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Comparative Relaxant Effects of Ataciguat and Zaprinast on Sheep Sphincter of Oddi

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Background: Relaxing the sphincter of Oddi (SO) is an important process during endoscopic retrograde cholangiopancreatography (ERCP) procedures. This issue suggests that the easier the sphincterotomy and cannulation, the more post-ERCP complications decrease.
Aims: To compare the relaxant effects of ataciguat (a novel soluble guanylyl cyclase activator) and zaprinast (an inhibitor of phosphodiesterase 5) on sheep SO *in vitro,* thus testing whether they can be used during ERCP.
Study Design: Animal experimentation.
Methods: Sheep SO rings were placed in tissue baths and their isometric tension to ataciguat and zaprinast were tested. We also tested their isometric tension against ataciguat in the presence of 1H-(1,2,4) oxadiazole (4,3-a) quinoxalin-1-one (ODQ) which is a soluble guanylyl cyclase inhibitor.
Results: Ataciguat and zaprinast both triggered concentration addicted relaxation on sheep SO rings (p=0.0018, p=0.0025 respectively) but the relaxation of the ataciguat was significantly greater than that of zaprinast at all concentrations (p=0.0024). It was observed that decreased relaxation responses were initiated by ataciguat in the presence of ODQ (p=0.0012).
Conclusion: Ataciguat and zaprinast both have relaxing effects on sphincter of Oddi, although that of zaprinast is lower. We believe that ataciguat and zaprinast can be used in ERCP procedures in order to relax the sphincter of Oddi and thus can be used locally in order to decrease complications.
Keywords: Ataciguat, Oddi's sphincter, zaprinast

The sphincter of Oddi (SO) has the ability to settle the drainage of bile into the duodenum, facilitates the filling of the gallbladder and stops reflux of the duodenal ingredients back into the common bile duct and pancreatic duct (1,2). SO is a complex structure formed by smooth muscle fibers and a neural network and its motility and function are mediated through hormonal and neural pathways (2). Nitric oxide (NO) has been shown to induce neurogenic relaxation in SO as a response to myenteric nerve stimulation in clinical and experimental studies (3,4). Disorders of the NO/cyclic guanosine monophosphate (NO/cGMP) pathway cause an inability to relax the sphincter and decrease the motility of the gastrointestinal system (5). Agents that act on the NO/cGMP pathway induce smooth muscle relaxation and reduce the contraction of sphincter smooth muscles (6).

Ataciguat, which is an aminobenzoic acid derivative and a new soluble guanylyl cyclase activator, activates guanylyl cyclase (GC) and induces the transformation of guanosine triphosphate (GTP) to cGMP and the activation of protein kinase (7). Activated protein kinase reduces the ionized calcium concentration and sensitivity in the smooth muscle cells, causing relaxation in smooth muscles (8,9). There are many studies in the literature which state that soluble guanylyl cyclase activators cause relaxation in all smooth muscles as well as SO and gastrointestinal smooth muscles (7,8).

Zaprinast, a phosphodiesterase 5 (PDE-5) inhibitor like the clinical drug sildenafil, is a precursor to the chemically-related cGMP-dependent (9,10). Zaprinast increases intracellular cGMP levels by inhibiting PDEs that reduce smooth muscle relaxation in many tissues, especially in the gastrointestinal

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system (11,12). Phosphodiesterase type 5 (PDE-5) has been defined in the esophageal sphincter, internal anal sphincter and pyloric sphincter in experimental studies (13,14). The sGC/cGMP signaling pathway plays a significant role in the gastrointestinal system (7,8).

Endoscopic retrograde cholangiopancreatography (ERCP) is an invasive procedure that is used for the diagnosis and treatment indifferent biliary and pancreatic canal pathologies (15). However, ERCP may lead to a number of serious complications including pancreatitis, hemorrhage, and perforation (15). Easy cannulation plays a critical role in both preventing these complications and shortening the duration of the procedure. Using pharmacological agents for relaxation of the SO can provide easier cannulation and therefore, a safer and shorter ERCP procedure (15,16).

There is insufficient evidence in the literature that compares the relaxant effect of ataciguat and zaprinast on isolated sheep SO rings. We aimed to examine the direct effects of ataciguat and zaprinast via the sGC/cGMP signal transduction system of sheep SO in this study in order to find new candidate agents for the ERCP procedure.

MATERIALS AND METHODS

Design of the study

Ten male adult Anatolian Akkaraman sheep weighing from 38 to 45 kg were used in the present study. The study was approved by the local ethical committee of Cumhuriyet University School of Medicine. The sheep were sacrificed by cervical cuts in the slaughterhouse. Their abdomens were opened with a midline incision and SO was quickly removed and taken to the laboratory in previously aerated (95% O_2 and 5% CO_2) Krebs' bicarbonate solution that consisted of 120 mmol/L NaCl, 4.6 mmol/L KCl, 2.5 mmol/L $CaCl_2$, 1.2 mmol/L $MgCl_2$, 22 mmol/L $NaHCO_3$, 11.5 mmol/L NaH_2PO_4 and 11.5 mmol/glucose. SO muscle rings were placed into in a 10 mL tissue bath that contained pre-aerated Krebs' bicarbonate solution at 37°C. The upper end of a 1.5 g SO ring was tied to an isometric transducer (Grass FT 03; MA, USA). In order to get a stable baseline measurement, SO muscle rings were sluiced every 15 min and left to establish equilibrium for about 60–90 min. Before each drug application, we washed the tissue at least twice and eliminated the drug by soaking it in an organ bath for at least half an hour. Once the tissue was free of the drug, a new set of drugs was applied.

Isometric measurements

After reaching equilibrium, submaximal concentration of carbachol (10^{-6} mol/L) was applied in order to get contraction in SO tissues. This concentration of carbachol was decided in previous experiments (6). After completing contraction studies performed using carbachol, we measured the relaxation responses to ataciguat (10^{-9}–10^{-4} mol/L) (a new soluble guanylyl cyclase activator) and zaprinast (10^{-9}-10^{-4} mol/L) (a phosphodiesterase-5 inhibitor) in a cumulative manner. We also measured relaxation responses to ataciguat in the presence of 1H-(1,2,4) oxadiazole (4,3-a) quinoxalin-1-one (ODQ) (an inhibitor of soluble guanylyl cyclase).

Statistical analysis

When alpha (α) levels are considered 0.05, the beta (β) level as 0.20 and 1-β level as 0.80, 10 sheep were recruited into each group. The analytical power of the test was found to be p=0.80310.

Carbachol-induced contractions were considered as reference values. Relaxation responses were expressed as a percentage of the carbachol-induced contractions. The relaxation effects of cumulative concentrations of ataciguat and zaprinast on carbachol-induced contractions in the absence or presence of ODQ were measured. Values for -log10 EC_{50} (pD_2) and mean maximal inhibition (E_{max}) were compared. The concentration-response data found in each individual experiment were planned as the response/concentration against the response, producing a linear association in each experiment, as predicted from the Scatchard equation for drug-receptor interaction: Response/concentration = -1 / EC_{50} x response + E_{max}/ EC_{50}.

After entering the data obtained from our study in the SPSS program (data 22.0), Oddi sphincter concentration-dependent relaxation levels, E_{max} and pD_2 values when parametric assumptions could not be performed (One sample Kolmogrov-Smirnov), the Friedman test and Bonferroni test were used for variations of groups among themselves. When groups were compared in terms of each single dose concentration, the Kruskal Wallis test was used. For all analyses, p values <0.05 were accepted as significant. Statistical analyses were performed using Statistical Package for the Social Sciences software 22.0 (IBM SPSS Statistics; Armonk, NY, IL, USA).

Drugs

Drugs included carbachol, ODQ, ataciguat, and zaprinast. Drugs were supplied by Santa-Cruz (carbachol, ODQ, ataciguat and zaprinast; Santa-Cruz Biotechnology; Texas, USA). Other than ODQ, which was dissolved in dimethylsulfoxide and then diluted with distilled water, all of the drugs were dissolved only in distilled water in order to obtain the reduced concentrations of these drugs. Drug-containing solutions were prepared only at the time of the experiments.

RESULTS

Ataciguat (10^{-9}-10^{-4} mol/L) induced concentration-dependent relaxations in SO rings pre-contracted by carbachol (p=0.0018). The relaxation responses induced by ataciguat were significantly decreased in the presence of ODQ (10^{-5} mol/L) beginning from the 10^{-8} -mol/L concentration (p=0.0012) (Figure 1). Zaprinast (10^{-8}-10^{-4} mol/L) induced concentration-dependent relaxation in SO rings was pre-contracted by carbachol as well (p=0.0025) (Figure 2). The relaxation of the ataciguat was significantly greater than the relaxation of zaprinast at all concentrations in SO rings (p=0.0024) (E_{maks} values 79.2±5.8 for ataciguat, 52.0±6.0 for zaprinast).

Table 1 presents the pD_2 and E_{max} values of ataciguat and zaprinast on SO rings previously contracted by carbachol. We could not find any significant difference between ataciguat and zaprinast in pD_2 values (p=0.091).

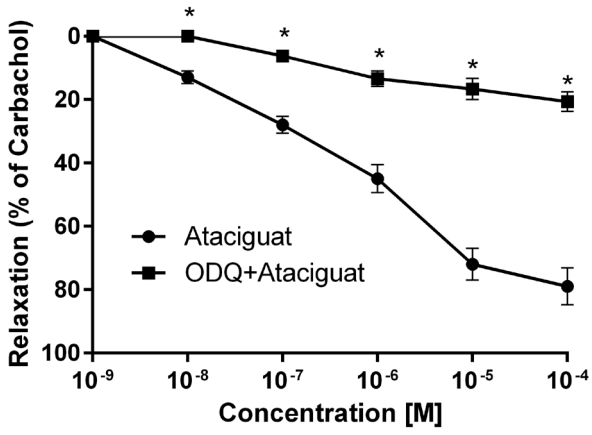


FIG. 1. Relaxation responses induced by ataciguat (10-9-10-4 mol/L) in the presence and absence of ODQ (10-5 mol/L) on isolated sheep SO rings. Relaxations are expressed as the percentage of carbachol (10-6mol/L)-induced pre-contraction and are shown as mean±SD.
*Statistically different from agent only (p=0.0012).

DISCUSSION

In this study, we aimed to establish whether the new agents affecting the sGC/cGMP pathway can be promising for SO relaxation. For this purpose, we used ataciguat, a new guanylate cyclase activator, and zaprinast, a phosphodiesterase type 5 inhibitor. Ataciguat and zaprinast induced concentration-dependent relaxations in SO rings previously contracted by carbachol. The relaxation effect of ataciguat was significantly greater than that of zaprinast at all concentrations in SO rings.

Endoscopic retrograde cholangiopancreatography is a complex and risky procedure used for the diagnosis and treatment of hepatobiliary and pancreatic diseases (15,16). It is an invasive procedure and short-term complications including hemorrhage, pancreatitis and perforation can occur (17,18). By facilitating cannulation and sphincterotomy with the help of appropriate pharmacological agents, it is possible to lower ERCP related complications.

Nitric oxide has the capacity to regulate the muscle tone of the pancreatobiliary junction, sphincter of the lower esophagus and pylorus. It is synthesized by NO synthase (NOS) from the L-Arginine molecule. It then activates guanylate cyclase in the cytosol of smooth muscle cells, which transforms GTP to cGMP. The cGMP activates protein kinase G and ion channels. As a result of these alterations, intracellular calcium decreases in the smooth muscle cells and the sphincter relaxes. The physiological effect of cGMP is terminated by the enzyme phosphodiesterase (1,2).

Guanylyl cyclase is an important part of the NO/cGMP pathway and its existence has been shown in many systems such as the cardiovascular, nervous, urogenital and gastrointestinal systems. GC activation results in increasing cGMP levels in cells and finally causes relaxation in smooth muscle cells of the cardiovascular and gastrointestinal systems (18). Some guanylyl cyclase activators are used in clinical practice for pulmonary hypertension, congestive heart failure and erectile dysfunction because of their relaxation effect on smooth muscles (19). GC is found in several locations in the gastrointestinal tract and it is denser, especially in the lower esophageal sphincter tissue and SO (20,21). It has been shown in nitric oxide-sensitive GC knockout mice that gastrointestinal

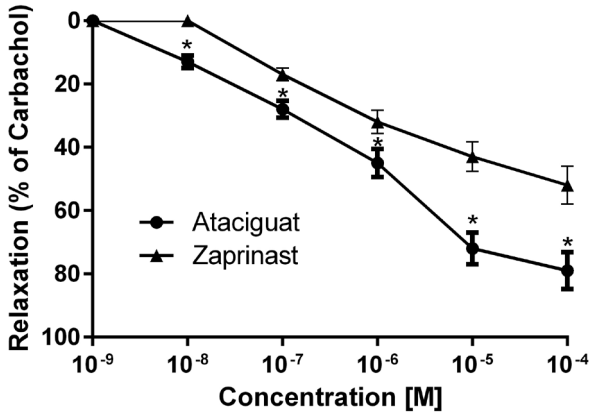


FIG. 2. Relaxation responses induced by ataciguat (10-9-10-4 mol/L) and zaprinast (10-9-10-4 mol/L) on isolated sheep SO rings. Relaxations are expressed as percentage of carbachol (10-6 mol/L)-induced pre-contraction and shown as mean±SD.
*Statistically different from zaprinast (p=0.0024).

TABLE 1. E_{max} and pD_2 values for the relaxant effects of ataciguat and zaprinast alone and the ODQ + Ataciguat (10^{-5} mol/L) on sheep SO rings pre-contracted by carbachol (10^{-6} mol/L)

ODQ + Ataciguat (10^{-5} mol/L) on Sheep SO Rings Pre-contracted by Carbachol (10^{-6} mol/L)

	Emaks (% of Carbachol)	pD_2
Ataciguat	79.2±5.8	6.52±0.10
ODQ + Ataciguat	20.6±3.0*	6.70±0.14
Zaprinast	52.0±6.0**	5.92±0.09

Data were presented as mean±SD (standard deviation).
*Statistically different from only ataciguat (p=0.0012).
**Statistically different from zaprinast when compared with ataciguat (p=0.0024).
E_{max}: maximum relaxant effect; pD_2: negative logarithm to base 10 of the EC50; ODQ: 1H-(1,2,4) oxadiazole (4,3-a) quinoxalin-1-one

motility is reduced, which results in gastroparesis and pyloric stenosis (22). Several previous studies have shown that some sGC activators and some drugs that affect NO/sGC/cGMP cause relaxation of sheep SO (6,22). Ataciguat is a new sGC activator. The vasodilator effect of ataciguat has been determined previously but no study regarding the effect of this drug on gastrointestinal system and sphincter of Oddi has been presented (23). In our study, a new sGC activator ataciguat induced significant and concentration-dependent relaxations in SO rings. Ataciguat-induced relaxations are significantly decreased in the presence of the GC inhibitor ODQ.

PDE-5 inhibitors prevent hydrolysis of cGMP leading to an increase in cGMP concentrations. Then, cGMP activates protein kinase and smooth muscle relaxation occurs (24). Several studies have shown that the inhibition of PDE-5 may be helpful in the cure of chronic anal fissure, erectile dysfunction, pulmonary hypertension and various vascular diseases (25-27). Experimental animal studies have shown that in the lower esophageal sphincter, internal anal sphincter and pyloric sphincter, zaprinast may increase cGMP levels, activate protein kinase and cause smooth muscle relaxation. For these reasons, since PDE-5 inhibitors can decrease motility and reduce basal sphincter pressure, these drugs have been found to be useful in medical therapy of dysfunction of SO and hypercontractile esophageal motility disorders (28,29). Sildenafil, a PDE-5 inhibitor, creates concentration-dependent relaxation in the gallbladder. Cheon et al. (29) found that the PDE-5 inhibitor vardenafil also inhibited SO motility and reduced the basal SO pressure. They have also shown that the administration of local vardenafil before the ERCP procedure may reduce the tone of SO and allow the doctor to perform easy cannulation. Easy cannulation would help to decrease the incidence of post-ERCP pancreatitis. Zaprinast is a PDE-5 inhibitor that has been recognized for many years. The relaxing effect of zaprinast on the esophageal lower sphincter has been determined by Barnette et al. (30), but its effect on the sphincter of Oddi has not been investigated until today. In our study, similarly, the PDE-5 inhibitor zaprinast induced significant concentration-dependent relaxations in SO rings.

In this study, when comparing the effects of ataciguat and zaprinast, the relaxant effect of ataciguat was more potent than zaprinast in sheep SO. The GC inhibitor ODQ significantly reduced the relaxation of ataciguat in SO rings. This confirms that relaxation caused by ataciguat may occur via the activation of sGC. Relaxations on the sphincter of Oddi induced by ataciguat and zaprinast in this study show parallel results with other studies regarding other sGC activators and PDE-5 inhibitors.

The strong and weak aspects of this study are as follows: the study needing to be *in vitro* is one of the weak aspects. Adjusting the study performed to *in vivo* may not always be possible. However, because the target drugs investigated in this study are used locally during ERCP, adjusting data obtained from this study to *in vivo* studies may be possible. Additionally, because the variables can be controlled by the investigator in an *in vitro* setting, much clearer comments concerning the mechanism of action of these drugs can be made, which is a strong aspect of the study.

In conclusion, our study results suggest that ataciguat, a new guanylate cyclase activator and to a lesser extent zaprinast, a phosphodiesterase-5 inhibitor, are preferable agents for the relaxation of SO. However, our results should be confirmed by further *in vivo* and clinical studies. Relaxed SO is one of the most important aspects in performing a successful ERCP procedure. We believe that this study will pave the way for the local use of these molecules providing faster ERCP procedures with fewer complications.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Cumhuriyet University School of Medicine (Date: 02.11.2011, Number: 2011-283).

Informed Consent: N/A.

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Author contributions: Concept - E.C., O.Y., I.B.; Design - E.C.; Supervision - O.Y.; Resource - E.C.; Materials - B.S., M.P.; Data Collection and/or Processing - E.C., C.C.; Analysis and/or Interpretation - I.B., M.P.; Literature Search - E.C.; Writing - E.C., O.Y.; Critical Reviews - E.C., O.Y., H.A.

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