The effect of budesonide on lymphoid and non-lymphoid cell profiles, and la-antigen expression in rats with experimental colitis

Mersetyawan HNE Soesatyo\(^1\) and Mary Palmen\(^2\)
\(^1\)Department of Histology, Faculty of Medicine, Gadjah Mada University, Yogyakarta, \(^2\)Department of Cell Biology-Immunology, Faculty of Medicine, Vrije Universiteit, Amsterdam

ABSTRAK

Mersetyawan HNE Soesatyo & Mary Palmen — Pengaruh budesonide terhadap profil sel limfoid dan non-limfoid serta ekspresi antigen la pada tikus dengan colitis eksperimental.

Kortikosteroid merupakan obat yang efektif terhadap penyakit pada jaringan kulit dan paru paru. Pemilihan ini bertujuan untuk mengetahui pengaruh pemberian kortikosteroid lokal, yakni budesonide colitis experimentalis pada tikus, khususnya pengaruhnya terhadap profesi sel limfoid dan nonlimfoid, serta ekspresi antigen la (molekul major histocompatibility complex (MHC) klas II). Dengan itu dilakukan pula penambahan molekul adhesi, seperti intercellular adhesion molecules (ICAM-1) dan lymphotoxins function associated antigen (LFA-1) serta pemberian obat tersebut.

Model colitis dibuat dengan pemberian secara intralokal sel hatap: 2,4,6-trinitrobenzen sulfonic acid (TNBS) dalam etanol, berdampak pada selulit standard. Obat budesonide dibikat dalam dosis tunggal dan gandang sebanyak 6,25 ml larutan 10\(^{-3}\) M secara lokal pada dalam kolon dengan menggunakan kateter. Populasi sel limfoid dan nonlimfoid di sepanjang mukosa dan submukosa, termasuk plak Peyers (PP) dan jaringan limfoid di dasar protozomat colon diperoleh dengan teknik immunohistologi. Ekspresi MHC klas II dan molekul adhesi dibikat menggunakan panel antibodi monoklonal (AbMa).

Dosis ganda budesonide (0,5) sangat efektif untuk menghindari colitis akut; ini dapat diaplikasikan pada pencegahan jika guna dalam hal-inflamatory bowel disease (IBD).

Key words: inflammatory bowel disease; TNBS colitis; budesonide; lymphoid and non-lymphoid cell populations — MHC class II expression — adhesion molecules.

(Bedah Ilmu Kedokteran Vol. 27, No. 1, Maret 1995)

INTRODUCTION

Treatment of chronic colitis, such as inflammatory bowel disease (IBD), is hampered by the fact that little is known about the underlying causes of the condition. To acquire deeper insights of the pathogenesis of this disorder, animal models for IBD are primarily important. Morris et al. have developed a model in rats by means of administration of a haptens: 2,4,6- trinitrobenzene sulfonic acid (TNBS), dissolved in ethanol, into the distal colon. The model for IBD is proved to be reproducible.

Glucocorticoids have been widely used as anti-inflammatory agents since 40 years. With respect to IBD, hydrocortisone enemas were introduced for the treatment of ulcerative colitis and other inflammatory processes of the distal colon. This drug, however, has high systemic influences. So, a new generation of cortico-
steroid, characterized by a high first pass metab-
olism in the liver, was developed.\(^5\) One of the
new corticosteroid is budesonide, which has high
topical potency but little systemic effect. Recently
budesonide has been on trials against IBD, in
particular ulcerative colitis and proctitis.\(^5\) IBF
is a chronic gastrointestinal disorder in
which its etiology remains unclear. Several
reports indicated the involvement of local
macrophages and dendritic cells (DC) in the
pathogenesis of such a disease.\(^1\) For instance,
differences in numbers and heterogeneity of
macrophages and DC were found between tissues
obtained from IBF patients and normal
individuals.

This study was aimed at examining the effect
of local corticosteroid, i.e. budesonide treatment
on TNBS-induced colitis in rats, with special
emphasis on the lymphoid and non-lymphoid cell
populations by using immunocytochemistry. In
addition, the expression of MHC class II antigens
and the adhesion molecules was also investigated.

**MATERIALS AND METHODS**

**Animals**

Male wistar rats, weighing about 250 g
(Harlan Sprague Dawley, Zeist, The Netherlands)
were used in the study. The animals were
maintained under standard laboratory conditions
with pelleted food formula and tap water ad
libitum.

**Drugs**

TNBS (2,4,6-trinitrobenzene sulfonic acid)
was purchased from Sigma Chemical Co., St.
Louis, MO, USA. Budesonide was kindly
provided by Dr. S.E. Svensjo, Astra Draco, Lund,
Sweden.

**Experimental design**

Induction of colitis

Colitis was induced by intracolonic
administration of TNBS in ethanol, as previously
described by Morris et al.,\(^1\) with slight modifi-
cations. Under Hypnovel\(^R\) (Janssen Pharma-
ceutica BV, Tilburg) anaesthesia, each rat received
30 mg TNBS dissolved in 0.25 ml 40% ethanol,
using a catheter inserted approximately 8 cm in
the colon from the anus. Subsequently, the rats
were checked daily to see their general conditions, body weight, and quality of stools.

The histological sections of the colon were
prepared and examined in the study on day 1, 7, 14
and 28 after the induction.

**Budesonide treatment**

The micropigmented-budesonide was diluted to
10\(^{-7}\)M in 100% ethanol, as stock solution. This
was then adjusted to the final concentration at
10\(^{-7}\)M dissolved in 0.9% saline as working
solution. Each dose contained 0.25 ml 10\(^{-7}\)M
budesonide and was administered locally through
a catheter into the colon. This drug was given
either once a day at day 1, or 3 times at day 1, 4
and 8 after TNBS administration.

Fifty two animals were divided into 4 groups.
TNBS-ethanol was given to group A, B and C.
Group A was treated with multiple doses of
budesonide 3 times a day on day 1, 4 and 8 after
TNBS treatment; group B with a single dose of
budesonide on day 1 after TNBS; and group C
received a placebo. Group D was non-IBD
control, which only received budesonide at day 1,
4 and 8. On day 9, 15 and 18 after the induction
of colitis, the rats were sacrificed. The effects of
budesonide 2 times daily administered 24 h prior
to colitis induction were also examined.

Morphological changes of the colon were
examined by at least 3 independent observers.
Any visible damage was scored on 0-5 scale, as
described by Morris et al.,\(^1\) in which, score 0
means no damage; score 1 to 5 represent different
severity of colonic lesions (see TABLE 3). The
inflamed and non-inflamed colon (including
proximal colonic lymphoid tissues, PCLT), a part
of small intestine (including Peyer’s patches, PP),
were collected and snap frozen in liquid nitrogen
for immunocytochemistry.

**Immunocytochemistry**

Cryostat sections of 8 \(\mu\)m were picked up on
slides, fixed in acetone and air-dried. The slides
were incubated for 60 minutes at room temperature with a solution of the first step of monoclonal antibodies (MoAbs, see TABLE 1) in 0.01 M phosphate-buffered saline (PBS), pH 7.4, with 0.5% bovine serum albumin (BSA). Afterwards, the slides were washed 3 times in PBS and then incubated with peroxidase conjugated rabbit antimouse serum, dilution 1:200 (Miles, Elkhart, USA) in PBS with 0.5% BSA and 1% normal rat serum, for 30 minutes. After being rinsed with PBS, the sections were stained for peroxidase activity with 3,3'-diaminobenzidine-tetra-hydrochloride (Sigma, St. Louis, Mo, USA) in 0.5 mg/ml TRIS-HCl pH 7.6, containing freshly added 0.01% H2O2. After washing again with PBS, the slides were lightly counterstained

TABLE 1. – Monoclonal antibodies (Mo Abs) used in immunocytochemistry

<table>
<thead>
<tr>
<th>MoAb</th>
<th>Detection</th>
<th>Antigen</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>ED1</td>
<td>monoclonal, almost all macrophages</td>
<td>cytoplasmic</td>
<td>10</td>
</tr>
<tr>
<td>ED2</td>
<td>tissue macrophages, mature macrophages</td>
<td>differentiation</td>
<td>10,11</td>
</tr>
<tr>
<td>ED3</td>
<td>macrophage subpopulation</td>
<td>membranous antigen</td>
<td>10,11</td>
</tr>
<tr>
<td></td>
<td>mainly in lymphoid organs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OX5</td>
<td>MHC class II gene products</td>
<td>Ia-antigens</td>
<td>13</td>
</tr>
<tr>
<td>OX8</td>
<td>suppressor T-cells</td>
<td>CD8</td>
<td>14</td>
</tr>
<tr>
<td>OX9</td>
<td>T-cells</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>W3/25</td>
<td>helper T-cells</td>
<td>CD4</td>
<td>16</td>
</tr>
<tr>
<td>WT-1</td>
<td>adhesion molecule LFA-1</td>
<td>CD11a</td>
<td>17</td>
</tr>
<tr>
<td>IA-29</td>
<td>adhesion molecule ICAM-1</td>
<td>CD54</td>
<td>18</td>
</tr>
</tbody>
</table>

TABLE 2. – The lymphoid and non-lymphoid cells in the colon after TNBS-administration.

<table>
<thead>
<tr>
<th>time (days)</th>
<th>mφ</th>
<th>PMN</th>
<th>DC</th>
<th>T-cells</th>
<th>B-cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>++</td>
<td>=</td>
<td>=</td>
<td>=</td>
</tr>
<tr>
<td>1</td>
<td>++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>28</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
</tbody>
</table>

mφ: macrophages; PMN: polymorphonuclear; DC: dendritic cells; # number of positive cells is the same as controls: +=1-5 positive cells per microscopic field more than in the controls; ++: 6-10 positive cells per microscopic field more than in the controls; +++: 11-15 positive cells per microscopic field more than in the controls; ++++: 16 positive cells per microscopic field more than in the controls.

TABLE 3. – Effect of single and multiple doses of budesonide (Bud) on the number of rats showing symptoms and signs related to colonic inflammation

<table>
<thead>
<tr>
<th>group</th>
<th>Bud + saline</th>
<th>Bud + 1x Bud</th>
<th>Bud + 3x Bud</th>
<th>3x Bud</th>
</tr>
</thead>
<tbody>
<tr>
<td>115</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>7</td>
</tr>
<tr>
<td>17</td>
<td>7</td>
<td>6</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>5</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>11</td>
<td>0</td>
<td>13</td>
<td>7</td>
</tr>
<tr>
<td>2</td>
<td>13</td>
<td>16</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

Damage score: 0 = no damage; 1 = localized hyperemia ≤ 1 cm, but no ulcer; 2 = one ulcer; 3 = one ulcer and area of inflammation; 4 = two or more sites of ulceration/inflammation; 5 = two or more sites of ulceration/inflammation and site > 1 cm length.
with haematoxylin, then dehydrated and mounted in Entellan® mounting medium (Merck, FRG). Slides that will be used for quantification of positive cells were counterstained with 10 times diluted nuclear fast red (Merck, FRG), and then were analysed with the Image analysis system (IBAS, Kontron Electronic, GMBH).

Quantification

The positive cells per microscopic field could be separated from the homogenous, relatively colorless background (objective magnification 40x). This was done semi-automatically, in which the threshold of the critical grey values was set interactively for each measurement. Thus, the degree of condensation of a specimen did not affect the morphometric measurements.

The percentage of positive cell staining was determined as follow,

$$\frac{\text{field area of positive cells}}{\text{total field area}} \times 100\%$$

Statistical analysis

The statistical significance of the differences was evaluated using Student’s t-test and non-parametric analysis. A level of $p < 0.05$ was considered significant.

RESULTS

General findings on TNBS-model

All animals treated with TNBS-ethanol developed both clinical and histopathological symptoms, such as diarrhea and transmural inflammation with or without ulceration. Damage scores from day 1 to day 28 are shown in TABLE 2. Colon wall thickening occurred in about 70% of the total IBD group. The animals with the most severe bowel lesion (damage score 5) reached to 60%.

Histologically, there was an influx of granulocytes and ED1⁺ and ED2⁺ macrophages during the acute phase of inflammation. In addition, ED2⁺ macrophages were found not only in the basal of the crypts, but also at the upper parts. After the induction with 'TNBS-ethanol, the ED3⁺ cells, which are normally present in the spleen and lymph nodes, were also found at low numbers in the colon. MHC class II expressions on dendritic cells around the crypts, and the upper region of lamina propria was increased, judged by their intensive staining with the corresponding MoAb. Interestingly, no such an expression was found on colonic epithelial cells during active disease. From day 7 and 14, the number of T and B cells, respectively, was increased (TABLE 2).

Budesonide treatment

After a single dose of 0.25 ml 10⁻⁵M budesonide, no apparent improvement of the inflammation occurred. The multiple doses of this drug, however, dampened signs of acute inflammation. The clinical symptoms disappeared, and the ulceration recovered. The damage scores declined significantly ($p < 0.05$) on day 15 and 18 compared to that which received a placebo (FIGURE 1). Local budesonide administration had no obvious effect on the colonic mucosa of normal control rats.

![FIGURE 1: Damage scores of the colon at day 15 and 18 after induction of colitis. (D) control animals which received a placebo; (E2) colitis animals which received budesonide 3 times daily. (***p < 0.001).]

On day 15 (7 days after the last budesonide treatment) the percentages of ED1⁺ and ED2⁺ macrophages in the submucosa decreased (FIGURE 2A and 2B). The number of ED3⁺ macrophages in the submucosa also decreased after
therapy. Among those three macrophage subpopulations, however, only the ED1+ cells decreased enormously.

With respect to MHC class II expression, budesonide reduced the intensity of this la staining in the mucosa and submucosa. Hardly any MHC class II expression seen on dendritic cells around the crypts. The reduction of la staining was also observed in the colon of the controls treated with this drug.

![Graphs showing percentage of different cell populations at day 15 after induction of colitis.](image)

**DISCUSSION**

This study describes the effect of budesonide on immunocompetent cells in TNBS-induced colitis. Although this drug has a potent topical influence, as previously demonstrated in the respiratory and skin diseases, a single dose of $10^{-3}$M budesonide presented locally in the colon did not have any effect on acute inflammation. In contrast to the multiple doses ($3 \times 10^{-5}$M), which markedly produced a therapeutic effect. The latter doses seemed to affect only on mucosal lesions, whereas it did not influence the normal colonic tissue.

After budesonide treatment, a decrease was observed in ED1+, ED2+ and ED3+ macrophage subpopulations. The reduction of ED3+ macrophages is in contrast to the findings of Damoiseaux et al., who reported that the addition of corticosteroid to bone marrow cultures strongly induced the ED3 expression. The different result is possibly due to difference in test system, i.e. in vitro vs in vivo. A decrease in the number of macrophages at the sites of inflammation and a concomitant reduction of circulating monocytes after steroid treatment have been reported by several authors. Goye and Munk3 described that monocytes and macrophages were among the most sensitive cells against anti-inflammatory effect of glucocorticoids. This drug could reduce the number of inflammatory cells in the inflamed airways to normal condition by inducing apoptosis, inhibiting cell migration, and by decreasing the production of various
cytokines. It is likely that similar actions take place in the mucosa of colon during IBD.

After the induction of TNBS-mediated colitis, the intensity of MHC class II staining increased, and subsequently this expression was down-regulated by badeconomic. Similar result were reported by Jevouika23 that the increment of expression of MHC molecules in autoimmune nephritis could be reduced by oral administration of corticosteroid.

Other interesting findings were accomplished, that budesonide modulated the expression of adhesion molecules, such as ICAM-1. This molecule was expressed on macrophages, dendritic cells and memory T cells and is the ligand receptor for LFA-1. Our study demonstrated the reduction of ICAM-1+ cells in the colon. This may be due to the reduction of ICAM-1 expression which in turn causes a reduction of neutrophil migration into the lesion.

The mucosal T lymphocytes outnumbered B cells after TNBS induction and their number decreased gradually following the budesonide treatment. The changes of lymphoid cells profiles compared to normal situation were, however, not significant. In this study, the PP- and PCLT-B cells were not affected by this corticosteroid, though several reports4,13,14 have found a depletion of B cells in the dome area of PP and in the germinal center of PCLT. These conflicting results were possibly due to the use of different species and different route of drug administration.

CONCLUSION

Budesonide has a potential effect for the treatment of experimentally-induced colitis, particularly to non-lymphoid cells, like the ED1+, ED2+ and ED3+ macrophage subpopulations and dendritic cells. These cells are suggested to play a role in the pathogenesis of IBD model through their MHC class II expression, and indirectly control the inflammatory cell migration by regulating the adhesion molecules for cellular interactions.

ACKNOWLEDGMENT

We thank Dr. E.P. Van Rees for critical reading of the manuscript.

This study was financially supported by Astra Pharmaceutical (Land, Sweden).

REFERENCES


