Modulation of Lung Inflammation in the Control of Bovine Respiratory Disease

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Introduction

Despite the use of preventive and therapeutic agents, respiratory disorders in cattle occur at an increasing frequency. This could be due to several factors such as an increase of the population density, an increase of the virulence of some pathogens, an increase of air pollution,...

Another explanation could be that, in the past, respiratory disorders have been analyzed only at a pathological but not at a functional level. In the light of new information on animal pulmonary function in health and diseases, it appears that, before the occurrence of irreversible lung damages, there are some reversible dysfunctions at the level of pulmonary ventilation, perfusion or gas diffusion. An early and appropriate correction of these dysfunctions seems to be of critical importance in order to decrease the rate of irreversible lung damages and mortality.

The purpose of this paper is to review the potential strategies which could improve the treatment of respiratory disorders in general and the modulation of lung inflammation in particular. Possible preventive treatments will not be discussed in this review.

Efficiency Factors of the Respiratory Therapy

As for the treatment of other dysfunctions, several factors may play a role in obtaining maximal efficiency, minimal toxicity and minimal residues problems.

An *accurate diagnosis* of the dysfunctions and injuries is necessary before selecting the most appropriate therapeutic strategy. For instance, a bronchodilator will have little effect if the major cause of an obstructive disorder is oedema or hypersecretion in the airways. On the other hand, the presence of diffuse irreversible damage will limit the potential effect of the treatment.

Moreover, *a detailed understanding of the mechanisms leading to inflammation* is important to open new therapeutic methods. Furthermore, the high specificity of some antagonists may lead to decrease some side effects.

The *method of administration of a drug* is also of critical importance in the treatment of respiratory disorders. For instance, a local treatment by aerosol therapy may show a much higher efficiency/toxicity ratio than a systemic treatment.

Finally, the control of respiratory disorders must be assessed more as a *modulable strategy* than as a single standardized act, in order to take into account the individual, breed and species peculiarities for each pulmonary dysfunction. This global and integrated strategy can theoretically be divided into three targets, i.e. suppression of the etiological agents, modulation of lung inflammation and correction of the mechanical disorders. This paper will investigate only the modulation of lung inflammation.

Nature of Lung Inflammation

Acute or chronic inflammation is, in fact, the basis of most pathology and occurs as a consequence of chemical, physical or microbiological damage to tissues[16,58] (Fig 1). For each pulmonary inflammation injury, there are multiple causes and it's becoming clear that the cause leaves its imprint on disease. Chronic bronchitis and Acute Respiratory Distress Syndrome (ARDS) are examples of this. This has practical implications. An anti-inflammatory agent may be useful in some etiological groups, not in others.[69]

Inflammation is the initial, nonspecific response to injury of any sort and its purpose is to eradicate or limit the spread of the injurious agent. The inflammatory response is subdivided in three major events:

-(1) changes in vascular flow and caliber
-(2) increased vascular permeability (vascular leakage)
-(3) cellular events: cells extravasation and phagocytosis.

During chemotaxis and phagocytosis, membrane perturbations occur and cell products are released not only within the phagolysosome or the granule but also potentially into the extravascular space. The chemical mediators that account for the inflammation events originate either from plasma or from cells (Table A).

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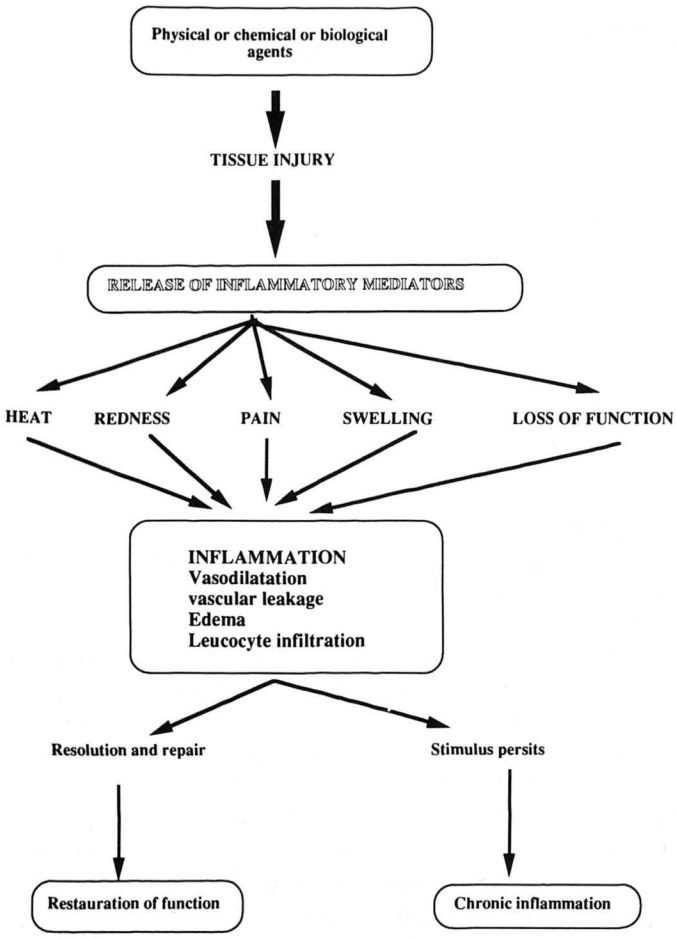

Figure 1. The inflammatory process.

Table A. Mediators of Inflammation

Plasma-derived mediators	Cell-derived mediators
The kinin system : triggered by contact activation of Hageman Factor	*Vasoactives amines* : Histamine and Serotonine (5-HT).
The complement system : the most critical step in the elaboration of biologic functions is the activation of C3 C3 → C3b + C3a ↑ C1 + IgG or IgM Microbial surfaces	*Cell membrane phospholipids-derived* : 1. Platelet-activating factor (PAF) 2. Eicosanoids : a) Cyclo-oxygenase derived products : Prostaglandins (PG) Prostacyclins (PI) Thromboxanes (TX) b) Lipo-oxygenase-derived products : Leukotrienes (LT)
The clotting system : it is a series of plasma proteins that can also be activated by Hageman Factor	*Cytokines* : "pro" and "anti" inflammatory cytokines
	NO
	Oxygen-derived free radicals : H_2O_2, O_2°
	Lysosomal products : lactoferrin, lysozyme alkaline phosphatase...
	Neuropeptides : Substance P, PDGF, TGF-β

Plasma-derived mediators are present in plasma in precursor forms that must be activated to acquire their biologic properties. Cell-derived mediators are normally sequestered in intracellular granules that need to be secreted or are synthesized *de novo* in response to a stimulus. The major cellular sources are platelets, neutrophils, monocytes/macrophages and mast cells (Fig 2).[20,87] Finally, the neurogenic component of the inflammatory response should not be ignored, since neuropeptides like calcitonin gene related peptide (CGRP) and substance P, released from unmyelinated primary afferent fibbers, possess properties which include vasodilatation, enhancement of vascular permeability, leukocyte adherence and migration and the release of histamine from mast cells. In addition, the peripheral sympathetic nervous system contributes to inflammation by enhancing plasma extravasation.[18,21]

Without any modulation, mediators from the inflammatory processes will bind and act through specific receptors of the target cells. The pain component of inflammation arises from sensitization of nociceptive (pain) receptors to the actions of released chemicals. Inflammation, if associated with infection, may be accompanied by fever, as the result of resetting of the "thermostat" in the hypothalamus. Thus, these changes are the four classical signs of inflammation, *rubor et tumor cum calore et delore*. To these Virchow added a fifth sign, *functio laesa* (loss of function) some centuries later.[58]

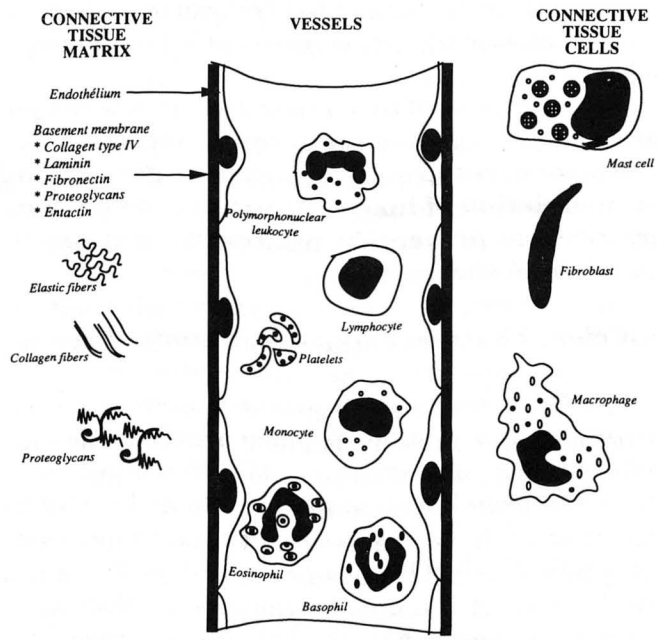

Figure 2. Intravascular cells and connective tissue martix and cells involved in the inflammatory response.

An *intact* inflammatory response is of a great importance. Indeed, inflammatory reaction is a protective mechanism: the rapid outpouring of plasma dilutes bac-

terial toxic products or foreign material and facilitates the accumulation of circulating antibodies. The emigration of leukocytes serves to initiate the phagocytosis and killing of invading bacteria. Their phagocytic function is assisted by the presence of specific opsonizing antibodies. The inflammatory response is, of course, closely intertwined with the process of repair. Inflammation sets into motion a series of events that, as far as possible, heal and reconstitute the damaged tissue. During repair, the injured tissue is replaced by regeneration of native parenchymal cells or by filling of the defect with fibroblastic tissue (scarring), or most commonly by a combination of these two processes.[87]

In fact, it is the magnitude and/or the duration of the inflammatory response that would lead the practitioner to use or not anti-inflammatory drugs. Indeed, a major systemic inflammatory response may lead to disordered function in vital organs such as the kidney or lung and a chronic inflammation can lead to important tissue damages due to proliferative response of cells such as fibroblasts which make a deposition of collagen in the lung. Functional damages are thus present in a irreversible manner.

Consequently, the aim of the modulation of lung inflammation is to block the production and/or the effects of the mediators and modulators of inflammation (i.e. compounds that amplify the actions of others mediators) which interact negatively with the gas exchange processes in the lungs. Moreover, the kinin system is the major responsible of pain and anti-inflammatory drugs can act as analgesic and antipyretic substances and thus these drugs improve the clinical status to the point where animals wish to eat and drink. In this way, nutritional and fluid requirements will be maintained. Species, such as cattle, which rely principally on respiration for temperature regulation, may be under particular stress from infections causing pyrexia. The antipyretic action of anti-inflammatory drugs may be particular useful in such species.[58]

In the bovine species, the anti-inflammatory endogenous system is weak and, therefore, the pulmonary inflammation is often aggressive. The use of anti-inflammatory agents, especially non-immunosuppressant agents, will reduce morbidity and mortality.[41]

Modulation of the Lung Inflammation

Introduction

The inflammatory process can be attenuated or blocked at many levels, such as:

1. Inhibition of the activation of inflammatory cells
2. Inhibition of cell membrane phospholipids-derived mediators
3. Modulation of neurogenic inflammation
4. Inhibition of pro-inflammatory cytokines
5. Miscellaneous.

Inhibition of the Activation of the Inflammatory Cells

The inhibition aims to contend substances which are able to stimulate the production of key-mediators involved in the inflammatory cascade. The inhibition of bacterial lipopolysaccharides (LPS) is justified in this context. Indeed, LPS are able to activate neutrophils and to induce the production of autacoides, oxygen radicals and proteases. This type of treatment will include anti-LPS antibodies,[116] LPS-receptor blockade[76] and inhibition of the activator signal transmission in the target cell.[90]

Other drugs such as cromolyn sodium are known for their stabilizing properties of mast cells.

The anaphylatoxines (C3a and C5a) neutralization, the cellular adherence mechanisms inhibition and the breakdown of the activator signal transmission way for the target cells by use of monoclonal antibodies are studied as possibilities in the inhibition of the activation of the inflammatory cells.[2,20,61,70,86] Actually, these kind of investigations are conducted *in vitro, ex vivo* and on experimental animals.

Inhibition of Cell Membrane Phospholipids-Derived Mediators

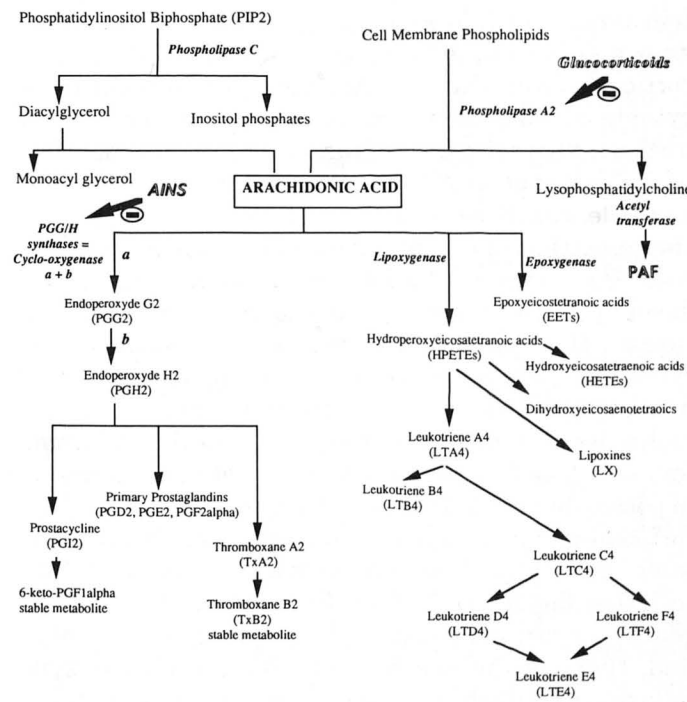

Figure 3. Cell membrane phospholipids

Two distinct families of autacoids that are derived from membrane phospholipids have been identified: the

eicosanoids which are formed from certain polyinsatured fatty acids (principally, arachidonic acid), include the prostaglandins, prostacyclin, thromboxane A2 and, the leukotrienes and modified phospholipids, currently represented by platelet-activating factor (PAF). The eicosanoids (they are called eicosanoids because most of these compounds contain 20 carbon atoms) are extremely prevalent and have been detected in almost every tissue and body fluid.[12,46,47,113] On the other hand, PAF is a potent platelet-aggregating agent and has important pro-inflammatory effects. PAF is generated by a variety of inflammatory cells including neutrophils, monocytes, macrophages, platelets as well as endothelial cells after exposure to activating stimuli.[47,106]

Anti-inflammatory drugs can act at different levels of the arachidonic acid metabolic pathway i.e. in inhibiting enzymes such as the PLA2 or lipo-oxygenase or cyclo-oxygenase.

Global Inhibition of the Arachidonate Metabolites

Steroids

The global inhibition of arachidonate metabolites may be obtained by anti-inflammatory steroids.

Action mechanisms

Actually, new works on mechanism of glucocorticoids activity suggest that a large part of this effect occurs because the drug stimulate production of a protein IκBα, which locks up a key activator of the genes known as NF-κB, so that it can't do its job. When glucocorticoids enter the cell, they bind to a receptor in the cytoplasm and form a complex that moves into the nucleus, where it acts as a transcription factor that turns genes either on or off. Among the genes turned on, for example, are those involved in stress reactions, such as the genes that make the enzyme needed to produce glucose. But many of the immune system genes turned down by glucocorticoids appear to lack a necessary feature for that regulation. These include genes encoding cytokines, such as interferons and interleukins, which activate immune cells, as well as those for cell adhesion molecules that draw immune cells into the inflammatory site. The fact that a wide range of these genes are in possession of the DNA sequence that binds the glucocorticoid-receptor complex suggests that this complex works indirectly. Glucocorticoids prevent another transcription factor, AP-1, from binding site to its target genes and turning them on. Among the genes so inhibited, there is the one for protein-dissolving enzyme collagenase, which is a major contributor to the tissue damage of inflammation. Despite that, the glucocorticoids' immune effect are not all explained by blocking AP-1. Indeed, in the presence of glucocorticoids, the amount of NF-κB that went into the nucleus was significantly diminished while IκBα concentrations were higher than expected. This suggested that glucocorticoids were working through IκBα. In fact, glucocorticoids stimulate production of another protein that inhibits NF-kB action, and the likely candidate is IκBα . Glucocorticoids increase IκBα concentrations within the cell, allowing the protein to hold NF-κB in inactive form in the cytoplasm even under conditions when it would normally be released to move into the nucleus (Fig 4).

The discovery of the central role of NF-κB inhibition in suppressing immunity is of great importance and opens new ways to improve anti-inflammatory drugs. Once the action mechanism is known, research can begin to set up rational screens for others NF-κB inhibitors and to look for a better safety-side effect profile.[4,6,66] Moreover, the induction of the inducible form of Nitric Oxide Synthase (NOS) is potently inhibited by glucocorticoids, the induction of the NOS is prevented by inactivation of NF-κB which appears to be the most important transcription factor in regulating inducible NOS gene transcription.[6,73] Glucocorticoids inhibit the synthesis of several inflammatory mediators such as inducible cyclo-oxygenase derivatives and this also appears to be via NF-κB activation. Glucocorticoids also inhibit the gene transcription of a form of phospholipase A_2 induced by cytokines. Whether steroids also modulate expression of 5-lipo-oxygenase has not yet been established, but studies of cysteinyl-leukotriene formation *in vivo* in patients with asthma indicate that doses of glucocorticoids which are effective clinically do not significantly reduce the excretion of LTE_4, the major metabolite of LTD_4. Thus LTB_4 and PAF activate AP-1 binding in inflammatory cells and this may be inhibited by steroids.[6]

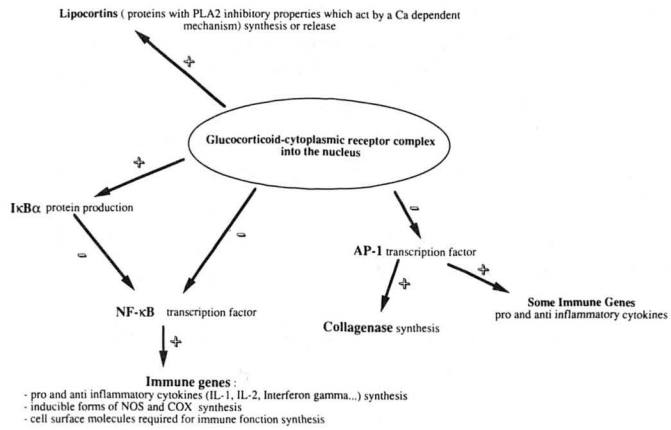

Figure 4. Possible anti-inflammatory mechanisms of corticosteroids.

General uses of steroids

Glucocorticoids are highly effective in the control of inflammatory process: their inhibitory effects on

cytokines are of particular relevance. Anti-inflammatory steroids are widely used for the therapy of immune and non-immune inflammatory conditions. A number of respiratory conditions, both allergic and non allergic, have been treated with systemic steroids. They include acute respiratory distress syndrome in cattle and chronic obstructive pulmonary disease in horses. Steroids have been used extensively in the therapy of anaphylactic, endotoxic, and hemorrhagic shock but there are several disadvantages. Steroids possess a slow onset of action and very large doses are required, making therapy in cattle and other large animal subjects often impractical and/or prohibitively expensive. Moreover, the immunosuppressant actions are very undesirable and where antimicrobial cover cannot be provided, such as many viral infections, corticosteroid therapy is generally totally contraindicated.[4,58,77]

Recently, Olaerts *et al*[79] had compared a nonsteroidal and a steroidal anti-inflammatory treatment in an experimental model of bovine pneumonic pasteurellosis. The results of this experiment suggested that modulation of lung inflammation in calf pneumonic pasteurellosis is more appropriate by using ketoprofen (non steroidal drug) instead of a dexamethazone solution.

Administration route

The inhaled route is considered to be much safer than the oral supply of steroid.[60] Consequently, corticosteroids such as beclomethasone dipropionate, bethamethasone valerate, budesonide, triamcinolone acetonide and flunisolide have been synthesized. They combine the characteristics of enhanced topical anti-inflammatory potency with little systemic activity.[102] During an experimental porcine septicaemia, nebulized beclomethasone dipropionate improved pulmonary function and outcome.[112]

Side effects

Anti-inflammatory steroids may lead to important side-effects such as an inhibition of the hypothalamic-pituitary-adrenal axis (i.e. sodium and water retention and resulting oedema and hydrogen and potassium loss leading to hypokalaemia and metabolic alkalosis), depression of the immune system and osteoporosis. Protein catabolism with long-term therapy causes muscle wasting and in the short term may delay wound healing. The lipolytic action of glucocorticoids is associated with redistribution of body fat with the characteristic "moon face" appearance in man and pendulous abdomen in animals. Moreover, glucocorticoids are insulin antagonists and may, therefore, exacerbate or precipitate diabetes mellitus and cessation of therapy abruptly after a prolonged course of treatment may reveal a serious degree of adrenal insufficiency.

Moreover, steroids are generally contraindicated in late pregnancy, since they may induce parturition, in subjects with corneal ulcers and more general, in viral infections. Gastro-intestinal side-effects include reduced motility, thinning of the gastric mucosa and reduced mucus production.[50,77]

Resistance to anti-inflammatory actions of steroids

Resistance to anti-inflammatory and immunomodulatory effects of glucocorticoids is described in several inflammatory and immune diseases such as rheumatoid arthritis and human immunodeficiency virus infection. This resistance differs from the familial glucocorticoid resistance and these patients are not Addisonian. Steroid resistance is often subdivided in 2 forms: primary and secondary steroid resistances. The possible mechanism of primary steroid resistance in asthma could be an increased activation of activator protein-1 (AP-1) that results in the complexing of glucocorticoid receptors, thus preventing the anti-inflammatory action of steroids. Secondary resistance may arise in the presence of cytokine-mediated inflammation through an interaction between the cytokine-activated transcription factors, such as AP-1, and the glucocorticoid receptor, resulting in a reduced availability of glucocorticoids receptor for control of the anti-inflammatory response. This can only be overcome by increasing the dose of glucocorticoid administered.[6]

Preventive Dietary Alimentation

On the other hand, modulation of the membrane fatty acid composition by dietary manipulation (for instance by dietary supplement with α-linoleic acid) may be adopted in order to decrease the production of proinflammatory mediators for instance in response to endotoxin. Such nutritional investigations were successfully carried out in rats,[17] dogs[62] and horses.[44,75]

Inhibition of the Cyclo-Oxygenase Pathway

Action mechanism

The major categories of nonsteroidal anti-inflammatory drugs (NSAIDs) include salicylates (aspirin), propionic acids (ibuprofen, fenoprofen, ketoprofen and naproxen), pyrazolones (phenylbutazone), anthranilic acids (meclofenamic acid), fenamates (piroxicam, meloxicam) and aminonicotinic acids (flunixin meglumine). The NSAIDs are a diverse group of compounds that are antipyretic, anti-inflammatory and analgesic agents. They share a basic mechanism of inhibiting cyclo-oxygenase, resulting in decreased production of prostaglandins.[50,107] However, it is well recognized that the anti-inflammatory and analgesic potency of these drugs does not always correlate with their activity as cyclo-oxygenase inhibitors.[18,71] For example, the propionic acid derivative carprofen has only weak enzyme inhibitory activity but demonstrable anti-

oedematous and analgesic actions.[18,56] At a clinical level impressions have been gained that some NSAIDs are superior to others in their analgesic properties, but the evidence is primarily anecdotal. Nevertheless, it is clear that flunixin provides excellent analgesia in equine colic, to the extent that developing symptoms may be masked for several hours.[18] Other mechanisms of action have also been proposed, i.e. capture of free radicals, decoupling of the oxydative phosphorylation, inhibition of kinin release, inhibition of proteoglycans degradation, inhibition of leukocyte migration, inhibition of neutrophil adhesion reactions,...[20,103] Recently, Kopp and Ghosh[54] found that activation of the transcription factor nuclear NF-κB is inhibited by aspirin and salicylate. Indeed, the presence of salicylates blocked the degradation of Ik-B protein, which suggests that they were interfering with a component of the signalling pathway, most likely a serine-threonine kinase. However, Frantz and O'Neill[31] had studied the effect of sodium salicylate and aspirin on NF-κB and suggest that the ability of salicylate to inhibit NF-κB is due to a non-specific inhibition of cellular kinases. However, newer results on NF-κB studies are of great interest since NF-κB is involved in the production of a large number of cytokines, acute phase response proteins, and adhesion molecules; it is an ideal target for inhibitors of an inflammation reaction.

These anti-inflammatory properties of NSAIDs probably explain the efficacy of NSAIDs during some acute respiratory diseases.[26,33] On the other hand, the lack of beneficial effects of NSAIDs during other respiratory syndromes may be explained by the fact that NSAIDs not only inhibit the production of prostaglandin (PG) $F_{2\alpha}$ and thromboxanes (TX), which have negative pulmonary effects, but they also inhibit the production of PGI_2 and PGE_2, which are beneficial for the pulmonary function.[26]

Recently two forms of human cyclo-oxygenase (COX) enzyme have been identified: COX-1 which is constitutively expressed in many tissues, and COX-2, which is associated with pro-inflammatory prostaglandins production in several tissues and cell types. The identification of an inducible form of the COX enzyme led to the hypothesis that selective inhibition of COX-2 would be anti-inflammatory without side effects as decrease of PGI_2 and PGE_2. Indeed, it seems that great differences exist between actual anti-inflammatory drugs in the ability of COX-2 inhibition.[37,67,72,100,108,115]

Furthermore, NSAIDs do not inhibit other proinflammatory mediators nor the lipoxygenase pathway. Consequently, given that the amount of available arachidonic acid is increased through the inhibition of the cyclo-oxygenase pathway, NSAIDs may enhance the production of leukotrienes[57] which may negatively act on the pulmonary function.[45]

Ketoprofen has recently been introduced for veterinary usage (in 1990), with claims that it inhibits both cyclo-oxygenase and 5-lipo-oxygenase and that, additionally, it possesses anti-bradykinin activity. Direct evidence for these claims has not, to date, been presented with recommended doses rates in animal species for which the drug is licensed.[18] The ketoprofen anti-bradykinin activity was investigated in comparison with phenylbutazone, and flunixin meglumine in horses[55] and the results showed that both flunixin meglumine and ketoprofen inhibited bradykinin-induced swelling and that neither drug had any effect on exudate leukotriene B4 concentration. However, the relative toxicity of phenylbutazone, flunixin meglumine and ketoprofen was studied in healthy adult horses[64] and the toxic potential of the 3 NSAIDs was greatest for phenylbutazone, less for flunixin meglumine and least for ketoprofen in clinically normal horses. However, in a model of anaphylaxis in the conscious guinea pig, ketoprofen increased slow reacting substance of anaphylaxis release and the drug's activity as an inhibitor of 5-lipo-oxygenase is therefore in doubt.[19,111] Moreover, Landoni and Less[55] had compared pharmacodynamics of flunixin, ketoprofen and tolfenamic acid in calves after intravenous administration. In this study, an acute inflammatory reaction was induced in tissue cages by intracaveal injection of carrageenan solution. None of these drugs (*i.e.* ketoprofen, tolfenamic acid and flunixin) affected the concentration of LTB_4 but the synthesis of serum TXB_2 and PGE_2 was inhibited by all the drugs. In another recent study, Twomey and Dale[103] examined the effects of several NSAIDs on the oxidative burst of neutrophils induced by PAF and the post receptor stimuli, fluoride and dioctanoylglycerol. These drugs could be classified into those which increase superoxide generation (meclofenamate, benoxaprofen), had no effect (aspirin) or suppressed the response (phenylbutazone, piroxicam). The effects of NSAIDs on neutrophil oxidative burst and on neutrophil migration are assumed to be mediated by a cyclo-oxygenase independent mechanism.[18] Until recently, the central actions of NSAIDs were poorly understood and therefore potentially underestimated. Malmberg and Yaksh[65] have demonstrated a direct spinal action of NSAIDs, inhibition of the central hyperalgesia induced by activation of spinal glutamate and substance P receptors. These authors concluded that spinal prostanoids are involved in augmenting the processing of pain information at spinal level, since the inhibitory action of NSAIDs is stereospecific for the enantiomer producing cyclo-oxygenase blockade.[18]

General uses

The major clinical use of NSAIDs is for pain suppression in acute tissue inflammation. A good analgesia and a reduction of oedema swelling can be obtained.

NSAIDs have been used to treat acute inflammatory conditions caused by microbial agents. The severe and acute pulmonary oedema associated with pneumonia in calves is suppressed.[80] In experimental endotoxaemia challenges, NSAIDs improve the clinical status of subjects and it is especially important when NSAIDs are given in pretreatment. PGE_2 is a pyretic agent released as a result of the action of endogenous pyrogens such as IL-1. The NSAIDs inhibition of PGE_2 synthesis accounts for their antipyretic action when diseases are accompanied by fever.[18] NSAIDs have been used in the treatment of respiratory disease produced by the parainfluenza type 3 virus in calves.[93]

Aspirin differs from the AINS in that its inhibitory action on cyclo-oxygenase is irreversible. As aspirin concentration falls the cell's ability to form the products of cyclo-oxygenase metabolism is restored. The platelet is anuclear and the enzyme is thus inactivated permanently, i.e. for the lifespan of the platelet. Partial inhibition may persist for several days since platelets are replaced at the rate of approximately 10 per cent per day. The full potential for the antithrombic action of aspirin in veterinary medicine has not yet been fully explored. The use of two or more NSAIDs in combination generally has no clear advantages over the administration of a single dose at higher dose rate. Most NSAIDs probably act by the same mechanisms and they are likely to be additive in their toxic effect as well as their therapeutic effects. However, there are potential advantages with some combination such as phenylbutazone and isopyrin: phenylbutazone may be a more potent anti-inflammatory drug whereas isopyrin is a more effective analgesic.[91]

Side effects

The toxic effects of most NSAIDs are probably caused primarily by inhibition of cyclo-oxygenase. Most common side-effects are irritation leading potentially to irritation of the gastrointestinal mucosa. All NSAIDs produce this effect at high dose rates and with most drugs some gastric irritation is associated with oral dosing at therapeutic dose levels. The vasodilatator eicosanoid PGI_2 may be a local hormone controlling blood flow to the gastrointestinal mucosa. By inhibiting the synthesis of PGI_2 NSAIDs may produce ischaemia and hence hypoxia of the mucosal surface. This may be the mechanism that causes erosion and hence ulceration of tract mucosa. Useful data on the ulcerogenic actions of particular NSAIDs in cattle and any relationship to age, does administered and route of administration are generally lacking. However, there is no reason to suppose that ruminant species are not susceptible to the ulcerogenic action of these drugs.

NSAIDs anti-inflammatory drugs may be nephrotoxic. At high dose rates they may produce tubular nephritis. In horses papillary necrosis has been described in animals that are receiving clinical doses of NSAIDs. However, this occurred only when horses on NSAID therapy also had restricted access to water. Cholestatic and parenchymal hepatotoxicity have also been reported in animals. Since most NSAIDs are metabolized in the liver, any toxicity to parenchymal cells might, potentially, lead to a cycle of drug accumulation and toxicity. Again, evidence for drug-induced hepatotoxicity has been described for horses receiving high doses rate of phenylbutazone, but whether similar effects occur in cattle has not been reported.

A number of blood dyscrasias have been reported in human subjects treated with NSAIDs, generally over long periods. Similar blood cells effects in dogs, cats and horses have been reported. However, with the use of clinical doses rates of NSAIDs, the incidence of blood dyscrasias in animals seems to be low.[18,50,58,85]

Specific inhibition of thromboxanes

Thromboxane A_2 (TXA_2) promotes platelet aggregation and vasoconstriction.[12] Drugs such as dazoxiben, that inhibit thromboxane synthetase preferentially,[84] have antithrombotic effects *in vivo*. Such inhibitors are more selective than salicylates. They inhibit the production of TXA_2 without interfering with PGI_2 production.[12] Thromboxane inhibitors were assessed to reduce vascular thrombi.[36] In endotoxic horses, a selective thromboxane synthetase inhibitor did not lead to any clinical improvement.[74]

Specific inhibition of leukotrienes

Among leukotrienes issued from the lipoxygenase pathway, leukotriene C_4 (LTC_4), LTD_4 and LTE_4 take part in the pulmonary inflammatory process and pulmonary dysfunction observed during respiratory disease.[45,99] The use of specific blockers seems to decrease the intensity of these phenomena.[15,45]

In cats, LTD_4/LTE_4 blockers enhanced the survival rate and prevented the acute pulmonary effects observed in endotoxinic shocks[84] although other investigations failed to point out any advantage of such a blockade.[1,82]

Specific Inhibition of Platelet Activating Factor (PAF)

Pathological functions of PAF have been widely investigated.[14,43] During acute respiratory distress syndrome, PAF seems to be responsible for several pulmonary dysfunctions.[13,48]

Many compounds have been described that selectively inhibit the actions of PAF *in vivo* and *in vitro*.[89] Furthermore, PAF-blockers are also able to modulate

the 5- and 12-lipoxygenase pathway.[81] The development of PAF antagonists is still in its early stages, and their clinical utility has not yet been established.[12] Some reports seem to forward beneficial effects on the pulmonary function[48,49] although this is refuted by others.[14]

The pulmonary dysfunctions generated by PAF perfusion are completely prevented by the administration of a PAF antagonist, *i.e.* WEB 2086, both in horses[29] and in cattle. Indeed, a PAF challenge induced severe and reversible changes in lung mechanics, heart rate and platelet count in unsedated healthy calves and selective blockade with WEB 2086 I.V. abolished PAF-induced dysfunctions.[105]

Modulation of Neurogenic Inflammation

The neuropeptides involved in the neurogenic inflammation, i.e. substance P, neurokinin A and calcitonin gene-related peptide,[5,22] could be inhibited by three different mechanisms, i.e. (1) inhibition of the activation of the C-fibbers by local anesthesia,[114] cromolyn sodium,[25] nedocromil sodium[51] and nebulized furosemide,[109] (2) inhibition of the sensory neuropeptide release by opioids,[8] γ-amino butyric acid[3] and α_2-agonist,[21] and (3) inhibition of sensory neuropeptide effects by specific tachykinin receptor blockade.[96]

Many other neuropeptides are involved in lung inflammation.[5] However, the usefulness of their inhibition by specific antagonists or antibodies during pulmonary diseases remains to be proven.

Modulation of Pro-inflammatory Cytokines

Starting inflammatory process: "pro" and "anti" inflammatory cytokines

The first mediators involved in the start of inflammation reaction are, indubitably, the vasoactive amines. The richest source of vasoactive amines are the mast cells that are normally present in the connective tissue adjacent to blood vessels. They are also found in blood basophils and platelets. The activation of the complement cascade, the Hageman factor and the factor XII by agents from the inflammatory site induces the bradykinin release and consequently causes locally the contraction of smooth muscle, dilatation of blood vessels and pain. Indeed, these mediators may act on the afferent nerve endings and are responsible of the inflammation associated pain. Moreover, they can also induce oedema and increase the local temperature. The complexity of the inflammatory response is due to the fact that mediators induce the release of others mediators making an autocrine maintenance of the inflammatory process. After this immediate reaction, the leukocytes and endothelial cells which were activated by the primary inflammation process release cytokines. Cytokines are short-acting soluble mediators and possess a wide spectrum of effects and some are produced by several different cell types. Classically, cytokines are subdivided in four functional categories: (1) cytokines that mediate natural immunity such as IL-1, TNFα, and IL-8, (2) cytokines that regulate lymphocyte growth, activation and differentiation such as IL-2, IL-4, IL-5 and IL-12, (3) cytokines that activate inflammatory cells such as IFNγ, TNFα, TNFβ and IL-8 and, finally (4) cytokines that stimulate hematopoiesis such as (GM)CSF and (G)CSF. It should be noted that many cytokines are pleiotropic in their effect. More recently, a new notion of functional effect of cytokines that act during the inflammatory process must be taken into account. Indeed, cytokines possess either pro-inflammatory or anti-inflammatory activity. The cytokines pro-inflammatory action is currently well known and new cytokines and their action are reported every day. The major role of pro-inflammatory cytokines are locally to induce the classical signs of inflammation i.e. *rubor, tumor, calore et dolore* via the induced release of membrane phospholipids mediators (TXA_2, PGH_2, LTC_4, PAF, . . .) from neutrophils, monocytes, platelets and endothelial cells. Free oxygen radicals, thrombin and vasoactive amines are also released at the level of inflammation site. The second step of the inflammation is the systemic reaction characterized by hematologic modifications, by a decrease in plasmatic iron and zinc concentrations, and by a specific decrease of some proteins called proteins of acute inflammatory phase. These proteins are synthesized principally by the liver. A wide range of cytokines act on hepatocytes to increase the release of acute inflammation phase proteins. However, some cytokines are anti-inflammatory agents such as IL-4 that inhibits the IL-1, IL-6, IL-8 and TNFα release from activated-macrophages. Likewise, IL-1 and TNF productions are diminished by IL-6. Lymphocytes T product the IL-10 which is able to inactivate the macrophages synthesis of IL-1, IL-6, IL-8 and TNF. The anti-inflammatory activity may reflect a decrease of pro-inflammatory cytokines receptors or the specific inhibitors action. However, it is interesting to note that soluble forms of receptors (particularly TNF receptors) is an important parameter to take into account in "pro" and "anti" inflammatory signals. Indeed, some of natural inhibitors of TNF were been identified as the soluble form of TNF receptor. Specific activation of neutrophils can allow neutrophils to release these surface receptors (Fig 5).[41] In conclusion, the cytokines network act at all levels of inflammation i.e. initiation, amplification, maintenance and disappearing of inflammatory process and is able to eliminate causal agent when coordination and regulation are in harmony.

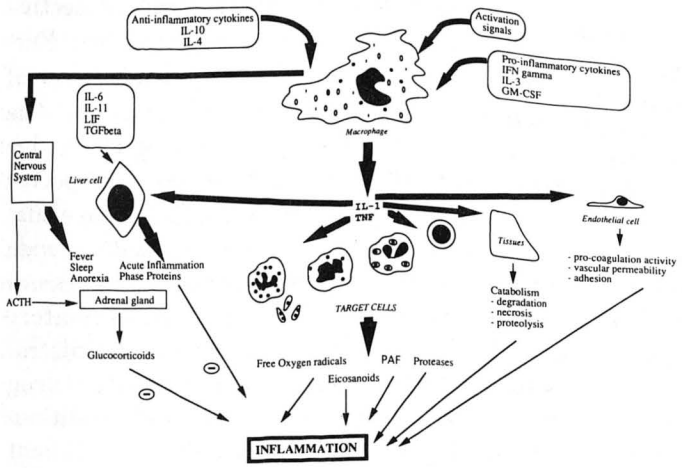

Figure 5. Neutrophils release of cytokines.

The use of an anti-inflammatory therapy is therefore necessary when this harmony is lost by the overworking of the anti-inflammatory cytokines and/or by a severe causal agent attack.

Inhibition of Pro-Inflammatory Cytokines

The inhibition of pro-inflammatory cytokines may be performed through an inhibition of their synthesis, an inhibition of their soluble form or an inhibition of their action on the target cell.[20]

A modulation of the cytokine synthesis may be achieved through an inhibition of the gene transcription,[38] a translation controlled by means of complementary anti-sense nucleotides,[11] an inhibition of the cytokine release,[88] or an inhibition of promediator transformation.[24]

Inhibition of soluble cytokine may be achieved through the use of anti-cytokine antibodies. In this field, anti-tumor necrosis factor antibodies were able to reduce the mortality from endotoxinic shock.[94] The soluble cytokine may also be blocked by exogenous soluble receptors[28] or by binding proteins.[52]

Recombinant interleukin-1-receptor antagonist (IL1RA), which specifically binds type I and II receptors,[27] has been successfully assessed during pulmonary inflammation processes[104] and during various septic shocks.[28]

Miscellaneous

Inhibition of cytolytic inflammatory products

Antiproteases

Proteases such as elastase and thrombin are responsible for pulmonary inflammatory injuries.[91]

In vitro results suggest that antiproteinases protect lung endothelial cells from endotoxin injury.[102]

In sheep, a single dose of aerosolised secretory leukoprotease inhibitor (rSLPI) results after 3 hours in a four-fold decrease of the antineutrophil elastase capacity in epithelial lining fluid. This effect has a half-life of 12 hours. This demonstrates that it is feasible to use aerosolised rSLPI to directly increase the antiprotease capacity of the lung, particularly on the pulmonary epithelial surface.[110]

Oxygen radical scavengers

Free radicals derived from oxygen during respiratory disorders are able to induce severe pulmonary injuries.[97] In dogs, inorganic particles phagocytised by lung tissues cause alveolar macrophages to generate reactive oxygen compounds extra- and intracellularly.[7]

Inhibition of these compounds may be obtained by enzymatic antioxidants such as catalase, superoxyde dismutase and gluthation peroxydase,[68,92] by non enzymatic antioxidants such as vitamin A, C and E, taurine,[64] N-acetylcysteine[9] and dimethylthiourea,[30] and by iron chelators such as desferioxamine.[42]

Inhibition of neutrophils and macrophages

Neutrophils and macrophages are required for the acute lung injury induced by many pathogens. In neutrophil-depleted calves, blood gas values, heart and respiratory rates, and numbers of circulating leukocytes do not change after inoculation with *Pasteurella haemolytica*.[95] Consequently, substances such as dapsone or inhibitors of proteine-kinase C could be useful to reduce neutrophil-mediated injury to pulmonary endothelial cells.[98]

Inhibition of autacoids

The pathways to the production of autacoids should be inhibited when these substances generated during the inflammatory process interact negatively with the defence system of the organism.[10]

Complement

The complement system consists of 20 plasmatic component proteins. This system functions in immunity for defence against microbial agents, culminating in lysing microbes by the so-called membrane attack complex. In the process, a number of complement components are elaborated that cause increased vascular permeability, chemotaxis, and opsonization.[87] Moreover, the release of leukotrienes and some smooth muscle fibbers are stimulated.[87] These mechanisms have been incriminated during some bovine respiratory diseases.[53]

Modulation of the proinflammatory properties of the complement could be obtained by the use of monoclonal antibodies or specific antagonists such as SC-41930.[32]

Histamine

Histamine, which is produced during the acute respiratory distress syndrome,[78] is able to induce a contraction of bovine respiratory smooth muscle.[39] However, the importance of anti-H_1 substances seems to be trivial in the therapeutic schedules of animal respiratory diseases.[40] However there is also limited knowledge of the pharmacokinetics of available drugs in veterinary medicine.[18]

Serotonin (5-Hydroxytryptamine)

The administration of exogenous 5-hydroxytryptamine in healthy unsedated calves induces a reversible bronchoconstriction and pulmonary vasoconstriction[23] which can be prevented by a 5-HT_2-receptor blockade.[59] In double-muscled calves, such a 5-HT_2-receptor blockade has been proved to be effective during both early and late stage of a naturally occurring Acute Respiratory Distress Syndrome.[33,34] Furthermore, economic, clinical and functional consequences of this blockade have been assessed during Shipping Fever pneumonia.[35]

Bradykinin

Bradykinin is a nonapeptide issue of the kinin system. This substance is a potent vasodilatator and permeability increasing agent. In addition, bradykinin activates nociceptors. Phospholipase A_2 is stimulated by bradykinin and prostaglandins are known to synergise with bradykinin in causing pain and oedema. BK1 and BK2 are the two bradykinin receptors actually identified and selective antagonists have been characterized for BK receptors and may prove to be of value in limiting certain aspects of the inflammatory response.[58]

Conclusions

The control of pulmonary dysfunction caused by respiratory diseases is of crucial importance for the health and production of cattle. Modulation of lung inflammation plays an important part in this control. The systematic and routine use of powerful anti-inflammatory drugs that act in a spectacular manner for the short term but possess long term important adverse side effects, must be replaced by more specific therapy. This will require precise identification of the etiologic agent, complete assessment of clinical status of the patient, and efficacy/toxicity matching of available anti-inflammatory drugs. This leads to some key questions such as: what are the appropriate clinical parameters for which an anti-inflammatory therapy is indicated? Indeed, the modulation of inflammation is of interest only if the negative effects of inflammation atteign its positive effects. For example, the organism possesses some protective systems such as the anti-inflammatory cytokines. Furthermore, for each type of disease which kind of anti-inflammatory drug is most appropriate to use? The number of specific antagonists is increasing every day but most of them are studied as pretreatment in specific clinical trials. To the practitioner in the field, the situation is very different and super infections with a wide range of etiologic agents are an ever present risk. Drugs combinations (*i.e.* antibiotics and anti-inflammatory agents) are made empirically. The route of administration, the timing of administration and the eventual drug interactions must be rigorously investigated. Additionally, environmental outcomes of therapeutic treatment in cattle are rarely taken into account. However, it seems necessary to investigate this latter aspect from an economical and ecological point of view.

In conclusion, bovine therapy in general, and use of anti-inflammatory drugs in particular, should be envisaged as a strategic therapy that gives the practitioner the necessary means to practice focussed and informed treatment that is adapted to each kind of pathology encountered in bovine medicine.

Summary

This thesis reviews the anti-inflammatory agents that could be available to the practitioner in the future in order to enhance the efficacy of treatment of respiratory diseases in cattle.

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The Development and Use of a Long Acting Oral Bolus Containing Baquiloprim for the Treatment of Calf Scours

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Ensuring a complete course of antibacterial treatment for calf scours in farms with large herds and limited labour can be aided by provision of long acting antibacterials. Traditionally only available with injectable antibacterials, the introduction of a four day oral bolus based on baquiloprim (Zaquilan™ 15g 4 Day Bolus for Calves) is the first available long acting oral antibacterial in Britain (Williams, P.C.W. 1994).

Baquiloprim's metabolic half-life at 10 times the elimination half-life of trimethoprim or amoxycillin gives high, persistent serum levels. Its mode of action is the same as that for trimethoprim and addition of a sulphonamide gives potentiation. This combination has a wide *in vitro* spectrum of activity against at least 15 genera of bacteria. Significantly higher *in vitro* efficacy than for trimethoprim was shown (White, G., Daluge, S.M., Sigel, C.W., Ferone, R., Wilson, H.R. 1993) in a mouse protection test in which mice were challenged with a strain of *E. coli* (Effective dose (ED_{50}) 9.5 ± 1.0 vs 19.9 ± 4.7).

A *Salmonella dublin* challenge trial in calves gave 92% survival for a 2 bolus compared with 17% for amoxycillin/clavulanic acid boluses (sensitive *in vitro*) and 50% for untreated controls. Field trials in calves with scours gave a 72% clinical response compared to 69% clinical response with amoxycillin/clavulanic acid. Use of multilayer formulation technology enabled development of a four day bolus with a slow release core and rapid release outer layer. The conclusions are that the high persistent serum levels achieved with baquiloprim gave efficacies as good as or better than the G.B. market leading twice daily product with a quarter the dosing with the 2 day bolus and an eighth the dosing with the 4 day bolus, thus ensuring complete and effective treatment on farm with reduced labour input.

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