

Adenosine Receptor Agonists: Suppression of Macrophage Inflammatory Proteins and Collagen Production Causing Arthritis

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Research Article

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ABSTRACT

The production of seasoned and anti-inflammatory cytokines is modulated via ligands of numerous adenosine receptor subtypes. In this study, we looked at how adenosine and special ligands for the adenosine receptor subtypes (A1, A2 and A3) affected the production of the chemokine Macrophage Inflammatory Protein (MIP) 1a in immune stimulated RAW macrophages *in vitro*. In addition, we investigated whether or not an A3 adenosine receptor agonist reduces MIP-1a manufacturing and impacts the path of irritation in collagen-caused arthritis.

The A3 receptor agonist N6-(3-iodobenzyl)-adenosine-5'-N-methyluronamide (IB-MECA) and, much less potently, the A2 receptor agonist 2-p-(2-carboxyethyl)phenethylamino-5'-N-ethylamino-five'-N-ethylamino-5'-N-ethylamino-five'-N Adenosine turned into a mild inhibitor, and the selective A1 receptor agonist 2-chloro-N-cyclopentyladenosine (CCPA, 1 200 M) proved ineffective. The manufacturing of MIP-1a is inhibited by using suppressing its consistent-state mRNA levels from being associated with A3 and A2 agonists. Based on our *in vitro* results, we finish that stimulation of A3 and to a lesser volume A2 adenosine receptors reduces MIP-1a expression. We investigated whether or not IB-MECA, the maximum potent inhibitor of MIP-1a expression, also impacts the production of different seasoned-inflammatory mediators.

IB-MECA (1300 M) reduced nitric oxide manufacturing in immune stimulated cultured macrophages in a dose-established manner with IL-12, IL-, and to a lesser volume. Because MIP-a is a chemokine that increases neutrophil recruitment to infected sites, the A3 agonist IB-MECA alters the path of inflammation, MIP-a production, and stages of neutrophil recruitment in arthritis. I checked if it changed into. With IB-MECA (5 mg/kg/day) decreased the degree of joint infection in a mouse version of collagen-caused arthritis. IB-MECA suppressed neutrophil infiltration and suppressed the production of MIP¹a, IL-12, and nitrotyrosine (a marker of reactive nitrogen species) inside the foot. We finish that adenosine receptor agonists, specially the A3 agonist IB-MECA, inhibit MIP-a synthesis and feature anti-inflammatory consequences. Therefore, stimulation of adenosine receptor subtypes A3 and A2 can be a promising method for treating acute and continual inflammatory illnesses.

INTRODUCTION

Adenosine is a purine nucleoside generated by using some of cells in reaction to metabolic stress or Adenosine receptor ligands A1, A2a, A2b, and A3 were located to inhibit the synthesis of proinflammatory mediators, lessen the recruitment of Polymorph Nuclear Leukocytes (PMNs), and showcase anti-inflammatory houses Furthermore, the potential to launch adenosine is related to the anti-inflammatory activities of medicines such methotrexate, sulphasalazine, and adenosine kinase inhibitors Both A1 and A2 receptor agonists have been stated to decrease TNF-a manufacturing in RAW cells in current research. 24.7 macrophages or human monocytes, and the rank order of agonist efficiency changed into now not standard of both A1 or A2 receptors implying that the A3 receptor may be concerned. Furthermore, adenosine, however now not adenosine, In human monocytes, selective A1 and A2 receptor agonists extended IL-10 manufacturing It was further confirmed that suppression of TNF-a technology by means of LPS-inspired U937 (human monocyte) cells was ordinarily an A3 receptor-mediated mechanism using particular A1, A2, and A3 receptor agonists and antagonists In the murine J774.1 macrophage cellular line, ADO receptor agonists prevented endotoxin activation of the TNF-a gene and protein expression in a dose-based way much like the A3 receptor. Although the above research showed that several adenosine receptor agonists can affect the manufacturing of pro and anti-inflammatory cytokines no facts on the change of the manufacturing of chemokines through adenosine receptor ligands turned into formerly published. MIP¹a (macrophage inflammatory protein) is a low-molecular-weight CC chemokine that has great inflammatory results. The enhancement of neutrophil recruitment is the key mechanism into inflammatory web sites ^[1-5].

We investigated whether or not adenosine receptor ligands affect the production of MIP¹a protein and mRNA in macrophages because MIP¹a is a critical mediator of acute inflammation and adenosine receptor ligands can exert robust anti-inflammatory results. We investigated whether the A3 agonist IB-MECA impacts MIP¹a manufacturing, the route of irritation, and neutrophil recruitment in a model of collagen-brought about arthritis after finding that adenosine receptor agonists, most drastically the A3 agonist, suppress MIP¹a expression.

MATERIALS AND METHODS

Adenosine agonists have an effect on MIP-Los Angeles manufacturing in RAN 264.3 cells

The mouse macrophage mobile line RAW 24.7 changed into grown. 1 mL of one mL of one mL of 1 mL of 1300 mM adenosine, the A1 receptor agonist 2-chloro-N-cyclopentyladenosine (CCPA), the A2 receptor agonist 2-p-(2-carboxyethyl) phenethylamino-five'-N-ethyl-carboxamidoadenosine (CGS-2180), and the A3 receptor agonist N-(three-iodobenzyl)-adeno. Research Biochemical Inc. furnished the adenosine agonists. After 30 minutes, cells were stimulated with LPS and supernatants or cells were gathered 1-3 hours later for MIP¹a protein or mRNA assays.

The effect of a 1 h pretreatment with the adenosine deaminase inhibitor erythro-nine-(2-hydroxy-three-nonyl) adenine (EHNA, 50 M) at the inhibitory effect of adenosine on MIP-1a production became investigated in a second set of checks. MIP-1a is a protein that was observed in the human frame. As previously described, the usage of murine ELISA kits offered from Genzyme) (detection restriction: 1.5 pg/ml) the test become not hampered by means of any check chemical substances [6,7].

Adenosine agonists' results on MIP-Los Angeles consistent-country mRNA stages

A guanidinium isothiocyanate/chloroform-based totally method (TRIZOL) was used to extract overall RNA from every nicely, followed with the aid of isopropanol precipitation. On a 1% formaldehyde gel, cytoplasmic RNA (15 g) become separated and transferred to a nylon membrane. The rat MIP-1a cDNA collection is 92% same to the mouse collection, indicating move-hybridization between the 2 species by random priming, this cDNA become radiolabeled with 32P-dCTP (unique hobby, 3,000). The radioactivity of probes became measured. The Northern blot was hybridised at 42°C for 1 hour after scintillation counting and 1.5107 c.P.M. have been applied. The hybridised filters have been washed serially at 53°C with a solution of 2 sodium citrate, sodium chloride, and zero.1 percent SDS (2SSC). Autoradiography became done the usage of Hodak-OMAT-AR movie at -70°C. Membranes have been stripped with boiling 5 mM EDTA and rehybridized with a 32P-radiolabeled oligonucleotide probe for 18S ribosomal RNA after probing for MIP-1a. In RAN 264. Three cells, the impact of IB-MECA on cytokine and NO manufacturing.

In the example of the A3 agonist IB-MECA, we desired to peer if it affected the production of IL-12, IL-, and NO in RAW 24.7 macrophages as well. The cells were pretreated with 1 three hundred M IB-MECA in those tests after half-hour, cells have been inspired with either LPS (10 g/ml⁻¹) or murine IFN (200 uml⁻¹; Genzyme, Boston, MA, USA). This LPS concentration is higher than that hired in investigations on the consequences of adenosine receptor agonists on MIP-1a generation. Our early studies have shown that more LPS concentrations are required to reach optimum NO technology degrees. Only the aggregate of a more dose of LPS and IFN-ended in detectable IL-12 manufacturing on this cellular type. After a 24-hour incubation length at 37°C, the lifestyle supernatant fluids were accumulated and stored at -70°C. ELISA kits unique for murine cytokines have been used to determine cytokines. ELISA kits from Genzyme had been used to quantify IL-12 (p40 and p70) and IL-a Spectramax 250 microplate reader was used to study the plates at 450 nm. IL-12 (p40) had detection limits of 10 pg ml⁻¹, IL-12 (p70) had detection limits of five pgml⁻¹, and IL-had detection limits of five pg ml⁻¹. Assays were executed as formerly described and mg/ml by using stirring at 4°C overnight. CII became dissolved and saved at -70°C until wished. The addition of *Mycobacterium tubeuculosis* H37Ra at a dose of 2 mg ml⁻¹ yielded Complete Freund's adjuvant (CFA). CII became emulsified with a same volume of CFA before injection. As formerly defined collagen-brought on arthritis became developed [8,9]. On day 1, mice were given a hundred I of the emulsion (containing 100 g CII) intradermally at the base of the tail. A second injection of CII in CFA was given on day 21.

Animals were given IB-MECA at a dose of 0.5 mg kg⁻¹ to stimulate A3 adenosine receptors. This dose was selected based totally on previous *in vivo* investigations. Starting on day 18, animals have been given both automobile (n=18) or IB-MECA (n=18; 0.5 mg kg⁻¹, i.P.) each 24 hours. The mice were checked for arthritis on an everyday basis. Employing a macroscopic scoring device that tiers from 0 to four (zero=no indications of arthritis, 1=swelling and/or redness of the paw or one digit, 2=joints involved, 3=more than two joints involved, and 4=excessive arthritis of the entire paw and digits). Each mouse's arthritic index was computed by way of adding the four precise

paw rankings. No animals were removed from the calculations, and severity indices had been decided for complete businesses of mice (car-treated or IB-MECA-dealt with) [10-19].

Nitrotyrosine immunohistochemistry and histology

Animals had been sacrificed underneath anaesthesia at the cease of the experiments (Day 35), and their paws and knees were taken and preserved for histological analysis by way of an investigator who changed into blinded to the treatment regimen. Joints have been embedded for nitrotyrosine immunohistochemistry. Snap frozen in liquid nitrogen in M1 medium. A microtome geared up with a carbide metallic knife was used to reduce cryostat pieces (m). By immunohistochemistry, joint slices were tested for the presence of nitrotyrosine, a peroxy-nitrite indicator for 15 mins, endogenous peroxidase become quenched with 0.3% H₂O₂ in PBS by incubating the slice in 2% normal goat serum in phosphate buffered saline for zero mins, non-specific adsorption became decreased. After that, the sections have been dealt with with a 1:500 dilution of number one anti-nitrotyrosine antibody overnight a biotin-conjugated goat anti-rabbit IgG and an avidin-biotin peroxidase complicated have been used to identify particular labelling. Anti-nitrotyrosine antibodies had been used to incubate sections on top of things studies. In the presence of 10 mM nitrotyrosine, antibody turned into produced. The nitrotyrosine staining within the figures turned into eliminated due to this intervention.

In paw extracts, cytokines, chemokines, and nitrotyrosine have been detected

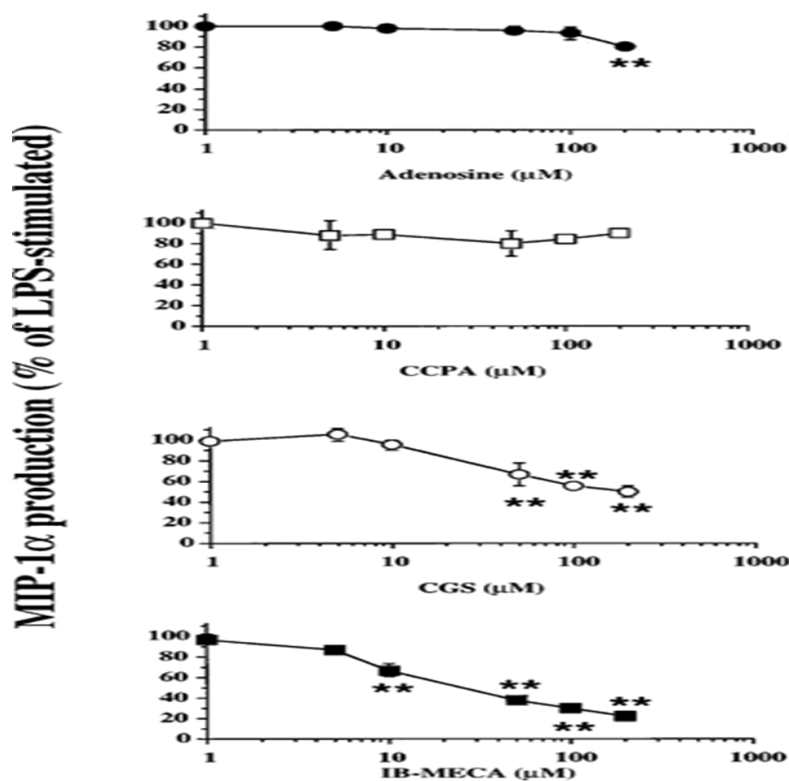
Aqueous joint extracts had been prepared from control animals and animals with 35 days of arthritis as described with the aid of homogenization in a lysis buffer within the presence of an aggregate of protease inhibitors (10 gml⁻¹ aprotinin, 20 gml⁻¹ leupeptin, 10 gml⁻¹ pepstatin A, and 1 mM PMSF, pH-7.25) ELISA) turned into used to determine the presence of IL-12, TNF- α , and MIP-1 α in the extracts, in addition to the presence of nitrated proteins the use of Western blotting Each pattern weighed thirty gramme heated to 95 °C for 3 minutes after being diluted in an equal quantity of treatment buffer [11,12]. After that, the samples had been positioned into a 1% Tris-Glycine answer. Gels had been operated for 2 hours at one hundred twenty volts, and then transferred to 0.45 m nitrocellulose at 30 volts for 0 minutes using the 1/2 Towbin buffer machine (1.45 g Tris, 7.2 g glycine, 800 ml di H₂O and 200 ml MeOH). The membrane became blocked in 1 percentage BSA: 1 percent nonfat milk in PBS-Tween (phosphate-buffered saline with 0.05% Tween 20) for 1 hour before being probed with rabbit anti-nitrotyrosine (1 g/ml in PBS-Tween) overnight at 4 °C. The blot was washed 3 instances with PBS-T and as soon as with H₂O before being incubated with the secondary antibody, goat anti-rabbit-HRP, for 1.5 hours (1:3000). After washing the blot 3 times in PBS-T and once in dil H₂O, 1.5 ml blended ECL chemiluminescence reagent changed into carried out for 1 minute. After that, the blot turned into uncovered to X-ray movie for zero seconds.

Measurements of myeloperoxidase in paw homogenates

In addition, the activity of myeloperoxidase, a marker of neutrophil infiltration, was evaluated within the paws as defined Paws had been homogenized in an answer of 0.5% hexa-decyl-trimethyl-ammonium bromide mixed in 10 mM potassium phosphate buffer (pH-7) and centrifuged at 20,000 g for half-hour at 4 °C. An aliquot of the supernatant was allowed to react with tetra-methyl-benzidine (1 mM) and 0.1 mM H₂O₂ in a solution. At a wavelength of 50 nm, spectrophotometry became used to decide the rate of trade in absorbance [13,14]. Myeloperoxidase the quantity of enzyme that degraded 1 mol of hydrogen peroxide in step with minute at 37 °C turned into measured in milliunits per mg protein (Figure 1).

Figure 1. Effect of Adenosine (ADO), the A1 agonist CCPA, the A2 agonist CGS, and the A3 agonist IB-MECA on the production of MIP-1 α at 3 h after stimulation with bacterial lipopolysaccharide LPS (10 ngml⁻¹) in RAW macrophages. MIP-1 α production in the absence of the inhibitors amounted to 6.6 ng ml⁻¹ \pm 1.3 ng ml⁻¹, and was considered 100%.

Note: n=6-9 wells from two to three independent experiments, *P<0.05 and **P<0.01 indicate significant inhibition of the production of MIP-1 α .



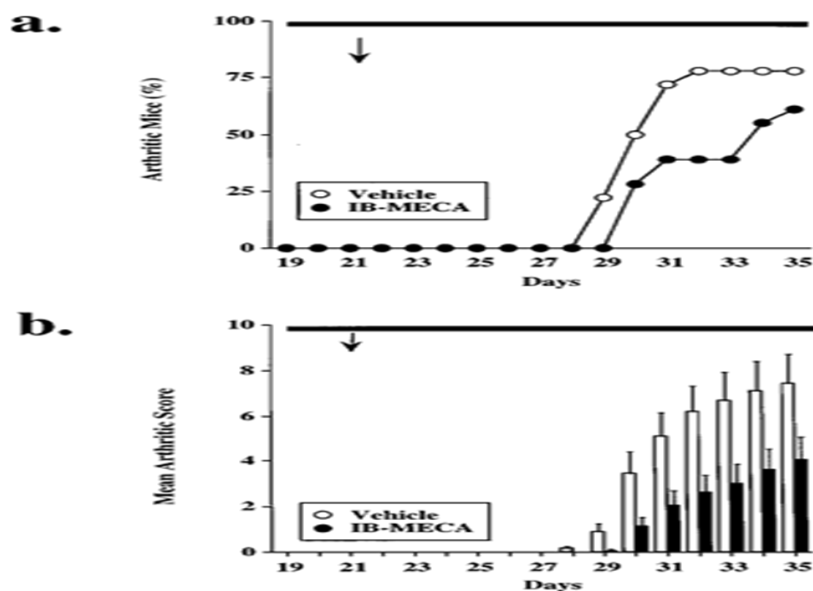
Analyzing and offering facts

All figures and textual content records are expressed because the suggest of n observations, in which n is the wide variety of wells (9 wells from two to a few impartial experiments) or the quantity of animals analyzed. One and two manner analysis of variance was used to analyze the statistics sets, and man or woman group averages have been as compared using Dunnett's check. The statistical variations within the arthritic indices have been tested the usage of the Mann-Whitney U-check (2-tailed, impartial) inside the arthritis research. Statistical importance changed into described as a P-value of less than 0.05. (a) IB-MECA suppresses collagen-induced arthritis in mice. The percentage of arthritic mice (mice showing clinical scores of arthritis >1) are represented. (b) Effect of IB-MECA on the severity of collagen-induced arthritis. Median arthritic score during collagen-induced arthritis. n=10-12. There was a significant increase in the arthritic score from day 28 (*P<0.01), and there was a significant suppression of the arthritic score by IB-MECA from day 30 (ϕ P<0.05). (a) IB-MECA suppresses collagen-induced arthritis in mice. The percentage of arthritic mice (mice showing clinical scores of arthritis >1) are represented. (b) Effect of IB-MECA on the severity of collagen-induced arthritis. Median arthritic score during collagen-induced

arthritis. n=10-12. There was a significant increase in the arthritic score from day 28 (*P<0.01), and there was a significant suppression of the arthritic score by IB-MECA from day 30 (ϕ P<0.05) (Figure 2).

Figure 2. IB-MECA suppresses collagen-induced arthritis in mice. (a) The percentage of arthritic mice (mice showing clinical scores of arthritis >1) are represented. (b) Effect of IB-MECA on the severity of collagen-induced arthritis. Median arthritic score during collagen-induced arthritis. n=10-12.

Note: There was a significant increase in the arthritic score from day 28 (*P<0.01), and there was a significant suppression of the arthritic score by IB-MECA from day 30 (ϕ P<0.05).



RESULTS

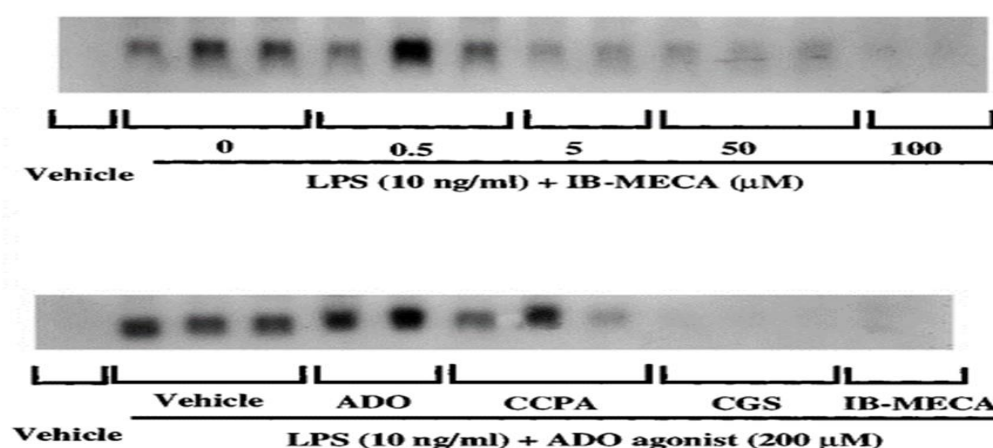
MIP-1a is suppressed via adenosine agonists in immune stimulated macrophages. MIP-1a turned into produced in significant quantities in reaction to LPS (10 ng/ml). The A3 agonist IB-MECA and, to a lesser volume, the A2 agonist CGS-2180 inhibited MIP-1a manufacturing, while the A1 receptor agonist CCPA had no effect. At two hundred M, adenosine had a minor inhibitory impact, ensuing in a 21.3 percent inhibition. The MTT test discovered that the adenosine agonists examined had no effect on mitochondrial respiratory.

Because adenosine deaminase degrades adenosine quickly, we wanted to see if inhibiting adenosine deaminase modified the degree of inhibition. There changed into no boom inside the inhibitory action of adenosine in the presence of the adenosine deaminase inhibitor EHNA (50 M) 24.5% inhibition at 200 M MIP-1a constant-kingdom mRNA levels upward push in a time-dependent way IB-MECA brought about a considerable discount in MIP-1a production, which turned into observed by means of a sizable, dose-structured reduction in MIP-1a mRNA. MIP-1a mRNA expression changed into unaffected by way of adenosine or the A1 agonist however steady state mRNA degrees have been reduced through the A2 agonist CGS-2180. Overall, the degree of inhibition of MIP-1a protein and MIP-1a mRNA expression by adenosine agonists had a fairly excessive affiliation. The diploma of suppression of MIP-1a mRNA ranges and protein stages, alternatively, did now not usually in shape. Using the ELISA approach, we determined that CGS (at 200 M) suppressed MIP-1a protein synthesis by kind of 50% whiles the inhibition of MIP-1a protein production through almost 50% [20-25].

LPS elicited the synthesis of mRNA became without a doubt completely removed differences in measurement length (protein become tested three hours after LPS, mRNA 2 hours after LPS) and/or sensitivity of the two assays. These disparities can be because of the methods utilised (ELISA as opposed to Northern blotting) (Figure 3).

Figure 3. Effect of Adenosine (ADO), the A1 agonist CCPA, the A2 agonist CGS, and the A3 agonist IB-MECA on the production of MIP-1a mRNA in response to stimulation with bacterial lipopoly-saccharide LPS (10 ng ml⁻¹) in RAW macrophages. (a) Dose-response showing the effect of 0.5, 5, 50 and 100 μM IB-MECA on the production of MIP-1a mRNA at 3 h after stimulation. (b) Effect of adenosine, CCPA, CGS and IB-MECA (200 μM each) on the production of MIP-1a mRNA at 3 h after stimulation.

Note: Representative blots of n=3-4 blots are shown; 18S mRNA was unaffected by any of the treatments.



IB-MECA inhibited MIP-1a production the most strongly of all the adenosine agonists tested

As an end result, we examined the impact of this agonist on the technology of different inflammatory mediators (IL-12, IL-, and NO) by means of activated macrophages and in an *in vivo* model of inflammation inside the following experiments. LPS (10 g ml⁻¹) or IFN (2 hundred u ml⁻¹) did not elicit measurable ranges of IL-12 (p40 or p70) in RAW 24.7 macrophages after a 24-hour remedy even though the aggregate of LPS (10 g ml⁻¹) and IFN (2 hundred u ml⁻¹) promoted the manufacturing of IL-12 p40 (Figure 4a), IL-12 p70 was no longer generated in measurable quantities.

Pretreatment of cells with IB-MECA half-hour before LPS + IFN-led to a concentration-dependent reduction of IL-12 p40 manufacturing, as measured at 24 hours (Figure 4a). As determined 24 hours after stimulation, LPS (10 g ml⁻¹) inspired the release of IL and nitrite (the breakdown fabricated from NO) inside the subculture supernatants of RAW 24.7 cells. IB-MECA, administered 30 minutes earlier than LPS, decreased the generation of IL and nitrite (Figure 4b and 4c). In all of those trials, IB-MECA had no effect on cell viability as assessed *via* the MTT assay (now not proven). IB-stronger MECA's inhibition of IL-12 and MIP-1a and much less discount of NO and IL manufacturing could be due to two awesome A3 receptor subtypes. The current research, however, IB-MECA therapy protects mice from collagen-brought about arthritis:

The majority of the car-treated mice advanced arthritis among days 2 and 35 after the primary collagen immunization treatment with IB-MECA (0.5 mg/kg once day, IP) decreased the occurrence of arthritis and reduced the sickness's severity. The paws of the car-handled arthritic animals confirmed proof of excessive suppurative

arthritis at day 35, with huge neutrophil, macrophage, and lymphocyte infiltration. In addition, there was extreme or mild necrosis, hyperplasia, and sloughing of the synovium, as well as inflammation spreading into the neighbouring musculature. The diploma of arthritis changed into dramatically decreased inside the IB-MECA dealt with mice, with slight cell infiltration, moderate to mild necrosis, and synovial hyperplasia. The reduction in neutrophil infiltration become measured with the aid of paw myeloperoxidase [26]. Arthritis precipitated a 991 mU/mg protein growth in myeloperoxidase levels (n=12). There becomes a massive growth in the wide variety of IB-MECA-treated animals. At the cease of arthritis, there has been a substantially decreased degree of upward push within the paw myeloperoxidase concentration (3 mU/mg protein; n=12, P0.01) [27-30].

At 35 days, there was a huge growth in MIP-1a and IL-12 p40 tiers within the aqueous extracts of the arthritic paws, but no detectable TNF-a or IL-12 p70 degrees in the extracts. IB-MECA therapy reduced the formation of LPS-stimulated cells or LPS-challenged animals, in line with the results in LPS-inspired cells or LPS-challenged animals. Increased levels of IL-12 p40 and MIP-1a within the joints. We detected the emergence of nitrotyrosine-nice staining in the inflamed joints using immunohistochemistry and Western blotting of proteins in aqueous joint extracts, however no longer in healthful manipulate animals. The degree of nitro tyrosine staining becomes (Figures 4 and 5).

Figure 4. IB-MECA suppresses the production of inflammatory mediators in immunostimulated cultured RAW macrophages. Cells were stimulated with LPS (10 µg/ml⁻¹) and IFN- α (200 mU/ml) for 24 h for the measurement of IL-12 (p40), with LPS (10 µgml⁻¹) for 24 h for IL-6 and nitrite measurements, or with LPS (10 ng/ml) and IFN- α (200 mU ml⁻¹) for 3 h for the measurement of MIP-1a. Absolute values of IL-12, IL-6 and nitrite in the absence of IB-MECA treatment amounted to 378 ± 23 pg ml⁻¹, 76 ± 4 ng ml⁻¹ and 49 ± 5 µM respectively, and were considered 100%. n=6-9 wells from two to three independent experiments.

Note: *P<0.05 and **P<0.01 indicate significant inhibition by IB-MECA treatment.

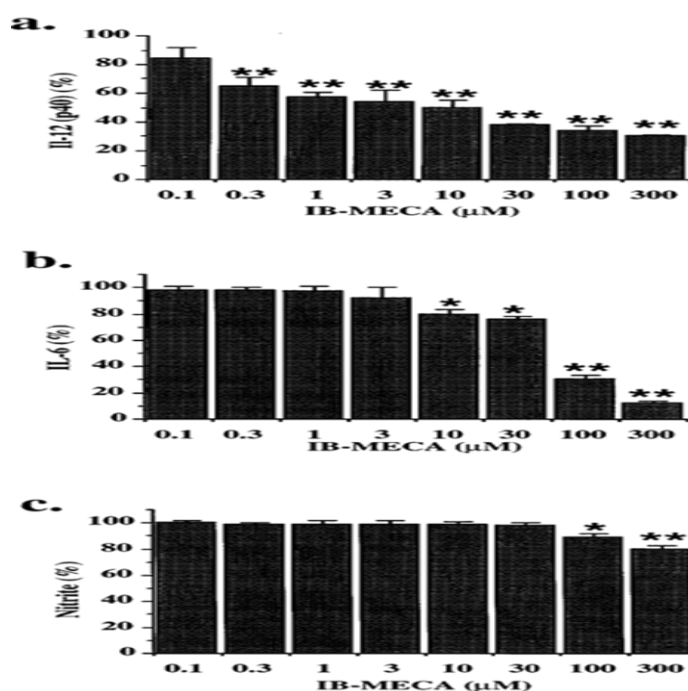
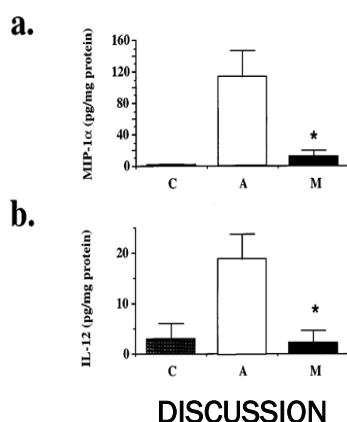


Figure 5. IB-MECA suppresses the production of inflammatory mediators in aqueous paw extracts of mice subjected to collagen induced arthritis. Levels of MIP-1a and IL-12 are shown in samples from control animals (a), from collagen-induced arthritis at 35 days (b), and IB-MECA treated mice subjected to collagen-induced arthritis (M) at 35 days. n=10-12.

Note: *P<0.05 indicates suppression of IL-12 and MIP-1a production in arthritis by IB-MECA.



Different adenosine receptor subtypes activate one of kind sign transduction techniques in extraordinary cells. In macrophages, however, A3 receptor stimulation does not require cyclic AMP, protein kinase or the transcription thing NF-kappa B, notwithstanding the fact that stimulation of this receptor subtype might exchange the make-up of the AP-1 transcription complicated further research is needed to determine the cellular signalling pathway by means of which A3 activation reduces cytokine or MIP-1a manufacturing.

In our settings, the A1 agonist had no considerable inhibitory effect on MIP-1a generation; however adenosine turned into a vulnerable inhibitor. Adenosine is often an A1 receptor agonist, with minor A2 and A3 agonist homes our studies with the adenosine deaminase inhibitor confirmed that adenosine deaminase, that's gift inside the foetal calf, degrades adenosine quickly. The low quantity of inhibition isn't attributable to serum. The decrease eAcacy of adenosine on MIP-1a manufacturing can be due to its decreased efficiency on A2 and A3 receptors, according to our findings.

In addition to inhibiting MIP-1a production, IB-MECA decreased IL-12, and NO production in immune inspired cultured macrophages, in line with the modern-day take a look at. Adenosine receptor agonists have recently been shown to prevent endotoxin-mediated stimulation of the TNF-a gene and protein expression within the murine J774.1 macrophage cell line in a dose-dependent manner function of the A3 receptor. We recently validated that stimulating A3 receptors with the selective A3 receptor agonist IB-MECA decreased plasma TNF-a while increasing IL-10 in LPS-treated mice. The modern-day observe's findings add to these earlier findings. The RAW 24.7 mobile studies are simply a rough approximation of the residences of primary or resident macrophages, or the *in vivo* state of affairs in arthritis, where resident macrophages play a role within the production of chemokines, cytokines, and NO. Despite this, The control of chemokine, cytokine, and NO manufacturing by means of adrenergic, purinergic receptors in monocytic/macrophage cells and primary monocytes/macrophages shows hanging parallels. The *in vivo* assessments of NO, IL-12, and MIP-1a manufacturing in joint extracts within the modern research were in addition in good settlement with our *in vitro* findings in RAW cells. In the arthritis model, it's possible that IB-MECA inhibits the synthesis of IL-12, MIP-1a, and NO, which are all giant anti-inflammatory mechanisms. It is well known

that IL-12 performs a key role within the early events of the arthritis induction segment. The research first-class showed this, showing that IL-12 may replacement mycobacteria and motive autoimmune arthritis in DBA/1 mice inoculated with chook CII in incomplete Freund's adjuvant. Furthermore, those researchers discovered that IL-12 ought to irritate the arthritis caused by fowl CII in CFA mice. The route of the disorder became suppressed in IL-12 deficient animals or mice treated with anti-mIL-12 antibodies. After IL-12 initiates the inflammatory method, macrophages and different cellular types come to be activated and launch a range of mediators along with TNF- α , IL-, IL-1, MIP-1 α , and NO, which maintain the inflammatory country alive. The chemokine MIP-1 α , which is generated by way of fibroblasts or macrophages, seems to be a key pathogenic element in the development of arthritis, attributable to its chemotactic motion on inflammatory cells. The decrease of paw MIP-1 α ranges may additionally contribute to the discount of neutrophil recruitment (as evidenced by means of the suppression of paw myeloperoxidase degrees inside the modern-day investigation). The A3 has an inhibiting action .Over production of this loose radical, as well as its reactive reaction product, peroxy nitrite, has been proven to make contributions to the pathogenesis of inflammatory joint disorder, consequently a receptor agonist on no release will be tremendous Although latest research postulated change mechanisms of tyrosine nitration, linked to myeloperoxidase-based conversion of nitrite to NO₂Cl and NO₂, nitrotyrosine manufacturing is basically stated as a selected 'footprint' of peroxy nitrite In the joints, nitrotyrosine could perform as a collective indication for the formation of reactive nitrogen species.

CONCLUSION

The contemporary work shows that stimulating the A3 and A2 receptors reduces MIP-1 α manufacturing. By way of inhibiting the expression of its mRNA Furthermore, the current observe suggests that an A3 receptor agonist reduces inflammation in collagen-prompted arthritis. Down-regulation of the pro-inflammatory mediators MIP-1 α , IL-12, and NO is one of the mechanisms underlying this anti-inflammatory action. The potential of medicine like methotrexate sulphasalazine and adenosine kinase inhibitors to launch adenosine at the web sites of inflammation is linked to their anti-inflammatory houses These anti-inflammatory moves may be related to A3 and A2, at the least in element, according to our speculation activation of receptors We trust that stimulating the adenosine receptor subtypes A3 and A2 could be a promising approach for the treatment of acute and chronic inflammatory illnesses.

REFERENCES

1. Standiford TJ, et al. Macrophage inflammatory protein-1 alpha mediates lung leukocyte recruitment, lung capillary leak, and early mortality in murine endotoxemia. *J Immunol.* 1995;155:1515-1524.
2. Szabó C, et al. Inhibition of poly (ADP-ribose) synthetase exerts anti-inflammatory effects and inhibits neutrophil recruitment. *J Exp Med.* 1997;186:1041-1049.
3. Szabó C, et al. Protection against peroxy nitrite-induced fibroblast injury and arthritis development by inhibition of poly (ADP-ribose) synthetase. *Puoc Natl Acad Sci.* 1998;95:3867-3872.
4. Tina C wan, et al. Selective adenosine A3 receptor stimulation reduces ischemic myocardial injury in the rabbit heart. *Caudiovasc Res.* 1997;33: 410-415.
5. Von Lubitz DH, et al. Adenosine A3 receptor stimulation and cerebral ischemia. *Euu J Phaumacol.* 1994; 263:59-67.

6. Wolberg G, et al. Effects of adenosine deaminase inhibitors on lymphocyte-mediated cytotoxicity. *Ann N Y Acad Sci.* 1985;451:215-226.
7. Al-Mughales J, et al. The chemoattractant activity of rheumatoid synovial fluid for human lymphocytes is due to multiple cytokines. *Clin Exp Immunol.* 1996;106:230-236.
8. John AA, et al. Selective activation of A3 adenosine receptors with N6-(3-iodobenzyl) adenosine-5'-N-methyluronamide protects against myocardial stunning and infarction without hemodynamic changes in conscious rabbits. *Circ Res.* 1997;80:800-809.
9. Bechman JS, et al. Nitric oxide, superoxide, and peroxynitrite: The good, the bad, and ugly. *Am J Physiol.* 1996;271:C1424-C1437.
10. Collis MG, et al. Adenosine receptor subtypes. *Trends Pharmacol Sci.* 1993;14:360-366.
11. Jane R Connor, et al. Suppression of adjuvant-induced arthritis by selective inhibition of inducible nitric oxide synthase. *Eur J Pharmacol.* 1995; 273:15-24.
12. Cronstein BN, et al. Adenosine, an endogenous anti-inflammatory agent. *J Appl Physiol.* 1994;76:5-13.
13. Cronstein BN, et al. Methotrexate inhibits neutrophil function by stimulating adenosine release from connective tissue cells. *Proc Natl Acad Sci USA.* 1991;88:2441-2445.
14. Cronstein BN, et al. The antiinflammatory mechanism of methotrexate. Increased adenosine release at inflamed sites diminishes leukocyte accumulation in an *in vivo* model of inflammation. *J Clin Invest.* 1993;92:2675-2682.
15. Cronstein BN, et al. The anti-inflammatory effects of an adenosine kinase inhibitor are mediated by adenosine. *Arthritis Rheum.* 1995; 38:1040-1045.
16. Dubyah GR, et al. Signal transduction *via* P2 purinergic receptors for extracellular ATP and other nucleotides. *Am J Physiol.* 1993;265:C577-C606.
17. Eiserich JP, et al. Formation of nitric oxide derivatives catalysed by myeloperoxidase in neutrophils. *Nature. Circ Res.* 1998; 391:393-397.
18. Firestein GS, et al. Protective effect of an adenosine kinase inhibitor in septic shock. *J Immunol.* 1994; 152:5853-5859.
19. Gadangi P, et al. The anti-inflammatory mechanism of sulfasalazine is related to adenosine release at inflamed sites. *J Immunol.* 1991; 156:1937-1941.
20. Gallo-Rodriguez C, et al. Structure-activity relationships of N6-benzyladenosine-5'-uronamides as A3-selective adenosine agonists. *J Med Chem.* 1994;37:636-646.
21. Germann T, et al. Administration of interleukin 12 in combination with type II collagen induces severe arthritis in DBA/1 mice. *Proc Natl Acad Sci USA.* 1995;92:4823-4827.
22. Halliwell B, et al. What nitrates tyrosine? Is nitrotyrosine specific as a biomarker of peroxynitrite formation *in vivo*? *FEBS Lett.* 1997;411:157-160.
23. Hasho G, et al. Regulation of cytokine and chemokine production by transmitters and co-transmitters of the autonomic nervous system. *Biochem Pharmacol.* 1998;56:1079-1087.
24. Hasho G, et al. Adenosine receptor agonists differentially regulate IL-10, TNF- α , and nitric oxide production in RAW 264.7 macrophages and in endotoxemic mice. *J Immunol.* 1996;157:4634-4640.

25. Hasho G, et al. Suppression of interleukin-12 production by phospho-diesterase inhibition in murine endotoxemia is interleukin-10 independent. *Eur. J Immunol.* 1998;28:468-472.
26. Hasho G, et al. The crucial role of IL-10 in the suppression of the immunological response in mice exposed to staphylococcal enterotoxin B. *Eur. J Immunol.* 1998;28:1417-1425.
27. Jutta S, et al. Dual role of IL-12 in early and late stages of murine collagen type II arthritis. *J. Immunol.* 1997;159:4094-4102.
28. Tsuyoshi K, et al. Interleukin-10 expression and chemokine regulation during the evolution of murine type II collagen-induced arthritis. *J Clin Invest.* 1995;2868-2876.
29. Harparkash K, et al. Evidence for nitric oxide-mediated oxidative damage in chronic inflammation, Nitrotyrosine in serum and synovial fluid from rheumatoid patients. *FEBS Let.* 1994;350:9-12.
30. Tsuyoshi K, et al. The role of chemokines in inflammatory joint disease. *J Leukocyte Biol.* 1996;59:6-12.