Effects of nimodipine administration on small bowel mucosa under conditions of laparotomy and consequent 48-hour starvation in a rat model


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Introduction

Operative procedures anywhere in the body could lead to surgical stress response and furthermore changes in human homeostasis. The intestine's role in the development of postoperative complications, such as sepsis, the systemic immune response syndrome (SIRS) and multiple organ failure syndrome (MOFS), has been well studied 1. The gut is thought to be highly susceptible to surgical stress and trauma. Laparotomy, mild intestinal handling 23 and starvation 45 could lead to derangements to the intestinal mucosal structure and function. The possible underlying mechanism for these drastic changes, following surgery and trauma, might be related to several factors, such as ischemia-reperfusion (I/R) and inflammatory mediators that could induce intestinal mucosal damages 3,36.

Splanchnic hypoperfusion is known to be a common finding in trauma. In systemic pathological stresses 1,3, some adaptive response exists, mediated by neuroendocrine 49, which results in selective splanchnic vascular spasm in order to maintain the normal supply of blood to vital organs. Consequent reduction in oxygenation, which affects the small bowel and particularly its mucosa, is greatly influenced by those alterations. After a period of intestinal ischemia, restoration of blood flow is necessary, in order to sustain cell function and

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BACKGROUND/AIMS: The combination of starvation and surgical trauma induces disturbances to the intestinal mucosal structure and function, as well as changes in mucosal barrier function in the rat small bowel. The aim of the present study was to evaluate the effects of nimodipine administration, on intestinal mucosal structural changes and enterocyte apoptosis, following laparotomy and subsequent postsurgical starvation (PSS) in the rat.

METHODS: Thirty Wistar rats were divided into two experimental groups: A: Control group (n=15), where the animal models underwent laparotomy and consequent 48-hours PSS and B: Nimodipine group (n=15), where the rats underwent laparotomy, followed by intraperitoneal nimodipine administration and consequent 48-hour (h) PSS. Small bowel mucosal structural changes and enterocyte epithelial apoptosis were determined 48 h following laparotomy.

RESULTS: Nimodipine rats (group B) demonstrated a significant decrease in small bowel villous height in jejunum (p=0.016) and ileum (p=0.002). Similarly, crypt depth decreased in jejunum (p<0.001) and ileum (p<0.001). Nimodipine group exhibited significantly higher apoptotic index in ileum compared to control rats (p=0.006).

CONCLUSION: Nimodipine did not protect the intestinal mucosa from damage caused by surgery and consequent PSS and had obvious damaging effects on intestinal mucosa with derangements to its structure and subsequent mucosal atrophy.

KEY WORDS: Apoptosis, Intestine, Ischemia-reperfusion, Nimodipine, Surgery.
viability. However, reperfusion might initiate a series of events that could potentially exacerbate tissue injury, via the formation of reactive oxygen species (ROS) and other toxic and inflammatory mediators. Necrosis had been thought to be equivalent to epithelial cell death after an ischemic insult in various tissues. Nevertheless, more recent reports have underlined the significance of apoptosis in cell death after I/R. Ischemia and/or I/R could provoke a cascade of apoptotic events in several tissues, including the brain, liver, kidney, myocardium, pancreas and stomach. More recently, the effects of I/R on enterocytic apoptosis in the intestine have been demonstrated.

Although a pleiad of studies and investigators have noted that the disturbances of the intestinal blood flow play an important role in the development of intestinal mucosal damage during and following surgery and trauma, the potential role of nimodipine treatment in this process has not yet been studied and still remains unclear. Nimodipine is a Ca^{2+} antagonist that affects the L-type calcium channels in the cell membrane, mainly in the smooth muscle, causing some vasodilation effects. The aim of the present study was to evaluate the effect of intraperitoneal nimodipine administration on structural mucosal changes in the small bowel induced by surgical trauma and subsequent PSS in a rat model and to elucidate the possible pathophysiological pathways, by which restoration of local circulation and surgical stress/trauma/laparotomy-induced intestinal ischemia influences intestinal structure.

**Methods**

**Surgical preparation**

After approval by the Directorate of Veterinary Services of the Prefecture of Athens, Attica, Greece, 30 male Wistar rats, weighting 250–450 g, were acclimatized at 21°C on 12-h day and night cycles for a minimum of 1 week before experimentation. The rats had free access to water and were fed with standard chow. Rats were housed in racks with wire floors and were fasted for 48 h after the experiment, but access to water was ad libidum. The rats were euthanatized and samples from the proximal jejunum and distal ileum were harvested.

The small bowel was excised quickly, washed with cold isotonic saline and divided into three segments: duodenum, jejunum and terminal ileum. Histological sections were prepared from the proximal jejunum and distal ileum. The samples of intestinal tissues were fixed in a 10% formaldehyde solution (2-3% methanol), clearly labelled, coded, embedded in paraffin wax, using standard techniques and finally sectioned.

**Intestinal mucosal parameters**

Sections (4 μm each) were cut and stained with hematoxylin and eosin. As a measure of mucosal atrophy, villous height and crypt depth were determined by randomly selecting at least 30 complete well-oriented crypt-villus units from each section. Crypt depth was determined by measuring the distance from the base of the crypt to the crypt-villus junction. Villous height was determined by measuring the distance from the crypt-villus junction to the villous tip. Slides were photographed using a Nikon Eclipse 80i microscope (Nikon Corp, Tokyo, Japan) attached to a 1600x1200 pixel resolution digital camera under a 20x magnification. The villous height and crypt depth for each specimen were digitally measured using Image ProPlus software (Image ProPlus v. 5.1.2.59 for WinXP, Media Cybernetics Inc, USA). Villous height and crypt depth data were derived from all rats in each group and each measurement consisted of the mean of 30 villi and crypts.

**Immunohistochemistry & villous cell apoptosis**

The apoptosis of ileal cells was detected by immunohistochemical (IHC) staining with anti ssDNA monoclonal antibody F-7-26 (CHEMICON International, Inc, USA). The procedure, according to manufacturer's instructions, included the following steps: 4 μm sections were obtained from the blocks and attached to SuperfrostPlus slides (Menzel-Glaser GmbH, Germany). After standard deparaffinization and rehydration, slides were immersed into phosphate buffered saline solution (PBS), incubated in saponine (0.1 mg/mL in PBS) for 20 min and in proteinase K (another 20 min in room temp), rinsed in distilled water, treated with in water bath with formamide in 56°C and transferred into ice cold PBS for 5 min. Non specific antibody binding was blocked by treating the slides in 3% non fat dry milk.
Villous height significantly decreased in jejunum (p=0.016) and ileum (p=0.002) in the nimodipine rats compared to control animals. Crypt depth showed a pattern similar to that of the villous height. The nimodipine group demonstrated a lower crypt depth in jejunum (p<0.001) and ileum (p<0.001) compared to control animals (Table II).

Ileum and jejunum sections from the control rats showed better architecture of the intestinal epithelium. Both ileum and jejunum from nimodipine rats demonstrated subepithelial space at villous tip, inflammatory cell infiltration extending through the wall, shortening and loss of villi, as well as reduction of crypt depth. Whereas, the effect of Nimodipine on microscopic bowel appearance, following laparotomy plus consequent PSS in rat, is shown in Figures 1-4.

**CELLULAR APOPTOSIS**

The AI increased following nimodipine administration in ileum (p=0.006) compared to control animals, as summarized in Table III.

**Results**

**MICROSCOPIC BOWEL APPEARANCE**

The homogenic intestinal response to nimodipine was suggested by the following strong statistically significant correlations (p<0.001):

(i) Mean crypt depth in the ileum positively correlated with mean crypt depth in the jejunum.

(ii) Mean villous height in the ileum positively correlated with mean villous height in the jejunum.

(iii) Strong positive correlations were detected between mean crypt depth and mean villous height in the ileum as well as in the jejunum.

These statistical observations are summarized in Table 1.

<table>
<thead>
<tr>
<th>Correlations</th>
<th>c.c</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crypt depth (Ileum) - Crypt depth (Jejunum)</td>
<td>0.835</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Villous height (Ileum) - Villous height (Jejunum)</td>
<td>0.702</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Villous height (Jejunum) - Crypt depth (Jejunum)</td>
<td>0.698</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Villous height (Ileum) - Crypt depth (Ileum)</td>
<td>0.648</td>
<td>p&lt;0.001</td>
</tr>
</tbody>
</table>

**TABLE II - Effect of Nimodipine on microscopic bowel appearance following laparotomy plus consequent PSS in rat.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n=15)</th>
<th>Nimodipine (n=15)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>VILLOUS HEIGHT (µm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jejunum</td>
<td>130.77±26.41</td>
<td>104.93±24.44</td>
<td>p=0.016</td>
</tr>
<tr>
<td>Ileum</td>
<td>129.99±34.03</td>
<td>91.11±20.11</td>
<td>p=0.002</td>
</tr>
<tr>
<td>CRYPT DEPTH (µm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jejunum</td>
<td>55.66±17.67</td>
<td>27.72±6.49</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Ileum</td>
<td>54.77±10.84</td>
<td>26.83±9.12</td>
<td>p&lt;0.001</td>
</tr>
</tbody>
</table>

Fig. 1
The immunostaining with anti-ssDNA antibody showed that nimodipine has damaging effects on intestinal mucosa, as it increased the frequency of apoptotic ileal epithelial cells. The ileal mucosal apoptosis in each group is shown in Figures 5 and 6.

### Discussion

Surgical stress response causes biochemical and physiologic changes resulting in perturbations of essentially every homeostatic axis. These responses include: sympathetic nervous system activation, hypothalamic-pituitary-adrenal (HPA) axis activation and immunologic-hematological changes.

Mesenteric hypoperfusion is a common finding in trauma and the small bowel mucosa is vulnerable to the ischaemic insults, which result in increased gut permeability, alteration of enteral immune function, mucosal edema and atrophy, epithelial necrosis and apoptosis, and even focal mucosa ulcer, which might contribute to bacterial and endotoxin translocation.

These responses could partly be attributed to the microvascular anatomy; meaning that the presence of a villous counter-current exchanger mechanism results in shunting of oxygen at the base of villi during low-flow states, causing hypoxia to villous tips. The high intestinal content of xanthine dehydrogenase is thought to be another important predisposing factor during ischaemia and produces oxygen-derived free radicals upon reperfusion.

Microscopically, ischemia appears to lead to some degree of detachment of the villous epithelium, possibly leaving denuded villi. In addition, significant vascular dilatation, congestion and hemorrhage is apparent in the lamina propria. In parallel, submucosal vessels show dilatation and congestion. Whereas, in I/R, villi could appear collapsed, shortened and broadened and there could be further detachment of villous epithelium. Reperfusion after a period of intestinal ischemia seems to play an important role in the maintenance of cellular function and integrity. However, the reintroduction of oxygen in the reperfusion phase could give birth to unwanted events, which could cause injury and apoptosis in small intestinal mucosa. Various mechanisms have been implicated in the initiation and progression of intestinal I/R injury, such as overproduction of ROS and reactive nitrogen species (RNS), increased lipid

### Table III - Effect of nimodipine administration on ileal enterocyte apoptosis in a rat model of laparotomy and (consequent postsurgical) starvation.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n=15)</th>
<th>Nimodipine (n=15)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>APOPTOTIC INDEX</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ileum</td>
<td>1.59±0.22</td>
<td>3.05±1.95</td>
<td>p=0.006</td>
</tr>
</tbody>
</table>

Fig. 2

Fig. 3

Figures 1-4: Low-power photomicrographs of the full-thickness hematoxylin-eosin stained sections of distal ileum (Fig.1, Fig.2) and proximal jejunum (Fig.3, Fig.4) in control and nimodipine group, respectively (100x magnification). Photos are showing the damage in the intestinal mucosa and the structural changes in the nimodipine group compared to control group. Mean villous height is significantly decreased and similarly decreased is also the crypt depth consisting mucosal atrophy.

Fig. 4
Peroxidation, increased expression of adhesion molecules and infiltration of leukocytes and production of inflammatory mediators. At the microcirculatory level, I/R injury is thought to be initiated by chemotactic accumulation of circulating leukocytes and their activation and interaction with the endothelium of postcapillary venules. In addition, oxygen free radicals are thought to play an important role in the pathogenesis of I/R injury. Following reperfusion, an increase in mucosal inflammatory cell infiltration and epithelial apoptosis occurs in crypts and villi.

The present study was designed to elucidate the effects of an intestinal vasodilator on the structural mucosal changes in the small bowel induced by surgery trauma and consequent PSS in a rat model and its effect on enteroctytic apoptotic death.

Nimodipine, a dihydropyridine calcium channel blocker, exhibits its effects on cerebral and peripheral vessels and seems to favor the capillary blood flow, which nourishes tissues, at the expense of the non-nutrient blood flow, especially in the skeletal muscles. Nimodipine has been reported to attenuate brain damage either from ischemia or high doses of pilocarpine. In a rat model with partially reversible cerebral ischemia nimodipine infusion reduced the infarct volume. In addition, nimodipine is clinically used to prevent and treat cerebrovasospasm in subarachnoid haemorrhage. Nimodipine, per se, induces vasodilation in the small bowel, myocardium, diaphragm and other muscles.

The absence of intestinal feeding the first postoperative 24-h periods is a common situation in abdominal surgery. Handling of the intestine is a usual procedure in any surgery involving the abdomen and postsurgical ileus is a well-studied complication. In addition, starvation alone has been shown to induce small bowel mucosal atrophy and also plays a pivotal role in enteroctytic apoptosis.

Our histomorphometric mucosal studies showed that the nimodipine group demonstrated a significant lower crypt depth in jejunum and ileum compared to control animals. In addition, villous height significantly decreased in jejunum and ileum in the nimodipine group compared to control group. In parallel, the immunohistochemical staining with anti single-stranded DNA antibody of the ileum showed that the frequency of apoptotic ileal epithelial cells was increased following nimodipine administration compared to control animals.

These data indicate that nimodipine infusion did not protect the intestinal mucosa from damage caused by surgery and consequent PSS in the rat laparotomy model. Our results demonstrate that nimodipine has obvious damaging effects on intestinal mucosa, as suggested by villous height and crypt depth, which are specific indices for the evaluation of mucosal damages.

The observed significantly decreased villous height and crypt depth in jejunum as well as ileum in this model support this conclusion. This impaired effect was accompanied by a significantly increased enterocyte loss via apoptosis. Possibly the aggressive reversal of the gut vaso-spasm/ischemia-induced by surgical stress/truma and the subsequent acute restoration of the intestinal blood flow and reoxygenation/reperfusion enhanced the mucosal atrophy, which was induced by laparotomy and consequent PSS. This aggravation might be associated with derangements of the autoregulation of intestinal blood flow on one hand, which occurs normally after an intestinal ischemic insult and results in redistribution of blood flow within the layers of the gut, preserving initially some mucosal blood flow and on the other hand, with an increase of the degree of the intestinal reperfusion injury, which occur physiologically during and following surgery.

In conclusion, nimodipine caused a marked intestinal...
mucosal injury in the rat laparotomy and consequent PSS model. The aforementioned disturbance resulted in increased epithelial cell apoptosis, which may be responsible for this negative effect. Exposure to nimodipine did not prevent surgery-induced ischemic damage, possibly via derangement of the autoregulation of intestinal blood flow.

Riassunto

La combinazione di trauma chirurgico e di non somministrazione di cibo nel postoperatorio, provoca delle alterazioni alla struttura ed alla funzionalità della barriera intestinale, all’intestino tenue di ratti sperimentati. Lo scopo del presente paper originale e sperimentale, fu quello di valutare, come la somministrazione di nimodipina, su modelli sperimentali di intestinali tenue di ratti, potesse provocare delle modifiche strutturali della mucosa da una parte ed una pronunciata apoptosi cellulare epiteliale dall’altra, se somministrata per via intraperitoneale, in seguito ad un intervento di laparotomia e prima che iniziasse il periodo postoperatorio di non somministrazione di cibo, della complessiva durata di 48 ore.

A detto scopo, 30 ratti da razza Wistar, sono stati divisi in 2 gruppi, di cui uno di controllo (Gruppo A), composto da 15 di essi, i quali furono sottoposti ad un intervento di laparotomia ed alla non somministrazione di cibo per le prime 48 ore nel immediato postoperatorio ed un altro (Gruppo B) sempre composto da 15 ratti, i quali sono stati sottoposti, alla pari dei ratti del gruppo di controllo ad un intervento di laparotomia e alla non somministrazione di cibo per le prime 48 ore nel postoperatorio, ma tra la fine dell’intervento di laparotomia ed il periodo postoperatorio di astinenza dal cibo, gli si e’ somministrata, per via intraperitoneale, la Nimodipina.

In entrambi i gruppi, 48 ore dopo l’intervento della laparotomia, sono state valutate sia le modifiche strutturali della mucosa intestinale che il grado, da essa giunto, di apoptosi cellulare.

In conclusione di detta sperimentazione, si e’ messo in evidenza che la nimodipina, non solo non protegge la mucosa intestinale, dal danno provocatole dall’intervento chirurgico laparotomico e dal successivo, all’intervento, di non somministrazione di cibo per 48 ore, ma provocava anche dei ben evidenti danni alla mucosa intestinale, in quanto, in seguito alla sua somministrazione per via intraperitoneale, si verificarono intense alterazioni dell’architettura della mucosa intestinale ed una sua consistente atrofia.

References


Il valore didattico del film in chirurgia

di Giuseppe Romagnolo, Biagio Trojanello

DVD- 21' 41"- Italiano

Pubblicato da : Salvatore Cafiero Audiovisi- 2001

Autore Corrispondente: Biagio Trojanello, Salita Piedigrotta 3, 80122 Napoli

Recensione:
Rassegna storica, ricamente corredata di iconografia classica, che ripercorre la storia della tecnica chirurgica moderna non semplicemente nelle tappe dei suoi progressi tecnici quanto nell’ottica della dimostrazione dei mezzi strumentali storici, della didattica finalizzata all’apprendimento.
Nella prima parte vengono esibiti disegni e figure statiche da affreschi e miniature classiche, per passare quindi ad una breve rassegna fotografica esemplificativa di patologie e situazioni cliniche. Vengono rievocati ritratti celebri di altrettanti celebri chirurghi nell’atto docente della dimostrazione anatomo-patologica e chirurgica.
L’arrivo dell’invenzione dei fratelli Lumière ha modificato radicalmente l’aspetto didattico della chirurgia non direttamente vissuta ed osservata, poiché essa venne adottata già due anni dopo della sua prima dimostrazione per dimostrare atti chirurgici su pellicola bianco e nero, con intervento del Prof. Roberto Alessandri.
Dopo un primo periodo euforico, in cui singoli chirurghi si avvalevano della tecnica chirurgica su pellicola – costosa - per dimostrare le personali capacità tecniche, la cinematografia chirurgica ha dimostrato sempre di più il suo valore didattico intrinseco. Ma la vera esplosione si è avuta con l’introduzione della tecnica di ripresa e postproduzione digitale, che ha consentito di confezionare prodotti didattici di grande valore. Prevedendo addirittura non solo la multimedialità ma anche l’interattività tra dimostrazione e spettatore discente.
L’interesse del DVD è di tipo storico, ma il suo valore intrinseco risiede anche nell’entusiasmo delle finalità dimostrate con convinzione e capacità dagli Autori.