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The effects of nifedipine and glyceryl trinitrate on anorectal muscle contractions: an "in vitro" study

The effects of various drugs on anorectal muscle

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Abstract

Aim: Numerous methods have been tried to reduce the anal sphincter spasm occurring in cases with anal fissure. One of these methods is chemical sphincterotomy performed with various drugs. We aimed to show and compare the "in vitro" effects of glyceryl trinitrate and nifedipine, which are among these drugs, in the organ bath, using the rat anal sphincter muscle. Material and Method: We used 18 Wistar-Albino rats in our experimental study. We used isolated experimental tissue chamber protocol. The sphincteric muscle tissue was placed in the tissue chamber containing Krebs solution, and the degree of contraction was measured as "mg". We evaluated the effects of drugs in both the baseline and the precontracted states. Results: Tissue relaxation response against nifedipine and glycerol trinitrate in both the baseline and the precontracted states were statistically significant. The relaxation response against nifedipine was higher than the one against glycerol trinitrate; however, it was not statistically significant. Discussion: We showed that both nifedipine and GTN were effective " in vitro" on muscle tissue relaxation in the organ bath. Both nifedipine and GTN were found to lead to significant reduction of tension at both the baseline and the precontracted states. Their effects were more significant in the precontracted muscle tissue than the tissue with baseline tension. Although clinical studies have revealed controversial results, since we have proven their efficacies "in vitro", we have the opinion that these two drugs may find more place for themselves in clinical use particularly with the purpose of chemical sphincterotomy, in other words, the relief of sphincter spasm, paying attention to their side effects.

Keywords

Organ Bath; Anal Fissure; Constipation; Chemical Sphincterotomy

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Introduction

Anal fissure, which occurs in chronic constipation in children, leads to reflex spasm of the anal sphincter. The child continuously postpones defecation due to the reflex spasm and this condition may lead to serious complications, starting with constipation refractory to medical treatment. There are various treatment methods for acute anal fissure and constipation in childhood; however, a chronic anal fissure may develop despite such treatments. Numerous medical and surgical techniques have been tried and are currently being tested to reduce the elevated sphincteric pressure and spasm, particularly in cases with a chronic anal fissure. Chemical sphincterotomy, performed by various drugs, is one of these methods [1,2].

Administration of various agents, such as nifedipine (calcium channel blocker), glyceryl trinitrate (GTN), and botulinum toxin, have gained importance in chemical sphincterotomy; however, the data related to their use in children are very limited [3-5].

The isolated organ bath provides a system in which the tissue maintains its survival *"in vitro"*. It is not possible to measure the potency of various drugs with clinically known responses of specific muscle tissue other than such systems. When we searched the medical literature, we found that very few studies have evaluated "in vitro" the anal sphincteric muscular responses against clinically used drugs. It was possible to show that nitric oxide, which is a by-product of GTN, did not have any effect on anal external sphincteric muscular tissue, and that it provided its effect by relaxation of the internal anal sphincteric tissue only by an organ bath study [6]. We met no organ bath study quantifying the response of anal sphincteric muscular tissue against nifedipine.

Given all this information, we aimed to determine the effects of the drugs such as GTN and nifedipine utilized in chemical sphincterotomy on the rat anal sphincteric muscular tissue "in vitro" and intended to base our determinations on tangible data in our study.

Material and Method

This study was performed in the Experimental Laboratory of Adnan Menderes University and approved by the Local Ethics Committee. Eighteen adult female Wistar rats were used.

Briefly, anesthesia was done with ketamine (50 mg/kg) and xylazine (3 mg/kg) injection intramuscularly. Following the dissection and excision of the anal channels in all rats, the obtained muscular tissue samples were inserted immediately in the carbogenized Krebs Henseleit solution (118.3 mM NaCl, 4.7 mM KCl, 1.2 mM MgSO4, 1.22 mM KH2PO4, 2.5 mM CaCl2, 25.0 mM NaHCO3and 11.1.mM glucose, Sigma-Aldrich). Each ring-shaped muscular tissue was suspended by two stainless steel hooks in the organ bath filled with Krebs Henseleit solution (May IOBS 99 Ankara-Turkey). The top hook was connected to the transducer (May GTA 0303 and Biopac Systems Inc. Model MP 100 – USA) with an appropriate-sized thread, and the contractions were measured in the unit of mg. Acq Knowledge was used as the computer software. Then, the standard Equilibrium phase was applied to each muscular tissue sample. The tissue was first stretched by 4 gr and waited for 10 minutes, then, the tension was increased to 6 gr and waited for 10 minutes, and finally, the tension was increased up to 8 gr and waited for 30 minutes. After the contraction curve was stabilized, 0.1 ml of 10 -4M (Molar Concentration) concentrated acetylcholine was administered by using amicropipette, and the contraction response was obtained. After reaching the peak contraction value and waited for 5 minutes, the relaxation response was obtained by administering 0.1 ml of 0.25 mg of atropine by using a micropipette. When the contraction curve became flattened, the bath was washed twice with Krebs solution and waited for approximately 30 minutes until the contraction value reached baseline level.

For evaluation of nifedipine, four drops (5 mg) of 10 mg nifedipine preparation was added to the bath, and the relaxation response within the bath was recorded. After the contraction curve was stabilized at baseline, the bath was washed three times with Krebs solution and waited for 15 minutes for stabilization of the contraction curve. The tissue was precontracted by administering 0.1 ml of 10 -4 M (Molar Concentration) concentrated acetylcholine (Ach) to the bath using a micropipette, and the contraction response was recorded. After reaching supramaximal concentration and waiting for 5 minutes, four drops (5 mg) of 10 mg nifedipine preparation was added to the bath, and the relaxation response at supramaximal concentration was recorded.

For evaluation of GTN, 0.2 mg (0.2 ml) of 1 mg/ml GTN preparation was added to the bath, and the relaxation response within the bath was recorded. After the contraction curve was stabilized at baseline, the bath was washed three times with Krebs solution and waited for 15 minutes for stabilization of the contraction curve. The tissue was precontracted by administering 0.1 ml of 10 -4 M (Molar Concentration) concentrated acetylcholine (Ach) to the bath using a micropipette, and the contraction response was recorded. After reaching supramaximal concentration and waiting for 5 minutes, 0.2 mg (0.2 ml) of 1 mg/ml GTN preparation was added to the bath, and the relaxation response at supramaximal concentration was recorded.

All data were statistically compared by using t-test general linear model and ANOVA Tukey's multiple comparison tests. The results were considered statistically significant when the p-value was less than 0.05.

Results

The differences between the pre- and post-administration values were found to be statistically significant for both nifedipine and GTN in the tissue samples with baseline tension in the organ bath, and therefore both nifedipine and GTN were considered to cause relaxation in the muscular tissues with baseline tension ($p<0.05$) (Fig 1). When nifedipine and GTN were administered to the tissue samples precontracted with Ach in the organ bath, the differences between the pre- and post-administration values were found to be statistically significant for both nifedipine and GTN. Therefore, both nifedipine and GTN were considered to cause relaxation in the pre-contracted state in muscular tissues ($p<0.05$).

When the relaxation responses in tissues with baseline tension and tissues at the pre-contracted state were compared, they were found to be statistically significantly higher in pre-contracted tissue samples regarding both nifedipine and GTN ($p<0.05$).

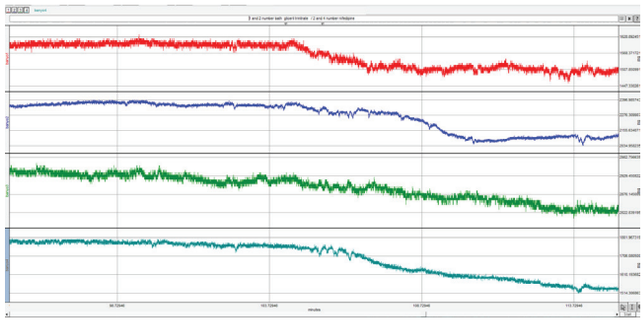

Fig 1.

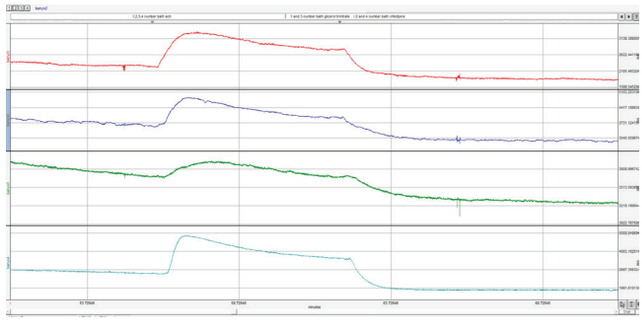

Fig 2.

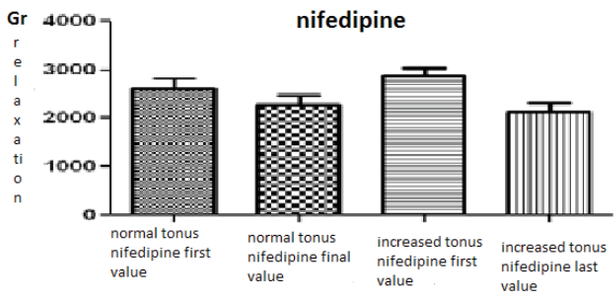

Fig 3.

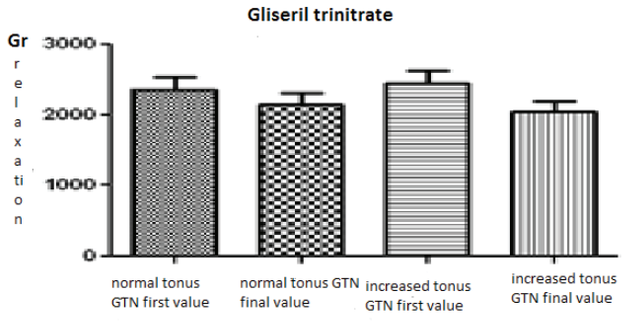

Fig 4.

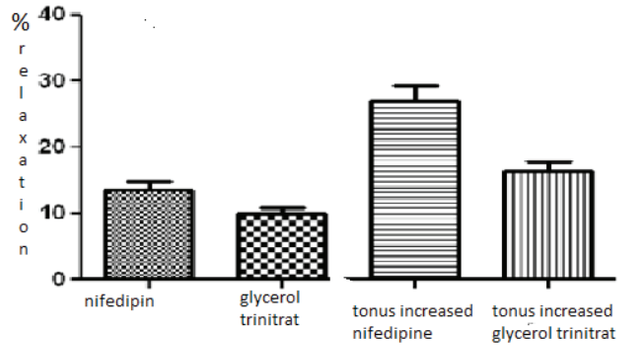

Fig 5.

When the effects of nifedipine and GTN were compared, nifedipine was determined to provide more relaxation than GTN in tissue samples with baseline tension; however, this difference was statistically insignificant ($p>0.05$) (Fig. 1-2). In tissue samples precontracted with Ach, the relaxation response obtained with nifedipine was statistically significantly higher than the response obtained with GTN (Fig. 3). Nifedipine was considered to provide more effective relaxation than GTN in precontracted tissue.

Discussion

The anal fissure is a common anorectal disorder, being even more common in children with chronic constipation and under three years of age. Typically, it leads to pain during defecation, eventually resulting in the spasm of the internal anal sphincter [7,8]. The continuously elevated baseline anal pressure causes disturbance of the intra-sphincteric blood perfusion, and relatively ischemic areas occur throughout the anal channel in children. This ischemic environment leads to prolongation of the spontaneous healing process of anal fissures. It has been suggested that the perfusion in the epithelium of the anal channel is improved with reduction of the anal pressure in general [9,10]. For this reason, the most significant purpose is increasing the blood flow to the ischemic region to facilitate the healing process, and this can be provided by the reduction of the resting pressure of the internal anal sphincter [8,11,12]. Previous studies have shown that in cases with low internal anal sphincter pressure, the healing rate of the fissure is above 90% [13].

Numerous drugs such as nifedipine, nitric oxide, botulinum toxin have been tried with the purpose of reducing the internal anal sphincter pressure in other words, for chemical sphincterotomy [14]. In our study, we aimed to make the “in vitro” quantification of the clinically observed effects of nifedipine and GTN. In the first phase of our study, we measured the effects of GTN and nifedipine “in vitro” on tissue samples obtained from the rat anal channel at baseline tension and investigated for a difference between them. We found that both nifedipine and GTN provided statistically significant relaxation at baseline tension. However, we did not determine any superiority of these drugs against each other.

Since a reflex contraction of the anal sphincteric muscle is present in the pathophysiology of anal fissure, for resemblance to the natural pathophysiological process, in other words, with the purpose of simulating the sphincter spasm, we first created a contraction in the anal channel muscular tissue samples (precontraction) in the second phase of our study. Then, we administered the drugs and obtained the muscular relaxation responses. We determined that, for both nifedipine and GTH, the tissue tension measured following administration of the drug was significantly lower than the tension at the pre-contracted state, before the administration. Also, we found that the relaxation responses against both drugs were significantly increased in the precontracted tissue samples when compared to the samples with baseline tension; this relaxation response difference was more significantly increased for nifedipine when compared to GTN.

In summary, both nifedipine and GTN were determined to lead to muscular relaxation response in both the tissue samples

with baseline tension and the tissue samples at pre-contracted state. Additionally, nifedipine was determined to cause more statistically significant relaxation response in the pre-contracted tissue sample when compared to GTN.

To our knowledge, no study showing the effects of nifedipine and GTN on muscular tissue "in vitro" has been published in the medical literature yet. Studies with contradictory results regarding the clinical use of topical GTN are presented in the medical literature. While some studies have reported the rate of success in treatment as 80%, some others have found no superiority of GTN against placebo [5,11,12,15-17]. We quantitatively showed the relaxation effect of GTN, clinical use of which has such diverse study results, on anal channel muscular tissue "in vitro", and determined that it created more effective relaxation in precontracted muscle tissue. Since precontracted muscular tissue simulates internal sphincter spasm, we consider that we have shown the presence of the clinical effectivity of GTN in this model.

The results of various studies have shown that with the aid of topical nifedipine, the anal pressure was effectively reduced, and recovery was achieved with a rate of 67-89.4% without any side effects. Also, it has been argued that due to its minimal systemic absorption, it had no side effect [5,15]. Çevik et al. in their study have conducted in pediatric patients, reported that nifedipine was significantly more effective than GTN and lidocaine, with fewer side effects and faster regression of the symptoms [16]. Recently conducted studies in both children and adults have also revealed that topical nifedipine use is effective in the healing process of anal fissures with minimal side effects [4,16,18]. Regarding the medical literature on topical nifedipine, studies showing that it causes sphincter relaxation in animal experiments and the adult population are present [19,20]. The statistically significantly increased relaxation response of the precontracted muscular tissue "in vitro", which simulates sphincter spasm, with nifedipine supports these clinical studies. In conclusion, we showed that both nifedipine and GTN were effective "in vitro" on muscle tissue relaxation in the organ bath. Both nifedipine and GTN were found to lead to significant reduction of tension at both the baseline and the precontracted states. Their effects were more significant in the precontracted muscle tissue than the tissue with baseline tension. Although clinical studies have revealed controversial results, since we have proven their efficacies "in vitro", we have the opinion that these two drugs may find more place for themselves in clinical use particularly with the purpose of chemical sphincterotomy, in other words, the relief of sphincter spasm, paying attention to their side effects. Nifedipine may be the first choice in chemical sphincterotomy since its "*in vitro*" relaxation effect is higher than GTN in the precontracted state, which simulates anal sphincter spasm seen in anal fissure.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

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