

Published in final edited form as:

Inflamm Bowel Dis. 2010 September ; 16(9): 1476–1487. doi:10.1002/ibd.21250.

Overexpression of Translocator protein TSPO in inflammatory bowel disease: potential diagnostic and treatment value

Mariano A Ostuni, PhD^{*}, Leeyah Issop, MSc^{*}, Gabriel Péranzi, PhD^{*}, Francine Walker, MD, PhD^{*}, Magali Fasseu, BSc^{*}, Carole Elbim, MD, PhD[§], Vassilios Papadopoulos, DPharm, PhD[#], and Jean-Jacques Lacapere, PhD^{*}

^{*}Unité INSERM U773, Centre de Recherche Biomédicale Bichat Beaujon CRB3, Université Paris 7 Denis Diderot, BP 416, F- 75018 Paris, France.

[§]Centre de Recherche des Cordeliers, Université Pierre et Marie Curie – Paris 6, UMR S 872, Paris, F-75006 France; Université Paris Descartes, UMR S 872, Paris, F-75006 France; INSERM, U872, Paris, F-75006 France.

[#]The Research Institute of the McGill University Health Center, Department of Medicine, McGill University, Montreal, Quebec, H3H 2R9, Canada.

Abstract

Background—Inflammatory bowel diseases (IBD), such as ulcerative colitis and Crohn’s disease, are chronic inflammatory disorders that increase the risk for colorectal cancer. The mitochondrial Translocator Protein (TSPO) is a high affinity drug- and cholesterol-binding protein expressed in the colon and its expression is increased in colon cancers. The aim of this study was to investigate TSPO expression in IBD biopsies and to establish an animal model of IBD to examine the role of TSPO. In addition, we evaluated the potential use of TSPO drug ligands in diagnosing and treating IBD.

Methods—TSPO expression in IBD biopsies was evaluated using immunohistochemistry. IBD was induced in a rat experimental model via treatment with dextran sodium sulfate (DSS). Colon morphology, TSPO expression, and proinflammatory cytokine production were evaluated in addition to the effect of TSPO drug ligands on disease pathology.

Results—TSPO protein levels were elevated in the enterocytes of IBD biopsies. TSPO expression was localized to the enterocyte mitochondria. DSS treatment induced a time-dependent phenotype mimicking IBD with tissue injury and subsequent tissue regeneration. Co-administration of DSS and the TSPO drug ligands PK 11195 or Ro5-4864 increased both the rate of colon ulceration and regeneration, whereas administration of the TSPO drug ligand flunitrazepam partially prevented this pathology. These data correlated with changes in pro-inflammatory cytokine plasma levels, as well as increased cytokine production and secretion from the colon.

Conclusions—TSPO may serve as a marker of the IBD repair process, and TSPO drug ligands should be further evaluated for IBD treatment.

Keywords

Peripheral benzodiazepine receptor; isoquinolines; benzodiazepines

Introduction

Ulcerative colitis (UC) and Crohn's disease (CD) are the primary forms of inflammatory bowel disease (IBD). The incidence of these diseases is increasing¹, and they have an unknown etiology. They are characterized by chronic relapsing inflammation of the gastrointestinal tract due to a deregulated mucosal immune response. Thus, IBDs represent a major public health problem and unfortunately, these diseases can be difficult to diagnose. Ten to fifteen percent of all colorectal IBD cases cannot be classified as UC or CD. Taking into account genetic background and environmental factors, the difficulties and differences in classification criteria between different countries may contribute to the reported geographical variations¹. The cascade of events leading to the development of IBD remains poorly understood; however, it is important to understand the mechanisms underlying chronic inflammatory diseases, as patients with these diseases are generally thought to be at an increased risk of developing cancer². In particular, both UC and CD impose an increased risk of developing colorectal cancer³. This association is thought to arise from the existence of an inflammation-dysplasia-carcinoma sequence⁴. More recently, a correlation between inflammation in the tumor microenvironment and metastasis has been described, adding a new hallmark of cancer⁵. Cytokines, including interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α), expressed in this microenvironment promote excessive inflammatory processes and augment the ability of tumor cells to metastasize⁶. Taken together, these observations underscore the importance of studying the proteins that contribute to IBD in order to better characterize IBD and its relationship to dysplasia, carcinoma, and metastasis and to identify potential IBD therapeutic drug targets.

The mitochondrial outer membrane Translocator Protein (18 kDa; TSPO)⁷, previously named peripheral-type benzodiazepine receptor (PBR), is expressed in healthy colon⁸ but is overexpressed in colon cancer⁹⁻¹¹ as well as in several other cancer types¹¹. This protein has been implicated in tumor growth and spread¹² in congruence with the observation that strong overexpression of TSPO is an independent unfavorable prognostic factor in stage III colorectal cancer¹³. A wide variety of endogenous molecules, including the peptide diazepam binding inhibitor (DBI) and its derived fragments, porphyrins and cholesterol, possess high affinity for TSPO⁷. Specific synthetic ligands have also been primarily developed from two chemical families: the benzodiazepines and the isoquinoline carboxamides⁷. These ligands, which bind to TSPO with nanomolar affinities, regulate cell growth¹⁴, apoptosis¹⁵, and downregulate inflammation¹⁶⁻¹⁷.

Despite the knowledge that TSPO is overexpressed in colon cancer and it regulates the growth of colorectal cancer cells, the role of TSPO in IBD and dysplasia has not yet been thoroughly investigated. Therefore, this study was designed to evaluate the extent of TSPO involvement in these inflammatory conditions in humans and to investigate TSPO and its drug ligands *in vivo* in a well-characterized animal model of inflammation. Animal models relying on chemical induction of IBD have been widely used¹⁸. These models have permitted the study of several parameters involved in the pathogenesis of IBD, including examination of proinflammatory cytokine profiles, which are a hallmark of cancer-related inflammation¹⁹. Thus, to examine the relationship between TSPO and IBD, we employed an inflammatory rat model obtained via dextran sulfate sodium (DSS) treatment¹⁸. This model involves extensive superficial mucosal ulceration with increased production of a complex mixture of inflammatory mediators. We evaluated the temporal relationship between secretion of inflammatory mediators, TSPO expression, and histological evolution in DSS-treated rats with or without concomitant treatment with TSPO drug ligands.

Methods

Human biopsies

IBD, colon cancer, and normal human biopsy specimens were obtained from the Department of Gastroenterology at Bichat-Claude Bernard Hospital, Paris (France) following patient consent. The research protocol was approved by the Bichat-Claude Bernard Hospital Review Board. Specimens of 5- μ m thickness were formalin fixed and stained with H&E-saffron. The samples were visually analyzed and diagnosed by two independent pathologists. Normal ileum and colonic mucosa biopsies and normal tissue adjacent to the tumor tissue were used as controls.

Animal studies

Adult male Wistar rats weighing 250–300 g (Iffa-Credo, L'Arbresle, France) were treated in accordance with European Committee guidelines concerning the care and use of laboratory animals. Colonic inflammation was induced by treating animals with drinking water supplemented with 5% (w/v) DSS, (MW 40,000; ICN Biomedicals, Aurora, OH) for up to 7 days¹⁸, and then animals were administered untreated water for 7 additional days. To determine the effects of the inflammatory agent on the colon, rats were sacrificed after 2, 4, or 7 days of DSS treatment. Control rats were administered water without DSS. In order to determine the effectiveness of TSPO ligand treatment in the DSS model, rats were treated with or without DSS in the presence or absence of PK 11195, Ro5-4864, or Flunitrazepam, which were administered by intraperitoneal (i.p.) injection of 1 mg/kg daily^{16,20} until sacrifice on days 2, 4, 7, 9, 11 and 14. Control groups were i.p. injected with vehicle (5% DMSO, 5% Tween-20 in sterile phosphate-buffered saline). The body weight, stool consistency and rectal bleeding were evaluated daily in each animal. At each time point, four animals were sacrificed and examined.

At the indicated time-points, the final 2 cm of distal colon was collected, longitudinally cut, fixed in 4% paraformaldehyde, and paraffin-embedded. Colon pieces (5 μ m) were collected every 600 μ m. Paraffin sections were rehydrated and then stained with H&E-saffron. The percentage of lesions in the sections was evaluated using a semi-morphoquantitative method²¹, according to the following anatomopathological criteria: inflammation; thickening of the mucosa with hyperemia; inflammatory infiltrates; erosion (superficial mucosal epithelial cellular necrosis); ulceration (extensive cellular mucosal necrosis with loss of normal crypt); regeneration (mucosal repair neighboring ulceration with gland hyperplasia and numerous mitotic cells); and re-epithelialization (ulceration surface colonized by epithelial cells).

Immunohistochemistry

Paraffin sections of human or animal tissues were immunostained with rabbit anti-TSPO peptide affinity-purified polyclonal antibody (1:100)²². Dual immunofluorescence staining for TSPO and voltage-dependent anionic channel (VDAC) and subsequent analysis were performed as previously described²². Control experiments included the absence of the primary antibody and immunoabsorption (8 μ g of recombinant 18 kDa TSPO per ml of diluted antibody^{22,23}).

Measurement of cytokine levels

Rat blood was collected after 2, 4, 7, 9, 11, and 14 days of treatment. Samples were placed into sterile tubes containing EDTA and centrifuged at 2000 \times g for 10 min at 4°C to avoid cytokine synthesis. Plasma was stored at -80°C for no longer than 15 days prior to the assay. Plasma levels of cytokine-induced neutrophil chemoattractant 1 (CINC-1, rat

homolog of human IL-8), and TNF- α were measured by ELISA (R&D Systems, Lille, France), following the manufacturer's instructions. The results are expressed as pg/mL.

Distal colons were collected after 2, 4, 7, 9, 11 and 14 days of treatment, washed in ice cold phosphate buffered saline, and stored at -20°C . Colon pieces were sliced and homogenized using a glass-potter in a solution containing 1 mM EDTA and 1 mM PMSF in PBS at pH 8 and then centrifuged at $10\,000 \times g$ for 15 min at 4°C . Supernatants were stored at 20°C until use. Tissue CINC-1 and TNF- α levels in the supernatants were measured by ELISA [R&D Systems and BD Biosciences (Pont de Claix, France), respectively] following the manufacturer's instructions. The results are expressed as pg/mL.

Extraction of total RNA and qRT-PCR analysis

Distal colons were collected after 2, 4, 7, 9, 11 and 14 days of treatment, and total RNA was extracted using Trizol reagent (Invitrogen, Cergy-Pontoise, France). Reverse transcription was performed using a master mix prepared with Thermo Scientific Verso cDNA (Thermo Scientific, Epsom Surrey, UK). The cDNA was diluted 5-fold and stored at -20°C until PCR amplification. Real-time PCR amplification was performed using a LightCycler @480 PCR system (Roche Diagnostics, Meylan, France). Quantitec primers assays were provided by Qiagen (Courtaboeuf, France). The PCR included 5 μl of cDNA, 10 μL of SYBR GREEN I master mix (Roche), 0.5 μM forward-reverse primer pair (Eurogentec, Angers, France) or 0.5 μM of primer (Qiagen) in a 20 μL final reaction volume. Standard cycling conditions were used: 95°C for 5 min; 95°C for 10 sec; 60°C for 30 sec; and 72°C for 30 sec, (40 cycles). The cycle threshold (CT) values were automatically calculated using LC480 SDS Software. CT values were converted into quantities relative to the normal (Qrel) and corrected for PCR amplification efficiency using the following formula: $Qrel = E^{-\Delta CT}$, where $\Delta CT = CT(\text{test sample}) - CT(\text{calibrator sample})$.

TSPO drug ligand binding

Rat intestinal total tissue extracts (T) and mitochondrial fractions (MF) were prepared as previously described²⁴. Protein levels were quantified using the dye-binding BioRad assay using bovine serum albumin as a standard. Binding of [^3H]PK 11195 (specific activity 83.5 Ci/mmol; Dupont-New England Nuclear, Boston, MA) to total tissue extracts was performed as previously described²⁴. The dissociation constants (K_d) and the number of binding sites (B_{max}) for PK 11195 were determined using saturation isotherm analysis. Experimental analysis of time-dependant changes of [^3H]PK 11195 binding to the total tissue extracts per days of DSS treatment were performed using 9 nM of [^3H]PK 11195. Results are representative of three independent experiments performed in triplicate. The standard error of the mean (SEM) values obtained for each data point were consistently less than 5% of the mean.

Statistics

Data represent the mean \pm SEM. Experimental data were analyzed using a one-way ANOVA. The Scheffe homogeneity test was performed to identify differences between the means of each experimental group. Differences were considered to be significant for p -values < 0.05 .

Results

TSPO expression in human intestinal pathology

In normal human colonic mucosa, TSPO immunoreactivity was only observed in the surface epithelial cells (Fig. 1a). Enterocytes strongly expressed TSPO, whereas goblet cells had no detectable TSPO expression. In the areas of low and high grade dysplasia adjacent to

invasive colon cancers, there were numerous TSPO-labeled enterocytes visible deep within the colonic crypts (Fig. 1b-c). In all invasive colon cancers, TSPO immunoreactivity was greatly increased but was still localized to the enterocytes (Fig. 1d-g). In the inflammatory areas of ileal CD tissue, especially near erosion or ulceration, enterocytes exhibited diffuse TSPO immunolabeling within the entire gland (Figs. 2b and c), as compared to the normal ileum where TSPO immunoreactivity was only observed in the enterocytes present at the top of the villi (Fig. 2a). Some immunoreactivity was also observed in mononuclear inflammatory cells. Similar results were observed in the colonic mucosa in patients with CD (Fig. 2b, c, e, and f) and UC (Fig. 2g and h).

TSPO expression in the rat colon

Similarly, immunohistochemical staining for TSPO in the rat colon revealed strong expression in enterocytes (Fig. 3a) but not in goblet cells. Confocal microscopy indicated that TSPO (Fig. 3b) colocalized with mitochondrial VDAC (Fig. 3c and d), which is a well characterized outer mitochondrial membrane protein^{7,15}. Radioligand binding studies with rat colon membrane preparations revealed the presence of a high affinity ($K_d = 3.0 \pm 0.5$ nM) and high capacity ($B_{max} = 6.5 \pm 0.50$ pmol/mg) [³H]PK 11195 binding site (Fig. 3e). Isolated mitochondria had a 2-fold enrichment of TSPO, as compared to homogenates (Inset of Fig. 3e). Pharmacological characterization of TSPO in the rat colon indicated that the isoquinoline carboxamide PK 11195 and the benzodiazepine flunitrazepam exhibited the highest affinities (IC_{50} of 4 and 25 nM, respectively) for TSPO. The benzodiazepines Ro5-4864 and diazepam exhibited an IC_{50} of 75 and 200 nM, respectively, for colon TSPO, whereas protoporhyrin IX demonstrated an IC_{50} of 3 μ M (Fig. 3f).

TSPO expression in a rat IBD model

The rats treated for 7 days with DSS had reduced body weights and increased gastrointestinal disease complications, including diarrhea and bloody feces¹⁸ (supplementary Table 1). Histological examination of the colon revealed progressive alterations, including edema, inflammatory cell infiltration, erosion, and deep ulceration, occasionally including the complete loss of crypts (Fig. 4a-d), which is consistent with previous reports²⁵. TSPO immunoreactivity, which was primarily observed in the surface epithelial cells of normal colon (Fig. 4a), was increased in the crypts following DSS treatment (Fig. 4b and c). TSPO immunoreactivity was also observed in the inflammatory cells correlating with ulceration. Quantitative analysis of TSPO expression in total colon tissue extracts from DSS-treated rats was performed using high affinity PK 11195 ligand binding studies. There was significantly increased colon-bound PK 11195 observed in a time-dependent manner following DSS treatment (Fig. 4e). Scatchard plot analysis of the saturation isotherms indicated an increase in the number of binding sites rather than a change in TSPO affinity (Fig. 4f).

Effect of TSPO drug ligands on rat IBD

TSPO drug ligands (PK 11195, Ro5-4864 and flunitrazepam) were administered daily by i.p. injection in conjunction with DSS treatment for 7 days, followed by another 7 days post-DSS treatment. Changes in body weight, rectal bleeding and stool consistency were measured as clinical parameters of disease progression (supplementary Table 1). Under our DSS-treatment conditions, the reduction in body weight was lower than previously reported²⁵. The TSPO drug ligand PK 11195 did not affect DSS-induced weight loss during the 7 days of treatment, whereas both benzodiazepine Ro5-4864 and flunitrazepam enhanced its effect. Moreover, Ro5-4864 increased rectal bleeding, whereas flunitrazepam diminished this response. Once DSS-treatment was arrested, there was no more rectal bleeding observed at 7 days post-DSS for animals treated with any of the TSPO drug ligands, whereas bleeding was still observed in the non-treated animals. Furthermore, after 7

days of recovery, only the animals treated with benzodiazepines still exhibited a reduction in their body weight. Histological examination and semi-quantitative morphometric analysis of rat colons after 7 days of DSS treatment (with and without TSPO drug ligands) and after 7 days of recovery (i.e., 14 days total) are summarized in Fig. 5 and supplementary Figure 1. Flunitrazepam-treated rats exhibited reduced histological damage after 7 days of DSS treatment, as compared to the rats treated with DSS alone (Fig. 5a). After 7 days of Ro5-4864 treatment, there was predominant erosion and ulceration with low levels of inflammation in the rat colons (Fig. 5a). These data suggest that animals achieved a disease state faster under these conditions. PK 11195 treatment enhanced the erosion and ulceration at 7 days in comparison to DSS alone, but the erosion was not as severe as that observed after Ro5-4864 treatment (Fig. 5a). After 7 days of recovery, the presence of PK 11195 and Ro5-4864 increased the colon regeneration process (Fig. 5b); however, flunitrazepam-treated rats exhibited a further reduction in histological damage, as compared to that observed in rats treated with DSS only or with DSS combined with the other TSPO drug ligands (Fig. 5b).

Effect of treatment on cytokine expression

Proinflammatory mediators, CINC-1, which is the rat homolog of human IL-8, and TNF- α , were measured in the blood and colon tissues from treated animals. In animals treated with DSS only, we observed a correlation between increased proinflammatory mediators and histological evidence of tissue damage (Figs. 5–7). CINC-1 and TNF- α levels reached maximal level after 7 days of treatment both in plasma and colon tissue. Administration of TSPO drug ligands reduced CINC-1 plasma levels, whereas it increased CINC-1 tissue levels. Flunitrazepam exerted the greatest effect on CINC-1 levels, which were reduced by as much as 70% in plasma (Fig. 6a) and increased by 125% in colon tissue (Fig. 6b). TSPO drug ligands similarly reduced plasma and tissue levels of TNF- α (Figs. 7a and 7b). Flunitrazepam reduced TNF- α plasma levels by 60% (Fig. 7a). PK 11195 and Ro5-4864 had differential effects on cytokine plasma and tissue levels: PK 11195 did not affect TNF- α levels but had a mild and strong effect on CINC-1 plasma and tissue levels, respectively, whereas Ro5-4864 reduced plasma and tissue TNF- α levels but had a strong and mild effect on CINC-1 plasma and tissue levels, respectively.

In order to further characterize the differences in cytokine plasma and tissue levels, we measured the mRNA expression of both CINC-1 and TNF- α in colon tissue. DSS treatment increased mRNA expression of these cytokines in tissue (Fig. 6c and 7c), which is consistent with measurements of cytokine protein levels. Benzodiazepines (Ro5-4864 and Flunitrazepam) exerted the greatest effect (2-fold increase) on CINC-1 and TNF- α mRNA expression. Ro5-4864 had an interesting effect; it delayed CINC-1 mRNA expression (Fig. 6c), which was consistent with the tissue CINC-1 expression profile (Fig. 6b). Conversely PK 11195 reduced the DSS-induced CINC-1 mRNA expression, whereas this TSPO ligand increased the colon tissue levels of CINC-1 (Fig. 6b) and had no effect on DSS-induced TNF- α mRNA and protein expression (Figs. 7b and 7c).

Quantitative analysis of TSPO levels using PK 11195 ligand binding in colon membrane preparations was performed after 7 and 14 days of treatment. DSS-induced a 1.7-fold increase in PK 11195 binding sites compared to control, and this effect was reduced with all TSPO drug ligands at day 7 but only with flunitrazepam at day 14 (Fig. 8).

Discussion

Human intestinal biopsies from UC and CD patients clearly show overexpression of TSPO, which is also evident in colon cancers that contain severely altered epithelium. In addition, this overexpression is found in the inflammatory regions adjacent to tumor tissue. In all

these cases, the intestinal epithelia were strongly modified; there is a reduction in the number of goblet cells, as compared to enterocytes, reinforcing the morphological findings of TSPO overexpression. The TSPO protein overexpression observed in our study is consistent with previous reports showing both protein and mRNA overexpression^{11,12,26}, as well as increased TSPO ligand binding in colon cancer^{9,10} and other cancer types^{11,27}.

To study the role of TSPO in this inflammatory process, we chose an animal model that closely mimics the modifications observed in the colonic epithelium of UC patients¹⁸. DSS-treated rats exhibit severe erosion with strong overexpression of TSPO that increases with the duration of DSS treatment and parallels the changes observed in plasma cytokine levels. In normal rat colon tissue, TSPO immunoreactivity is primarily observed in the mitochondria of enterocytes, and there is a 2-fold enrichment in TSPO drug ligand binding sites in mitochondrial membrane preparations similar to that observed for other tissues and cell types^{22,24, 28-29}. The overexpression of TSPO under inflammatory conditions suggests the involvement of mitochondria, which is consistent with previous studies on various disease processes and aging⁷. Under inflammatory conditions, mitochondria generate reactive oxygen species (ROS) that activate the nuclear factor NFB, which subsequently modulates proinflammatory cytokine production³⁰. In DSS-treated rats, this nuclear factor has been demonstrated to initiate a cascade of events leading to histological manifestation of the disease, including increased expression of proinflammatory cytokines³¹, as reported in our study. The mechanism underlying DSS-induced TSPO overexpression is not clearly understood and might not involve the NFB pathway, since it has been previously reported that PMA-induced TSPO overexpression occurred through the PKC ϵ signal transduction pathway in different cell lines³².

TSPO high affinity drug ligands exert differential effects on the extent of mucosal damage and plasma cytokine levels in DSS-treated rats. The benzodiazepine flunitrazepam protects intestinal mucosa against DSS-induced damage, whereas the other benzodiazepine Ro5-4864 and the isoquinoline PK 11195, increases the rate of appearance of initial damage but also increases epithelial regeneration. The molecular mechanisms responsible for these effects are not clear but may reflect the differential actions of various TSPO ligands. TSPO has been characterized as a component of different mitochondrial complexes that perform different functions and exhibit different pharmacological profiles^{33,34}. PK 11195 has been reported to exclusively bind to the 18-kDa TSPO protein, whereas Ro5-4864 requires the presence of the 30-kDa VDAC for optimal binding^{35,36}. Moreover, flunitrazepam acts as a partial agonist of TSPO in the presence of the TSPO endogenous ligand DBI³⁷. Interestingly, we identified immunoreactive DBI in the rat colon (data not shown).

We observed a strong correlation between histological observations and plasma cytokine levels following TSPO ligand treatment. TSPO ligands reduced CINC-1 plasma levels but increased expression of CINC-1 in the colonic mucosa, suggesting either cytokine retention in the tissue or transcriptional regulation, which was previously observed³⁸. Indeed, LPS-induced secretion of IL-8 in polymorphonuclear leukocytes is blocked by the benzodiazepine midazolam when IL-8 mRNA expression is slightly increased³⁸. There are multiple cytokine secretory pathways involving different release mechanisms that are not yet fully understood³⁹, and these can be regulated by TSPO drug ligands. TSPO drug ligands can modulate the cytokine mRNA levels, resulting in accumulation of cytokines in the tissue. Production and secretory pathways are not identical for all cytokines, for example those that lack a secretory signal sequence, such as TNF, have been suggested to be secreted through exovesicle formation⁴⁰. In agreement with this, we observed dramatic differences between CINC-1 and TNF levels and changes in the presence of TSPO drug ligands, suggesting that different production and secretion regulatory mechanisms were present.

Further work is needed to characterize the processes underlying cytokine regulation, in particular the involvement of calcium mobilization¹⁷, the regulation of ROS generation⁴¹, and oxysterol production^{42,43}, as these mechanisms have been reported to confer protective effects against induced-colitis in the rat.

Generally, the integrity of the intestinal mucosal surface barrier is rapidly re-established, even after extensive destruction, due to the enormous regenerative capability of the mucosal surface epithelium⁴⁴. Overexpression of TSPO has been reported to contribute to the regenerative process following renal ischemia reperfusion injury by increasing mitochondrial viability⁴⁵. The central role of mitochondria in ischemia reperfusion injury might be due to their contribution to energy production (ATP), ROS generation, and apoptosis. In this latter case, TSPO has been reported to be functionally associated with proapoptotic Bax and antiapoptotic Bcl-2⁴⁶, and the TSPO antagonist SSR180575 has been reported to regulate oxidative stress through the modulation of expression of TSPO binding partners⁴⁷. In addition, TSPO drug ligands may affect the regeneration process by inducing mitochondrial membrane biogenesis, which is a prerequisite for accelerated cell proliferation⁴⁸.

In summary, the results presented in this study demonstrate that TSPO is overexpressed during early stages of inflammation in IBD and suggest that this protein may serve as a marker of the repair process. The enhancement of intestinal repair mechanisms by TSPO ligands may provide future approaches for the treatment of diseases that are characterized by epithelial tissue injury.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Grant support:

This work was supported by the Institut National de la Santé et de la Recherche Médicale (INSERM), the Centre National de la Recherche Scientifique (CNRS) and by grants from the Agence Nationale de la Recherche (ANR BLAN-0190-01 to J.J.L.) and National Institutes of Health (ES-07747 to V.P.). M.A. Ostuni has a postdoctoral fellowship from INSERM, and L. Issop has a fellowship from the Ministère de la Recherche Française.

References

1. Gismera CS, Aladren BS. Inflammatory bowel diseases: a disease (s) of modern times? Is incidence still increasing? *World J Gastroenterol.* 2008; 14:5491–5498. [PubMed: 18810764]
2. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. *Nature.* 2008; 454:436–444. [PubMed: 18650914]
3. Rubin DT. The changing face of colorectal cancer in inflammatory bowel disease: progress at last! *Gastroenterology.* 2006; 130:1350–1362.
4. Zisman TL, Rubin DT. Colorectal cancer and dysplasia in inflammatory bowel disease. *World J Gastroenterol.* 2008; 14:2662–2669. [PubMed: 18461651]
5. Mantovani A. Inflaming metastasis. *Nature.* 2009; 457:36–37. [PubMed: 19122629]
6. Kim S, Takahashi H, Lin W-W, Descargues P, Grivennikov S, Kim Y, Luo J-L, Karin M. Carcinoma-produced factors activate myeloid cells through TLR2 to stimulate metastasis. *Nature.* 2009; 457:102–106. [PubMed: 19122641]
7. Papadopoulos V, Baraldi M, Guilarte TR, Knudsen TB, Lacapere J-J, Lindemann P, Norenberg MD, Nutt D, Weizman A, Zhang MR, Gavish M. Translocator protein (18kDa): new nomenclature for the peripheral-type benzodiazepine receptor based on its structure and molecular function. *Trends Pharmacol Sci.* 2006; 27:402–409. [PubMed: 16822554]

8. Bribes E, Carriere D, Goubet C, Galiegue S, Casellas P, Simony-Lafontaine J. Immunohistochemical assessment of the peripheral benzodiazepine receptor in human tissues. *J Histochem Cytochem.* 2004; 52:19–28. [PubMed: 14688214]
9. Katz Y, Eitan A, Amiri Z, Gavish M. Dramatic increase in peripheral benzodiazepine binding sites in human colonic adenocarcinoma as compared to normal colon. *Eur J Pharmacol.* 1988; 148:483–484. [PubMed: 2838309]
10. Katz Y, Eitan A, Gavish M. Increase in peripheral benzodiazepine binding sites in colonic adenocarcinoma. *Oncology.* 1990; 47:139–142. [PubMed: 2156202]
11. Han Z, Slack RS, Li W, Papadopoulos V. Expression of peripheral benzodiazepine receptor (PBR) in human tumors: relationship to breast, colorectal, and prostate tumor progression. *J. Recept Signal Transduct Res.* 2003; 23:225–238. [PubMed: 14626449]
12. Maaser K, Grabowski P, Oezdem Y, Krahn A, Heine B, Stein H, Buhr HJ, Zeitz M, Scherübl H. Up-regulation of the peripheral benzodiazepine receptor during human colorectal carcinogenesis and tumor spread. *Clin Cancer Res.* 2005; 11:1751–1756. [PubMed: 15755996]
13. Maaser K, Grabowski P, Sutter AP, Hopfner M, Foss HD, Stein H, Berger G, Gavish M, Zeitz M, Scherübl H. Overexpression of the peripheral benzodiazepine receptor is a relevant prognostic factor in stage III colorectal cancer. *Clin. Cancer Res.* 2002; 8:3205–3209. [PubMed: 12374690]
14. Li W, Hardwick MJ, Rosenthal D, Culty M, Papadopoulos V. Peripheral type benzodiazepine receptor overexpression and knockdown in human breast cancer cells indicates its prominent role in tumor cell proliferation. *Biochem Pharmacol.* 2007; 73:491–503. [PubMed: 17126818]
15. Decaudin D, Castedo M, Nemati F, Beurdeley-Thomas A, dePinieux G, Caron A, Pouillard P, Wijdenes J, Rouillard D, Kroemer G, Poupon M-F. Peripheral benzodiazepine receptor ligands reverse apoptosis resistance of cancer cells in vitro and in vivo. *Cancer Res.* 2002; 62:1388–1393. [PubMed: 11888910]
16. Bribes E, Bourrie B, Casellas P. Ligands of the peripheral benzodiazepine receptor have therapeutic effects in pneumopathies in vivo. *Immunol Lett.* 2003; 88:241–247. [PubMed: 12941483]
17. Choi HB, Khoo C, Ryu JK, vanBreemen E, Kim SU, McLarnon JG. Inhibition of lipopolysaccharide-induced cyclooxygenase-2, tumor necrosis factor- α and calcium responses in human microglia by the peripheral benzodiazepine receptor ligand PK11195. *J Neurochem.* 2002; 83:546–555. [PubMed: 12390516]
18. Wirtz S, Neufert C, Weigmann B, Neurath MF. Chemically induced mouse models of intestinal inflammation. *Nature Protocols.* 2007; 2:541–546.
19. Brown SJ, Mayer LM. The immune response in inflammatory bowel disease. *Am J Gastroenterol.* 2007; 102:2058–2069. [PubMed: 17561966]
20. Torres SR, Fröde TS, Nardi GM, Vita N, Reeb R, Ferrara P, Ribeiro-do-Valle RM, Farges RC. Anti-inflammatory effects of peripheral benzodiazepine receptor ligands in two mouse models of inflammation. *Eur J Pharmacol.* 2000; 408:199–211. [PubMed: 11080527]
21. Barbier M, Attoub S, Joubert M, Bado A, Laboisse C, Cherbut C, Galmiche JP. Proinflammatory role of leptin in experimental colitis in rats benefit of cholecystokinin-B antagonist and beta3-agonist. *Life Sci.* 2001; 69:567–580. [PubMed: 11510951]
22. Ostuni M, Tumilasci OR, Péranzi G, Cardoso EML, Contreras LN, Arregger AL, Papadopoulos V, Lacapère J-J. Effect of translocator protein (18-kDa) ligand binding on neurotransmitter-induced saliva secretion in rat submandibular glands. *Biol Cell.* 2008; 100:427–439. [PubMed: 18269350]
23. Ostuni M, Ducroc R, Peranzi G, Tonon M-C, Papadopoulos V, Lacapère J-J. Translocator protein (18 kDa) ligand PK11195 induces transient mitochondrial Ca²⁺ release in HT-29 human colon cancer cells. *Biol Cell.* 2007; 99:639–647. [PubMed: 17561806]
24. Ostuni M, Marazova K, Peranzi G, Vidic B, Papadopoulos V, Ducroc R, Lacapère J-J. PBR ligands mediate chloride current through calcium signaling in rat gastric mucosa. *Am J Physiol.* 2004; 286:G1069–G1080.
25. Buyse M, Radeva G, Bado A, Farinotti R. Intestinal inflammation induces adaptation of P-glycoprotein expression and activity. *Biochem Pharmacol.* 2005; 69:1745–1754. [PubMed: 15885661]

26. Kronigsrainer I, Vogel UF, Beckert S, Coerper S, Braun A, Lember N, Steurer W, Kronigsrainer A, Kupta S. Increased translocator protein (TSPO) mRNA levels in colon but not in rectum carcinoma. *Eur Surg Res.* 2007; 39:359–363. [PubMed: 17652962]
27. Galieges S, Tinel N, Casellas P. The peripheral benzodiazepine receptor: a promising therapeutic target. *Cur Med Chem.* 2003; 10:1563–1572.
28. Nayyar T, Mukherjee S, Das SK. Modulation of binding characteristics of peripheral benzodiazepine receptors in vitamin A-deficient guinea pig lung. *Biochem Pharmacol.* 1996; 51:1203–1209. [PubMed: 8645344]
29. Costa B, Salvetti A, Rossi L, Spinetti F, Lena A, Chelli B, Rechichi M, Da Pozzo E, Gremigni V, Martini C. Peripheral benzodiazepine receptor: characterization in human T-lymphoma Jurkat cells. *Mol Pharmacol.* 2006; 69:37–44. [PubMed: 16189298]
30. Hughes G, Murphy MP, Ledgerwood EC. Mitochondrial reactive oxygen species regulate the temporal factor κ B to modulate tumour necrosis factor-induced apoptosis: evidence for mitochondria-targeted antioxidants. *Biochem J.* 2005; 389:83–89. [PubMed: 15727562]
31. Reed KL, Fruin AB, Gower AC, Gonzales KD, Stucchi AF, Andry CD, O'Brien M, Becker JM. NF- κ B activation precedes increases in mRNA encoding neurokinin-1 receptor, proinflammatory cytokines, and adhesion molecules in dextran sulphate sodium-induced colitis in rats. *Dig Dis Sci.* 2005; 50:2366–2378. [PubMed: 16416193]
32. Batarseh A, Giatzakis C, Papadopoulos V. Phorbol-12-myristate 13-acetate acting through protein kinase C ϵ induces translocator protein (18kDa) *Tspo* gene expression. *Biochemistry.* 2008; 47:12886–12889. [PubMed: 18975922]
33. Veenman L, Papadopoulos V, Gavish M. Channel-like functions of the 18-kDa translocator protein (TSPO): regulation of apoptosis and steroidogenesis as part of the host-defense response. *Curr Pharm Des.* 2007; 13:2385–2405. [PubMed: 17692008]
34. Ostuni M, Peranzi G, Ducroc R, Fasseu M, Vidic B, Dumont J, Papadopoulos V, Lacapère J-J. Distribution, pharmacological characterization and function of the translocator protein peripheral-type benzodiazepine receptor in rat intestine. *Biol Cell.* 2009 doi :10.1042/BC20080231.
35. Lacapère JJ, Delavoie F, Li H, Péranzi G, Maccario J, Papadopoulos V, Vidic B. Structural and functional study of reconstituted peripheral benzodiazepine receptor. *Biochem Biophys Res Commun.* 2001; 284:536–541. [PubMed: 11394915]
36. Garnier M, Dimchev AB, Boujrad N, Price JM, Musto NA, Papadopoulos V. In vitro reconstitution of a functional peripheral-type benzodiazepine receptor from mouse Leydig tumor cells. *Mol Pharmacol.* 1994; 45:201–211. [PubMed: 8114671]
37. Papadopoulos V, Berkovich A, Krueger KE, Costa E, Guidotti A. Diazepam binding inhibitor and its processing products stimulate mitochondrial steroid biosynthesis via an interaction with mitochondrial benzodiazepine receptors. *Endocrinology.* 1991; 129:1481–1488. [PubMed: 1651852]
38. Galley HF, Dubbels AM, Webster NR. The effect of midazolam and propofol on interleukin-8 from human polymorphonuclear leukocytes. *Anesth Analg.* 1998; 86:1289–1293. [PubMed: 9620522]
39. Eder C. Mechanisms of interleukin-1 β release. *Immunobiology.* 2009; 21:543–553. [PubMed: 19250700]
40. Obregon C, Rothen-Rutishauser B, Gerber P, Gehr P, Nicod LP. Active uptake of dendritic cell-derived exovesicles by epithelial cells induces the release of inflammatory mediators through a TNF- α -mediated pathway. *Am J Path.* 2009; 175:696–705. [PubMed: 19628765]
41. Mustafa A, El-Medany A, Hagar HH, El-Medany G. Ginkgo biloba attenuates mucosal damage in a rat model of ulcerative colitis. *Pharmacol Res.* 2006; 53:324–330. [PubMed: 16458529]
42. Péliissier MA, Muller C, Hill M, Morfin R. Protection against dextran sodium sulfate-induced colitis by dehydroepiandrosterone and 7 α -hydroxy-dehydroepiandrosterone in the rat. *Steroids.* 2006; 71:240–248. [PubMed: 16371229]
43. Hennebert O, Pelissier MA, Le Mee S, Wulfert E, Morfin R. Anti-inflammatory effects and changes in prostaglandin patterns induced by 7 β -hydroxy-epiandrosterone in rats with colitis. *J Steroid Biochem Mol Biol.* 2008; 110:255–262. [PubMed: 18502118]

44. Sturm A, Dignass AU. Epithelial restitution and wound healing in inflammatory bowel disease. *World J Gastroenterol.* 2008; 14:348–353. [PubMed: 18200658]
45. Favreau F, Rossard L, Zhang K, Desurmont T, Manguy E, Belliard A, Fabre S, Liu J, Han Z, Thuillier R, Papadopoulos V, Hauet T. Expression and modulation of translocator protein and its partner by hypoxia reoxygenation or ischemia and reperfusion in porcine renal models. *Am J Physiol Renal Physiol.* 2009; 297:F177–F190. [PubMed: 19386723]
46. Veenman L, Shandalov Y, Gavish M. VDAC activation by the 18 kDa translocator protein (TSPO), implications for apoptosis. *J Bioenerg Biomembr.* 2008; 40(3):199–205. [PubMed: 18670869]
47. Kunduzova OR, Escourrou G, De la FARGE F, Salvayre R, Séguélas MH, Leducq N, Bono F, Herbert JM, Parini A. Involvement of peripheral benzodiazepine receptor in the oxidative stress, death-signaling pathway, and renal injury induced by ischemia-reperfusion. *J Am Soc Nephrol.* 2004; 15:2152–2160. [PubMed: 15284300]
48. Carreras MC, Poderoso JJ. Mitochondrial nitric oxide in the signalling of cell integrated responses. *Am J Physiol Cell Physiol.* 2007; 292:C1569–C1580. [PubMed: 17496232]

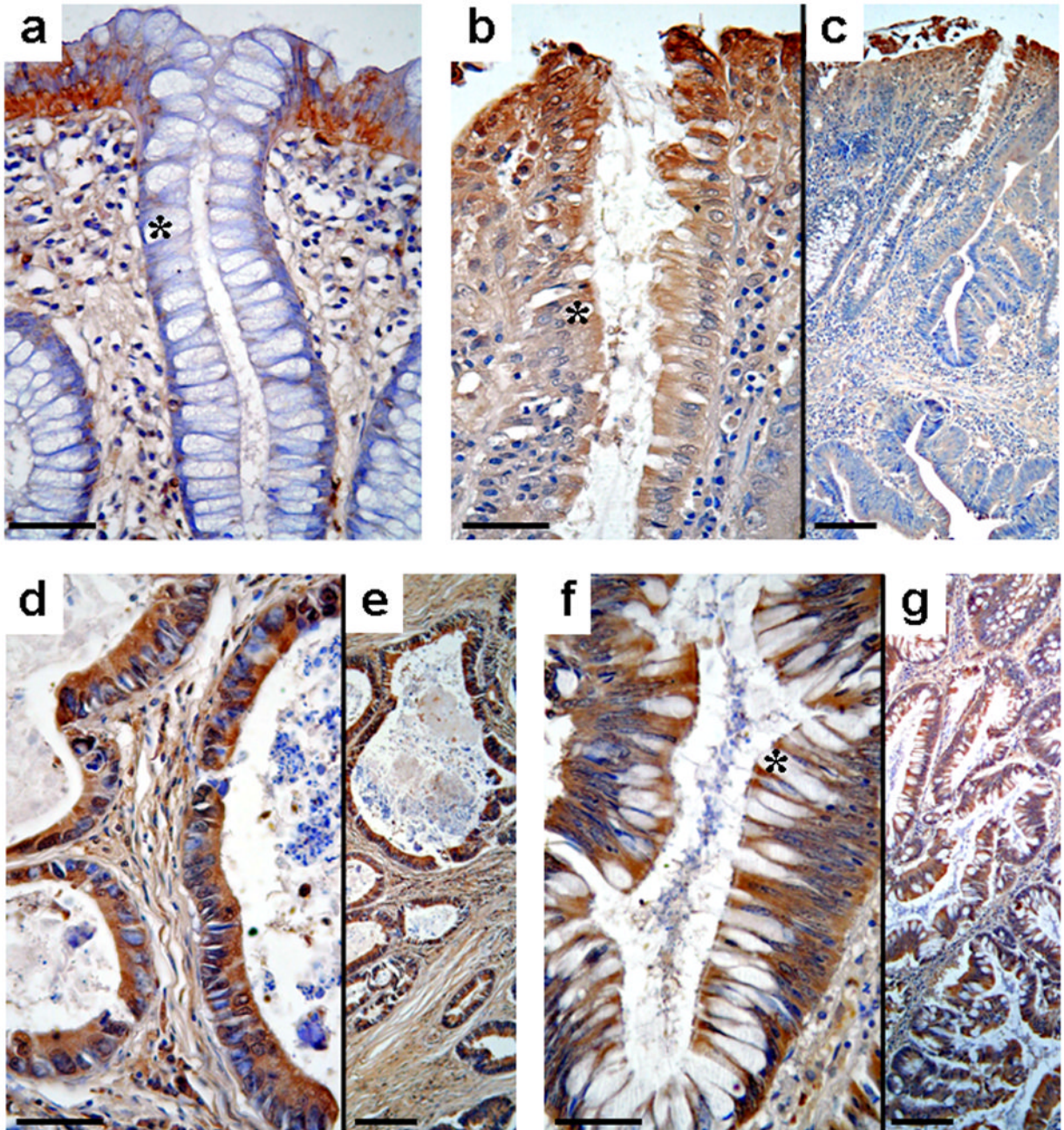


Figure 1.

TSPO immunoreactivity in human normal colon and colon tumor tissue specimens. Intense cytoplasmic immunoreactivity for TSPO is observed in the surface epithelial cells of normal colon (a), as well as in the dysplastic area neighboring the invasive tumor tissue (b, c). TSPO-labeled cells are observed throughout the colon crypts. Four colon cancer samples; intramuscular tumoral invasive glands (d, e) and mucosecretant cancer (f, g) are provided. Enterocytes, but not goblet cells (asterisk), are strongly labeled with peroxidase-conjugated anti-TSPO antibody, except for mucosecretant cancer (f). In this case, we observe a high rate of cell proliferation with strong labeling. Attribution of labeling to one single cell type is

difficult; the labeling at the base of goblet cells (f) may differ from normal colon (a). Bars a, b, d, and f = 50 μm . Bars c, e, and g = 100 μm .

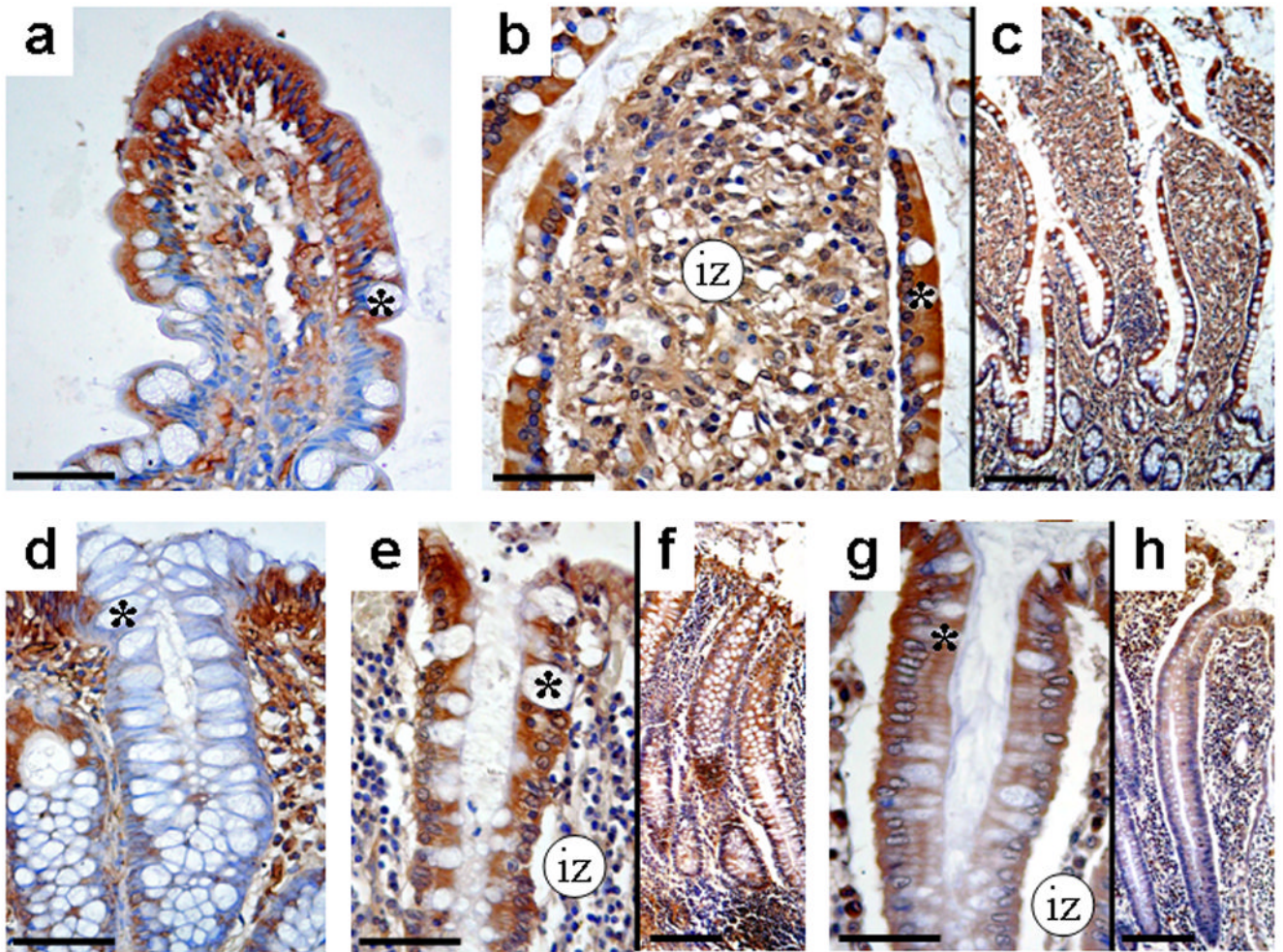


Figure 2.

TSPO immunoreactivity in the ileum and colon of chronic IBD patients. Intense TSPO immunoreactivity is observed in enterocytes within the epithelium of biopsies from CD (b, c, e, and f) and UC (g and h) patients. TSPO-labeled cells are distributed along the entire epithelial villi or along the colonic crypt in ileum (a) and colon (d). The two different CD biopsies are ileal (b and c) and recto-sigmoidal (e and f). The UC biopsy (g and h) corresponds to distal colon. Typical inflammatory zones (denoted as “iz” in white circles) are provided for each case. Goblet cells are indicated by an asterisk. Bars a, b, d, e, and g = 50 μ m. Bars c, f, and h = 100 μ m.

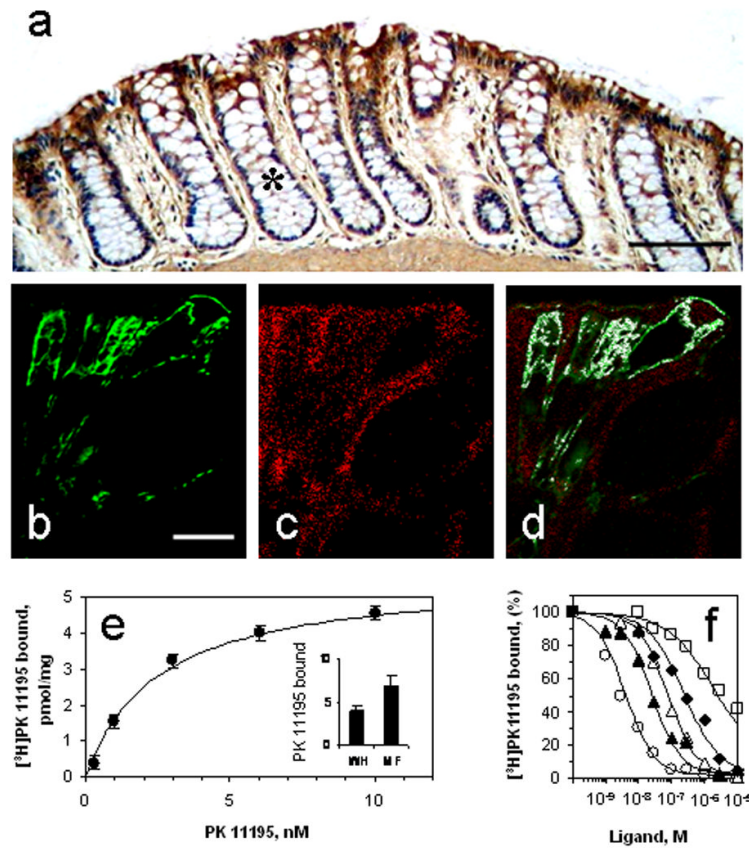


Figure 3.

Characterization of TSPO expression in the rat colon. (a) Intense TSPO immunoreactivity in the surface epithelial cells where enterocytes, but not goblet cells (asterisk), are labeled. Confocal microscopy shows localization of TSPO (b) and mitochondrial VDAC (c). (d) Merged images show colocalization of TSPO and mitochondria as white dots. (e) Saturation isotherm of [³H]PK 11195 binding to total tissue extracts ($K_d = 3 \pm 0.5$ nM, $B_{max} = 6.5 \pm 0.5$ pmol/mg and $nH = 0.95 \pm 0.025$). Inset shows the specific binding of [³H]PK 11195 (3 nM) to the mitochondrial fraction (MF), as compared to total tissue extracts (T). (f) Pharmacological characterization of TSPO ligand binding in total tissue extracts. Open circles represent PK 11195 data; closed triangles represent Ro5-4864; open triangles indicate flunitrazepam; closed diamonds indicate diazepam; and open squares show protoporphyrin IX. Bars in micrographs are: 100 μ m in panel (a) and 10 μ m in panels (b-d).

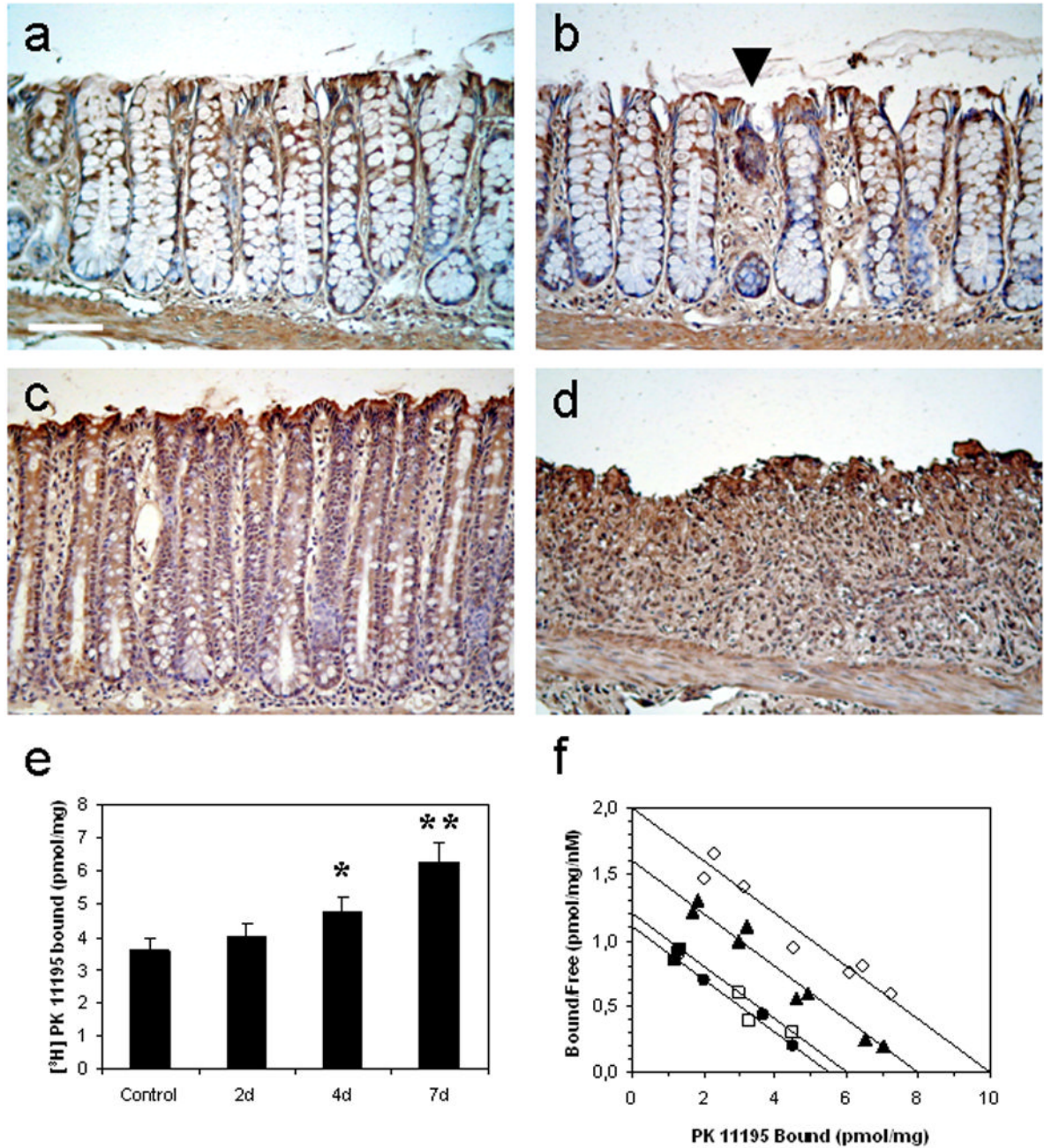


Figure 4.

Characterization of TSPO expression in the colon of DSS-treated rats. TSPO immunoreactivity at different time-points following DSS treatment: (a) control; (b) 2; (c) 4; and (d) 7 days. A focal superficial erosion (arrow head in b), mucosal thickening with edema, numerous inflammatory cells in the lamina propria, loss of goblet cells (c), and deep ulceration of the mucosa (d) can be observed. All panels are the same scale; the bar = 100 μ m. TSPO ligand binding to total tissue extracts as a function of time (e). (f) Scatchard plots of [3 H]PK 11195 binding to tissue extracts as a function of time following DSS treatment. Closed circles (0 days); open squares (2 days); closed triangles (4 days); and open diamonds (7 days). * $P < 0.05$; ** $P < 0.01$ ($n=4$)

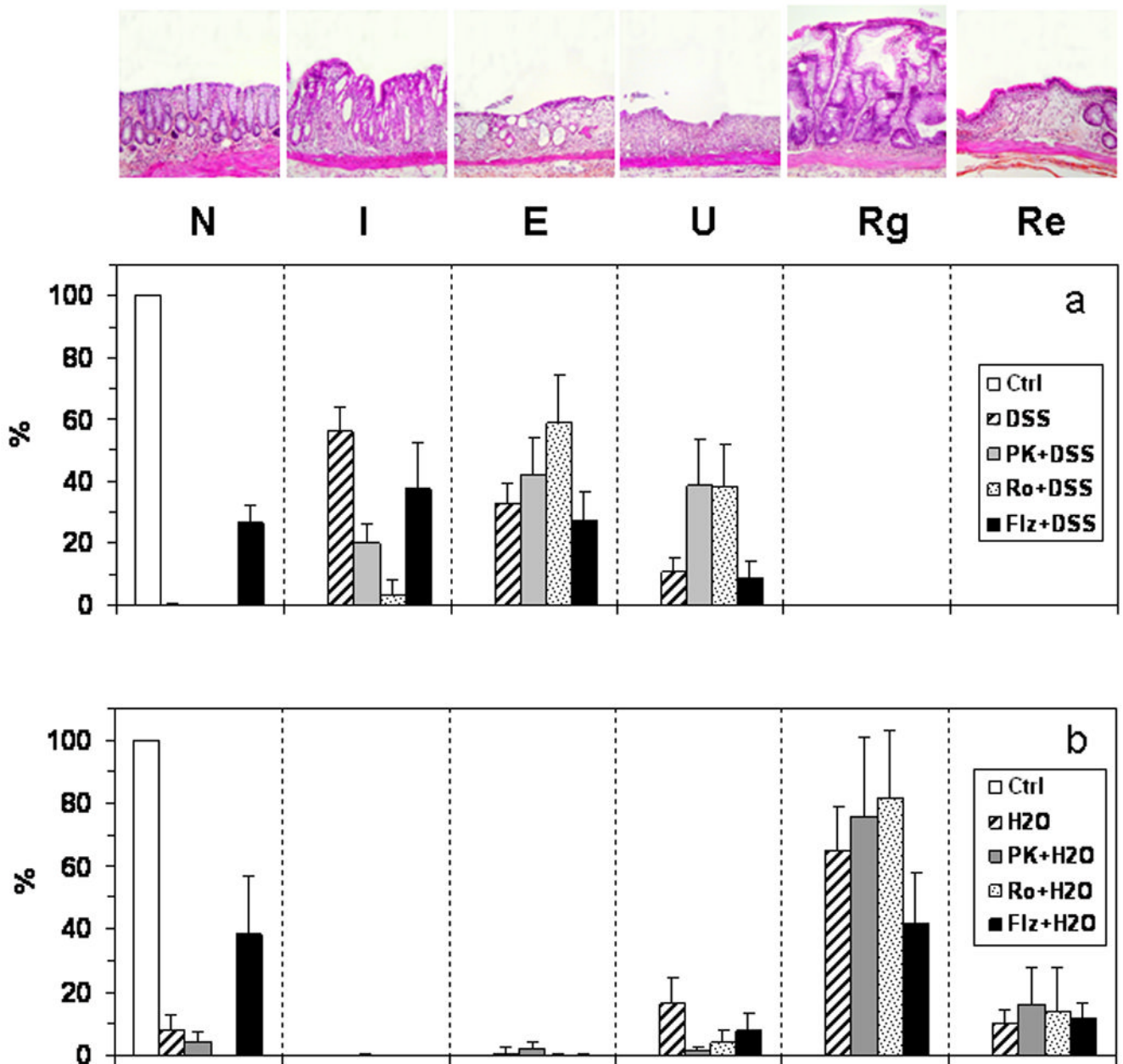


Figure 5.

Effect of TSPO drug ligands on the progression of DSS-induced inflammation in the rat colon. Rats were treated with DSS in the presence or absence of TSPO drug ligands for 7 days and then examined (a), or followed by an additional 7 days of drug treatment alone (b). The top panels show the typical histological changes observed in the rat colon upon treatment: normal tissue (N), inflammation (I), erosion (E), ulceration (U), regeneration of mucosa adjacent to ulcer (Rg), and re-epithelialization of ulcer (Re). Pathological aspects were determined as a percentage of the colon displaying lesions (see criteria description in Methods). Data from experiments with control treatment with vehicle (open bars); DSS alone (hatched bars); DSS and PK 11195 (gray bars); DSS and Ro5-4864 (dotted bars); and DSS and flunitrazepam (filled bars) are provided. Results represent means \pm 2SD (n=3).

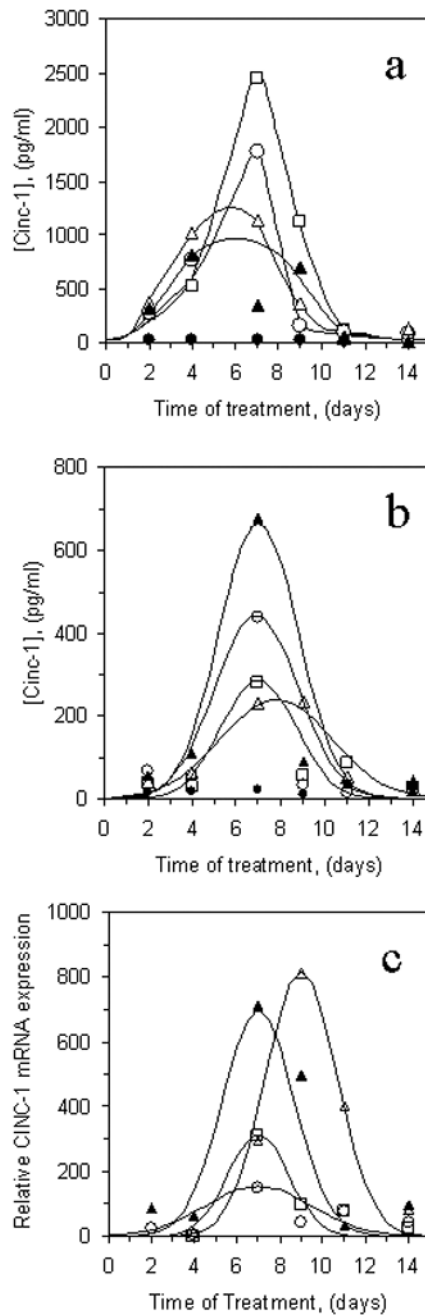


Figure 6.

Effects of DSS and TSPO drug ligands on CINC-1 expression. Rats were treated with DSS with or without concomitant TSPO drug ligand treatment for 7 days, followed by 7 days of drug treatment alone. Blood and tissue samples were collected at the indicated time-points, and levels of the cytokine CINC-1 (a,b for blood and tissue, respectively), as well as mRNA levels (c), were determined. Results represent means \pm SEM (n=4–9). Controls (closed circles); DSS alone (open squares); DSS and PK 11195 (open circles); DSS and Ro5-4864 (open triangles); and DSS and flunitrazepam (closed triangles).

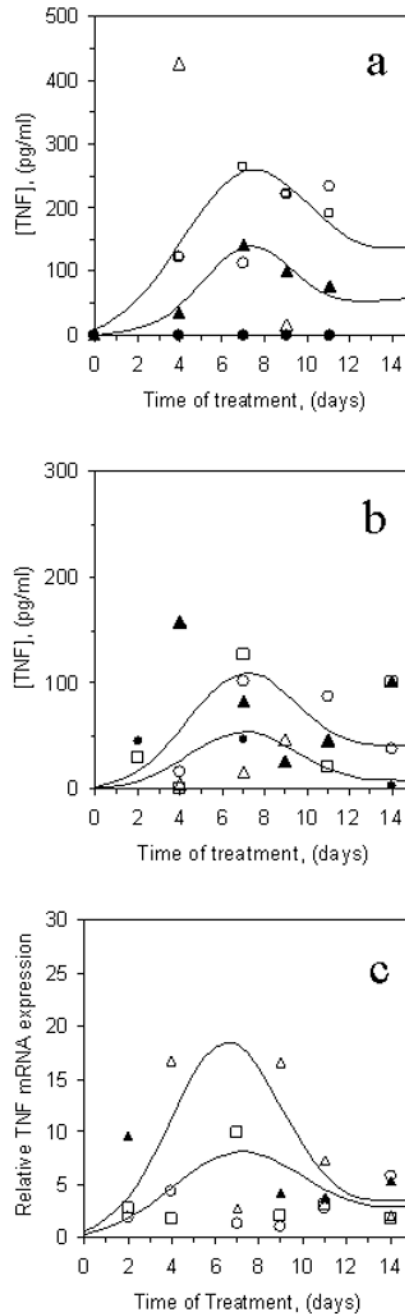


Figure 7.

Effects of DSS and TSPO drug ligands on TNF- α expression. Rats were treated with DSS with or without concomitant TSPO drug ligand treatment for 7 days, followed by 7 days of drug treatment alone. Blood and tissue samples were collected at the indicated time-points, and levels of the cytokine TNF- α (a,b for blood and tissue, respectively), as well as mRNA levels (c), were determined. Results represent mean \pm SEM (n=4–9). Controls (closed circles); DSS alone (open squares); DSS and PK 11195 (open circles); DSS and Ro5-4864 (open triangles); and DSS and flunitrazepam (closed triangles). Lines have been drawn to highlight the differences between the two data groups: DSS treatment alone or with PK 11195 and DSS treatment with benzodiazepines.

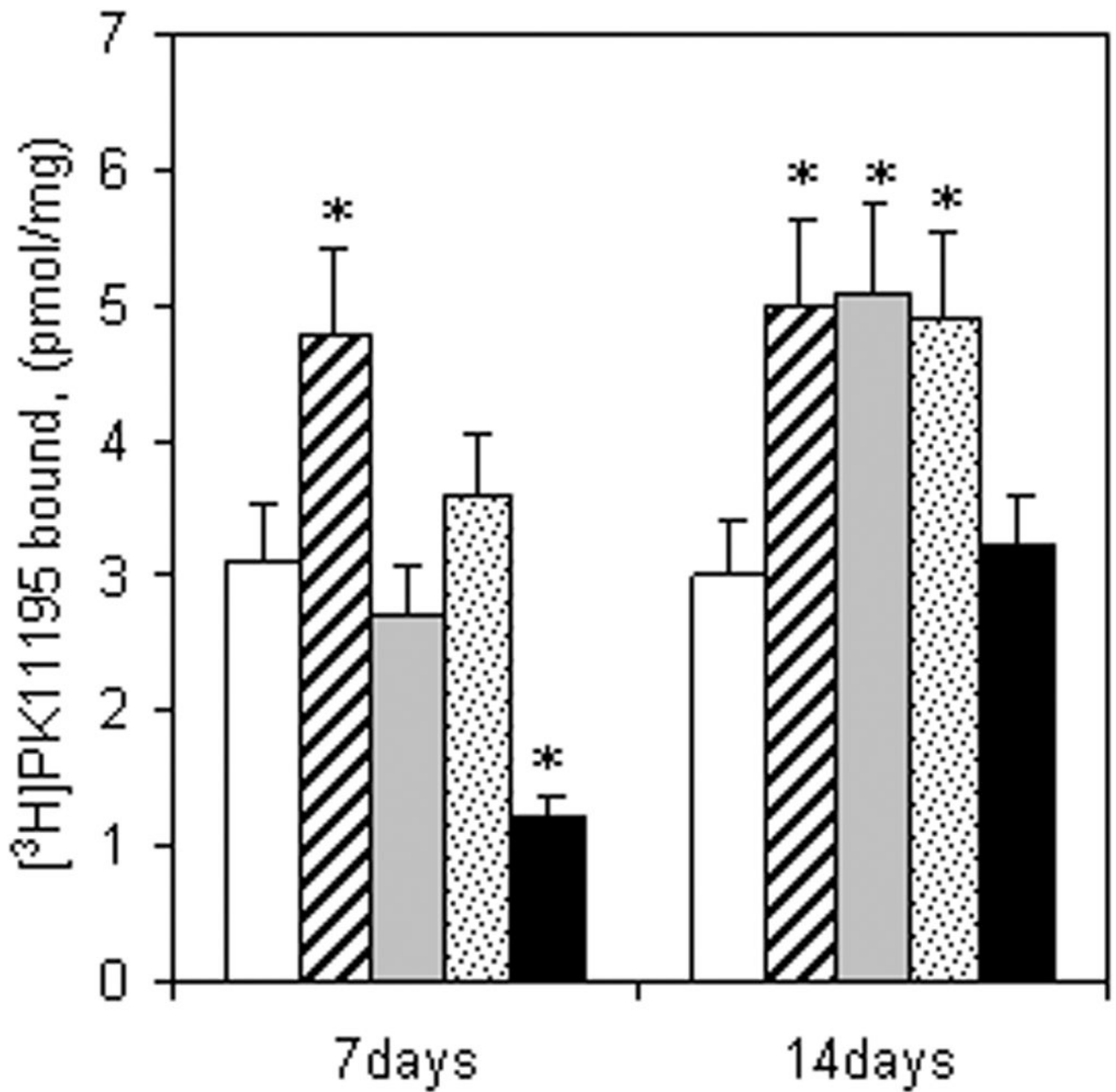


Figure 8.

Effects of DSS and TSPO drug ligands on TSPO expression. PK 11195 binding sites in total colon tissue extracts isolated from rats treated with DSS alone or with concomitant TSPO drug ligands were measured at 7 and 14 days. Data from control treatment with vehicle (open bars); DSS alone (hatched bars); DSS and PK 11195 (gray bars); DSS and Ro5-4864 (dotted bars); and DSS and flunitrazepam (filled bars) are shown. * $P < 0.05$ ($n=4$).