An evaluation of some lipid derivatives in treating irritable bowel syndrome in isolated rat intestine model

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Key words: irritable bowel syndrome, malonic acid, peppermint oil, citric acid

Abstract

Irritable bowel syndrome IBS remains one of the most widely distributed disorder that can interfere with human health, habitats and activities and hence causes a considerable health, social and economic burden. As a trial to solve this problem an isolated piece of rat intestine was prepared as an in vitro model of IBS by which some lipid derivatives were assessed for their effects on intestinal strain of contraction which was recorded with a highly sensitive physiograph connected to the piece of intestine through a mechanical transducer. Malonic acid, citric acid and peppermint oil were evaluated in comparison to a standard mebeverine and amlodipine calcium reducer agents. There was a significant increase in intestinal contraction induced with malonic acid as compared with basal, amlodipine and mebeverine associated contractions (80, 50 and 50 mg respectively) at P< 0.05. however, peppermint caused no significant increase in strain whereas citric acid caused a highly significant relaxing effect down to 8 mg strain at P< 0.01 which could be implemented in human IBS. In a conclusion, citric acid showed a promising therapeutic intestinal relaxation that may be of benefit in human IBS.

Introduction

Irritable bowel syndrome (IBS) or spastic colon, is a functional bowel disorder (1), recently modified Rome criteria (Rome II criteria) as the presence for at least 12 weeks (not necessarily consecutive) in the preceding 12 months of abdominal discomfort or pain that cannot be explained by a structural or biochemical abnormality and that has at least two of following three features: (1) pain is relieved with defecation, and its onset is associated (2) with a change in the frequency of bowel movements (diarrhea or constipation) or (3) with a change in the form of the stool (loose, watery, or pellet-like).
Although the Rome II criteria were not designed to be a management guideline, IBS, and at least a subset of functional dyspepsia, are related disorders that can manifest in the same patient at different times or that can coexist in up to 90% of patients (4).

The aggregate cost of irritable bowel syndrome in the United States has been estimated at $1.7-$10 billion in direct medical costs, with an additional $20 billion in indirect costs, for a total of $21.7-$30 billion (5). Compared with healthy subjects, in irritable bowel there is an increased motility in response to various stimuli, such as psychological stress, meals, and balloon inflation in the gut (6). Ninety-five percent of serotonin is in the GI tract, within enterochromaffin cells, neurons, mast cells, and smooth muscle cells. When released by enterochromaffin cells, serotonin stimulates extrinsic vagal afferent nerve fibers and intrinsic enteric afferent nerve fibers, resulting in such physiologic responses as intestinal secretion and the peristaltic reflex and in such symptoms as nausea, vomiting, abdominal pain, and bloating (7). Preliminary evidence suggests that patients with IBS have increased serotonin levels in plasma and in the rectosigmoid colon. (Other neurotransmitters that may play a role in IBS include calcitonin gene-related peptide, nitric oxide, and vasoactive intestinal peptide.

**Materials and Methods**

<table>
<thead>
<tr>
<th>Drug and Chemical agent:</th>
<th>Apparatus:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1- Diazepam ampoule 10mg/2ml</td>
<td>1- Cylinder flask (1000ml)</td>
</tr>
<tr>
<td>2- Tyrode solution which compose</td>
<td>Electrical balance</td>
</tr>
<tr>
<td>from:</td>
<td>Hung and stand</td>
</tr>
<tr>
<td>a- Sodium chloride (NaCl) 8 gm.</td>
<td>Recorder (Marco bio-system, INC, Houston-Texas).</td>
</tr>
<tr>
<td>b- Potassium chloride (KCl) 2 gm.</td>
<td>Organic path (Scientific and Research instrument LTD, England).</td>
</tr>
<tr>
<td>c- Magnesium chloride (MgCl2) 0.1 gm.</td>
<td>Transfuse and Air pump.</td>
</tr>
<tr>
<td>e- Calcium chloride (CaCl2) 0.2 gm.</td>
<td>Medical Scissors.</td>
</tr>
<tr>
<td>g- Sodium bicarbonate (NaHCo) 31 gm.</td>
<td></td>
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<tr>
<td>h- Sodium phosphate (NaH2Po4) 0.05 gm.</td>
<td></td>
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<tr>
<td>i- Glucose 1 gm.</td>
<td></td>
</tr>
<tr>
<td>j- Gas (O2).</td>
<td></td>
</tr>
<tr>
<td>K- Distilled water.</td>
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<tr>
<td>3- Test Agent:</td>
<td></td>
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<tr>
<td>a- Citric acid</td>
<td></td>
</tr>
<tr>
<td>b- Peppermint oil</td>
<td></td>
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<tr>
<td>c- Malonic acid</td>
<td></td>
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<tr>
<td>d- Amlodipine</td>
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<tr>
<td>e- Mebeverine</td>
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</tbody>
</table>

**Procedure:**

1- We prepared tyrod solution that used to keep the life small intestine parts. This solution was prepared by take the weight of each constituent by used electrical balance.
such as take 8gm of NaCl , 2gm of KCl , 0.1gm of MgCl2 , 0.2gm of CaCl2 , 0.05gm of NaH2PO4 , 1gm of NaHCo3 ,1 gm of Glucose. Then all dissolve in 1000 ml of distal water except MgCl2 dissolve alone, then mix all them together in cylinder flask.

2- we made the equipment as shown in fig (3) and the organ bath was filled by distilled water then heat the organ bath to 25°C and kept the temperature of distilled water at 25°C then empting the distilled water from the incubation beaker then add tyrode solution to the incubation beaker.

3-we gave the rat killing dose of diazepam by intraperitoneal rout (4 mg/2ml) then isolate part from small intestine (3cm) then put the isolated part in tyrode solution then cleaning this part from waste material.

4- The isolated part was fixed in incubation beaker in organ bath by bind one side of isolated part to transfuser hook then to recorder while other side bind to air pump hook, then put the isolated piece in tyrode solution.

5- The contraction of small intestine muscle was recorded by use recorder and this result was represent the first base result(basal).

6-The first test agent is peppermint oil (0.15mg/5ml)was injected beside the wall of incubation beaker then record the muscle contraction as shown in figure(1).

7-we emptied the tyrode solution that contain peppermint oil, then fill the test beaker by new tyrod solution, then record the result which represent the second base result.

8-add the second test agent which is malonic acid ,then record the result and remove the tyrod solution ,then add other test agent with continuous record the result.

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**Figure (1) Organ bath apparatus of testing effect of different agents on intestinal contractions.**

**Results**

Table (1) The means of physiograph recorded strains in milligrams of isolated rat intestine that were induced with different lipid derivatives in comparison with the conventionally used meberverine and amlodipine to relax smooth muscles with their statistical t test assessment at P< 0.05.
The materials | Tonicity of intestinal contraction induced with different test agents | Statistical t test of significance
---|---|---
Basal | 0.05+/-0.001 g | -
Mebeverine | 0.05+/-0.001 g | -
Malonic acid | 0.08+/-0.001 g | Significant increase
Amlodipine | 0.01+/-0.001 g | Significant decrease
Citric acid | 0.008+/-0.001 g | Significant decrease
Peppermint oil | 0.06+/-0.001 g | Not significant increase

Figure (2) shows the means of isolated rat intestinal strains of contractions in mg in relation to different test agents in organ bath test model.

**Discussion**

Irritable bowel syndrome (IBS) is characterised by abdominal pain, distension, and an abnormality of bowel habit. The precise cause of IBS is still unclear but it is likely to be multifactorial (8). The two most consistent characteristics found in these patients are visceral hypersensitivity and psychological morbidity.

The diagnosis of IBS should be based on typical parameters rather than extensive investigations to exclude other disorders (9). Standardised diagnostic criteria may be helpful. Lifestyle and dietary advice should also be considered (10). Although an increase in dietary fiber is often advocated, many patients find wheat bran makes their symptoms worse. Current drug treatments for IBS are of limited value and have a poor
evidence base. However, specific symptoms may respond in some patients. If drugs are used they should be targeted at the predominant symptom(s): abdominal pain, diarrhoea or constipation (11). IBS is still a social and health problem that it has prevalence rate upto 22%. This is because different diagnostic criteria are used and at least 50% of patients with IBS never consult a doctor. Symptoms occur more often in women than men (12,13). From results of research, a variety in histograms have been noticed between materials that were tested on rat intestine piece to study their effects in figure (2), that mebeverine caused the same basal strain (50 mg) and maintained the relaxation of intestine in a stabilized form, so that mebeverine of choice to treat colon or intestine spasm. Malonic acid and peppermint oil, when used and contributed to a high intestinal tonicity expressed as an increased strain (80, 60 mg respectively) similar to that in irritable bowel syndrome. While citric acid slowed a highly significant decrease in strain (only 8 mg at P< 0.05) even more potent intestinal relaxant than mebeverine. Amlodipine decreased the tension of intestine which may be attributed to its effect of blocking calcium channel in smooth muscle cells leading to reduce contraction of rat intestinal piece so, reduce spasm that could be further evaluated in human irritable bowel. The results of this current research were similar to other studies done by different physicians (14).

Recently, the patients with irritable bowel syndrome or neuronal colon (common name by populations) take or use many medications were described by physicians but the most effective one was mebeverine over other gastro-intestinal drugs (15). Mebeverine is a musculotropic antispasmodic drug without atropic side-effects whose major therapeutic role is in the treatment of irritable bowel syndrome. It is also indicated for treatment of gastrointestinal spasm secondary to organic disorder (15). It was first registered in 1965 and manufacture by Solvay Pharmaceuticals in Netherland under trade name Duspatalin and strength 135mg/tab, which given a good results when used by patients and a higher potency over other trade names by other companies, like colospasmine by EIPICO in Egypt, Dupaline by Asia in Syria, and Mevir by Micro in India. Therapeutic trial in treating IBS

Antispasmodic drugs relieve cramps or spasms of the stomach and intestines.

Peppermint Oil (Mentha Piperita) Organic Essential Oil.

Peppermint oil is considered by aromatherapists as one of the more indispensable essential oils. The oil should be a part of every traveler's first aid kit - it can work wonders for motion sickness and general nausea for some people. It is often taken internally for this; whereas ginger oil can be diluted and rubbed into the abdomen. Medical research has found Peppermint oil to be effective for irritable bowel syndrome (peppermint oil should be taken in enterically coated capsules). Further, French literature suggests Peppermint for asthma due to its liver strengthening and regenerating properties.

Peppermint oil is steam distilled from the partially dried tops of the plants. Growers will harvest just before the herb goes to flower to bring out the best of the oil's aroma. When allowed to mature further, the quality of the resulting oil may suffer, with a sharper, less-sweet and complex aroma.

The main constituent of peppermint oil is menthol, a potent chemical unto itself which causes a quick physical response when inhaled or applied to the skin. Menthol produces a sensation of coolness which the body reacts to by producing its own warming effect, with as blood flowing to the area of application. This physical sensation is responsible for peppermint's long history of use as medicine. Today menthol is often found in sports creams and chest rubs, such as the well-known 'Halls Mentholyptus' cough drops - the oil is excellent for opening the sinus passages, though should be used
with caution in this respect. Even a small amount of the oil coming into direct contact with the delicate membranes inside the nasal passages can result in a temporary burning sensation. The oil has shown favorable results in clinical testing for the treatment of headaches, without side effects at the dosages used. The oil has also been touted to possibly help arthritis, depression, skin conditions, food poisoning, headaches (rub a small amount on the temples) and may increase the receptivity of the sensory system for some people. Peppermint oil should not be used with children under two years of age; instead, Spearmint oil, peppermint's milder, sweeter cousin, is an excellent choice.

Malonic acid

Malonic acid (systematic name: propanedioic acid Other names is methanedicarboxylic acid) , Chemical formula is C₃H₄O₄ , pKₐ is 2.83(pK₁) 5.69 (pK₂), Molecular mass is 104.03 g/mol , Melting point is 135 - 137 °C , Solubility in water is Completely Soluble is a dicarboxylic acid with structure CH₂(COOH)₂. The ionised form of malonic acid, as well as its esters and salts, are known as malonates. For example, diethyl malonate is malonic acid's ethyl ester. The name originates from Latin malum, meaning apple. The calcium salt of malonic acid occurs in high concentrations in beet root. A classical preparation of malonic acid starts from acetic acid. This acid is chlorinated to chloroacetic acid.

Mebeverine

Mebeverine HCI is amusculotropic antispasmodic drug without atropic side-effects whose major therapeutic role is in the treatment of irritable bowel syndrome. It is also indicated for treatment of gastrointestinal spasm secondary to organic disorder. It was first registered in 1965 and manufacture by Solvay Pharmaceuticals.

Mebeverine HCI presented in tablets (100mg or 135mg), capsules (200mg slow release), and liquid (10mg/ml).Mebeverine HCI belongs to a group of compounds called musculotropic antispasmodics. These compounds act directly on the gut muscles at the cellular level to relax them. Mebeverine is also an inhibitor of calcium-depot replenishment. Therefore, mebeverine has dual mode of action which normalizes the small bowel motility.

Conclusion

There was a promising antispasmodic effect induced by citric acid that could be evaluated further as a beneficious alternative therapy for human irritable bowel syndrome.

References


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