prevalence of swallows in each subtype in ISDN group and NSS group only after the first dose but not after the second dose of test agents. In addition, we also found that the prevalence of simultaneous contraction was significantly decreased by ISDN, compared to NSS, only after the first dose but not the second dose. Conversely, the prevalence of esophageal peristalsis contractions was significantly increased by ISDN only after the first dose but not the second dose. All of these findings replied the primary research question that NO played a major role in helping patients with distal esophageal spasm restore their normal peristalsis because the prevalence of esophageal peristalsis contractions were significantly increased after the first dose of ISDN. However, the results from this study showed that there were no significant differences in prevalence of esophageal peristalsis contractions after the second dose of ISDN compared to NSS. These indicated that the proportion of esophageal peristalsis contraction was increased overtime after HRM catheter insertion in symptomatic patients with simultaneous contraction of the distal esophagus. Thus, we could not answer the secondary research question as whether the effect of NO to restore the normal peristalsis from the simultaneous contraction was dose-dependent. On the other hand, ISDN significantly im-

proved esophageal peristalsis contractions earlier than NSS. We proposed the role of central nervous system, esophageal adaptation, and exogenous NO to local stimulus on the restoration of esophageal peristalsis contractions in patients with distal esophageal spasm.

Among the manometric parameters, our study also demonstrated that the mean DCI of S3 and the mean DCI of S2 and S3 were significantly diminished in ISDN group than NSS group. Furthermore, the mean residual UES relaxation pressure was significantly higher in ISDN group than NSS group. On the other hand, ISDN did not affect the PFV of S1, S2 and S3, the 4-second IRP, and the UES resting pressure. These data suggested that ISDN significantly decreased the DCI or force of the contractions, and increased the residual UES relaxation pressure. Besides, the adverse events including headache and palpitation occurred in 2 patients after administration of ISDN spray whereas adverse event was not observed in patients receiving NSS spray.

This study had some limitations. Firstly, there were a small number of patients in this study due to low prevalence of distal esophageal spams in general population and short duration of the study. However, we designed this study as a randomized cross-over fashion to solve this problem. Secondly, the patient's discomfort from HRM catheter insertion might affected cooperation during underwent HRM. Thirdly, patients could know the kind of test agents that they received during the study because of different taste and smell between ISDN and NSS spray. However, the data of esophageal contraction from HRM was objective and this bias could be overcome.

Conclusion

Based on our analysis in symptomatic patients with diffuse or segmental simultaneous contraction of the esophagus, proportion of esophageal peristalsis contraction was increased overtime after HRM catheter insertion. ISDN significantly improved esophageal peristalsis contractions earlier than NSS but the effect of ISDN was not dose-dependent. Additionally, ISDN also decreased the DCI or force of the contractions and increased the residual UES relaxation pressure. Nonetheless, ISDN did not affect the PFV of S1, S2 and S3, the 4-second IRP, and the UES resting pressure. This study suggested the role of central nervous system or esophageal adaptation to local stimulus and exogenous NO on the restoration of esophageal peristalsis contractions in patients with distal esophageal spasm.

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