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Review Article

New Drug Discovery of Anti-inflammatory Drugs: Insights in Animal Models

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Abstract

Inflammation is a fundamental contributor to morbidity in the modern era, occurring in many acute and chronic severe disorders. Inflammation, along with respiratory, autoimmune, and cardiovascular illnesses, promotes the growth of rheumatoid arthritis, cancer, Alzheimer's disease, diabetes, and atherosclerosis if left unchecked. A complex network of many mediators, a wide range of cells, and the execution of numerous pathways all play a part in inflammation. There are following models used in this – *In-vitro* Models: Evaluation of no production in LPS/IFM-γ- costimulated and unstimulated RAW-264.7 murine macrophage cells, Inhibition of oxidative stress by reducing the production of reactive oxygen species (ROS), Inflammasome activation blocking, Induction of autophagy, Cellular degranulation, Measurement of cytokine expression in murine macrophages, Measurement of inflammatory gene expression, Adhesion assays, FMLP-induced Adhesion of PMN to HUVEC, Interleukin 1beta-stimulated human articular chondrocytes, Cyclooxygenase (COX) assays, In-vivo models: UV-β-induced Erythema in Guinea Pigs, Ear Edema Model, Carrageenan-induced Paw Edema Model, Carrageenan-induced Rat Pleurisy, Cotton Pellet-induced Granuloma, Air Pouch Model, Adjuvant Arthritis, Papaya Latex-induced Arthritis, Collagen-induced Arthritis(CIA), Cyclophosphamide-induced Cystitis in Rodents, Trinitrobenzene Sulfonic Acid (TNBS)-induced Experimental Colitis. Conclusions: The mention models are used for novel anti-inflammatory drug discovery.

Keywords: Anti-inflammatory; In-vitro Animal models, In-vivo Animal Models

Introduction

Autoimmune illnesses (such as rheumatoid arthritis), osteoarthritis, asthma, COPD, interstitial cystitis, prostatitis, inflammatory bowel disease, multiple sclerosis, allergic rhinitis, infectious diseases, several forms of malignancies, and cardiovascular diseases are inflammatory diseases [1]. Therefore, inflammation is described as a ubiquitous host defensive process involving complex cell-cell, cell-mediator, and tissue interactions. These inflammatory events may appear to be the same in different inflammatory illnesses, but there are underlying variances in paracrine signalling mechanisms that are regulated by differences in chemokines, cytokines, growth factors, lipids, and genetic influences [2,3]. Although inflammation is the common denominator in many diseases, the therapeutic approach for each inflammatory condition is often distinct. Each disease population has unique therapeutic requirements that are

not being met by current preventative and therapeutic efforts. As a result, newer strategies for discovering novel drugs are required. Inflammation occurs when the body's non-specific defense mechanisms are overwhelmed by a microbial invasion or tissue damage [4]. Following infection, the immune system is stimulated, and communication and coordination between different types of immune cells occur, as well as immune cell activity that causes inflammation. Inflammation is generally carefully regulated by the body and is the beginning of the body's self-repair process, which is begun by the body's protection against pathologic assaults; nonetheless, it occasionally goes horribly awry, resulting in physiological chaos and mortality [5].

Inflammation develops in reaction to a vast array of external and endogenous stimuli that have the potential to cause harm. Endogenous stimuli might be immunological, neurological, or genetic in nature. Exogenous stimuli can be physical, chemical, mechanical, dietary, or biological. The inflammation may be acute, subacute, or chronic. Chronic inflammation can linger for weeks, months, or years, whereas acute inflammation is transient. The typical triad of acute inflammation consists of (a) pain, fever, and swelling, which are all generated by increased blood flow, (b) enhanced migration of leukocytes, neutrophils being the first, into the affected tissue area, and (c) increased capillary permeability. Chronic inflammation, which is characterised by tissue growth, granuloma development, and healing, frequently develops from acute inflammation. In recent years, leukocyte migration has received increased attention. The rolling of leukocytes on the vascular wall, which is mediated by selectins and glycoproteins containing the Sialyl Lewis's moiety [6], is the first step in leukocyte recruitment. P-selectin, one of the selectins, is responsible for the adhesion between activated platelets and neutrophils. Adhesion assays are used in drug screening to measure how well a drug inhibits the adhesion of human platelets to neutrophils after the platelets have been activated by thrombin. Vascular cell adhesion protein 1 (VCAM-1) and intracellular cell adhesion molecule (ICAM-1) are very important for the development and maintenance of inflammatory disorders [7].

Accumulating research evidence demonstrates that inflammation is an orchestra comprised of several players, including histamine, prostaglandins (PGE2 and prostacyclins), leukotrienes (LTB4), cytokines (IL-1, IL-6, IL-8, TNF-α), bradykinin, serotonin and growth factors. [6], lysosomal contents of neutrophils, adipokines (leptin, a ROS generated in the endoplasmic reticulum and mitochondria participate in autophagy, A highly conserved housekeeping mechanism that serves a critical function in the elimination of damaged or aged intracellular organelles. ROS are comprised of hydroxyl radical (OH), hydrogen peroxide (H_2O_2), superoxide (O_2^-), and singlet oxygen. Emerging as regulators of inflammation [8] are gas transmitters such as hydrogen sulfide (H_2S), nitric oxide (NO), and carbon monoxide (CO). Based on a greater comprehension of the role of key mediators and the processes involved in the inflammation triad, new, safer, and more effective anti-inflammatory medications are being developed.

The genomic era has emphasised the significance of aberrant gene expression in the pathogenesis of inflammation and immunological diseases. Numerous exogenous stimuli, including bacterial lipopolysaccharide (LPS), and endogenous stimuli, including cytokines (IFN- γ, IL-6), activate transcription factors that bind to regulatory elements in many genes encoding proinflammatory enzymes (e.g., COX-2, iNOS) [9] and acute phase proteins and cytokines (e.g., TNF- α). We have found consensus sequences for NF-$_k$B, AP-1, and STATI in the promoters of iNOS and COX-2[8]. By activating ERK1/2, c-Jun, N-terminal kinases, and p38 kinases, TNF-α and LPS stimulate the mitogen-activated protein (MAP) kinase pathways, which in turn activate the transcription factors AP-1 and NF-kB, which control the expression of immediate early genes involved in immune, acute phase, and inflammatory responses.

Cytokines have been demonstrated to have critical roles in inflammatory disorders such as psoriasis, septic shock, and rheumatoid arthritis [10], and inhibiting or activating their action is a tried-and-true approach to disease modulation. During inflammation, immune cells increase the secretion of diffusible growth factors and heparin-binding chemokines by resident tissue [11]. Chemokine expression has been shown to precede inflammatory cell infiltration [12]. Certain hyperinflammatory states are associated with inflammasome activation, which facilitates caspase-1 and interleukin IL-1β processing by autophagy, resulting in an amplified inflammatory response [13-15]. During autophagy, cell size increases and there is an increase in the number of membrane vacuoles known as autophagosomes. Autophagy can reduce inflammasome activity by destroying inflammasomes via lysosomes. Autophagy can thus be described as a double-edged sword because autophagy's destruction of inflammatory cells such as macrophages and inflammasomes is desirable in the control of inflammation [16], but unchecked autophagy of differentiated cells could have serious consequences [17] in ageing and degenerative diseases.

Up until recently, corticosteroids and other nonsteroidal anti-inflammatory medicines were the mainstays in the treatment of inflammatory illnesses. However, mediator antagonists, either alone or in combination, as well as gene therapy, are currently being studied as potential alternatives. Potentially useful as novel anti-inflammatory drugs include inhibitors that operate by blocking the activation of transcription factors involved for disease-related gene expression [18-21] or by interfering with components of various intracellular signaling cascades. Inducible reporter gene vectors with natural promoters of inflammatory genes (COX-2, iNOS, and TNF- α) [18–21] or binding sites [20–22] for specific transcription factors are made the search of novel inflammatory signaling (NF-$_k$B, AP-1, glucocorticoid receptor, STATSs) inhibitors. Several standard in vitro tests for anti-inflammatory effects show that the test compound blocks inflammatory pathways in an unusually strong way. If a candidate drug does well in in vitro tests, it is then tested in whole animal models of acute, subacute, and chronic inflammation.

Animal Models in New Drug Discovery of Anti-inflammatory drugs:

In-Vitro Method

Evaluation of NO production in LPS/IFM-γ- costimulated and unstimulated RAW264.7 murine macrophage cells,

In this method of in vitro screening, Potential of the test medication to inhibit NO, through enhanced vasodilation, one of the early mediators of inflammation is investigated. RAW264.7 macrophage cell line is maintained at 37°C in Dulbecco's modified eagle medium (DMEM) containing 10% foetal bovine serum (FBS), penicillin (100 units per ml), and streptomycin sulphate (100 g/ml) in a humidified atmosphere containing 5% carbon dioxide [23]. The cells are stimulated with 1 g/ml LPS for 4 hours or 20 ng/ml IFN-γ, and 100 l of Griess reagent is added to 100 l of each supernatant from LPS or IFN-γ stimulated cells. Before addition to the cell line, inhibitory test compounds are dissolved in DMSO; the final concentration of DMSO is maintained at 0.1% or less. DMSO-only controls are also run alongside the test drugs. The protein determination is performed using the Bradford assay. The plates are read at 550 nm against a sodium nitrite standard curve. Griess reaction serves as an indicator of NO production in the medium by measuring nitrite accumulation. Hydrocortisone, a well-known steroidal anti-inflammatory drug, is used as a positive control in such experiments, and potential candidates' efficacy is measured in comparison to hydrocortisone.

Inhibition of Oxidative Stress by Reducing the Production of Reactive Oxygen Species (ROS)

Excess ROS production has been linked to several diseases, including inflammatory ones. The murine macrophage cell line RAW264.7 is treated with the test drugs at various concentrations for 24 hours before being incubated with the fluorescent marker 5 μM carboxy-2,7'-dichlorodihydrofluorescein diacetate for 30 minutes at 37 degrees Celsius. Prior to staining with 5 M carboxy-2.7'-dichlorodihydrofluorescein diacetate, drug-treated cells and positive control cells were exposed to hydrogen peroxide H_2O_2 (0.03%). The treated cells are washed in PBS, and fluorescent cells are counted using flow cytometry. A decrease in the percentage of fluorescent cells compared to a positive control indicates ROS production. Another in vitro model of superoxide (O_2^-) generation by polymorphonuclear cells PMNs was used for drug screening by Dianzani et al. (2006) [24]. To maximize O_2^- production, these researchers cultured PMNs and challenged them with a concentration of 10-7M n-FMLP (N-formyl methionyl-leucyl-phenylalanine). Superoxide production was measured spectrophotometrically by measuring the superoxide dismutase-inhibitable reduction of cytochrome C per 106 PMNs per minute.

Inflammasome Activation Blocking

Caspase-1 resides in the cytoplasm as an inactive pro-form enzyme that is activated by multimolecular protein complexes known as inflammasomes [17–19]. This experiment can be conducted with freshly obtained mouse peritoneal macrophages and mast cells or with at least one night-old cell lines such as RAW264.7 and HMC-1. Cultured cells are treated to test drugs at doses of 0.1, 1, and 10 μg/ml for 1 hour, followed by 24 hours of exposure to LPS (100 ng/ml) [24] or a proinflammatory cytokine (interleukin 1beta, ILIβ, 5 ng/ml). The cells and supernatants are then collected for further analysis. This decrease can be quantified by western blotting of pro- and processed caspase-1 in drug-treated tissue homogenates [25]. The effectiveness of medications in preventing inflammasome activation is determined by a decrease in the active form of caspase-1 relative to a comparator drug, and this decrease can be measured by a decrease in pro- and processed caspase-1 in drug-treated tissue homogenates.

Induction of Autophagy

Recent studies on autophagy have made extensive use of RAW264.7 macrophage cells that either stably or transiently express an LC3 protein linked with green fluorescence protein (GFP) (microtubule associated protein I light chain 3). Using multiwell plates, test medications and positive controls are applied to macrophage cells stably expressing GFP-LC3 on the RAW264.7 strain. The redistribution of GFP-LC3 is a hallmark of autophagy-inducing medications [16], and it results in a transition from diffuse fluorescence throughout the cytoplasm to many punctate vesicles with concentrated fluorescent signal. The effect of potential anti-inflammatory drugs on the redistribution of fluorescence is evaluated using confocal microscopy [25]. Immunoblot examination of the relative expression levels of LC3-II and LC3-I offers a quantitative measure of autophagy induction [16].As cells grow, they develop an increasing number of autophagosomes, membrane vacuoles, and autophagy occurs in stages. The autophagosome finally unites with a lysosome to produce an auto phagolysosome, where cargo is degraded by lysosomal hydrolases. To generate LC3-II for the autophagy drug screening, GFP-LC3 is removed from the cytosol and integrated into the autophagosome, where its C terminus is exposed to proteolysis and lipidation. Light chain 3B (LC3B), autophagy protein 5 (ATG5), beclin 1 (BCL-2) and LAMP-2 are proteins involved in autophagy, and their expression can be used as a surrogate for the efficacy of a medication [26].

Cellular Degranulation

Re-exposure to an allergen causes mast cells to degranulate, resulting in the release of cytokines, chemokines, proteases, and histamine, which all contribute to the inflammatory response [27]. In the past, peritoneal mast cells were extracted to determine the ability of test drugs to suppress mast cell degranulation. Recent research achieved this by combining phorbol 12-myristate 13-acetate (PMA) with A23187 to replicate the activity of the HMC-1 mast cell line [27]. Mast cell line is exposed to test drugs for 30 minutes before stimulation with PMACI (20 nM of PMA plus 1 μM of A23187) and 6 hours of incubation at 37 degrees Celsius. By centrifuging the cells at 400 rpm for 5 minutes at 4°C, the released histamine is separated from the cells. Using a spectrofluorometer, the concentration

of histamine in the supernatant is then measured by measuring the fluorescence intensity at 460 nm (excitation at 355 nm). After treating the cell suspension with Triton X-100, the total content is evaluated. The determined proportion of release is regarded as an indicator of anti-inflammatory activity.

Measurement of Cytokine Expression in Murine Macrophages

Macrophages play a crucial role in chronic inflammation and are an excellent starting point for investigating possible therapies for this condition. Injecting 2 ml of 4% thioglycolate broth intraperitoneally into female CD-1 mice between 5 and 10 weeks of age yields primary macrophages. Four days following injection, peritoneal macrophages are collected and treated according to Ding's procedure. [28]. After planting 2 105 cells per well in 96-well plates, cells are either left untreated or treated for 48 hours with 2 ng/ml IFN- γ. After treatment, mediums from drug-treated and untreated cells can be examined for cytokines using ELISA or multiplexing [29]. In addition, mRNA expression of proinflammatory reserve was assessed using RT-PCR or quantitative PCR [23,30] to detect the effect of the studied medications.

Measurement of Inflammatory Gene Expression

As previously described, macrophages are extracted from the peritoneum of mice and treated with test drugs with or without LPS (5 g/mL) for 18 hours. The Westens Blot method is utilised to determine the effect of test drugs on inducible NOS (iNDS), COX-2 [31], microsomal PGE synthesis-1 protein, and p38 MAPK phosphorylation [32]. Immunofluorescence is a method for observing how drugs influence the translocation of $NF\text{-}_KB$ into the nucleus.

Adhesion Assays

The vascular adhesion molecules VCAM-1, ICAM-1, and E-selectin are being examined in primary cells derived from umbilical vein (HUVEC) and a microvascular cell line due to their essential roles in controlling leukocyte extravasations (HMEC-). In vitro adhesion assays utilise human umbilical vein endothelial cells (HUVECs) or human dermal microvascular endothelial cells (HDMECs) to simulate circumstances [33]. The following day, endothelial cells are treated with TNF-α (20 ng/ml) for 24 hours while being exposed to test drug concentrations ranging from 106 to 2105 M for 20 minutes. The expression of intercellular adhesion molecule (ICAM)-1 can be increased by tumour necrosis factor alpha (TNF-α), and this experiment can be conducted using either TNF-α for 24 hours or LPS at 4 mg/L for 6 hours as an alternate unpleasant stimulus. [34]. 2 105 neurophills intravenously labelled with 2', 7'-bis-(carboxyethyl)-5-(and-6)-carboxyfluorescein (BCECF) are plated on top of TNF-α activated endothelial cells after 20 minutes at 37 degrees Celsius. In place of neutrophils, fluorescently labelled THP-1 human monocytic leukaemia cell line cells can also be utilised. After washing with PBS, adherent cells are lysed with 0.5% CTAB (cetyl-trimethyl-ammonium bromide), and their adherence is measured using an ELISA reader to detect fluorescence at 490 nm.

FMLP-induced Adhesion of PMN to HUVEC

The bacterial peptide FMLP solely activates the machinery responsible for PMN adhesion. Dianzani et al. (2006) selected this stimulus to investigate the anti-inflammatory effects of several drugs on PMN adherence to HUVEC [24]. When there is nearly the maximal quantity of FMLP (10^{-7}M), PMN activity is at its peak. PMNs and HUVEC can also be challenged with additional inflammatory stimuli such as platelet activating factor (PAF), interleukin-1 (IL- Iβ), tumour necrosis factor (TNF-α), and phorbol myristate acetate (PMA) to prevent the adhesion phenomena that occurs during inflammation. At the concentration employed, PAF (10^{-7}) inhibited PMN adhesion more than other stimuli. c

a. C Paulet-neutrophil adhesion: Human platelets that have been activated by thrombin are combined with a medication (10^7–10^4 M) at 20°C for 10 minutes, followed by a 10:1 ratio of neutrophils. As an indicator of activity, neutrophils with two or more adhering platelets (called "number positives") and neutrophils with one or no adherent platelets (called "number negatives") are counted. Unlike controls, the test medication inhibits adhesion.

b. Neutrophil adherence to hypoxia-induced swine aortae: Fresh pig aortae are stimulated by placing them in PBS containing N_2 and securing them between a Teflon block and a perforated stainless-steel plate. Neutrophils and the test medication (100 to 10 M) are added to the luminal side for 90 minutes. The test medication prevents cells from adhering to one another, and the associated cells are shattered so that the MPO activity can be detected using light.

c. Interleukin 1beta-stimulated human articular chondrocytes.

Isolating chondrocytes (CHs) from individuals with osteoarthritis [35] is a valuable screening method for potential treatments for the condition. Like macrophages, the effect on NO and cytokine expression is evaluated by stimulating isolated cells with a proinflammatory cytokine (interleukin-1beta, IL1beta, 5 ng/ml).

Cyclooxygenase (COX) Assays

Arachidonic acid is converted into prostaglandins by the enzyme COX. COX is currently recognised to exist in two isoforms COX-1 and COX-2, which are constitutive and inducible in nature, respectively. Thus, constitutive form is related with physiological function whereas inducible form plays a harmful role (large part in inflammation) (major role in inflammation). The in vitro assays for these enzymes may be carried out as below.

Purified recombinant human COX-1 (50 µl of 1µg/ ml in 100 mm Tris-HCL, pH 8.0, 5 mm EDTA, 1 mm phenol, I µm hematin) [36] is preincubated for 15 minutes with 2 µl of the test drug solution. To initiate the reaction, 5 µl of 1 M arachidonic acid is added to reach a final concentration of 0.1 µM. After 7 minutes of incubation at room temperature, the reaction is stopped by adding 5 l IM HCl and 50 2 µl acetonitrile. Using the PGE_2 enzyme immunoassay, 50 µl aliquots

of each reaction mixture are examined for substrate conversion to PGE_2.

COX-2 assay: Drugs can be evaluated for COX-2 inhibitory activity by measuring the velocity of oxidation of N, N, N, N'-tetramethyl-p-phenylenediamine (TMPD) spectrophotometrically [37]. TMPD is oxidised when PGG_2 is reduced to PGH_2. The assay mixture contains 100 mM sodium phosphate, 1 µM hematine, 1 mg/ml gelatin, 2-5 µg/ml pure COX-2, and 4 µl of the test material dissolved in DMSO. Total assay mixture volume is 180 µl. This is then preincubated for 15 minutes at 22 degrees Celsius, after which 20 l of a solution containing 1 mM arachidonic acid and 1 mM TMPD in the assay buffer is added. Except for hematin and enzyme, the assay buffer contains the assay solution. During the first 36 seconds, the absorbance at 610 nm is measured and the percentage of inhibition is determined. Also seen and subtracted from the activity in the presence of COX-2 is the nonenzymatic oxidation of TMPD.

In Vivo Methods

Animal models are the means in which the results from simpler in vitro research may be tested in "intact" biological systems. Animal studies can increase trust in a novel medicine before it goes through human trials because of the obvious parallels between the two settings. Animal studies can be considered hierarchical in nature as research utilising rats and small laboratory animals are relatively simpler to undertake and maintain than studies done using larger animals. Research done with small mammals is less expensive, more accessible and less time consuming to carry out. However, none of the currently used models fully imitate the chain of events causing inflammation in patients, even though they use a wide range of phlogistic drugs that cause inflammation of varied degrees and durations. Different models used so far have their own advantages and disadvantages, and results should ideally be replicated in multiple mammalian species before being applied to humans. Clinical translation of findings from certain mouse models is less certain, as reported in a recent PNAS paper [38]. This is especially true for mouse models of inflammatory diseases. Within the revised chapter, rat models were prioritised over mouse models. Using rats also allows for more accurate pain behaviour modelling, and their larger size is more amenable to surgical manipulation. When trying to separate model-specific results from generic principles that might be used to predict clinical efficacy, it is crucial to take these into account.

It is advised to use multiple in vivo methods simultaneously, as this will better replicate the whole range of acute, subacute, and chronic inflammatory processes, including but not limited to redness, heat, plasma exudation, edema, pain, leukocyte migration, tissue growth, and partial necrosis. Complete animals like guinea pigs, rats, mice, rabbits, and even dogs can be utilized for this. There are several in vivo models of inflammation, but the most well-known ones include croton oil-induced mouse ear edema, carrageenan-induced edema [39], carrageenan-induced rat pleurisy [40], and cotton pellet-induced rat granuloma [41]. Inducing models of edema are used to test compounds for their capacity to inhibit vasodilation and edoema formation, while models of pleurisy and granuloma are used to assess the compounds' efficacy against the exudative and proliferative phases of inflammation, respectively. Table 1 lists several in vivo models of inflammation. To evaluate neutrophil migration and free radical damage, researchers in animal models measure myeloperoxidase (MPO) and superoxide dismutase (SOD) and malondialdehyde (MDA) levels, respectively.

Table 1: Various in vivo models of inflammation.

Sr. No.	Model of inflammation	Site of application/injection of inflammogen	Animal species	Reference(s)
1.	Papaya latex-induced arthritis	Subplantar region	Rats	[42]
2.	Cotton pellet-induced granuloma	Subcutaneously through the skin incision, groin region, flanks	Rats, mice	[41]
3.	Carrageenan-induced paw edema	Subplantar region	Rats, mice	[43-47]
4.	UV-B-induced erythema	Depilated skin	Guinea pig	[28,43,45]
5.	Collagen/LPS-induced accelerated arthritis	Intradermal injection at the base of the tail	Mice	[48-49]
6.	Cyclophosphamide-induced cystitis	Systemic injection	Rats, mice	[9,33,44,50]
7.	TNBS-induced colitis	Rectal application	Rats, mice	[51]
8.	Freund's adjuvant arthritis	Subplantar region	Rats	[52]
9.	Pleural exudation	Pleural space	Rats	[40]
10.	Ear edema	Topical application on ear	Mice	[39,41]
11.	Air pouch	Dorsal surface	Rats, mice	[48,53]
12.	Prostatitis	Intraprostatic application	Rats, mice	[31,44]

Papaya Latex-induced Arthritis

Conventional experimental models have failed to demonstrate considerable activity for slow-acting antirheumatic medicines (SARDS) such gold, chloroquine, levamisole, penicillamine, etc. It has been shown that lysosomal enzymes are crucial in adjuvant-induced arthritis. Research has shown that these enzymes are harmful to tissues. With the pathogenesis of the disease in mind, an experimental rheumatoid arthritis model generated by papaya latex has been created to evaluate the anti-inflammatory effects of SARDs. caricain, a cysteine protease present in papaya latex, is responsible for the degradation of cartilage in this model. The rat's hind paw is injected with 0.1 ml of a 0.25% solution of papaya latex (made in 0.05 M sodium acetate buffer, pH 4.5 containing 0.01% thymol) [42]. After 3 hours, the effect is at its strongest, and it continues to last for at least another 5 hours. Aspirin, ibuprofen, and other nonsteroidal anti-inflammatory medications (NSAIDs) do not exhibit significant action in adjuvant produced arthritis or other models of inflammation, but this method is sensitive to their effects.

Cotton Pellet-induced Granuloma

This method is used a lot to learn about the leaking and growing parts of inflammation. People use 5 mg of sterile cotton pellets that have been soaked in 0.4 ml of a 5% ampicillin solution in water [41]. Under ether anaesthesia, pellets are put under the skin of the animal's back through a cut in the skin (rats and mice). The drug treatment starts 2 hours after the cotton pellet is put in, and it lasts for 5 days straight. Animals in the control group get drug for the same amount of time that animals in the test group get normal saline. On the fifth day, the animals are killed, the granulomas are taken out and dried at 60°C for 24 hours. The dry weights are then measured. By subtracting the initial weight from the final dry weight of the cotton pellets, you can figure out how much protection the drug provides as a percentage. People have also used bigger cotton pellets (30 mg) that were put under the skin in the groyne area. Soaking cotton pellets in turpentine, carrageenan, or other irritants can make them even more likely to cause inflammation (Figures 1-7).

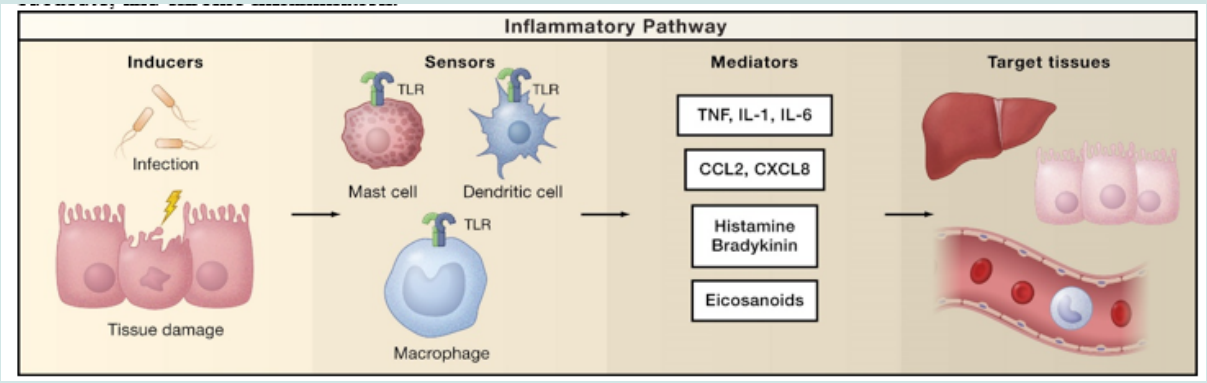

Figure 1: Inflammatory Pathway Route leading to inflammation Composed of Components, Inducers, Sensors, Mediators, and Target Tissues, the Inflammatory Pathway also includes Target Tissues. Sensors detect the causes of inflammation. TLRs are expressed on macrophages, dendritic cells, and mast cells. These components stimulate the production of cytokines, chemokines, bioactive amines, eicosanoids, and bradykinin. These inflammatory mediators work on target tissues to optimise adaptation to inflammatory-causing unfavourable situations (such as infection or tissue injury). The following are examples of inflammatory sensors, mediators, and target tissues:
Ruslan Medzhitov Inflammation 2010: New Adventures of an Old Flame. Cell 140, March 19, 2010 ©2010 Elsevier Inc. 771-776. https://doi.org/10.1016/j.cell.2010.03.006

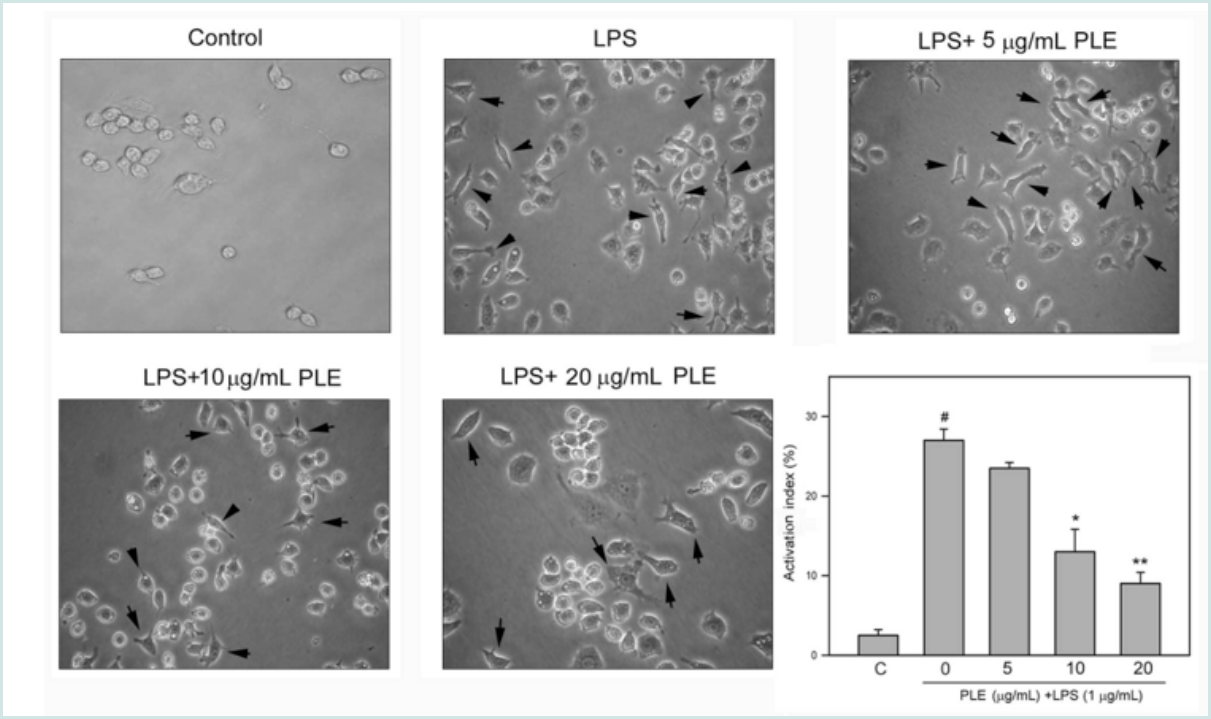

Figure 2: Effects of PLE on dendritic transformation of LPS-stimulated RAW264.7 cells. Cells were pre-incubated with the indicated concentrations of PLE in the DMEM culture medium for 4 h, and then stimulated with 1 μg/ml LPS for 24 h. Cell morphology was monitored under a light microscope at x400 magnitude . Activated RAW264.7 cells are indicated by arrows. The activation index percentage was expressed as the number of cells with activated morphology relative to the total number of cells, quantified in 5 random fields (number of total cells >100). # P<0.05 as compared to the control (C; untreated cells); * P<0.05 as compared to LPS alone. PLE, Perilla frutescens leaf extract; LPS, lipopolysaccharide.

Bee-Piao Huang, Chun Hsiang Lin, Yi-Ching Chen, Shao-Hsuan Kao Anti-inflammatory effects of Perilla frutescens leaf extract on lipopolysaccharide-stimulated RAW264.7 cells. Molecular Medicine Reports 2014 Aug;10(2):1077-83. PMID: 24898576. doi: 10.3892/mmr.2014.2298. Epub 2014 Jun 5.

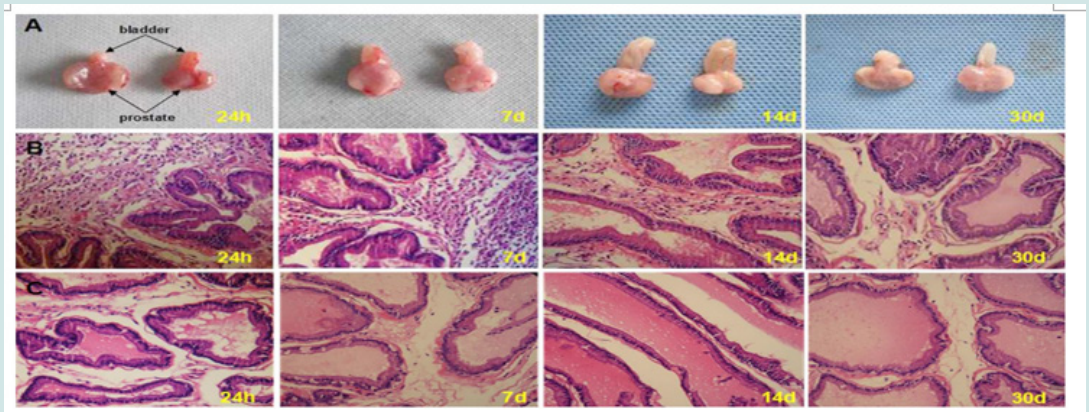

Figure 3: After intraprostatic injection of carrageenan, the histology detection.

A: 24 hours, 7 days, and 14 days after intraprostatic injection of 3%λ -carrageenan, prostatic edema occurred, however at 30 days following injection, there was no significant difference between the groups (left: inflamed group; right: control group). B: Injection of 3%λ -carrageenan intraprostatically induced a rapid accumulation of inflammatory cells at 24 hours, 7 days, and 14 days later. As of 30 days post-injection, very few inflammatory cells were visible. C: In the control group, there was no accumulation of inflammatory cells.

Feng Zeng, Hequn Chen, Jinrui Yang, Long Wang, Yu Cui, Xiao Guan, Zhao Wang, Jiping Niu, Xiongbing Zu, Lin Qi, Xiangyang Zhang, Zhengyan Tang, Longfei Liu(2014) Development and Validation of an Animal Model of Prostate Inflammation-Induced Chronic Pelvic Pain: Evaluating from Inflammation of the Prostate to Pain Behavioral Modifications. PLoS ONE 9(5): e96824. doi:10.1371/journal.pone.0096824. https://doi.org/10.1371/journal.pone.0096824.g001

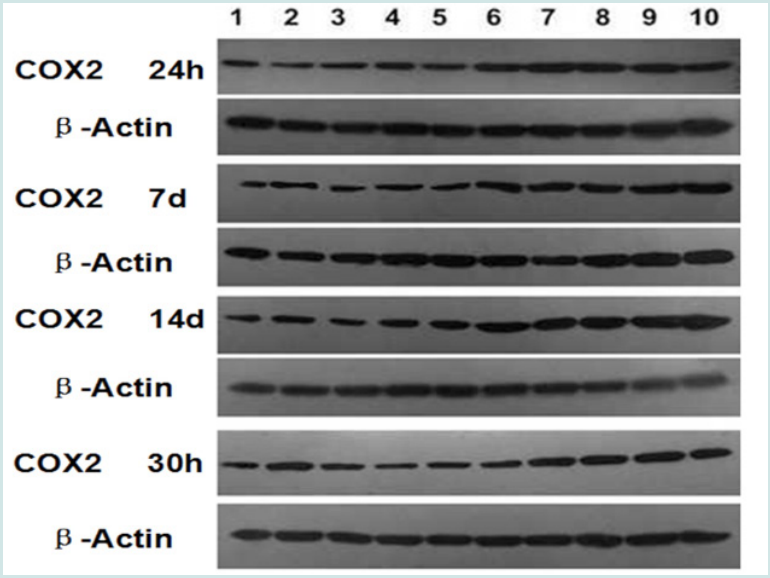

Figure 4: COX-2 expression is compared.
1–5: represents the control group; 6–10: represents the inflamed group. At 24 hours, seven days, and fourteen days following intraprostatic injection of carrageenan, the expression of cyclooxygenase-2 (COX2) increased significantly.
Feng Zeng, Hequn Chen, Jinrui Yang, Long Wang, Yu Cui, Xiao Guan, Zhao Wang, Jiping Niu, Xiongbing Zu, Lin Qi, Xiangyang Zhang, Zhengyan Tang, Longfei Liu(2014) Development and Validation of an Animal Model of Prostate Inflammation-Induced Chronic Pelvic Pain: Evaluating from Inflammation of the Prostate to Pain Behavioral Modifications. PLoS ONE 9(5): e96824. https://doi.org/10.1371/journal.pone.0096824.g002

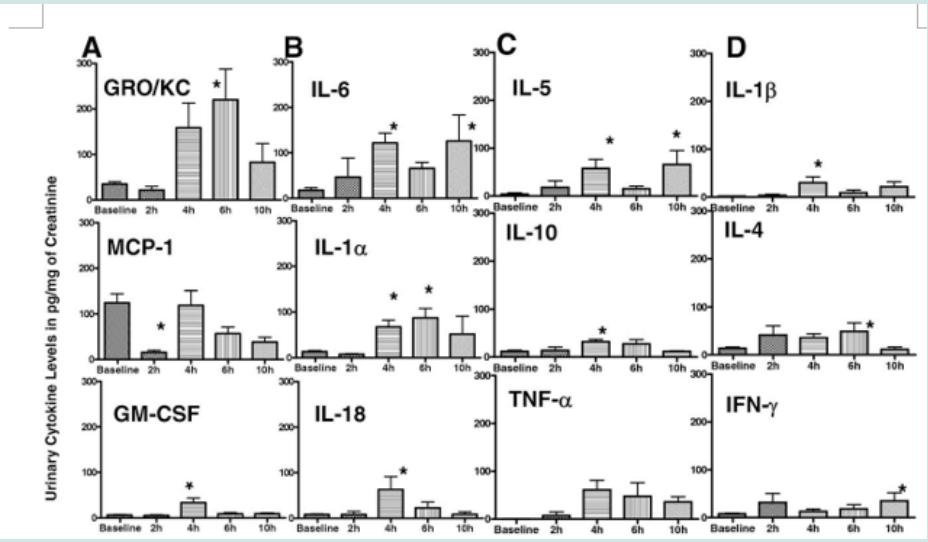

Figure 5: 51 Temporal profile of urine cytokines following injection of cyclophosphamide (CYP). Normalized cytokine levels in urine to creatinine excretion in urine at baseline and 2, 4, 6, and 10 hours following CYP injection. (A, B) At 4 hours, growth-related oncogene/keratinocyte-derived chemokine (GRO/KC), interleukin (IL)-6, IL-1α, and granulocyte-macrophage colony-stimulating factor (GM-CSF) levels increased five- to sixfold relative to baseline, whereas IL-18 levels increased tenfold (*P< .05). At 2 hours, the levels of monocyte chemotactic protein (MCP)-1 decreased 8-fold relative to baseline (*P<05; Fig. A, middle). (C, D) At 4 hours, IL-5 and IL-1 levels had increased 15-fold relative to their initial levels (*P<05). At 4 and 6 hours, the levels of IL-10 and IL-4 have increased by a factor of 3 relative to their initial values (*P< .05). (Bottom) In contrast, the elevation of tumour necrosis factor (TNF)-α by 60-fold at four hours was not statistically significant. At P< .05, values given as mean ± SEM were deemed significant relative to baseline (n = 8).
Smaldone MC, Vodovotz Y, Tyagi V, Barclay D, Philips BJ, Yoshimura N, Chancellor MB, Tyagi P. Multiplex analysis of urinary cytokine levels in rat model of cyclophosphamide-induced cystitis. Urology. 2009 Feb 1;73(2):421-6. DOI: https://doi.org/10.1016/j.urology.2008.07.03

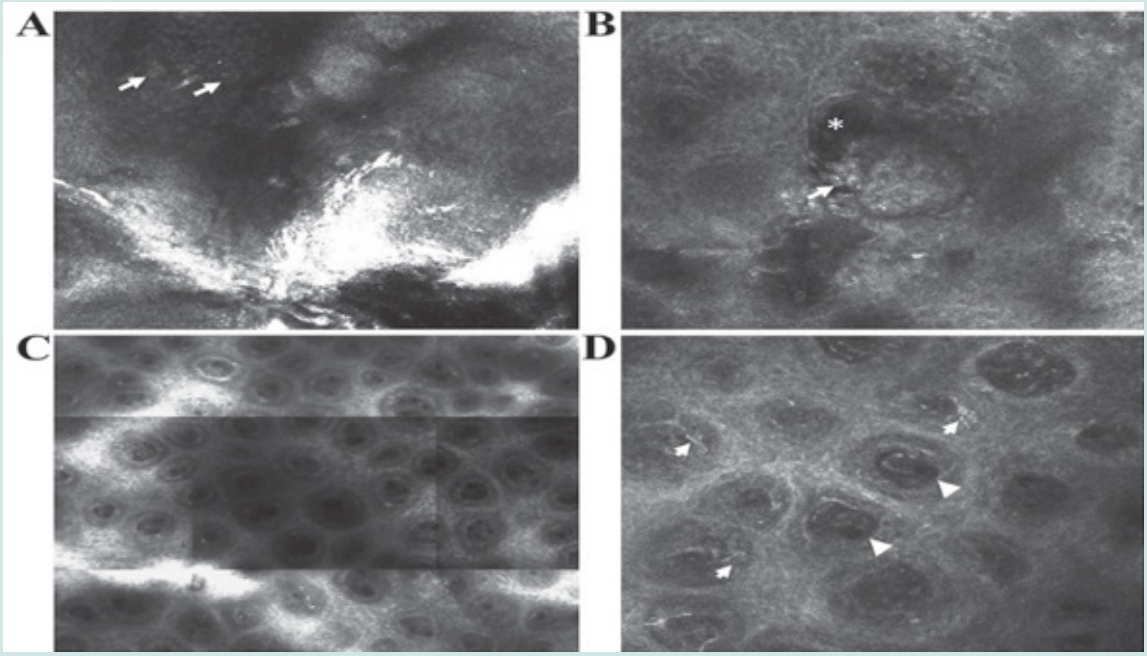

Figure 6: Psoriasis RCM characteristics in vivo. (A) RCM picture (0.5x0.5 mm) demonstrating round to polygonal, brightly nucleated cells (→) in the cornified layer that correspond to parakeratotic keratinocytes. (B) Clustered refractile roundish structures (→) and dark patches (*) indicate to aggregation of leukocytes in the cornified layer (Munro micro abscesses) or the upper portion of the spinous layer, respectively, in RCM image (0.5x0.5 mm) (spongiform pustules of Kogoj). (C) RCM mosaic (1x1 mm) showing increased density of dermal papillae. (D) An RCM image (0.5x0.5 mm) shows enlarged dermal papillae without a bright ring of basal cells, which are filled with dilated blood vessels (▶) and surrounded by moderately refractile dendritic inflammatory cells (→).

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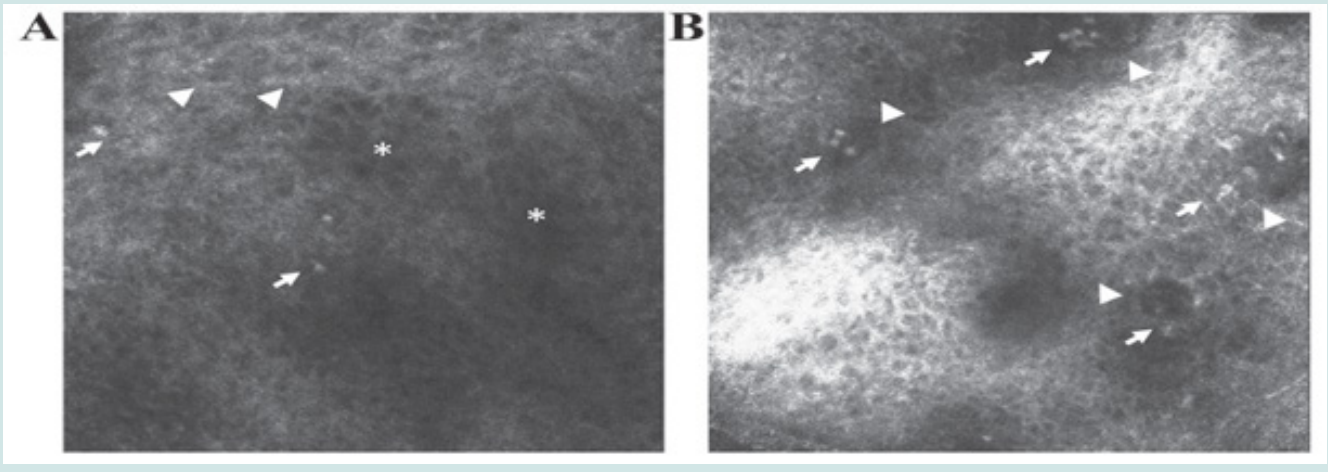

Figure 7: Lichen planus's in vivo RCM features. (A) RCM picture (0.5x0.5 mm) of the granular-spinous layer demonstrating increased intercellular gaps (spongiosis) (*), huge, polygonal cells (hypergranulosis in a wedge-shaped pattern corresponding to Wickham's striae) (▶), and inflammatory cells that appear as roundish brilliant patches (→). (B) RCM image (0.5x0.5 mm) at the level of the epidermal-dermal junction showing non-edged and non-rimmed dermal papillae (▶) due to inflammatory cell infiltrate (→).

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Carrageenan-induced Paw Edema Model

Carrageenan is a common irritant, inflaming, or phlogistic agent used to examine the early stages of inflammation in rodents (rats and mice) [42]. It is a sulphated polysaccharide derived from seaweeds and has several chemical properties that make it interesting. A series of events starting with the experimental tissue harm caused by this irritant culminate in the development of exudates. It causes inflammation, although the effects are two-fold [47]. When carrageenan is injected, it stimulates the infiltration of inflammatory cells and the expression of the cytokine cyclooxygenase-2 (COX-2). The key pathogenic cause of symptoms is prostaglandins generated from COX-2, especially prostaglandin E2[16-17,19-20]. The well accepted approach of Winter et al., 1962[44] is used. Carrageenan is injected subplantarly into the left hind paw (typically 0.1 ml in rats and 0.025-0.05 ml in mice) as a 1% w/v suspension in normal saline [45]. Animals used as controls had only vehicle injected into them. For best results, the test medication should be given either orally or intraperitoneally, based on body weight, either immediately before the carrageenan challenge or a half hour to an hour before (depending on the estimated peak effect). Each mouse or rat has an ankle joint branded. Using a mercury-filled plethysmograph, we compare the paw volume of drug-treated and untreated groups before and 3 hours after carrageenan challenge [54]. However, rat paw edema has been recorded more than 3 hours after carrageenan challenge. In the present day, sophisticated electronic instruments are being utilised to record the paw volume of rodents. The percentage decrease in edema is calculated using the following formula:

$$\% \text{ Reduction in edema} = \frac{\text{Mean edema in untreated control group - Mean edema in drug treated group}}{\text{Mean edema in control group}} \times 100$$

The method is simple, easy, quick, and can be done again and again. But it's not very specific and hard to measure. It is also hard to look at cells and how anti-inflammatory drugs change them. Because it hurts so much, you shouldn't inject the irritant into both animal's back paws. The carrageenan causes pain and deformities that can't be fixed. In this model, carrageenan can be swapped out for other irritants that cause inflammation, such as formalin, mustard oil, snake venom, dextran, polyvinylpyrrolidone, etc.

UV-B-induced Erythema in Guinea Pigs

Erythema (redness) is the earliest sign of inflammation, not yet accompanied by plasma exudation and edema. Guinea pigs are the frequently used animals to study the anti- inflammatory activity of drugs in this model [43]. In albino guinea pigs, the pure erythema reaction appears 2 h after exposure of the depilated skin to ultraviolet irradiation [28]. Guinea pigs are pretreated with the test drugs half an hour before UV-exposure from a UV lamp that emits radiation in the wavelength of 180-200 nm. This model can be used as a pure measure of the vasodilatory phase in the inflammatory reaction. However, the test suffers from the drawback that shaving of skin is required before application of irritant. The test also depends on the skin thickness and the intensity of erythema. It is difficult to quantify and requires a skilled investigator. However, the method is easily translatable in clinic and in recent studies it has been applied to Human volunteers [45].

Collagen/LPS-induced accelerated arthritis

This mice model of arthritis is produced by immunisation with type II collagen in FCA, which promotes an autoimmune illness directed against the cartilage in the joints [48-59]. Joint damage and inflammation in CIA are T cell and B cell specific, unlike other models. Clinical benefits of anti-(TNF-α) antibodies in rheumatoid arthritis were accurately predicted by the model. Type II chicken collagen [48-49] is emulsified in FCA at a 1:1 (v/v) ratio after being solubilized in 0.05 N acetic acid at 10°C overnight. Each female DBA/1 Lac J mouse (aged 12-14 weeks and weighing 22-26 g) is inoculated with 100 g of chicken type II collagen through intradermal injection at the base of the tail using 0.1 ml emulsion. Subcutaneous injection of 200 g of LPS solubilized in 0.2 ml PBS at the base of the neck 17 days later augments the body's immunological response to collagen. Microcalipers are used every day to measure the thickness of each affected paw. On a periodic basis, we use a scale from 0 to 3 to rate the clinical severity of inflammation (redness, swelling, joint deformity) in all four limbs: 0 = normal, 1 = mild swelling and/or erythema, 2 = severe edematous swelling, and 3 = joint rigidity. Since each mouse's score is the sum of its limb grades, each mouse can receive a maximum of 12 points. Three separate attempts were made to replicate this experiment. The prevalence and incidence of arthritis are determined by the following formulas:

$$\text{Frequency} = \frac{\text{Number of affected presses}}{\text{Total number of paws per group}}$$

The advantages of this method are:

a. This model is specifically well suited for high throughput screening to identify novel inhibitors of integrin VLA-1, the very late antigen-4.

b. The end point of the model is reached within 21 days whereas in other models it takes weeks to months.

c. Inflammation is not self-limiting as with other models and therefore mimics clinical arthritis.

d. It limits the amount of test compound required for the study by administration of the test compound at appropriate time (these investigators have administered antibody to VLA-4 on day 16-22 (effector phase of the response). Therefore, the animals do not need to be treated from the time of collagen immunization.

e. Administration of LPS causes a highly synchronous response with reduced variability.

f. **Cyclophosphamide-induced Cystitis in Rodents**

Cystitis, or inflammation of the urinary bladder, has been modelled in rodents by the long-term, systemic injection of the chemotherapeutic agent cyclophosphamide (CYP) [9,50]. To create a model of chronic bladder inflammation in mice or rats, a single or repeated systemic injection of CYP is sufficient. The liver and kidney convert cyclophosphamide into acrolein, the active agents in bladder injury and inflammation. Injecting 100–150 mg/kg of CYP intraperitoneally (ip) causes acute cystitis in mice. Submucosal edema, infiltration of inflammatory cells, telangiectasia (prominent dilated blood vessels engorged with red blood cells), and a rise in proinflammatory cytokine gene expression are all hallmarks of bladder inflammation. Edema and bleeding both contributed to a heavier bladder and thicker bladder wall. The levels of IL-18, IL-6, MCP-1, and VCAM-1 in bladder tissue, as well as PGE2 in the urine, were all elevated. The increased bladder wall thickness, macroscopic damage, and levels of cytokines IL-1ß, IL-6, and PGE2[54] were all ameliorated after oral administration of anti-inflammatory medications such as aspirin and ibuprofen. Due to the bladder inflammation model's non-invasive nature, the same animal can be used for many medications efficacy tests without sacrificing any additional animals. Different from the male model, females benefit more from CYP [9,50]. This model can assess the effectiveness of both local and systemic interventions.

rat modal of prostatitis: while cystitis is more common in women, prostatitis—which involves inflammation of the prostate—is a disease that is stereotypically associated with men. Over the past 30 years, researchers have reported a variety of animal models for prostatitis, including those caused by chemical agents, by bacterial infections, by hormones, and in other ways. To produce chemical prostatitis, the prostate of a male rat is surgically exposed and then injected with irritating chemicals. So far, researchers have utilised chemicals like capsaicin, 5% formalin [33], and 3% carrageenan [44] in saline.

For intraprostatic injection, rats are sedated with isoflurane (5% for induction and 3% for maintenance), the lower abdomen above the penis is shaved, and three administrations of a 10% povidone-iodine solution are used to disinfect the skin in this area. In the sterile region, a tiny midline incision is done to expose the bladder and surrounding prostate near the bladder outlet. 50 ul of an irritating chemical is injected with a 30-gauge needle into both the right and left ventral lobes of the prostate gland. In comparable areas for the control group, sterile normal saline is injected. Following the injection, the surgical wound is closed with sutures and the animal is allowed to recover from anaesthesia. At various time intervals (24 hours, 7 days, 14 days, and 30 days following injection), rats are slaughtered, and their prostates are extracted. A portion of the prostate is fixed in 10% buffered formaldehyde for 24 hours, then embedded in paraffin, sectioned on a microtome, and stained with haematoxylin-eosin. Under low-power microscopy, each slide is randomly analysed in four distinct locations containing inflammatory cells.

Intraprostatic injection of chemicals causes prostatic edema and increased accumulation of inflammatory cells in comparison to saline-injected prostate. Prostate inflammation without bacterial infection causes mild tissue necrosis and damage. Formalin-induced prostatitis can be used for up to a month to test interventions, while capsaicin-induced prostatitis only lasts for a week. Prostatitis caused by bacteria is called bacterial prostatitis, and it can be created in male rats by injecting 1010 CFU/ml of E. coli into the urinary tract [55]. Immediately prior to injection, a 10 CFU concentration of the bacterial isolate is cultivated from frozen stock. The urethra of sedated male rats is put into a sterile polyethylene tube (0.9 mm outer diameter, 2.5 cm length) and 0.2 ml of E. coli suspension is infused into the prostatic urethra with insulin syringes. Maintaining anesthesia for 1 hour allows germs enough time to infiltrate to the inside of the prostate by blocking urine excretion. 4 weeks after receiving an E. coli injection, a McConkey culture test can be run on collected urine or a prostate biopsy can be performed to confirm the absence of germs. Because of its ability to account for long-term effects, this model is useful for evaluating medications having such effects. Experimental autoimmune prostatitis can also be induced in mice by subcutaneously injecting them with prostate antigen [56] or spermine binding protein (p25) peptide [57], which both lead to immune-mediated prostatic inflammation. However, diabetes and other organ-specific autoimmune disorders are frequently seen in these immune mediated mouse models of prostatitis, which can impede the investigation of medicines targeting prostatic inflammation.

Trinitrobenzene Sulfonic Acid (TNBS)-induced Experimental Colitis

Normal intestinal mucosal function depends on a finely tuned balancing act between inflammatory cells, with feedback mechanisms and regulatory T-cells keeping a close eye on cytokine generation and cytokine-induced signal transduction pathways. When TNBS is administered to rats, the animals develop colitis, an inflammation of the colon that is characterised by a major shift/imbalance in the production profile of cytokines at various stages of the illness process. To give 2,4,6-trinitrobenzene sulfonic acid (50 mg/ml) diluted in 50% ethanol (v/v) intracolonically, a PE-90 catheter with its tip positioned around 6 cm proximal to the anal margin is used (total volume 0.5 ml) [58]. Rather than actual medication, sham animals receive 0.5 cc of normal saline. After 14 days, we check for inflammation in the most distal 8 centimetres of the colon from the rats. TNBS causes a dramatic rise of cytokines, which play a crucial role in both the innate and adaptive arms of the immune response in mucosal inflammation. Rats given TNBS exhibit diarrhoea, weight gain, and higher levels of free radicals in the colon, as well as damage to the colon, weight gain, and adhesions (both macroscopic and microscopic) in the colon (nitric oxide and lipid peroxidation).

Freund's adjuvant arthritis

The rat model of chronic inflammation described as adjuvant arthritis is the one that has been studied the most because of its

prevalence and its potential to provide light on the mechanisms of immune-mediated chronic inflammation. This model accurately represents the features of clinical rheumatoid arthritis. Results can vary depending on factors such as the type of rat used, the adjuvant's preparation (emulsion vs. suspension; emulsion generates both primary and secondary lesions in greater percentage of animals), the injection site, and the time of assessment of primary and secondary lesions [52]. Injecting mycobacteria suspended in oil or Freund's complete adjuvant (FCA) subcutaneously causes arthritis. When injected subcutaneously (under the skin), this suspension causes a localised immunological reaction in the paw (the "primary") and then a systemic immune illness (the "secondary"). TNF-α and IL-I2 are produced in response to the mycobacterial components in FCA, which in turn contributes to the development of arthritic abnormalities. Within 24 hours of the injection, local swelling develops in the paw that was given the drug. The edema peaks at around day 4 or 5, and then stabilises around day 6 to 11. Systemic illness typically manifests as edema of the non-injected limb on day 7 after injection. Comparisons between the drug treatment group and the control group can be made by measuring and recording changes in foot thickness and body weight. Drugs against inflammation that are given on a long-term basis can be tested using this animal. Drugs are given once daily beginning the day before the adjuvant is injected. This model is superior to others for evaluating the efficacy of all possible anti-rheumatic medicines because of the delayed systemic response to the Freund's adjuvant.

Ear Edema Model

Croton oil is obtained from the expression of the seeds of *Croton tiglium*. Apart from its role as a promoting agent in chemical carcinogenesis, it is a widely used agent to induce ear edema in mice. Croton oil ear edema (induced by topical application) is a predictable model to detect the activity of topical anti-inflammatory drugs. In this test, the inflammatory response is quantified by measuring the increase in earplug weight at single time interval (peak at 6 h) after croton oil application [41]. The method can be used to test both steroidal and nonsteroidal drugs. Both the rats and mice can be used for this test.

A total of 15 µl of an acetonic solution containing 75 µg of croton oil is applied to the outer and inner surface of the right ear of each mouse (about 1 cm^2 area). The left ear remains untreated. Control animals receive only the irritant while indomethacin (100 µg/ear) serves as the reference. Varying dose levels of the test drug are applied to the inner surface of the right ear of each mouse either 1 h before drug administration or by dissolving the drug in croton oil solution. The animals are sacrificed by cervical dislocation 6 h later and a plug (6 mm in diameter) is removed from both the treated and untreated ear. The difference in weight between the two plugs is taken as a measure of edematous response [43]. The inhibition percentage was calculated by the following equation:

$$\text{Inhibition (\%)} = (E_{control} - E_{treated)} \div E_{control} \times 100$$

where $E_{control}$ and $E_{treated}$ is the extent of edema from the control group and treated groups. Possible mechanism of action is determined by measuring MPO activity in harvested tissue.

Air Pouch Model

Over the past 30 years, the air pouch model of acute inflammation has been employed for screening anti-inflammatory drugs [53]. A good model of non-immune subacute exudative inflammation can be created by injecting air into the dorsal surface of a rat or mouse and then applying an appropriate irritant. Selye (1953) created the air pouch concept, which has since been tweaked by many researchers. It is common practise to produce subcutaneous dorsal pouches in sedated mice by injecting 5 ml of air [53]. Injections are repeated every three days; on day 6, 1 ml of 1% w/v carrageenan in sterile saline is administered, and on day 7, the pouches are re-injected. In the absence of any treatment, saline is given to the animals serving as controls. Mice are put to sleep and then killed 24 hours after being given carrageenan. In this study, we examine the anti-inflammatory effect of various dosages of the test chemical by administering it orally 30 minutes before, 8 hours after, and 20 hours after carrageenan. Indomethacin (5 mg/kg) orally is maintained as the standard and supplied to the control group at the same times as the drug vehicle (controls). After being rinsed with 1ml of saline, the pouches are placed on ice to chill the exudates, at which point their volume is measured. After staining with erythrosine B, you can determine how many leukocytes have migrated into the pouch, and then you can centrifuge the leftover exudate at 3,000 rpm for 10 minutes at 4°C, storing the supernatant in the refrigerator. While the precise chemical mediators of this inflammation are still a mystery, we do know that granuloma formation requires both protein synthesis and the production of kinin.

Carrageenan-induced Rat Pleurisy

Injecting various irritants into natural cavities causes non-immune acute inflammation (pleural and peritoneal cavities, knee joints). Anti-inflammatory medication testing has been accomplished with success using the pleural cavities of rats and guinea pigs. This model makes it simple to calculate the exudates' volumes as well as their protein, mediator, and leukocyte contents. Carrageenan [40], turpentine, Evan's blue, Arabic gum, glycogen and dextran, enzymes, antigens, microorganisms, mast cell degranulators, etc. can all be injected intrapleurally to cause pleurisy in rats. With the help of this model, it is possible to quantify the anti-inflammatory activity as well as look into the mechanism of new test medications' actions. The technique works for both steroidal and nonsteroidal anti-inflammatory drug identification.

Experimental pleurisy is induced by injecting 0.1 ml of turpentine oil into right pleural cavity in rats under light anaesthesia as described by Spector. The test medications are given intraperitoneally in graduated doses 1 h before turpentine administration. The rats are beheaded, and pleural exudate is collected half an hour after turpentine therapy. The exudate is eliminated. preferably by washing the pleural cavity with a specified volume of Hank's solution to assure complete recovery of the exudate and integrity of the

cells. Volume of the exudate is assessed as an index of activity of the test medication.

Conclusion

It is clear from the reviewed research literature that many new experimental approaches have been created in recent years to assess the efficacy of anti-inflammatory medications. The candidate drugs could be discovered using *in vitro* experiments and then put through additional testing *in vivo*. One of the goals of using these techniques is to find a medicine to treat inflammation. It's important to interpret the data within the constraints of the model, but the results may still drive medical progress and understanding. Findings from any animal model should be thoroughly verified before being used to people. The level of validation required for translation will depend on how well the selected model reproduces the disease in issue.

Most antirheumatic medications now on the market have demonstrated anti-inflammatory efficacy in carrageenan-induced edema, which utilises the transudative and exudative phases of inflammation. Hydrocortisone, a key steroidal anti-inflammatory medication, failed to demonstrate anti-inflammatory effect in the pleural exudation approach. Therefore, this is not the most suggested method for measuring inflammatory activity. For the detection of anti-inflammatory activity in a new chemical, one can therefore rely on either the suppression of the proliferative phase of the inflammatory reaction, i.e., the inhibition of granulation tissue formation, or the decrease of the exudative phase, carrageenan-induced edema. Carrageenan-induced edema seems most ideal for screening anti-inflammatory medications since it is convenient, less time-consuming, and detects activity in all clinically important pharmaceuticals. The previous procedures are more time-consuming and require higher quantities of drugs. Due to the accurate prediction of the clinical efficacy of TNF- α antibodies using this model, collagen-induced arthritis is a good model for developing novel biotechnology-based medicines for arthritis.

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