EFFECT OF AMLODIPINE ON ENTEROPATHY INDUCED BY INDOMETHACIN IN RATS

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ABSTRACT

Objective: Non-steroidal anti-inflammatory drugs (NSAIDs) have become well known for causing gastric duodenal mucosal damage. In addition, they are also known to affect the small intestine in humans. Amlodipine is a third-generation dihydropyridine-type calcium channel blocker; it can inhibit inflammatory cytokines and enhance antioxidant defenses. The aim of this study was to evaluate the effect of Amlodipine on indomethacin-induced enteropathy in rats.

Methods: Enteropathy was induced by subcutaneous indomethacin (Indo) prepared in 5% sodium bicarbonate administrated at a dose rate of 9 mg/kg for two days at 24h intervals. Amlodipine (10 mg/Kg body weight po) was administrated for seven consecutive days beginning 24 h after the first Indo injection. Rats were sacrificed under ether anesthesia on the 8th day. The small intestinal injury was assessed by body weight loss, small intestine weight/length ratio, macroscopic damage, histological study, as well as by biochemical measurement of reduced glutathione (GSH), lipid peroxides and superoxide dismutase (SOD) activity in the small intestine tissue.

Results: The results showed that Amlodipine didn’t decrease body weight loss, it decreased small intestine weight/length ratio, macroscopic and microscopic small intestinal damage scores caused by administration of Indo. It also increased SOD activity and decreased lipid peroxidation. The effect on the level of GSH wasn’t observed. No statistical significance was observed when previous findings were compared to Indo induced enteropathy group (p>0.05).

Conclusion: Amlodipine didn’t produce an obvious enhancement in enteropathy induced by Indo in rats.

Keywords: NSAIDS, Enteropathy, Amlodipine, Histopathology study, Superoxide dismutase activity, Reduced glutathione, Lipid peroxides

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are some of the most commonly prescribed drugs in the world. As a result of their anti-inflammatory, analgesic, and antiplatelet effects [1].

Nonsteroidal anti-inflammatory drugs (NSAIDs) have become well known for causing gastrointestinal mucosal damage. In addition, they are also known to affect the small intestine in humans [2]. NSAID-induced enteropathy has gained much attention due to the introduction of new emerging diagnostic modalities, capsule endoscopy (CE) and device-assisted enteroscopy as well as due to the increased use of aspirin and NSAIDs. Previous attention or studies have focused primarily on upper GI events but recent studies have shifted to the small bowel and colon during chronic NSAID use [3]. NSAIDs can cause a variety of functional and structural abnormalities in the small intestine, such as increased intestinal permeability, intestinal inflammation, protein loss, blood loss, ulceration, perforation, diaphragm-like strictures, and ileal dysfunction [4]. Several factors are involved in the pathogenesis of NSAID-induced enteropathy, including a deficiency in prostaglandins (PGs), bile acid, bacterial flora, and nitric oxide (NO) [5].

NSAIDs have recently increased; therefore, increased awareness of the gastrointestinal side effects is needed. However, effective prevention and treatment of the side effects of NSAIDs in the small intestine have not yet been determined [6].

In spite of relatively intense research, there is still no effective, safe and tolerable drug treatment available in the market for the management of NSAID-enteropathy [7].

Amlodipine is a third-generation dihydropyridine-type calcium channel blocker commonly used for the treatment of hypertension [8]. Experimental studies have shown that Amlodipine can inhibit inflammatory cytokines and enhance antioxidant defenses [9].

However, the effect of Amlodipine on NSAIDs induced enteropathy has not yet been studied.

MATERIALS AND METHODS

Animals

Female and male wistar albino rats weighing 160-280 g were purchased from the Scientific Research Center, Damascus, Syria. The animals were provided with ad libitum food and water. The animals were kept at controlled environmental conditions (temperature 23±2°C, humidity 55±15%, lighting regimen of 12h light: 12-h dark). They were acclimatized for one week before any experiment.

Experimental design

Rats were divided into three groups:

Group I: normal control group (6 rats in this group) received oral vehicle (physiological saline).

Group II: Indo control group (7 rats in this group) received subcutaneous Indo prepared in 5% sodium bicarbonate, administered at a dose rate of 9 mg/kg for two days at 24h intervals. It also received an oral vehicle (physiological saline).

Group III: Amlodipine treated group (6 rats in this group) received Amlodipine dissolved in physiological saline (10 mg/kg body weight po) for seven consecutive days beginning 24 h after the first Indo injection. Indo (9 mg/kg/day) was given at a dose rate of 9 mg/kg for two days at 24h intervals in order to induce enteropathy.

Tissue collection and preparation

On day eight, each subgroup of animals across all groups was sacrificed. The small intestine was removed and opened longitudinally along their antimesenteric borders, tissues were washed in saline solution, and any macroscopic change was checked.

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A precise evaluation of the lesions was made after each specimen was fixed in 10% formalin.

Intestinal tissue from jejunum was collected and stored at -80 °C till further analysis.

Clinical findings

During the study, rats were checked daily for body weight, behavioural changes, food intake, intestinal bleeding and stool consistency. The bodyweight of animals was measured at regular time intervals from day 0 to 7. Change of body weight (%) was calculated.

Small intestine weight/length ratio

The length and weight of the small intestine were measured for the estimation of:

Weight of the intestine (g)/length of the intestine (cm) ratio

Macroscopic characters [10]

| Score | Macroscopic score |
|-------|-------------------|
| 0     | No visible change |
| 1     | Hyperemia at sites |
| 2     | Lesions having diameter 1 mm or less |
| 3     | Lesions having diameter 2 mm or less (number<5) |
| 4     | Lesions having diameter 2 mm or less (number 5-10) |
| 5     | Lesions having diameter 2 mm or less (number>10) |
| 6     | Lesions having diameter more than 2 mm (number<5) |
| 7     | Lesions having diameter more than 2 mm (number 5-10) |
| 8     | Lesions having diameter more than 2 mm (number>10) |

Histopathological observations

A portion of the distal small intestine (jejunum) specimen from each rat was fixed with 10% formalin, embedded in paraffin wax and cut into sections of 5 mm thickness. The sections were stained with hematoxylin and eosin (H and E) dye for histopathological observations. The following histological features were examined by an unbiased pathologist (AM) blinded to the experimental design: grade and type of inflammation, an extension of inflammation throughout the gastrointestinal wall (mucosa, submucosa, muscular layer and serous membrane), presence of Lymphocytic aggregate/Follicle, Necrosis, Granuloma, Cryptitis, Crypt abscess and epithelial lesions (erosions, ulcers) [11].

Biochemical estimations

Accurately weighed tissues from jejunum were homogenized in cold phosphate-buffered saline [pH 7.4, 50 mmol] to prepare 10% homogenate and the suspension was divided into three portions. One part of tissue suspension was mixed with 0.2 ml 5% trichloroacetic acid to give a compound that absorbs at 412 nm (Ellman’s method). In short, each sample cuvette containing 0.375% TBA, 15% trichloroacetic acid and 0.25 N HCl. Samples were boiled for 15 min, cooled and centrifuged. Absorbance of the supernatant was measured by spectrophotometer measured at 532 nm. The concentration of MDA was calculated by the absorbance coefficient of MDA–TBA complex (1.56 × 10^-5 M/cm) and expressed in μmol/100 g of tissue [14, 15].

Lipid peroxidation, an indicator of mucosal injury induced by reactive oxygen species was measured as thiobarbituric acid reactive substance. Briefly, 0.5 ml of small intestinal tissue homogenates prepared were reacted with 2 ml of TBA reagent containing 0.375% TBA, 15% trichloroacetic acid and 0.25 N HCl. Samples were boiled for 15 min, cooled and centrifuged. Absorbance of the supernatant was measured by spectrophotometer measured at 532 nm. The concentration of MDA was calculated by the absorbance coefficient of MDA–TBA complex (1.56 × 10^-5 M/cm) and expressed in μmol/100 g of tissue [14, 15].

Statistical analysis

Data analyses were achieved using a software program Graph Pad Prism version 8. Data were expressed as mean±SEM, and different groups were compared using one-way analysis of variance (ANOVA) followed by Sidak test for multiple comparisons for parametric data, and Kruskal–Wallis test followed by Dunn test for multiple comparisons for non-parametric data and parametric data that have shown non-normal distribution. P values less than 0.05 were considered significance statistically.

RESULTS

Clinical findings, general observation and body weight change

After 24 h of administration first dose of Indomethacin, animals developed soft feces, weakness, decreased food intake and progressively body weight loss. All these symptoms reached a maximum at three days from first dose of Indomethacin, and then these symptoms started to decrease gradually. Compared with that of the normal control group, which revealed an increase in body weight (1.63%), the bodyweight of the Indomethacin control group at the end of the experiment was reduced by (-4.58%) with no statistical significance comparing with the normal control group (p=0.1891) (table 2).
Table 2: Effect of amlodipine on the body weight in Indo induced enteropathy in rats

| Parameter group     | Initial body weight | Final body weight | Body weight change % |
|---------------------|---------------------|-------------------|----------------------|
| Normal control      | 194.2±8.29          | 196.8±7.76        | 1.63±2.76            |
| Indo control        | 204.1±13.14         | 194.9±14.03       | -4.58±2.28*          |
| Amlodipine treated  | 216.7±9.09          | 197.7±6.03        | -8.49±1.94           |

Values are given as mean±SEM values are statistically significant at *P<0.05 between Normal and Indo control groups

Small intestine weight/length ratio

Small intestine weight/length ratio is a reliable indirect marker of the small intestinal inflammation. It was observed an increase in this ratio in the Indo control group; there was statistical significance comparing with normal control group (p=0.0372).

Amlodipine treated group revealed decrease in small intestine weight/length ratio with no statistical significance comparing with Indo control group (p=0.1521) (fig. 1).

Macroscopic score

The most sections of distal small intestine in normal control group didn’t reveal any morphological changes. In contrast, subcutaneous injection of Indo produced damage in the distal small intestine. Adhesions, erosion, edema, hemorrhagic spots were noticed. These lesions have a diameter greater than 2 mm, thus the morphological score in the Indo control group was significantly increased (p=0.0006) as compared to normal control group.

Amlodipine treated group revealed decrease in the severity of the gross lesion (fig. 2) (table 3), but there was no statistical significance comparing with Indo control group (p=0.4172) (fig. 3).

Histopathological study

The distal small intestine specimen of 50 % of rats in the normal control group revealed an intact architecture, while the distal small intestine specimen of 50 % of rats from this group revealed increased inflammatory cells infiltration, the inflammation was mild to moderate.

On the other hand the distal small intestine specimen of Indo control group revealed increased inflammatory cell infiltration, transmural inflammation, lymphocytic aggregate, cryptitis and ulcerations. There was statistical significance comparing with normal control group (p=0.0084).

Administration of Amlodipine as therapy revealed in some rats reduce the severity of the injury of the distal small intestine (table 4) (fig. 4) with no statistical significance comparing with Indo control group (p=0.3382) (fig. 5).

Fig. 1: Effect of amlodipine on small intestine weight/length ratio in Indo induced enteropathy in rats. Data are expressed as mean±SEM ▲ Significant difference as compared to normal control group at p<0.05

Table 3: Macroscopic score of different experimental groups

| Group macroscopic score | Normal control | Indo control | Amlodipine treated |
|-------------------------|----------------|-------------|--------------------|
| 0                       | 5 (83.33 %)    | 1 (16.67 %) | 1 (16.67 %)        |
| 1                       | 1 (16.67 %)    |             |                    |
| 2                       | 2             |             |                    |
| 3                       | 3             |             |                    |
| 4                       | 4             |             |                    |
| 5                       | 5 (85.71 %)    |             |                    |
| 6                       | 6 (85.71 %)    |             |                    |
| 7                       | 7 (14.02 %)    |             | 5 (83.33 %)        |
| 8                       | 8             |             |                    |
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Fig. 2: Macroscopic appearances of the distal small intestine in Indo induced enteropathy in rats. a-Normal control group (score 0), b-Indo control group (score 7), c-Amlodipine treated group (score 1), d-Amlodipine treated group (score 6)

Fig. 3: Effect of Amlodipine on the macroscopic score in Indo induced enteropathy in rats. Data are expressed as mean±SEM ▲▲Significant difference as compared to the normal control group at p<0.001

Table 4: Microscopic score of different experimental groups

| Group microscopic score | Normal control | Indo control | Amlodipine treated |
|-------------------------|----------------|-------------|--------------------|
| 0                       | 3 (50 %)       |             | 2 (33.33 %)        |
| 1                       | 1 (16.67 %)    | 2 (28.57 %) | 1 (16.67 %)        |
| 2                       | 2 (33.33 %)    | 2 (28.57 %) | 2 (33.33 %)        |
| 3                       |               | 2 (28.57 %) | 1 (16.67 %)        |
| 4                       |               |             | 2 (33.33 %)        |
| 5                       |               | 3 (42.86 %) |                    |
| 6                       |               |             |                    |
| 7                       |               |             |                    |
| 8                       |               |             |                    |
| 9                       |               |             |                    |
| 10                      |               |             |                    |
| 11                      |               |             |                    |
Fig. 4: Histological appearance of jejunum tissue sections, original magnification ×10, a-Normal control group (grade 0) shows an intact architecture, b-Indo control group (grade 5) shows focal cryptitis, transmural inflammation and ulceration, c-Amlodipine treated group (grade 0) shows an intact architecture, d-Amlodipine treated group (grade 4) shows transmural inflammation and lymphocytic aggregate

Fig. 5: Effect of Amlodipine on the microscopic score in Indo induced enteropathy in rats. Data are expressed as mean±SEM, ▲▲Significant difference as compared to the normal control group at p<0.01

Table 5: Effect of Amlodipine on lipid peroxides, GSH and SOD activity in Indo induced enteropathy in rats

| Group parameter         | SOD activity | GSH levels (μm/g of tissue) | Lipid peroxides (μmol/100 g of tissue) |
|-------------------------|--------------|----------------------------|----------------------------------------|
| Normal control          | 0.588±0.058675 | 1.925±0.213239             | 44.33±4.33333                         |
| Indo control            | 0.175±0.05719  | 1.391±0.137207             | 79.71±16.53238                        |
| Amlodipine treated      | 0.319±0.190946 | 1.133±0.162714             | 72.16±12.53107                        |

Data are expressed as mean±SEM, * Significant difference as compared to normal control group at p<0.05

Biochemical assays

Indo increased oxidative stress in the small intestine; it was evaluated by lipid peroxidation, SOD activity and GSH levels. Indo increased the levels of lipid peroxides, decreased SOD activity and GSH levels in the distal small intestine; there was statistical significance comparing with the normal control group (SOD: p=0.0413, GSH: p=0.0223, Lipid peroxides: p=0.0294).

Amlodipine treated group revealed a decrease in the levels of lipid peroxides, an increase in SOD activity. The levels of GSH in the distal small intestine tissues were decreased. There was no statistical significance comparing with the Indo control group (SOD: p=0.6746, GSH: p=0.5996, Lipid peroxides: p=0.6308) (table 5).

DISCUSSION

The ability of nonsteroidal anti-inflammatory drugs (NSAIDs) to damage the gastric mucosa and exacerbate preexisting ulcers in the stomach and duodenum is well established. Over the past decade, there has been increasing recognition of the damaging effects of NSAIDs on more distal regions of the small intestine [16]. Indo is a potent non-steroidal anti-inflammatory drug of proven effectiveness in man and in animals. Similar to other anti-
inflammatory agents, Indol has been reported to produce gastrointestinal irritation and ulceration in man as well as in animals [17]. Indol induces small intestinal and colonic ulceration in a dose-dependent fashion in rodents [18]. Depending on the dose and route of Indol administration, as well as the strain of rat, Indol-induced jejunalitis and intestinal inflammation usually resolved spontaneously and completely within 1–2 w [19]. YAMADA et al. have shown that one injection of Indol (7.5 mg/kg) produced acute injury and inflammation in the distal jejunum and proximal ileum that were maximal at three days and completely resolved within one week. Two daily subcutaneous injections of Indol produced a more extensive and chronic inflammation that lasted in an active form in more than 75% of the rats for at least two weeks [20]. This study has shown that the administration of two daily subcutaneous injections of Indol (9 mg/kg) was suitable to induce the injury in the small intestine.

Small bowel injury induced by NSAIDs is usually developed via COX-dependent decreases in prostaglandin production and there is a very close association between NSAID-induced apoptosis and oxidative stress [21]. Thus, NSAIDs are known to significantly increase intracellular ROS production [21]. The administration of Indol results in the generation of free radicals in enterocytes, possibly as a result of mitochondrial dysfunction produced and the infiltration of neutrophils into the mucosa [22].

In this study, Indol-induced enteropathy, as evidenced by body weight loss, reduction in food intake, increase in small intestine weight/length ratio, changes in biochemical parameters which include depletion of GSH, increased lipid peroxides levels and decreased SOD activity in small intestinal tissues. The macroscopic results revealed adhesions, erosion, edema, and hemorrhagic spots, as well as the microscopic score revealed increased inflammatory cells infiltration, transmural inflammation, lymphocytic aggregate, cryptitis and ulcera- tions.

The present study highlights the therapeutic effects of Amlodipine a third-generation dihydropyridine-type calcium channel blocker on Indol-induced enteropathy in rats.

The effect of Amlodipine on bodyweight improvement wasn’t observed. It decreased small intestine weight/length ratio with no statistical significance comparing with Indol control group. Administration of Amlodipine attenuated microscopic and macroscopic scores with no statistical significance comparing with Indol control group. That was against the paper of Morsy et al. which showed that Amlodipine has an attenuating effect in ulcerative colitis in rats [9]. Also Kim et al. who demonstrated that Azehdinidine, a novel calcium channel blocker, ameliorates severity of colitis in DSS-induced colitis in mice [23]. As well as this study is in agreement with the study of Rainhard, which showed that Amlodipine possess a significant reduction in inflammation against acetic acid-induced ulcerative colitis in mice [24].

Regarding the anti-oxidant effect of Amlodipine, it decreased lipid peroxidation and increased the activity of SOD, but it didn’t affect on GSH levels in this study. There was no statistical significance comparing with Indol control group. This is in agreement with several authors who described the antioxidant properties of calcium channel blockers (CCBs) as being due to either a direct scavenging effect or the preservation of the SOD activity. Under controlled experimental conditions; they may inhibit lipid peroxide formation at concentrations present in plasma. This antioxidant activity is found with high lipophilic CCBs when their chemical structure facilitates proton donating and resonance-stabilization mechanisms that quench the free radical reaction [25]. In addition the finding of this study isn’t in harmony with the study of Arati S. Mahajan et al. which showed that Amlodipine improved the status of oxidative stress as shown by a decrease in MDA and increase in SOD levels in essentials hypertension patients. It, therefore, has antioxidative action in addition to the antihypertensive action [26]. Also the finding of this study isn’t in agreement with the study of Morsy et al. which showed that Amlodipine exerts antioxidative effects in vitro and in vivo by inhibiting the oxidizability of the cell membrane and low-density lipoproteins. This effect was mediated by quenching free radicals due to its highly lipophilic properties and its chemical structure [9]. In addition, it isn’t in agreement with the study of Pronobesh et al. which reported that Amlodipine acts as an antioxidant, regulates membrane fluidity and raises the activities of mitochondrial antioxidant enzymes [27].

CONCLUSION

In this study conclude that Amlodipine didn’t produce obvious enhancement in enteropathy induced by Indomethacin.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The authors report no conflict of interest. The authors alone are responsible for the content and writing of this paper.

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