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Inflammation Research

The adenosine A_{2A} receptor agonist CGS 21680 fails to ameliorate the course of dextran sulphate-induced colitis in mice

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Abstract. *Objective:* In this study we investigated the effect of CGS 21680 (2-*p*-(2-Carboxyethyl)phenethylamino-5 -N-ethylcarboxamidoadenosine hydrochloride), an adenosine A_{2A} receptor agonist, in a model of dextran sulphate sodium (DSS)-induced colitis.
Methods: NMRI mice were fed 5 % (w/v) DSS, and were treated intraperitoneally with 0.5 mg/kg CGS 21680 or vehicle for 10 days. Changes of bodyweight, colon length, the incidence of rectal bleeding, levels of macrophage inflammatory protein (MIP)-1α, MIP-2, interferon γ, interleukin (IL)-1β, IL-12 and tumour necrosis factor-α from homogenates of colon biopsies, and the release of [^{3}H]acetylcholine (ACh) from longitudinal muscle strip were determined.
Results: DSS significantly decreased bodyweight, colon length, and it increased the incidence of rectal bleeding and levels of MIP-1α, MIP-2 and IL-1β compared to DSS-untreated animals. CGS 21680 had no effect on these changes. No change could be observed in release of ACh in DSS-induced colitis with or without CGS 21680.
Conclusion: In summary, CGS 21680 is ineffective in ameliorating DSS-induced colitis in mice.

Key words: Dextran sulphate – Colitis – Adenosine A_{2A} receptor – CGS 21680

Introduction

Crohn's disease and ulcerative colitis, collectively termed inflammatory bowel disease (IBD), are characterised by chronic inflammation of the gastrointestinal tract with unknown etiology. The recent establishment of animal models for IBD has provided evidence that immune dysregulation and altered cytokine secretion patterns may play an important role in the pathology of IBD [1]. Some of the most widely used animal models of colitis comprise the administration of either toxic chemicals or immune reactive substances [2–6]. One of these animal models of IBD is colitis induced by the oral administration of dextran sulphate sodium (DSS) [7]. Mice with colitis induced by DSS exhibit lymphoid hyperplasia, inflammatory cell infiltration, focal crypt damage as well as epithelial injury and ulceration [4, 7, 8]. Although the murine DSS model of IBD differs from the human disease in some ways, it is a widely used preclinical model for testing the efficacy of treatments for IBDs [4, 9, 10].

It is well recognised that certain naturally occurring purines are effective modulators of the immune system. Among these purines adenosine is the best characterised, and has been shown to affect almost all aspects of an immune response [11–16]. Adenosine and its analogs can ameliorate the course of a variety of inflammatory diseases including endotoxin shock [17], rheumatoid arthritis [18], pleural inflammation [19], uveitis [20], but adenosine can lead to the exacerbation of inflammation and damage [21]. The beneficial effects of adenosine are partly mediated by the inhibition of deleterious immune-mediated processes, including the release of injurious proinflammatory cytokines and free radicals [22–28]. Adenosine, following its release from cells or after being formed extracellularly, diffuses to cell membrane of surrounding cells where it interacts with four receptors: A_1, A_{2A}, A_{2B} and A_3. [29, 30]. All of these receptors are members of the G protein-coupled family of receptors [29], and are expressed on the surface of macrophages and lymphocytes [31–36]. The most potent anti-inflammatory and immunosuppressive effects of adenosine are generally attributed to occupancy of A_{2A} receptors expressed on immune cells [16]. Because relatively little is known about the effect of A_{2A} receptor stimulation on the development of IBD, in this work we studied the therapeutic efficacy of A_{2A} receptor activation in DSS-induced colitis in mice.

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Materials and methods

Materials

Reagents were obtained from the following sources: dextran sulphate sodium salt (DSS; MW 36,000–50,000) was from ICN Pharmaceuticals (Costa Mesa, CA); CGS 21680 (2-*p*-(2-Carboxyethyl)phenethylamino-5 -N-ethylcarboxamidoadenosine hydrochloride), dimethyl sulfoxide (DMSO), NaF, NaCl, Na-deoxycholate, Na_3VO_4, Tris-HCl, NP-40, phenylmethanesulfonyl fluoride (PMSF), aprotinin, leupeptin, pepstatin were from Sigma (St. Louis, MO); NMRI mice were from TOXICOOP (Budapest, Hungary); and specific cytokine ELISA kits were from R&D Systems (Minneapolis, MN).

Induction of colitis and treatment

Male NMRI mice, 8 wk of age, weighing 25–27 g were used for these studies. Mice were kept in individual cages in the Animal Unit for 7 days before starting the experiments. Animals received food and water *ad libitum*, and lighting was maintained on a 12-h cycle. All procedures were carried out in accordance with the European Community guidelines for the use of experimental animals and those of the institutional ethics committee.

Mice were fed 5 % (w/v) DSS (molecular mass 36–50 kDa), dissolved in the drinking water, throughout the experiment [37]. CGS 21680 was administered intraperitoneally at a dose of 0.5 mg/kg/day throughout the entire study. Control mice were treated with vehicle which was 10 % (v/v) DMSO in saline.

Evaluation of colitis severity and drug effects

Parameters recorded in the experiments were bodyweight, colon length, and bleeding from the rectum as determined by ocular inspection. Mice were weighed on every day and colitis-induced weight change was expressed as a percentage of the original weight. Mice were killed by cervical dislocation, and the colon resected between the ileocecal junction and the proximal rectum, close to its passage under the pelvisternum. The colon was placed on a non-absorbent surface and its length measured with a ruler.

Cytokine production

A colon biopsy was removed and snap frozen in liquid nitrogen. The samples were then homogenised (100 mg/ml) in modified RIPA buffer containing 50 mM Tris-HCl pH 7.4, 1 % NP-40, 0.25 % Na-deoxycholate, 150 mM NaCl, 1 mM EDTA, 1 mM Na_3VO_4, 1 mM NaF, and protease inhibitors (1 mM PMSF, 1-1 µg/ml aprotinin, leupeptin and pepstatin). Samples were centrifuged at 15,000 × *g* for 15 min, and the supernatants frozen at −80 °C until assay. Cytokine levels were determined using ELISA.

[^{3}H]acethylcholine (ACh) release

Experiments were performed at 37 °C in modified Krebs' solution containing (mM) 113 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 25 $NaHCO_3$, and 11.5 D-glucose, which was continuously saturated with carbogen gas (95 % O_2 + 5 % CO_2). Longitudinal muscle strips were incubated for 45 min in 1 ml of modified and saturated Krebs' solution in the presence of 4 µCi/ml [^{3}H]ACh (82.0 Ci/mmol; Amersham Pharmacia Biotech, Arlington Heights, IL). After incubation the strips were transferred to thermoregulated (37 °C) organ baths (internal volume, 0.2 ml). The tissue was perfused at a rate of 1 ml/min. The perfusing solution contained 10 µM hemicholinium-3 to inhibit reuptake of [^{3}H]choline originating from hydrolysis of [^{3}H]ACh. After 60 min of preperfusion, the outflow was collected in 10-ml (10-min) fractions for an additional 60 min. Tissues were stimulated at 30 V, 5 min, 2 Hz, 2 msec (600 pulses), then 30 V, 1 min, 10 Hz, 2 msec (600 pulses), via platinum electrodes using a Grass S88 stimulator during the 3rd and 6th collection periods (S_1 and S_2). At the end of the perfusion period, the tissue was removed from the organ bath and homogenised in 500 µl of 10 % trichloroacetic acid. To determine radioactivity released from the tissue, aliquots (0.5 ml) of the perfusate samples were assayed, and a 100-µl aliquot was assayed for tissue radioactivity. Radioactivity was determined in a liquid scintillation counter (Packard 1900; Packard, Meriden, CT). The outflow of tritium was expressed as fractional release (FR), i.e., as the percentage of the amount of radioactivity in the tissue at the time of the release. To calculate electrical field stimulation-induced overflow, the mean of the basal release determined before and after the stimulation was subtracted from the total efflux of radioactivity from the tissue in response to electrical stimulation.

Statistical analysis

Results are presented as mean ±SD; statistical analysis was performed using one-way ANOVA followed by unequal N HSD post hoc analysis, or Fisher's exact test as appropriate, with a *P* value of <0.05 considered significant.

Results

Effect of CGS 21680 on the clinical signs of colitis

Treatment of NMRI mice with 5 % DSS in their drinking water for 10 days resulted in many clinical signs of colitis compared with mice receiving regular drinking water. At earlier stages, all mice, independently from the presence or

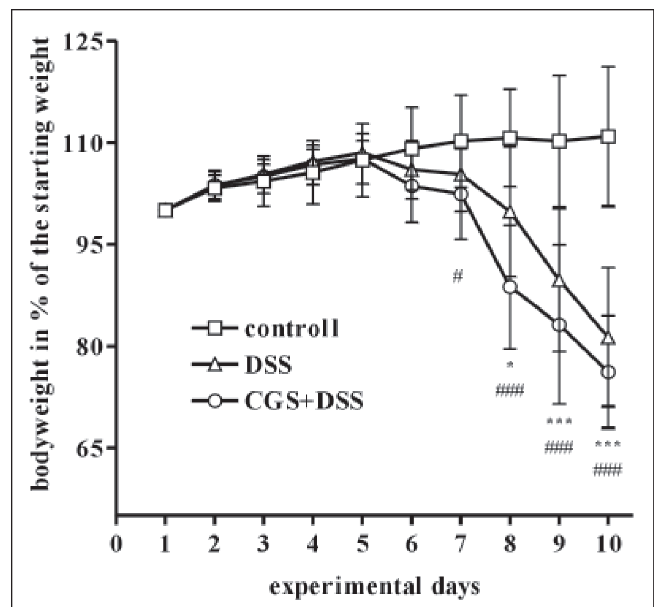

Fig. 1. DSS induces a progressive decrease in bodyweight both in the presence and in the absence of CGS 21680. Mice were exposed to DSS *ad libitum* for 10 days, treatment with CGS 21680 (0.5 mg/kg/day) started on *day 1*. Results are expressed as means ±SD from 8–14 mice. * $P < 0.05$, *** $P < 0.001$ when compared DSS-treated animals *vs.* control animals; # $P < 0.05$, ### $P < 0.001$ when compared CGS+DSS-treated animals *vs.* control animals.

absence of DSS in their drinking water, gained weight. After *day 8*, DSS-treated animals exhibited progressive weight loss (Fig. 1). Treatment with CGS 21680 starting on *day 1* could not reverse this weight loss; moreover, it slightly increased it (Fig. 1).

We next recorded the effect of CGS 21680 on colon length. DSS-treated mice had significantly shortened colons than those of control counterparts (3.62 ± 0.51 cm in DSS-treated mice *vs.* 5.96 ± 0.56 cm in control group, $P < 0.001$). CGS 21680 did not reverse this shortening effect of DSS on the colon length (3.62 ± 0.51 cm without CGS 21680 *vs.* 3.69 ± 0.47 cm with CGS 21680, $P > 0.05$; Fig. 2).

Rectal bleeding first appeared on *day 4* or *5*, and its incidence progressively increased during DSS-treatment. Although this increase in the incidence of rectal bleeding was moderated by CGS 21680 at earlier points (P = 0.0642 on *day 6*, P = 0.0516 on *day 7*, P = 0.049 on *day 8*), there was no difference in the incidence of rectal bleeding between CGS 21680-treated and untreated groups at the end of the experiment (P > 0.1 on *day 9 and 10*, Fig. 3).

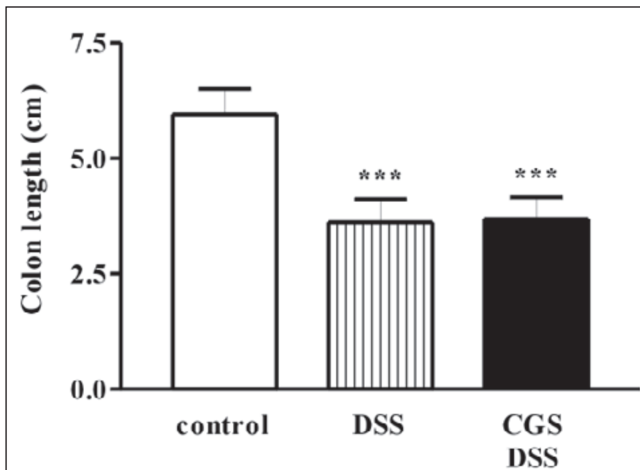

Fig. 2. CGS 21680 does not prevent shortening of colon length during DSS-induced colitis. Mice were exposed to DSS *ad libitum* for 10 days, treatment with CGS 21680 (0.5 mg/kg/day) started on *day 1*. Results are expressed as means ±SD from 9–12 mice. *** $P < 0.001$ *vs.* control.

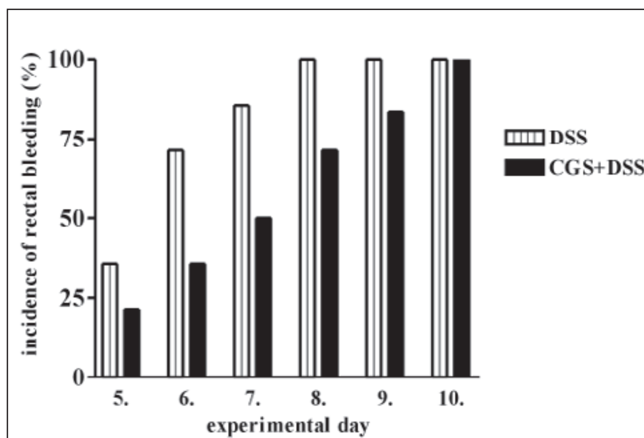

Fig. 3. The increase of the incidence of rectal bleeding is more moderate at early but not late stages of colitis in CGS 21680-treated animals. Mice were exposed to DSS *ad libitum* for 10 days, treatment with CGS 21680 (0.5 mg/kg/day) started on *day 1*. * $P < 0.05$.

Effect of CGS 21680 on chemokine and cytokine production in colon during DSS-induced colitis

Since the underlying mechanism by which colitis is induced involves increased production of chemokines and proinflammatory cytokines, we determined levels of the chemokines macrophage inflammatory protein (MIP)-1α and MIP-2, as well as four proinflammatory cytokines, which included interferon gamma (IFNγ), interleukin (IL)-1β, IL-12 and tumour necrosis factor-alpha (TNF-α). DSS-treated mice showed elevated levels of MIP-1α, MIP-2 and IL-1β (Fig. 4), while the concentration of IFNγ, IL-12 and TNF-α did not change in these animals compared to control ones (Table 1). CGS 21680 failed to decrease the DSS-induced elevations of MIP-1α, MIP-2 and IL-1β levels (Fig. 4). In addition, CGS 21680 did not have any effect on the levels of IFNγ, IL-12 and TNF-α (Table 1).

[^{3}H]ACh release from longitudinal muscle strip preparation of mice with colitis

As cholinergic mechanisms play an important role in the regulation of gastrointestinal motility, we studied the release of ACh, the major neurotransmitter of the enteric nervous system, from longitudinal muscle strip preparation of mice with colitis. No change could be observed in release of ACh in DSS-induced colitis both in the presence and in the absence of CGS 21680 (Table 2).

Table 1. Unchanged colonic production of IFNγ, IL-12p70, and TNF-α in DSS-induced colitis.

	Cytokines (pg/mg protein)		
	IFNγ	IL-12p70	TNF-α
control	1.749 ± 0.693	2.655 ± 1.243	0.947 ± 0.423
DSS*	1.492 ± 0.538	2.388 ± 0.501	1.291 ± 0.734
CGS**+DSS	2.123 ± 1.454	2.908 ± 0.893	1.375 ± 0.455

* 5 % (w/v)
** 0.5 mg/kg/day
Results are expressed as means ±SD from 9–12 mice.

Table 2. [^{3}H]ACh release from longitudinal muscle strip preparation of mice with colitis.

	FRS2/FRS1 ratio
control	0.94 ± 0.18
DSS*	0.95 ± 0.24
CGS**+DSS	0.98 ± 0.20

* 5 % (w/v)
** 0.5 mg/kg/day
Results are expressed as means ±SD from 3–4 samples.

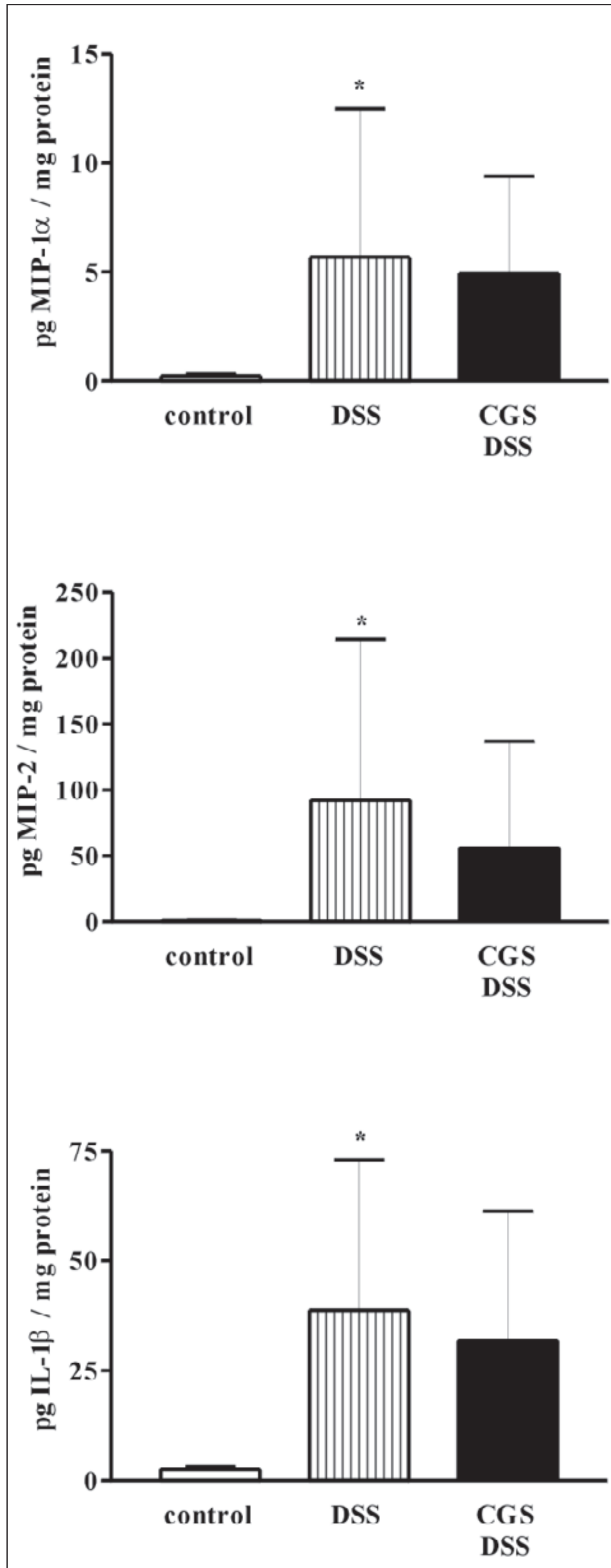

Fig. 4. Effect of CGS 21680 on colon MIP-1α (*A*), MIP-2 (*B*) and IL-1β (*C*) levels in colitis. Cytokine levels were determined in colon biopsies following treatment for 10 days with DSS + CGS 21680 (0.5 mg/kg/day) or DSS + vehicle. Results are expressed as means ±SD from 9–12 mice. * $P < 0.05$ *vs.* control.

Discussion

In this work, we investigated the effect of activation of adenosine A_{2A} receptors by the selective agonist CGS 21680 in DSS-induced colitis in mice. CGS 21680 failed to affect DSS-induced changes in bodyweight and colon length, incidence of rectal bleeding as well as the excessive production of MIP-1α, MIP-2, IL-1β, and the release of [^{3}H]ACh. Thus, despite promising results with A_{2A} agonists in other IBD models [38], A_{2A} receptor stimulation is not protective in the DSS model of colitis in mice.

CGS 21680 did not exacerbate weight loss in our DSS-treated mice. Chronic administration of CGS 21680 does not appear to have other toxic effects. For example, administering CGS 21680 to transgenic mice suffering from Huntigton's disease did not have any adverse effects and attenuated symptoms of the disease [39]. Also, repeated administration of CGS 21680 for 3 weeks was without toxic side effects in spontaneously hypertensive rats, and it normalized the blood pressure of the animals [40]. Finally, our results indicate that injection of CGS 21680 for 3 weeks to mice with streptozotocin-induced diabetes has no systemic toxic effects (data not shown).

Our finding of a lack of efficacy of A_{2A} receptor stimulation in DSS-induced colitis is somewhat unexpected, because adenosine A_{2A} receptors are generally considered to have a non-redundant role in the attenuation of inflammation and tissue damage [41]. Previous studies, including ours [42] and a study by Gomez and Sitkovsky [43] have shown that 0.5 mg/kg CGS 21680 effectively reduces inflammatory cytokine (TNF) production. The selectivity of the effect of CGS 21680 in decreasing TNF production was confirmed by using A_{2A} knockout animals, because CGS 21680 was no longer able to inhibit TNF production in these animals, whereas CGS 21680 decreased TNF production in wild-type mice [43]. Furthermore, Odashima et al. have recently demonstrated that the activation of A_{2A} adenosine receptors with ATL-146e, a novel selective A_{2A} receptor agonist [44], significantly reduces inflammation in the intestinal mucosa of SAMP/YitFc mice, a strain that develops disease spontaneously [38]. One obvious explanation of the differential effect of A_{2A} receptor stimulation in DSS-induced mice and SAMP/YitFc mice is the difference in the pathophysiology of the two models. While colitis in SAMP/YitFc mice appears to be dependent on T lymphocytes and the anti-inflammatory action of A_{2A} is targeted to this cell compartment [38], DSS-induced colitis is independent of lymphocytes, because DSS-induced colitis occurs in severe combined immunodeficient mice [45].

On the other hand, evidence exists that A_{2A} receptor stimulation with CGS 21680 does not moderate all aspects of inflammation, and its effect may depend on the tissue environment or the function studied. Support for this notion comes from a recent study in which we have demonstrated that CGS 21680 ameliorates shock-induced lung injury but fails to prevent gut injury [46]. In addition there is evidence that while A_{2A} receptor stimulation with CGS 21680 had no effect on the bronchoalveolar lavage fluid inflammatory cell influx or TNF-alpha, KC and MIP-2 levels, CGS 21680 potently inhibited neutrophil activation, as measured by bronchoalveolar lavage fluid elastase levels in a lipopolysaccharide-driven model of lung inflammation [47]. This observation indicates that CGS 21680 does not inactivate macrophage inflammato-

ry processes in every model *in vivo*. Since macrophages appear to be central to the pathogenesis of DSS-induced colitis [7, 48], the inefficacy of CGS 21680 to suppress macrophage activation *in vivo* may provide an explanation for its lack of protective effect in DSS-induced mice.

While A_{2A} receptor stimulation does not prevent colitis in DSS-induced mice, the A_3 receptor agonist IB-MECA (N^6-(3-iodo-benzyl)adenosine-5'-N-methyl-uronamide) is protective in this model [49]. In addition, we have shown previously that inosine, a naturally occurring anti-inflammatory purine [50–55] that can bind A_3 receptors [56], effectively suppressed the development of DSS-induced colitis in mice [57]. Inosine attenuated disease parameters, increased survival, and it reduced colon levels of chemokines (MIP-1α and MIP-2) and Th1 cytokines (IL-1, IL-6, IL-12, and TNF-α). These observations underscore the proposition that different adenosine receptors might be responsible for alleviating inflammation in different experimental models [16].

In turn, in a more recent study, A_{2A} receptors were shown to mediate inhibitory actions of endogenous adenosine on colonic motility in a rat model of experimental colitis induced by DNBS (2,4-dinitrobenzenesulfonic acid). In this model, CGS 21680 exerted inhibitory effects on electrical cholinergic contractions, acting with greater efficacy on colonic preparations from inflamed rats [58]. As intestinal motility is impaired in IBD patients, and intestinal dysmotility may result in abnormal growth of intestinal flora [59], which plays a role in the pathogenesis of IBDs [60], the inhibitory effect of CGS 21680 on intestinal motility may interfere with its anti-inflammatory effect. However, in our model, the release of acetylcholine was not affected by DSS-induced colitis either in the presence or in the absence of CGS 21680.

Our further interesting observation is that CGS 21680 can decrease the incidence of rectal bleeding at the early but not late stages. It is possible that the early inflammatory response is decreased by CGS 21680 thereby decreasing the incidence of rectal bleeding. It is not clear why CGS 21680 loses its efficacy later during the inflammatory process. One possibility is that A_{2A} receptor expression is down-regulated on inflammatory cells, as has been shown in both monocytes [36] and endothelial cells [61] that have been exposed to an inflammatory environment.

In conclusion, our results demonstrate that the adenosine A_{2A} receptor selective agonist CGS 21680 fails to ameliorate symptoms of DSS-induced colitis in NMRI mice. These observations indicate the necessity of further investigations to clarify the exact role of adenosine receptors in the regulation of the pathogenesis of IBDs.

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References

[1] Boismenu R, Chen Y. Insights from mouse models of colitis. J Leukoc Biol 2000; 67: 267–78.
[2] Kitajima S, Takuma S, Morimoto M. Histological analysis of murine colitis induced by dextran sulfate sodium of different molecular weights. Exp Anim 2000; 49: 9–15.
[3] Gaudio E, Taddei G, Vetuschi A, Sferra R, Frieri G, Ricciardi G et al. Dextran sulfate sodium (DSS) colitis in rats: clinical, structural, and ultrastructural aspects. Dig Dis Sci 1999; 44: 1458–75.
[4] Cooper HS, Murthy SN, Shah RS, Sedergran DJ. Clinicopathologic study of dextran sulfate sodium experimental murine colitis. Lab Invest 1993; 69: 238–49.
[5] Zea-Iriarte WL, Makiyama K, Goto S, Murase K, Urata Y, Sekine I et al. Impairment of antioxidants in colonic epithelial cells isolated from trinitrobenzene sulphonic acid-induced colitis rats. Protective effect of rebamipide. Scand J Gastroenterol 1996; 31: 985–92.
[6] Aube AC, Cherbut C, Barbier M, Xing JH, Roze C, Galmiche JP. Altered myoelectrical activity in noninflamed ileum of rats with colitis induced by trinitrobenzene sulphonic acid. Neurogastroenterol Motil 1999; 11: 55–62.
[7] Okayasu I, Hatakeyama S, Yamada M, Ohkusa T, Inagaki Y, Nakaya R. A novel method in the induction of reliable experimental acute and chronic ulcerative colitis in mice. Gastroenterology 1990; 98: 694–702.
[8] Dieleman LA, Palmen MJ, Akol H, Bloemena E, Pena AS, Meuwissen SG et al. Chronic experimental colitis induced by dextran sulphate sodium (DSS) is characterized by Th1 and Th2 cytokines. Clin Exp Immunol 1998; 114: 385–91.
[9] Elson CO, Sartor RB, Tennyson GS, Riddell RH. Experimental models of inflammatory bowel disease. Gastroenterology 1995; 109: 1344–67.
[10] Bennett CF, Kornbrust D, Henry S, Stecker K, Howard R, Cooper S et al. An ICAM-1 antisense oligonucleotide prevents and reverses dextran sulfate sodium-induced colitis in mice. J Pharmacol Exp Ther 1997; 280: 988–1000.
[11] Sipka S, Nagy K, Szegedi G. Adenosine induced delay of expression of AIDS virus, HIV, in H9T cells. Acta Biochim Biophys Hung 1988; 23: 75–82.
[12] Sipka S, Szentmiklosi AJ, Nagy A, Taskov V, Szegedi G. Inhibition of the zymosan-induced chemiluminescence of human phagocytes by adenosine, polyadenylic acid and agents influencing adenosine metabolism. Allergol et Immunopathol 1989; 17: 209–12.
[13] Apasov S, Koshiba M, Redegeld F, Sitkovsky MV. Role of extracellular ATP and P1 and P2 classes of purinergic receptors in T-cell development and cytotoxic T lymphocyte effector functions. Immunol Rev 1995; 146: 5–19.
[14] Cronstein BN. Adenosine, an endogenous anti-inflammatory agent. J Appl Physiol 1994; 76: 5–13.
[15] Hasko G, Szabo C. Regulation of cytokine and chemokine production by transmitters and co-transmitters of the autonomic nervous system. Biochem Pharmacol 1998; 56: 1079–87.
[16] Hasko G, Cronstein BN. Adenosine: an endogenous regulator of innate immunity. Trends Immunol 2004; 25: 33–9.
[17] Hasko G, Nemeth ZH, Vizi ES, Salzman AL, Szabo C. An agonist of adenosine A3 receptors decreases interleukin-12 and interferon-gamma production and prevents lethality in endotoxemic mice. Eur J Pharmacol 1998; 358: 261–8.
[18] Szabo C, Scott GS, Virag L, Egnaczyk G, Salzman AL, Shanley TP et al. Suppression of macrophage inflammatory protein (MIP)-1alpha production and collagen-induced arthritis by adenosine receptor agonists. Br J Pharmacol 1998; 125: 379–87.
[19] Schrier DJ, Lesch ME, Wright CD, Gilbertsen RB. The antiinflammatory effects of adenosine receptor agonists on the carrageenan-induced pleural inflammatory response in rats. J Immunol 1990; 145: 1874–9.
[20] Marak GE jr., de Kozak Y, Faure JP, Rao NA, Romero JL, Ward PA et al. Pharmacologic modulation of acute ocular inflammation. I. Adenosine. Ophthalmic Res 1988; 20: 220–6.
[21] Blackburn MR. Too much of a good thing: adenosine overload in adenosine-deaminase-deficient mice. Trends Pharmacol Sci 2003; 24: 66–70.
[22] Sipka S, Kovacs I, Szanto S, Szegedi G, Brugos L, Bruckner G et al. Adenosine inhibits the release of interleukin-1beta in activated human peripheral mononuclear cells. Cytokine 2005; 31: 258–63.
[23] Hasko G, Kuhel DG, Chen JF, Schwarzschild MA, Deitch EA, Mabley JG et al. Adenosine inhibits IL-12 and TNF-[alpha] pro-

duction via adenosine A2a receptor-dependent and independent mechanisms. FASEB J 2000; 14: 2065–74.
[24] Nemeth ZH, Lutz CS, Csoka B, Deitch EA, Leibovich SJ, Gause WC et al. Adenosine augments IL-10 production by macrophages through an A2B receptor-mediated posttranscriptional mechanism. J Immunol 2005; 175: 8260–70.
[25] Nemeth ZH, Leibovich SJ, Deitch EA, Sperlagh B, Virag L, Vizi ES et al. Adenosine stimulates CREB activation in macrophages via a p38 MAPK-mediated mechanism. Biochem Biophys Res Commun 2003; 312: 883–8.
[26] Pinhal-Enfield G, Ramanathan M, Hasko G, Vogel SN, Salzman AL, Boons GJ et al. An angiogenic switch in macrophages involving synergy between Toll-like receptors 2, 4, 7, and 9 and adenosine A(2A) receptors. Am J Pathol 2003; 163: 711–21.
[27] Nemeth ZH, Leibovich SJ, Deitch EA, Vizi ES, Szabo C, Hasko G. cDNA microarray analysis reveals a nuclear factor-kappaB-independent regulation of macrophage function by adenosine. J Pharmacol Exp Ther 2003; 306: 1042–9.
[28] Hasko G, Szabo C, Nemeth ZH, Kvetan V, Pastores SM, Vizi ES. Adenosine receptor agonists differentially regulate IL-10, TNF-alpha, and nitric oxide production in RAW 264.7 macrophages and in endotoxemic mice. J Immunol 1996; 157: 4634–40.
[29] Fredholm BB, AP IJ, Jacobson KA, Klotz KN, Linden J. International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors. Pharmacol Rev 2001; 53: 527–52.
[30] Ralevic V, Burnstock G. Receptors for purines and pyrimidines. Pharmacol Rev 1998; 50: 413–92.
[31] Sajjadi FG, Takabayashi K, Foster AC, Domingo RC, Firestein GS. Inhibition of TNF-alpha expression by adenosine: role of A3 adenosine receptors. J Immunol 1996; 156: 3435–42.
[32] Xaus J, Mirabet M, Lloberas J, Soler C, Lluis C, Franco R et al. IFN-gamma up-regulates the A2B adenosine receptor expression in macrophages: a mechanism of macrophage deactivation. J Immunol 1999; 162: 3607–14.
[33] Mirabet M, Herrera C, Cordero OJ, Mallol J, Lluis C, Franco R. Expression of A2B adenosine receptors in human lymphocytes: their role in T cell activation. J Cell Sci 1999; 112 (Pt 4): 491–502.
[34] Gessi S, Varani K, Merighi S, Ongini E, Borea PA. A(2A) adenosine receptors in human peripheral blood cells. Br J Pharmacol 2000; 129: 2–11.
[35] Gessi S, Varani K, Merighi S, Morelli A, Ferrari D, Leung E et al. Pharmacological and biochemical characterization of A3 adenosine receptors in Jurkat T cells. Br J Pharmacol 2001; 134: 116–26.
[36] Khoa ND, Montesinos MC, Reiss AB, Delano D, Awadallah N, Cronstein BN. Inflammatory cytokines regulate function and expression of adenosine A(2A) receptors in human monocytic THP-1 cells. J Immunol 2001; 167: 4026–32.
[37] Nemeth ZH, Deitch EA, Szabo C, Mabley JG, Pacher P, Fekete Z et al. Na+/H+ exchanger blockade inhibits enterocyte inflammatory response and protects against colitis. Am J Physiol Gastrointest Liver Physiol 2002; 283: G122–32.
[38] Odashima M, Bamias G, Rivera-Nieves J, Linden J, Nast CC, Moskaluk CA et al. Activation of A2A adenosine receptor attenuates intestinal inflammation in animal models of inflammatory bowel disease. Gastroenterology 2005; 129: 26–33.
[39] Chou SY, Lee YC, Chen HM, Chiang MC, Lai HL, Chang HH et al. CGS21680 attenuates symptoms of Huntington‘s disease in a transgenic mouse model. J Neurochem 2005; 93: 310–20.
[40] Casati C, Monopoli A, Dionisotti S, Zocchi C, Bonizzoni E, Ongini E. Repeated administration of selective adenosine A1 and A2 receptor agonists in the spontaneously hypertensive rat: tolerance develops to A1-mediated hemodynamic effects. J Pharmacol Exp Ther 1994; 268: 1506–11.
[41] Ohta A, Sitkovsky M. Role of G-protein-coupled adenosine receptors in downregulation of inflammation and protection from tissue damage. Nature 2001; 414: 916–20.
[42] Hasko G, Szabo C, Nemeth ZH, Kvetan V, Pastores SM, Vizi ES. Adenosine receptor agonists differentially regulate IL-10, TNF-alpha, and nitric oxide production in RAW 264.7 macrophages and in endotoxemic mice. J Immunol 1996; 157: 4634–40.
[43] Gomez G, Sitkovsky MV. Differential requirement for A2a and A3 adenosine receptors for the protective effect of inosine in vivo. Blood 2003; 102: 4472–8.
[44] McPherson JA, Barringhaus KG, Bishop GG, Sanders JM, Rieger JM, Hesselbacher SE et al. Adenosine A(2A) receptor stimulation reduces inflammation and neointimal growth in a murine carotid ligation model. Arterioscler Thromb Vasc Biol 2001; 21: 791–6.
[45] Dieleman LA, Ridwan BU, Tennyson GS, Beagley KW, Bucy RP, Elson CO. Dextran sulfate sodium-induced colitis occurs in severe combined immunodeficient mice. Gastroenterology 1994; 107: 1643–52.
[46] Hasko G, Xu DZ, Lu Q, Nemeth ZH, Jabush J, Berezina TL et al. Adenosine A2A receptor activation reduces lung injury in trauma/hemorrhagic shock. Critical care medicine 2006; 34: 1119–25.
[47] Bonneau O, Wyss D, Ferretti S, Blaydon C, Stevenson CS, Trifilieff A. Effect of adenosine A2A receptor activation in murine models of respiratory disorders. Am J Physiol Lung Cell Mol Physiol 2005.
[48] Ohkusa T, Okayasu I, Tokoi S, Araki A, Ozaki Y. Changes in bacterial phagocytosis of macrophages in experimental ulcerative colitis. Digestion 1995; 56: 159–64.
[49] Mabley J, Soriano F, Pacher P, Hasko G, Marton A, Wallace R et al. The adenosine A3 receptor agonist, N6-(3-iodobenzyl)-adenosine-5‘-N-methyluronamide, is protective in two murine models of colitis. Eur J Pharmacol 2003; 466: 323–9.
[50] Barankiewicz J, Cohen A. Purine nucleotide metabolism in resident and activated rat macrophages in vitro. Eur J Immunol 1985; 15: 627–31.
[51] Garcia Soriano F, Liaudet L, Marton A, Hasko G, Batista Lorigados C, Deitch EA et al. Inosine improves gut permeability and vascular reactivity in endotoxic shock. Crit Care Med 2001; 29: 703–8.
[52] Hasko G, Kuhel DG, Nemeth ZH, Mabley JG, Stachlewitz RF, Virag L et al. Inosine inhibits inflammatory cytokine production by a posttranscriptional mechanism and protects against endotoxin-induced shock. J Immunol 2000; 164: 1013–9.
[53] Liaudet L, Mabley JG, Soriano FG, Pacher P, Marton A, Hasko G et al. Inosine reduces systemic inflammation and improves survival in septic shock induced by cecal ligation and puncture. Am J Respir Crit Care Med 2001; 164: 1213–20.
[54] Liaudet L, Mabley JG, Pacher P, Virag L, Soriano FG, Marton A et al. Inosine exerts a broad range of antiinflammatory effects in a murine model of acute lung injury. Ann Surg 2002; 235: 568–78.
[55] Marton A, Pacher P, Murthy KG, Nemeth ZH, Hasko G, Szabo C. Anti-inflammatory effects of inosine in human monocytes, neutrophils and epithelial cells in vitro. Int J Mol Med 2001; 8: 617–21.
[56] Hasko G, Sitkovsky MV, Szabo C. Immunomodulatory and neuroprotective effects of inosine. Trends Pharmacol Sci 2004; 25: 152–7.
[57] Mabley JG, Pacher P, Liaudet L, Soriano FG, Hasko G, Marton A et al. Inosine reduces inflammation and improves survival in a murine model of colitis. Am J Physiol Gastrointest Liver Physiol 2003; 284: G138–44.
[58] Antonioli L, Fornai M, Colucci R, Ghisu N, Blandizzi C, Tacca MD. A2a receptors mediate inhibitory effects of adenosine on colonic motility in the presence of experimental colitis. Inflamm Bowel Dis 2006; 12: 117–22.
[59] Kinoshita K, Sato K, Hori M, Ozaki H, Karaki H. Decrease in activity of smooth muscle L-type Ca2+ channels and its reversal by NF-kappaB inhibitors in Crohn’s colitis model. Am J Physiol Gastrointest Liver Physiol 2003; 285: G483–93.
[60] Fiocchi C. Inflammatory bowel disease: etiology and pathogenesis. Gastroenterology 1998; 115: 182–205.
[61] Nguyen DK, Montesinos MC, Williams AJ, Kelly M, Cronstein BN. Th1 cytokines regulate adenosine receptors and their downstream signaling elements in human microvascular endothelial cells. J Immunol 2003; 171: 3991–8.