Inhibitory effect of sildenafil on gastrointestinal smooth muscle: Role of NO-cGMP transduction pathway

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Nitric oxide (NO) is an important neurotransmitter in the gut and has been demonstrated to be a key physiological mediator of non-adrenergic non-cholinergic (NANC) relaxation of gastrointestinal smooth muscle. In the present study the effect of PDE 5 inhibitor sildenafil on the gastrointestinal function (gastric emptying and intestinal transit) has been demonstrated in mice. Sildenafil (0.5-2 mg/kg, po) did not alter the percent gastric emptying however, in higher doses (5, 10 and 30 mg/kg, po) it inhibited the gastric emptying. On acute administration (0.5-5 mg/kg, po) it did not alter the intestinal transit but in higher doses (10 and 30 mg/kg, p.o.) delayed the intestinal transit. Further, the inhibitory effect of sildenafil was significantly blocked by L-NAME (10 mg/kg, ip), a non-selective NOS inhibitor and methylene blue (1 mg/kg, ip), a guanylate cyclase inhibitor. These findings suggest the participation of NO-cGMP transduction pathway in the inhibitory effect of sildenafil (higher doses) on the gastrointestinal smooth muscles and its potential application in patients with nutcracker oesophagus, hypertensive lower oesophageal sphincter (LOS), achalasia and diabetic gastroparesis or colitis where there is a loss of nNOS.

Keywords: Gastric emptying, Intestinal motility, Non-adrenergic non-cholinergic transmission, Sildenafil

Nonadrenergic noncholinergic (NANC) mediators and other transmitters like vasoactive intestinal peptide (VIP), and calcitonin gene-related peptide (CGRP) have been suggested in the relaxation of gastrointestinal smooth muscle. Nitric oxide (NO) is the principle inhibitory neurotransmitter released after deglutition by NANC inhibitory neurons. It is responsible for hyperpolarization and regulation of peristaltic contractions in gastrointestinal smooth muscles via elevation of cyclic guanosine mono phosphate (cGMP) and activation of cyclic GMP-dependent protein kinase (PKG) system. NANC-mediated relaxation is significantly antagonized by nitric-oxide biosynthesis inhibitors Nω-nitro-L-arginine (L-NNA) and Nω-nitro-L-arginine-methyl ester (L-NAME).

The amplitude and duration of the cGMP signal in the smooth muscle is regulated mainly by cGMP-specific cyclic nucleotide phosphodiesterase (PDE5). Sildenafil, a PDE5 inhibitor influences NO-cGMP pathway by blocking PDE5 enzyme that degrades NO stimulated cGMP. PDE5 a predominant cGMP-specific PDE is expressed in skeletal, cardiac, pylorus and smooth muscle of the gut.

The NO-cGMP has been shown to cause relaxation of lower oesophageal sphincter and regulates peristaltic contractions in animals and humans. In vivo studies in human oesophagus have focused on the consequences of reducing the effect of NO. The efficacy of PDE5 inhibitors in the treatment of esophageal motility associated with achalasia (a disease with decreased NO levels) has been reported. PDE5 inhibitors (sildenafil) activates the NO-cGMP pathway and relaxes the gut smooth muscle cells.

The present study has been aimed to investigate the possible effect of acute administration sildenafil on NO-cGMP pathway in gastric emptying and gastrointestinal motility in albino mice.

Materials and Methods

Animals—Swiss albino mice (20-30 g), of either sex (Central Animal House, Panjab University, Chandigarh, India) were housed under standard...
laboratory conditions and kept under a 12:12 hr L:D cycle. Experiments were carried out between 0900 and 1800 hrs. All the experimental protocols were approved by the Institutional Animal Ethics Committee.

**Gastric emptying**—Gastric emptying was determined by as per by Scarpi et al. A test meal (0.05% phenol red in a 1.5% aqueous methylcellulose) of 0.25 ml/mice was given through an intragastric tube. After 30 min the animals were sacrificed, stomach was removed and homogenized in 100 ml of 0.1 N NaOH. Stomach tissue proteins (in 5 ml homogenate) were precipitated with 0.5 ml of trichloroacetic acid (20% w/v) and centrifuged. The supernatant was mixed with 4 ml of 0.5 N NaOH and absorbance of the sample was read at 560 nm wavelength. Phenol red recovered from the stomach of mice sacrificed immediately after administration of the methylcellulose meal served as standard stomach.

**Charcoal meal test**—The charcoal meal test was used to assess the effect of sildenafil on intestinal transit. Briefly, mice were starved for 24 hr prior to the experiment but had free access to water. Thereafter, the test drug was administered alone. A charcoal meal (0.25 ml/mouse consisting of 10% charcoal in 5% gum acacia) was administered orally. The animals were sacrificed 30 min after charcoal meal administration. The abdomen was opened and the entire small intestine starting from the pyloric end was removed and placed on the blotting paper. All care was taken to prevent any damage to the gut and the distance traveled by the charcoal was measured and expressed as percent gastrointestinal (GI) transit.

The gastric emptying for each mouse was calculated according to the following formula:

\[
\text{Gastric emptying (\%)} = \frac{\text{amount of phenol red recovered from stomach}}{\text{average amount of phenol red recovered from standard stomach}} \times 100
\]

Six to seven animals were used per dose to determine the dose response curve. Gastric emptying or gastrointestinal motility effect was expressed as percent gastric emptying or GI transit inhibition in comparison to that observed in the saline treated controls.

**Statistical analysis**—Results were expressed as the mean ± SE. Difference between the mean values in groups of each drug treatment and control was analyzed by the nonparametric Kruskal Wallis, one-way analysis of variance with multiple range test.

**Results**

**Effect of sildenafil on gastric emptying in mice**—Acute administration (0.5, 1, 2, and 5 mg/kg, po) of sildenafil to mice did not alter the gastric emptying as compared to control group. However, high (10 and 30 mg/kg, po) produced a significant (P<0.05) decrease in gastric emptying (Fig. 1).

Sildenafil-induced decrease in emptying was significantly blocked by L-NAME (10 mg/kg, ip) and methylene blue (1 mg/kg, ip) (Fig 2), which on per se also delayed the gastric emptying in mice (Fig. 2).

**Effect of sildenafil on gastrointestinal motility in mice**—Acute administration (0.5, 1, 2, and 5 mg/kg, po) of sildenafil did not alter the intestinal motility as compared to the control group. However, high doses (10 and 30 mg/kg, po) significantly (P<0.05) delayed the intestinal transit (Fig. 1).

Sildenafil-induced delay in intestinal transit was significantly blocked by L-NAME (10 mg/kg, ip) and methylene blue (1 mg/kg, ip) (Fig 2), which on per se administration also delayed the intestinal transit time (Fig 2).

![Fig. 1](image-url) —Effect of sildenafil (Sil; 0.5-30 mg/kg, po) on gastric emptying and gastrointestinal transit in mice. Values are as mean ± SE from 6 to 8 mice per group. *P <0.05 as compared with control.
Discussion

Phosphodiesterases (PDEs) are a large group of structurally related enzymes that catalyse the hydrolysis of 3', 5'-cyclic nucleotides to the corresponding inactive nucleotide 5'-monophosphate by cleaving the phosphodiester bond between the phosphorus and oxygen atoms at the 3' position\(^{19,20}\). The levels of cGMP and cAMP in gastrointestinal smooth muscle are determined by the synthetic activities of soluble guanylyl cyclase (GC) and adenylyl cyclase (AC) respectively, and the degradative activities of specific PDEs, mainly cAMP-prefering PDE3, cAMP-specific PDE4 and cGMP-specific PDE5, respectively\(^{21}\). Eleven families (PDE1-11) of PDEs have been identified with each family being the product of a separate gene and usually comprise several isoforms. PDE5 is a predominant cGMP-specific PDE, expressed in skeletal, cardiac and smooth muscles\(^{22}\) and is responsible for the hydrolysis of cGMP\(^{23}\). Sildenafil, a potent, selective and reversible phosphodiesterase 5 inhibitor\(^{24}\) increases the effect of cyclic GMP, which displays an inhibitory effect on the smooth muscle cells. The inhibitory effect of sildenafil is due to the blockade of PDE5, which inactivates the intracellular cGMP stimulated by NO.

The NO released by the non-adrenergic non-cholinergic (NANC) inhibitory neurons of the myenteric plexus is also the principal inhibitory neurotransmitter in the gut smooth muscle through the production of cGMP. Molecular biological studies have indicated that the mechanisms for NO-mediated neurotransmission in intestine has inhibitory effects.

It is well documented that peristaltic phase leading to interdigestive motility of gastrointestinal tract is characterized by the cyclic occurrence of the ‘migrating motor complex’ consisting of three phases of motor activity; Phase I of motor silence, Phase II of
increasing irregular motility, and Phase III of sequence of propulsive motor waves, followed by phase I. The recent clinical findings suggested that sildenafil inhibited gastroduodenal motility by virtue of activation of NO-cGMP pathway which prolongs the phase I and prevents the occurrence of phase III of the normal peristaltic phase.

In the present study sildenafil in lower doses (0.5-5 mg/kg, po) neither altered gastric emptying nor gastrointestinal motility which may be due to the insufficient production of cGMP to alter the normal peristaltic movements. However, in higher doses it significantly delayed the gastric emptying and intestinal motility in both the models. The delayed effect of sildenafil at higher doses may be due to its inhibitory effect on gastrointestinal tract as PDE5 enzyme is present in pylorus and throughout the gastrointestinal smooth muscles. Sildenafil may be altering the peristaltic movement in higher doses by prolonging the phase I of motor silence, and inhibiting the phase III throughout the gastrointestinal tract. Further, the evidence of major participation of NO-cGMP pathway in the inhibitory effect of sildenafil may be ruled out as L-NAME and methylene blue significantly inhibited the inhibitory effect of sildenafil on gastric emptying and intestinal motility. Based on these findings it is suggested that sildenafil in lower doses did not alter the gastric function in mice but at higher doses it showed inhibitory effect.

The inhibitory effect of sildenafil on the oesophagus and the gastrointestinal smooth muscles may have deleterious or beneficial effects depending upon the clinical conditions and the dose employed. The inhibitory effect of sildenafil lowers LOS pressure and propulsive forces in the oesophageal body in healthy subjects and in patients with nutcracker oesophagus, hypertensive LOS, and achalasia. However, the inhibitory effect on the gastrointestinal tract may be responsible for the dyspeptic complaints. Interestingly, it may have beneficial effect in patients with dumping-type syndromes or accelerated gastric emptying or in conditions of diabetic gastroparesis or colitis where there is loss of nNOS that is altered with delayed gastric emptying and intestinal transit. Further, it is necessary to monitor the side effects associated with sildenafil due to inhibitory effects in clinical conditions.

References