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The Hydrogen Peroxide Scavenger, Catalase, Alleviates Ion Transport Dysfunction in Murine Colitis

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Abstract

Reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2) contribute to epithelial damage and ion transport dysfunction (key events in inflammatory diarrhea) in inflammatory bowel disease (IBD). The aim of this study was to identify if H_2O_2 mediates suppression of colonic ion transport function in the murine dextran sulfate sodium (DSS) colitis model by using the H_2O_2 degrading enzyme, catalase. Colitis was induced by administering DSS (4%) in drinking water for 5 days followed by 3 days on normal H_2O. Mice were administered either pegylated-catalase or saline at day −1, 0 and +1 of DSS treatment. Ion transport responses to the Ca^{2+}-dependent agonist, carbachol (CCh), or the cAMP-dependent agonist, forskolin, were measured across distal colonic mucosa mounted in Ussing chambers. Parameters of DSS-induced inflammation (loss in body weight, decreased colon length, altered stool consistency), were only partially alleviated by catalase while histology was only minimally improved. However, catalase significantly reversed the DSS-induced reduction in baseline ion transport as well as colonic I_{sc} responses to CCh. However, ion transport responses to forskolin were not significantly restored. Catalase also reduced activation of ERK MAP kinase in the setting of colitis, and increased expression of the Na^+-K^+-$2Cl^-$ cotransporter, NKCC1, consistent with restoration of ion transport function. *Ex vivo* treatment of inflamed colonic mucosae with catalase also partially restored ion transport function. Therefore, catalase partially prevents, and rescues, the loss of ion transport properties in DSS colitis even in the setting of unresolved tissue inflammation. These findings indicate a prominent role for ROS in ion transport dysfunction in colitis and may suggest novel strategies for the treatment of inflammatory diarrhea.

Keywords

Chloride secretion; inflammation; reactive oxygen species; diarrhea; epithelium; mucosa; NKCC1; ERK phosphorylation

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INTRODUCTION

A primary symptom of inflammatory bowel disease (IBD) is diarrhea. IBD-associated diarrhea can be attributed primarily to a suppressed ability of colonic surface epithelial cells to absorb Na^+, Cl^- and fluid entering from the ileum. The colonic epithelium is responsible for the active absorption and secretion of salt and water via the activity of ion transporters, exchangers and selective ion channels.[1, 2] Epithelial ion transport can be influenced by a variety of external factors such as neurohumoral and inflammatory mediators, bacterial products or toxins, and paracrine factors.[1, 2] Indeed, many inflammatory mediators such as eicosanoids, histamine and complement factors can activate ion transport in isolated intestine.[3–5] However, a reduced ability of the colonic epithelium to transport ions is a feature of both resected human tissues from IBD patients, and tissues isolated from several murine IBD models including the DSS colitis model.[6–13]

A number of studies indicate that chronically-inflamed colonic tissue is subject to significant oxidative stress due to sustained overproduction of reactive oxygen metabolites that arise from a dysregulated immune response.[14–16] One of the most important reactive oxygen species (ROS) produced during inflammation is H_2O_2, due to its relative stability and its role as a precursor for more toxic species such as hydroxyl radical and hypochlorous acid (HOCl).[16] This is of clinical relevance to IBD as the elevated numbers of activated phagocytes found in ulcerative colitis could secrete millimolar concentrations of H_2O_2 in close proximity to colonic epithelial cells.[17] Accordingly, a number of studies have investigated the effects of H_2O_2 on intestinal secretion. While *ex vivo* exposure of rat colonic tissue or treatment of T_{84} colonic epithelial cell monolayers with H_2O_2 results in a small and indirect increase in mucosal Cl^- secretion, the major effect of H_2O_2 on ion transport appears to be suppression of the capacity for stimulated electrolyte transport.[10, 18–20]

We previously demonstrated that H_2O_2 can inhibit Ca^{2+}-dependent Cl^- secretion in colonic epithelial cell monolayers by a mechanism involving MAPK activation and inhibition of the Na^+,K^+,$2Cl^-$ cotransporter, NKCC1.[20] The antioxidant catalase is a key regulator of H_2O_2 levels and reduces cellular H_2O_2 levels by dismutating H_2O_2 to H_2O and O_2.[21] In chronic inflammatory conditions such as Crohn's disease, patients exhibit reduced levels of catalase activity in mononuclear cells.[22] Reduction of H_2O_2 levels with catalase has previously been shown to reduce inflammation in rodent colitis models.[23, 24] In this study, we translated our findings to an animal model of colitis to investigate if the H_2O_2 scavenger, catalase, can reduce ion transport defects associated with colitis, and to determine some of the molecular targets mediating any effect.

RESULTS

Body weight loss in DSS colitis is partially inhibited by Peg-catalase

Prior to determining the effect of *in vivo* catalase administration on ion transport dysfunction in the DSS model, we investigated whether pre-treatment with pegylated catalase (Peg-catalase; days −1, 0 and +1 of DSS treatment) had any effect on overall pathology in mice treated with DSS (Figure 1). We used Peg-catalase as it has a longer half-life *in vivo* than the

unpegylated form with a plasma half-life of 13.6 hrs.[23, 25] DSS caused a significant decrease in the body weight of recipient mice over time from day 5 to termination of the experiment on day 8 compared with control mice ($p<0.001$; n=5; Figure 2A). Intraperitoneal (i.p.) administration of Peg-catalase partially protected against this loss of body weight on day 7 and 8 ($p<0.05$, $p<0.001$ respectively; Figure 2A). Control mice receiving saline i.p. or Peg-catalase both exhibited indistinguishable increases in body weight over this time frame. Quantification of the peak change in body weight demonstrated a significant, but incomplete, degree of protection by Peg-catalase pre-administration compared with mice receiving DSS plus saline ($p<0.05$, Figure 2B).

Partial reduction in markers of inflammation in DSS colitis by Peg-catalase pre-treatment

To identify if Peg-catalase affected markers of inflammation associated with DSS colitis, alterations in colon length, histology and stool consistency were assessed. DSS treatment induced a significant decrease in colon length compared with control mice ($p<0.001$; n=4; Figure 3A). Pre-treatment with Peg-catalase partially prevented this decrease in colon length induced by DSS ($p<0.001$; Figure 3A). H&E staining showed that DSS alone caused colonic ulceration but that this was restricted by Peg-catalase pre-treatment, while the overall inflammation score comprising epithelial and crypt damage as well as immune cell infiltration induced by DSS was partially, but significantly, reduced by Peg-catalase (Figure 3B, 3C). With respect to a readout of intestinal dysfunction, the fluidity of the stool was increased by DSS treatment ($p<0.001$ vs. control; n=4; Figure 3D) and this was significantly, albeit partially, reversed by Peg-catalase pre-treatment ($p<0.05$; Figure 3D). Overall, these data indicate that Peg-catalase pre-treatment partially reduces the severity of DSS-induced colitis in mice.

Peg-catalase restricted ion transport defects in DSS colitis

[8, 12, 13]To determine whether Peg-catalase pretreatment could mitigate ion transport defects associated with DSS colitis, colonic tissues were isolated from control and DSS-treated mice with or without Peg-catalase pre-treatment. Tissues were mounted in Ussing chambers and electrophysiological parameters were measured. DSS suppressed baseline colonic mucosal short-circuit current (I_{sc}) (Fig 4A). This was significantly alleviated by Peg-catalase thus indicating that the suppression of ion transport in DSS colitis was mediated in part by H_2O_2. Intriguingly, control mice treated with Peg-catalase also exhibited an increase in baseline I_{sc}, suggesting that background production of ROS may exert a tonic suppressive influence on murine colonic ion transport (Fig 4A). Previous work from our group and others showed that H_2O_2 can inhibit both calcium-dependent and cAMP-dependent ion transport responses across intestinal epithelial cells *in vitro*.[19, 20] Here we investigated the effect of Peg-catalase pre-treatment on calcium- and cAMP-dependent ion transport responses across colonic mucosa from DSS-treated mice. Peg-catalase almost completely prevented H_2O_2-induced suppression of transport responses to the Ca^{2+}-dependent agonist, carbachol ($p<0.05$; n=7; Fig 4B). These data are consistent with our previous *in vitro* findings.[20] However, catalase pre-treatment did not significantly improve ion transport responsiveness to the cAMP-dependent agonist, forskolin (Fig 4C). Nevertheless, our data indicate that degradation of H_2O_2 by catalase protects against the inhibitory effects DSS-induced colitis on intestinal ion transport capacity.

Catalase treatment reduced H_2O_2 levels and rescued ion transport function in inflamed mouse colon *ex vivo*

To confirm that acute catalase treatment could also rescue ion transport function in inflamed intestine, colonic tissues were isolated from control and DSS-treated mice and mounted in Ussing chambers. Tissues were subsequently treated with either saline or increasing concentrations of Peg-catalase (0, 50, 100, 300 U/ml) for 30 minutes prior to administration of carbachol or forskolin. Treatment of tissues from DSS mice with catalase *ex vivo* (100 U/ml) significantly increased I_{sc} compared with tissues from control mice ($p<0.05$; Fig 5A). *Ex vivo* Peg-catalase exposure also improved Ca^{2+}- and cAMP-dependent ion transport responses ($p<0.05$; Fig 5B, C, respectively) in inflamed tissues, without a significant effect on ion transport responses in normal tissues. To confirm that Peg-catalase treatment of intestinal tissue from DSS-exposed mice actually suppressed H_2O_2 levels, colonic tissues were isolated from control and DSS-treated mice and incubated with catalase or saline for 30 minutes at 37°C. H_2O_2 levels in bathing media were detected and quantified using a Bioxytech quantitative H_2O_2 assay. Colonic tissues from DSS-treated mice exhibited a significant increase in secreted H_2O_2 levels compared with control mouse tissue ($p<0.05$; n=3; Fig 5D). However, in both normal and inflamed samples treated with Peg-catalase, H_2O_2 was undetectable in the bathing media (Figure 5D). These data suggest that suppression of H_2O_2 levels by catalase is likely responsible for the correction of ion transport defects in inflamed colon.

Catalase pre-treatment prevented changes in protein activation and expression associated with suppression of epithelial chloride secretion

We previously demonstrated that H_2O_2 suppression of ion transport responses in intestinal epithelial cells *in vitro* involved increased phosphorylation of extracellular signal regulated kinase (ERK), a key member of the mitogen-activated protein kinase (MAPK) family, and down-regulation of NKCC1, a critical contributor to epithelial electrolyte balance and a component of the chloride secretory machinery.[1, 20, 26, 27] To identify whether these molecular targets of H_2O_2 suppression of ion transport *in vitro* were also affected *in vivo*, we determined the effects of DSS and/or Peg-catalase pre-treatment on NKCC1 expression levels and ERK (p44/42) activation (as determined by phosphorylation of Thr202/Tyr204 residues). DSS reduced NKCC1 expression and conversely increased ERK activation in keeping with our prior *in vitro* data (Figure 6A).[20] On the other hand, Peg-catalase blocked the reduction in NKCC1 levels and restricted the induction of ERK phosphorylation (Figure 6B,C). These data suggest that preservation of NKCC1 activity and ERK phosphorylation levels may mediate the protective effects of catalase on colonic electrolyte transport.

DISCUSSION

In this study we have shown that short-term administration of the H_2O_2 scavenger, catalase, can reduce the impact of DSS colitis on electrophysiological transport processes in murine intestine. This protective effect positively influenced baseline short-circuit current as well as electrogenic ion transport responses to calcium-dependent stimuli. Ion transport responses to cAMP were restored when Peg-catalase was applied *ex vivo*, but not in the *in vivo* experiments. The reason for this discrepancy is not known but likely involves the effects of

residual inflammation. While H_2O_2 has been shown to suppress both Ca^{2+}- and cAMP-dependent chloride secretion *in vitro* (likely a primary contributor to the I_{sc} responses to carbachol and forskolin in the current study), we cannot entirely rule out a possible contribution of electrogenic Na^+ absorption mediated by the epithelial sodium channel (ENaC) following at least forskolin exposure.[19, 20] This is consistent with expression of ENaC in mouse distal colon.[8, 28] The protective effect of catalase can be explained in part by some inhibition of the overall inflammatory response to DSS, although H&E staining in Figure 3 show that this inhibition was clearly only partial. This reflects the complexity of relying on specific ROS inhibitors to alleviate intestinal inflammation in an experimental or clinical setting.[23, 29, 30] However, the treatment protocol does provide some understanding of the kinetics of H2O2 involvement in the ion transport defects in DSS colitis and identifies that early and acute administration of catalase was sufficient to partially reduce the consequences of DSS on ion transport. In fact, our studies of tissues isolated from DSS-treated mice and exposed to catalase *ex vivo* strongly indicate that catalase can act acutely to reverse ongoing suppression of both Ca^{2+}- and cAMP-dependent ion transport capacity in inflamed intestine (c.f. Fig 5b,c). This effect was independent of a reversal of macroscopic inflammatory parameters, thus demonstrating not only that H_2O_2 mediates gross inflammation, but also more subtly manipulates the regulation of existing transport proteins.[31]

Despite studies showing that a number of inflammatory mediators can stimulate chloride secretion in *ex vivo* intestinal tissue, the overwhelming effect of inflammation on intestinal electrolyte transport in murine colitis models as well as IBD patients appears to be suppression of ion transport. It is not fully clear how this contributes to chronic inflammatory conditions such as IBD, but one possible consequence is that it may alter the capacity of intestinal crypts to secrete fluid that is essential for the ‘flushing’ mechanisms that maintain relative crypt sterility, and also modulate the composition of the microbiome.[1, 32, 33] This hypothesis is supported by studies showing that CFTR knockout mice that lack the capacity for normal intestinal chloride secretion exhibit increased bacterial association with the intestinal epithelium as well as an altered intestinal microbiota and mucosal inflammation.[32–34] Our *in vivo* and *ex vivo* data are also consistent with our earlier studies identifying signaling pathways co-opted by H_2O_2 to suppress electrogenic Cl^- secretion across intestinal epithelial monolayers.[20] In those studies, we observed functional suppression of the basolateral cotransporter NKCC1, a critical facilitator of electrogenic Cl^- secretion.[26] Here, NKCC1 expression was reduced in the colon of mice administered DSS, but preserved in mice pre-treated with Peg-catalase, indicating that inflammation-associated H_2O_2 negatively regulates expression of this transporter *in vivo*. The observed effect of DSS on NKCC1 differs from a previous study that showed no effect of DSS on NKCC1 mRNA expression.[35] However, given our previously published work showing that H_2O_2 can suppress NKCC1 activity, in part via colitis-induced activation of ERK MAP kinase (which was also alleviated in catalase pre-treated mice) the data in the current study suggest that H_2O_2 may have both acute and longer-term inhibitory effects on overall NKCC1 functionality.[20] H_2O_2 is capable of prolonging signaling events through suppression of protein tyrosine phosphatases (PTP) that predominantly serve to deactivate tyrosine kinase signaling.[36, 37] Thus, H_2O_2 activation of ERK phosphorylation may also involve suppression

of PTPs including T-cell protein tyrosine phosphatase (TCPTP) which is capable of dephosphorylating ERK activation by inflammatory stimuli.[38] This phosphatase represents an appealing future direction for our studies given that siRNA knockdown of TCPTP in intestinal epithelial cells facilitates suppression of calcium-dependent chloride secretion, while the gene encoding TCPTP, protein tyrosine phosphatase non receptor type 2 (PTPN2) is a validated IBD candidate gene.[39–41]

With respect to other studies of the effects of H_2O_2 on colonic transport, our findings differ slightly from those of Mayol and colleagues.[42] They observed that luminal exposure of colonic tissues to H_2O_2 induced a very substantial increase in basal I_{sc} across unstripped rat colon yet inhibited ion transport responses to forskolin, but not carbachol.[42] Some key characteristics of our study that may account for these differences include the use of mouse colon (*vs.* rat) and stripped mucosal preparations (*vs.* unstripped) that enhance access of serosally-applied carbachol to basolateral muscarinic receptors, as well as the more complex inflammatory environment of DSS colitis *vs.* acute exposure to H_2O_2 alone.

In conclusion, we have shown that Peg-catalase pre-treatment only partially reduced markers of colonic inflammation caused by DSS but had a more pronounced protective effect on ion transport function in DSS colitis. This was in keeping with the ability of Peg-catalase to restore ion transport responsiveness of colonic tissues from DSS-treated mice when applied to the tissues *ex vivo*. The protective effect of Peg-catalase was associated with reduced ERK activation and preservation of NKCC1 levels. Thus, our data indicate that epithelial transport properties can be rescued from the suppressive effects of reactive oxygen species in spite of the continued presence of mucosal inflammation. They also support the idea that an improvement in transport function is not always dependent on fully resolving mucosal inflammation.[10, 20] These findings may have implications for antioxidant-based therapeutic approaches to restore intestinal physiology in conditions, such as inflammatory diarrhea, where mucosal healing may be incomplete.

METHODS

Materials

Dextran sulfate sodium (M.W. 36,000 – 50,000) (ICN Biomedicals, Inc., Aurora, OH), carbachol and forskolin (Sigma, St. Louis, MO) were obtained from the sources indicated. All other reagents were of analytical grade and were acquired commercially.

Induction of colitis

Acute colitis was induced in male Balb/c mice (8–10 weeks old; body weight range 20–24 g) by administering 4% DSS in drinking water for five days followed by three days on normal drinking water.[43] Control mice received normal drinking water. DSS-treated mice were sacrificed at the completion of the treatment cycle (Day 8). Balb/c mice were administered either pegylated-catalase (40 units/g/0.01ml; i.p.) or an equal volume of saline vehicle at day −1, 0 and +1 of DSS treatment (Figure 1). Experiments were conducted with the approval of the Institutional Animal Care and Use Committee at the University of California, San Diego.

Assessment of colitis

Body weight was recorded prior to and after completion of the DSS or normal water treatment cycle. The peak change in body weight was determined by calculating the maximum decrease in body weight from day 0 of DSS treatment (Fig 1). After eight days, mice were killed by cervical dislocation and the colons removed. Change in colon length, a marker of colonic inflammation in DSS colitis, was recorded.[12, 13] Pieces of distal colon were removed and fixed in 4% formalin, dehydrated through graded alcohols, and embedded in paraffin, and transverse sections were cut and stained with hematoxylin and eosin.[8] Stool consistency was assessed using an established scoring scale: 0 (normal), 2 (loose stool), and 4 (diarrhea).[44] H+E stained sections of distal colonic tissues were examined for epithelial damage, cellular infiltration, and immune infiltration of smooth muscle using light microscopy by an investigator who was blinded to the sample groups according to a previously reported scale.[45] Colonic epithelial damage was assigned scores as follows: 0 = normal; 1 = hyperproliferation, irregular crypts, and goblet cell loss; 2 = mild to moderate crypt loss (10–50%); 3 = severe crypt loss (50–90%); 4 = complete crypt loss, surface epithelium intact; 5 = small- to medium-sized ulcer (<10 crypt widths); 6 = large ulcer (≥10 crypt widths). Infiltration with inflammatory cells was assigned scores separately for mucosa (0 = normal, 1 = mild, 2 = modest, 3 = severe), submucosa (0 = normal, 1 = mild to modest, 2 = severe), and muscle/serosa (0 = normal, 1 = moderate to severe). The sum of these individual histology scores comprised a microscopic score for each colon with a total range of 0–12 where 0 = normal and 12 = maximally affected.

Colonic epithelial ion transport studies

A 3 cm segment of distal colon was stripped of muscle layers, and cut into smaller sections that were mounted on specially designed Ussing chamber inserts with a window area of 0.07 cm^2. Tissues were bathed in oxygenated Ringer's solution at 37°C, with a composition (in mM): 140 Na^+, 5.2 K^+, 1.2 Ca^{2+}, 0.8 Mg^{2+}, 120 Cl^-, 25 HCO_3^-, 2.4 $H_2PO_4^-$, 0.4 HPO_4^{2-}, and 10 glucose. The tissues were short-circuited by an automated voltage clamp and the short-circuit current (I_{sc}, expressed in $\mu A/cm^2$) across the tissues was monitored at intervals as an indication of net active ion transport. Tissues were allowed to equilibrate for a 20 min period, at which point baseline potential difference (PD), I_{sc} and tissue conductance were measured prior to administration of any reagents. Ion transport responses to the Ca^{2+}-dependent agonist, carbachol (CCh, 300 μM basolaterally), or the cAMP-dependent agonist, forskolin (20 μM, bilaterally), were measured as the change in short-circuit current (ΔI_{sc}). In some experiments, Peg-catalase (50, 100, 300 U/ml), or an equal volume of Ringer's solution, was administered bilaterally to tissues mounted in Ussing chambers for 30 min prior to treatment with secretagogues.

Western blotting

Isolated distal colonic tissues were stripped of muscle layers and placed in 0.5 mL of ice-cold lysis buffer (1% Triton X-100, 1 μg/ml leupeptin, 1 μg/ml pepstatin, 1 μg/ml antipain, 100 μg/ml phenylmethylsulfonyl fluoride, 1 mM Na^+-vanadate, 1 mM sodium fluoride and 1 mM EDTA in phosphate buffered saline). Tissues were homogenized using a Tissue-Tearor (Biospec Products, Bartlesville, OK), and samples were centrifuged at 12,000 rpm for 10

min and the pellet discarded. Samples were assayed for protein content (Bio-Rad protein assay kit) and adjusted so that each sample contained an equal amount of protein. A sample of lysate was mixed with an equal volume of 2× gel loading buffer (50 mM Tris, pH 6.8, 2% SDS, 200 mM dithiothreitol, 40% glycerol, 0.2 bromophenol blue) and boiled for 4 min prior to separation by SDS-polyacrylamide gel electrophoresis. Resolved proteins were transferred onto polyvinylidene membranes (NEN Life Science Products Inc., Boston, MA). After transfer, the membrane was pre-blocked with a 1% solution of blocking buffer (Upstate Biotechnology Inc.) for 30 min followed by a 1 h incubation with the appropriate concentration of primary antibody in 1% blocking buffer (mouse anti-NKCC1 (T4) – a gift from Dr. Christian Lytle (University of California, Riverside) and Dr. Bliss Forbush III (Yale University); rabbit anti-phospho-ERK (p44/42 MAPK)(Thr201/Tyr204) and rabbit anti-ERK (both from Cell Signaling Technology, Danvers, MA). After washing (5 × 10 min) in Tris-buffered saline with 1% Tween (TBST), membranes were incubated for 30 min in horseradish peroxidase-conjugated secondary antibody (anti-mouse or anti-rabbit IgG; Transduction Laboratories, Lexington, KY) in 1% blocking buffer. After washing in TBST (5 × 10 min), immunoreactive proteins were detected using an enhanced chemiluminescence detection kit (Roche Molecular Biochemicals, Indianapolis, IN). Densitometric analysis was performed using ImageJ software (NIH).

H_2O_2 assay

For measurement of H_2O_2 in mouse distal colon, excised tissues were rinsed in PBS, stripped of underlying muscle layers, cut into 1 cm^2 pieces and then placed in individual wells containing 400 μL of either oxygenated Ringer's solution or catalase (100 U/mL) in Ringer's. Tissues were incubated at 37°C to maintain viability. After 30 minutes, tissues were removed and washed in ice-cold Ringer's solution prior to lysis. Bathing media was collected from the individual wells. H_2O_2 release from the tissues into the bathing media was measured using a Bioxytech quantitative H_2O_2 assay, which detects oxidation of Fe^{2+} ions to Fe^{3+} by H_2O_2 and Fe^{3+} binding to an indicator dye, xylenol orange. This complex was detected colorimetrically at 560 nm.

Statistical analysis

Data are expressed as means ± standard error of the mean (S.E.M.) for n experiments. All comparisons between multiple treatment groups were performed by ANOVA followed by the Student-Newman-Keuls post-test. Comparisons between data from paired samples were performed using Student's t-test. All data were analyzed using GraphPad Instat version 3.0, with $p<0.05$ considered significant.

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Abbreviations used in this paper

cAMP	cyclic adenosine monophosphate
CCh	carbachol
DSS	dextran sulfate sodium
ERK	extracellular signal-regulated kinase
Fsk	forskolin
IBD	inflammatory bowel disease
NKCC1	sodium/potassium/2 chloride cotransporter 1

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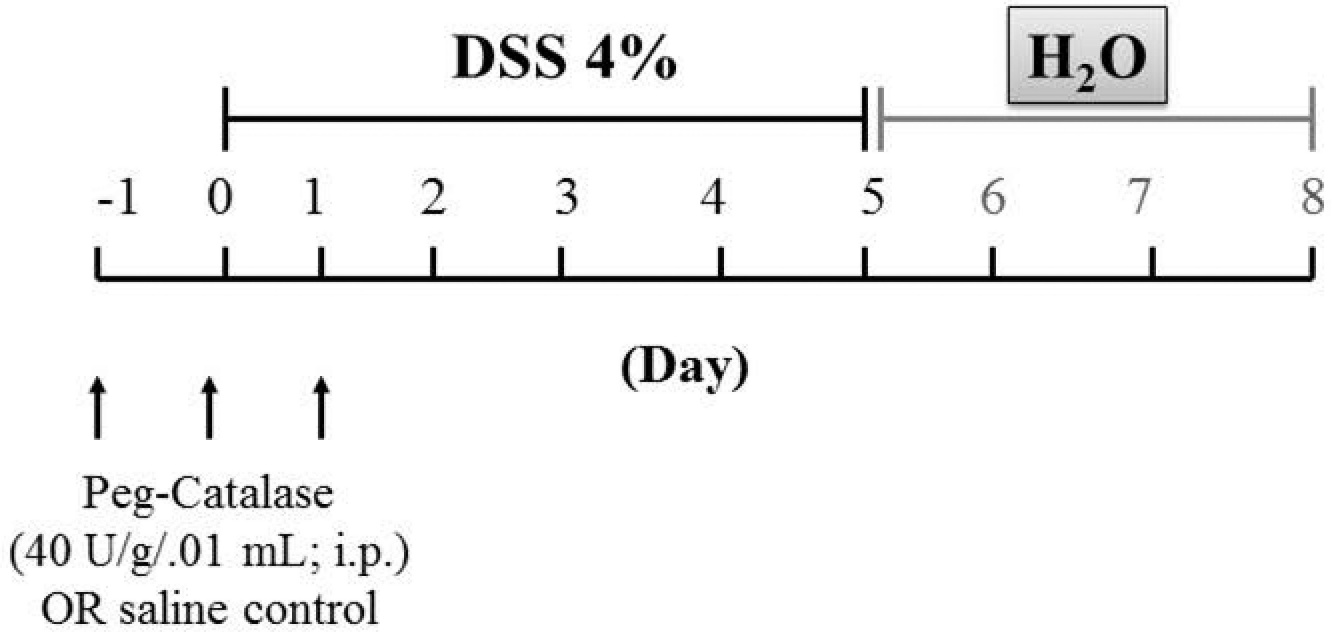

Fig. 1. Peg-catalase and DSS treatment protocol in mice

Colitis was induced in 8–10 week old male Balb/c mice by administering DSS (4%) in drinking water for 5 days followed by 3 days on normal H_2O. Paired mice (control and DSS) were administered either pegylated-catalase (40 units/g/0.01ml; i.p.) or an equal dose of saline (i.p.) at day −1, 0 and +1 of DSS treatment. Mice were sacrificed at Day 8 post-DSS treatment.

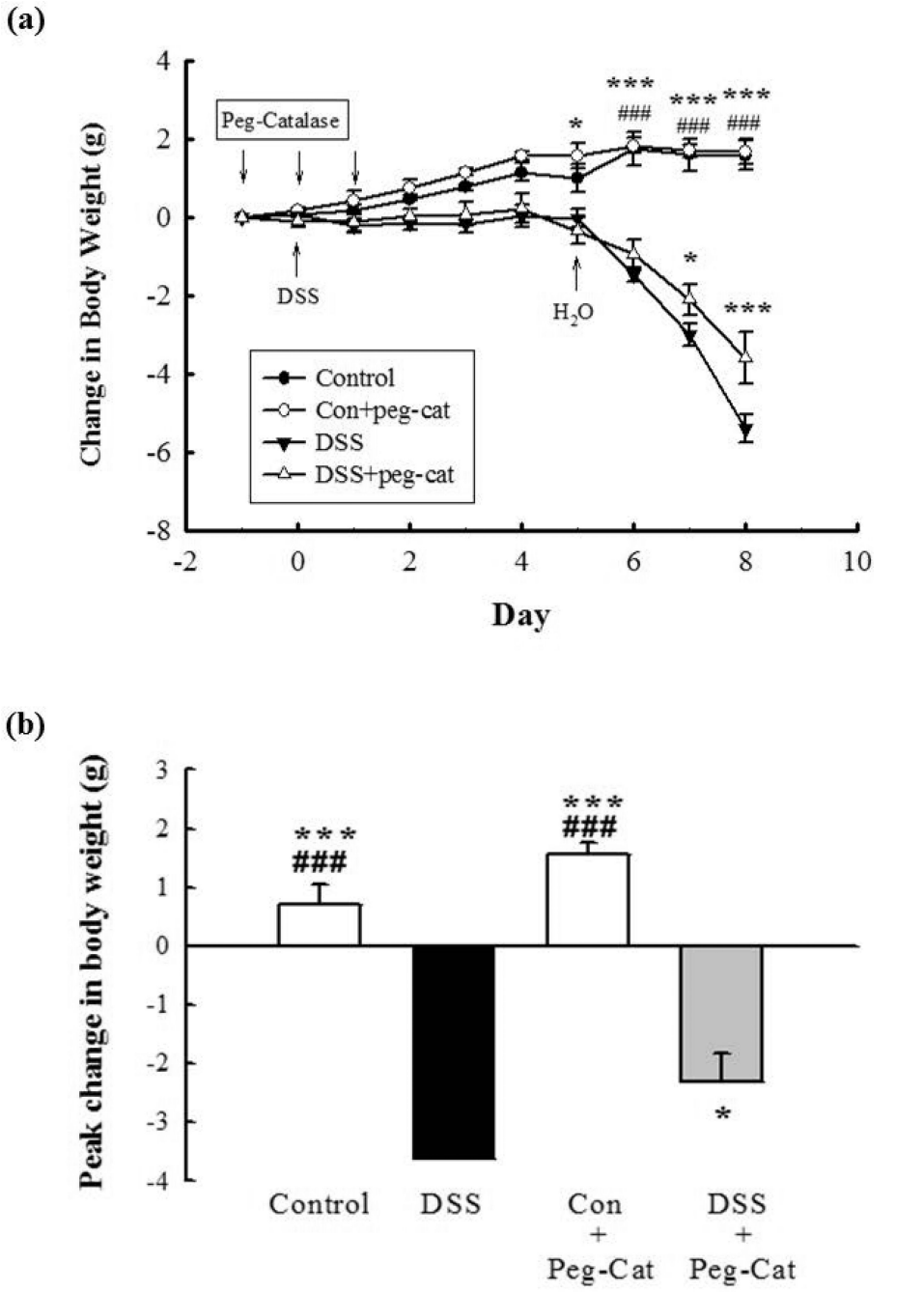

Fig. 2. Catalase pre-treatment partially protects against DSS-induced loss of body weight

Mice were weighed at 24hr intervals from initiation of Peg-catalase or saline (i.p.) with or without DSS treatment. Change in body weight (in grams) from Day −1 was calculated over the course of the treatment protocol and plotted as (a) the change over time, and (b) the peak change in body weight. *, $p<0.05$, ***, $p<0.001$ vs. DSS, ###, $p<0.001$ vs. DSS + Peg-catalase; n=7–12. DSS treatment caused a significant decrease in body weight that was only partially alleviated by Peg-catalase pre-treatment. Data are expressed as mean ± standard error (SEM) and analyzed by ANOVA and Student-Newman-Keuls post-hoc test.

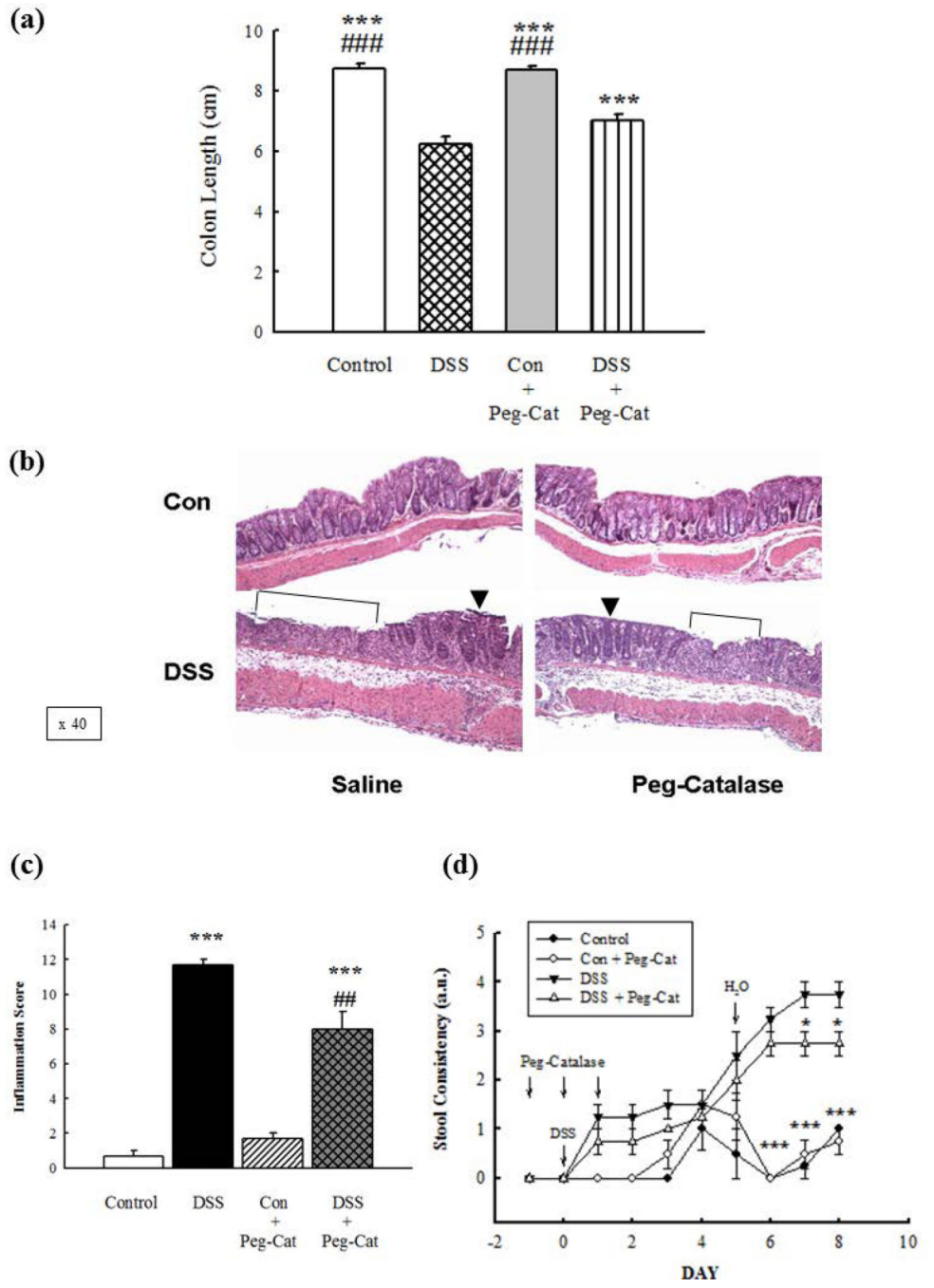

Fig. 3. Catalase partially reduces markers of DSS-induced inflammation

(a) Colon length was measured and expressed in cm. (b) H&E staining showed areas of immune cell infiltration, crypt deformation (marked by lines), and epithelial damage observed in DSS-treated mice with or without Peg-catalase. Areas of healthy tissue are marked by arrowheads. (c) Quantification of inflammation score (based on 0–12 scoring system) of H&E stained colonic tissues from each mouse group (n=3). (d) Change in stool consistency over the course of the experimental protocol (Day −1 to +8). &&&, $p<0.001$ vs. Control; *, $p<0.05$, **, $p<0.01$, ***, $p<0.001$ vs. DSS; ###, $p<0.001$ vs. DSS + Peg-catalase;

n=7–8. Data are expressed as mean ± standard error mean (SEM) and analyzed by ANOVA and Student-Newman-Keuls post-hoc test.

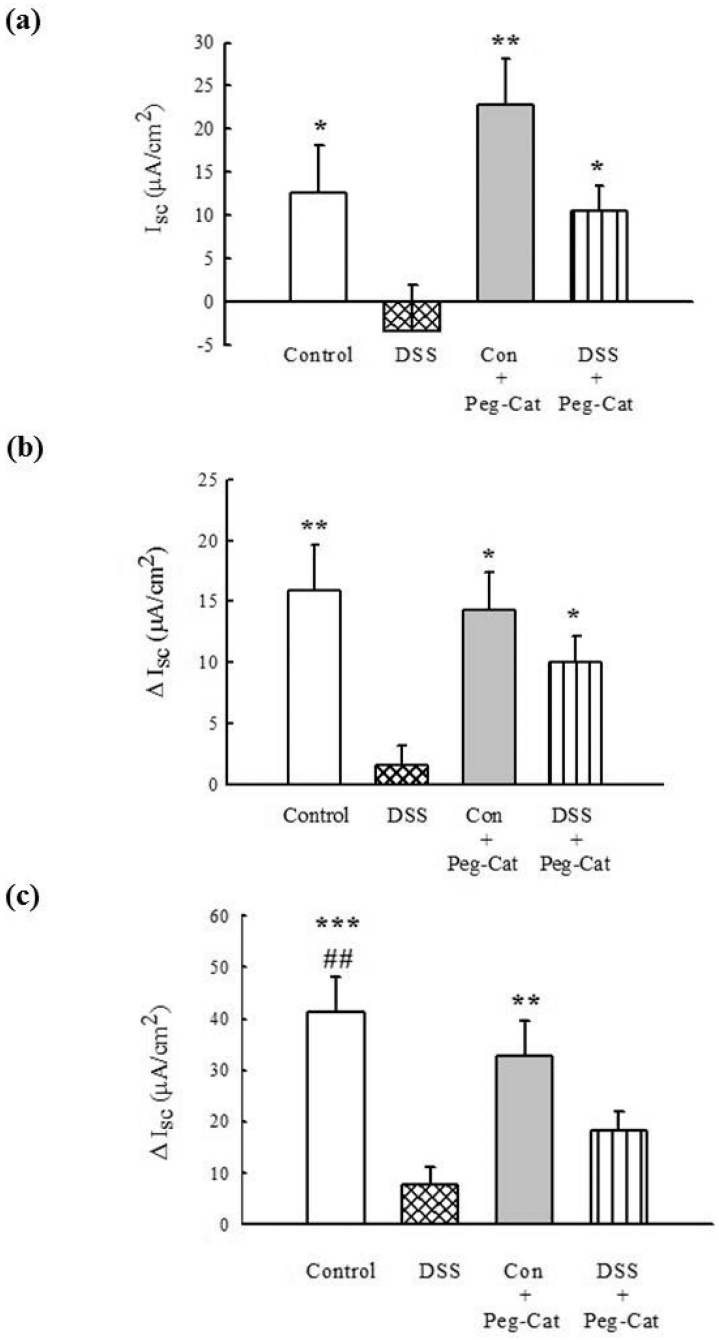

Fig. 4. Peg-catalase pre-treatment prevents the loss of baseline and Ca^{2+}-dependent, but not cAMP-dependent, ion transport responses in colonic tissues from mice treated with DSS

(a) Colonic tissues were stripped of underlying muscle layers and mounted in Ussing chambers. Tissues were allowed to equilibrate for 20 minutes prior to recording of basal I_{sc} (in μA/cm^2). (b) Carbachol (300 μM) was added to the serosal side of mounted tissues and the peak change in I_{sc} (ΔI_{sc}) was calculated. (c) Forskolin (20 μM) was added bilaterally and the peak change in I_{sc} (ΔI_{sc}) was calculated. (*, $p<0.05$, **, $p<0.01$, ***, $p<0.001$ vs. DSS, ##, $p<0.01$ vs. DSS + Peg-catalase; n=7–12). Data are expressed as mean ± standard error (SEM) and analyzed by ANOVA and Student-Newman-Keuls post-hoc test.

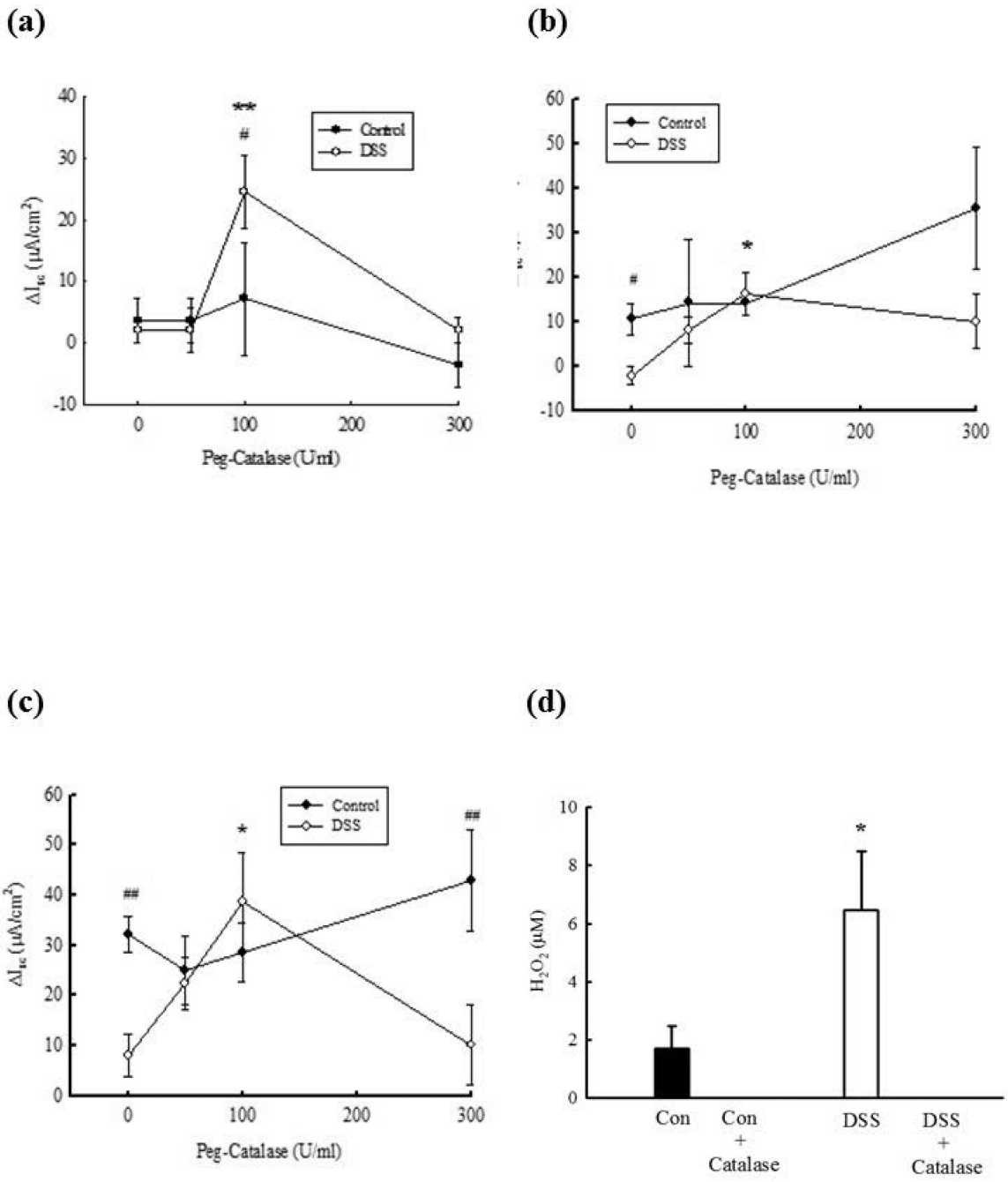

Fig. 5. Catalase treatment *ex vivo* rescues the loss of baseline, Ca^{2+}-dependent and cAMP-dependent ion transport responses in colonic tissues from mice with DSS colitis

(A) Colonic tissues from control and DSS-treated mice were stripped of underlying muscle layers and mounted in Ussing chambers. Tissues were allowed to equilibrate for 20 minutes prior to recording of basal I_{sc} (in $\mu A/cm^2$). Paired tissues from each mouse were then treated with a different dose of catalase (0, 50, 100, 300 U/mL) for 30 minutes and peak changes in I_{sc} (ΔI_{sc}) were determined. Tissues were then treated with (b) carbachol (300 μM) or (c) forskolin (20 μM) and the peak change in I_{sc} (ΔI_{sc}) was calculated. The asterisk (*) indicates a significant difference with 100 U/ml Peg-catalse treatment *vs.* 0 U/ml Peg-catalase within

the DSS condition, while the '#' indicates a significant difference between control *vs.* DSS tissues at the respective concentration of Peg-catalase. (d) Colonic tissues were stripped of underlying muscularis layers and incubated with Ringer's solution with or without catalase (100 U/mL) for 30 min. H_2O_2 release into the bathing media was assessed using a Bioxytech quantitative H_2O_2 assay (n=5). (*, $p<0.05$, **, $p<0.01$, vs. DSS + 0 U/ml catalase, #, $p<0.05$ ##, $p<0.01$ vs. DSS; n=7). Data are expressed as mean ± standard error (SEM) and analyzed by ANOVA and Student-Newman-Keuls post-hoc test, or paired Student's t-test where appropriate.

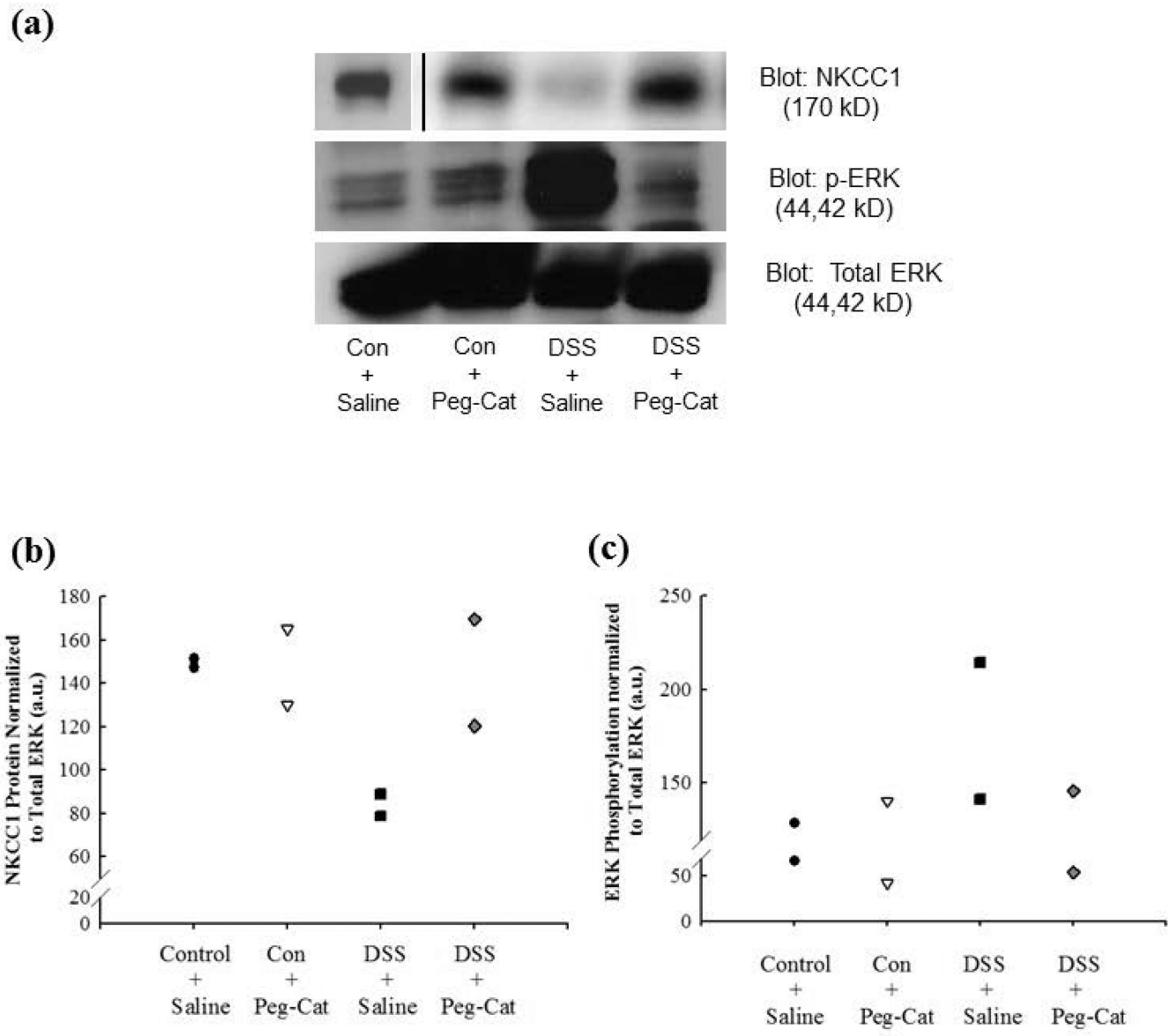

Fig. 6. Catalase reduces mucosal ERK activation and protects against loss of NKCC1 in DSS colitis

Colonic tissues from control and DSS-treated mice with or without Peg-catalase pre-treatment) were stripped of underlying muscle layers and proteins in mucosal lysates were resolved by SDS-PAGE. (a) Proteins were transferred to PVDF membranes and expression of NKCC1, p44/42 ERK, and phospho-p44/42 ERK (Thr202/Tyr204) was determined by Western blotting using appropriate antibodies. The black line indicates that the gel has been cropped at this position. Densitometric quantification of (b) NKCC1 and (c) phosphorylated ERK (p44/42) was performed and both proteins were normalized to total ERK protein levels for n=2 experiments.