**Teaching Unit 12**

**THERAPEUTIC EFFECTS OF INTRAVENOUS IMMUNOGLOBULINS, CORTICOSTEROIDS, AND NONSTEROIDAL ANTI-INFLAMMATORY DRUGS**

The term "immunomodulation" is usually defined as the alteration of immune response which may decrease or increase the immune responsiveness. Enhancement in the immune responsiveness is called immunostimulation, while reduction in the immune responsiveness is called immunosuppression. Thus, an immunomodulators are agents which can modulate the activity of the immune system by acting immunosuppressive (suppressing the immune response) or immunostimulating (increasing the immune response).

In transplantation and autoimmune diseases, the goal of therapy is to suppress the immune response. However, in some cases, such as tumors and immunodeficiency disorders, the goal of therapy is to enhance the immune response. Some substances can act both immunosuppressive and immunostimulating by binding to different receptors on different cellular targets of the immune system.

**INTRAVENOUS IMMUNOGLOBULINS**

Immunoglobulins or antibodies are molecules synthesized by В lymphocytes-plasma cells. They recognize a wide range of specific antigenic determinants, and represent the main effector mechanism of humoral immunity. The unique structure of immunoglobulin provides a wide range of specificities of this molecule.

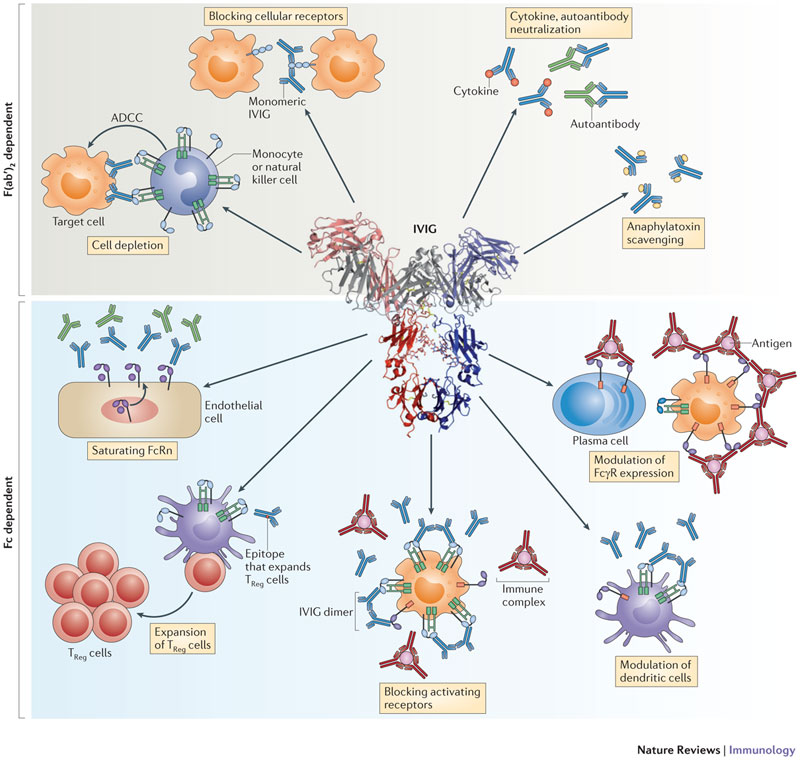
More than 70 years ago, it was noted that a fraction of human serum isolated by ethanol could be used in the therapy of some infections. By improving this method, it was shown that immunoglobulins are the active principle of this therapy and particularly effective if they are obtained from people who have overcome an infection. These immunoglobulins can prevent or alleviate the symptoms and clinical course of an infectious disease in person infected with the same microorganism.

Purification of immunoglobulins from serum is obtained a preparation that can also be administered intravenously (hence the name intravenous immunoglobulins). Immunoglobulin preparations are prepared from pooled plasma obtained from healthy blood donors (intravenous immunoglobulins) or from donors who have a high titer of relevant antibodies in the serum (hyperimmune gammaglobulins) that can be given in the prophylaxis of specific infections.

Intravenous immunoglobulins (IVIGs) are a collection of pooled IgG class immunoglobulins received from minimum of 1000 different plasma donors. IgM and IgA are present in trace amounts. IVIGs can exert numerous immunomodulatory and anti-inflammatory effects (Figure 1) in two ways: by binding different antigens through their Fab region and by performing effector functions through their Fc region.

IgG monomer comprises more than 90% of the proteins in an IVIG preparation, and it is the principal component required for the therapeutic effect of IVIGs. IgM and IgA are present in trace amounts. The others additives, such as sugars, amino acids or albumin, are used to stabilize the IVIG preparation. Originally IVIGs was used to treat immunodeficiency diseases. Later on, the use of IVIGs extended to autoimmune diseases as well. IVIGs exert different effector functions via its variable regions (two Fab regions), as well as constant Fc region.

For more than 25 years, immunoglobulins have been used as replacement therapy (passive immunization) in patients with primary immunodeficiency (e.g. hypogammaglobulinemia and agammaglobulinemia) to reduce risk of infections. IVIG preparations contain antibodies with a large repertoire of specificity for various antigens with which donors have been in contact during their lives. In addition, most intravenous immunoglobulin preparations contain intact immunoglobulins with a half-life of 3 weeks.

**Figure 1**

**MECHANISM OF ACTION OF INTRAVENOUS IMMUNOGLOBULINS**

Intravenous immunoglobulins predominantly consist of pooled polyclonal IgG and it is used therapeutically for many conditions including immunodeficiency as well as autoimmune and inflammatory disease.

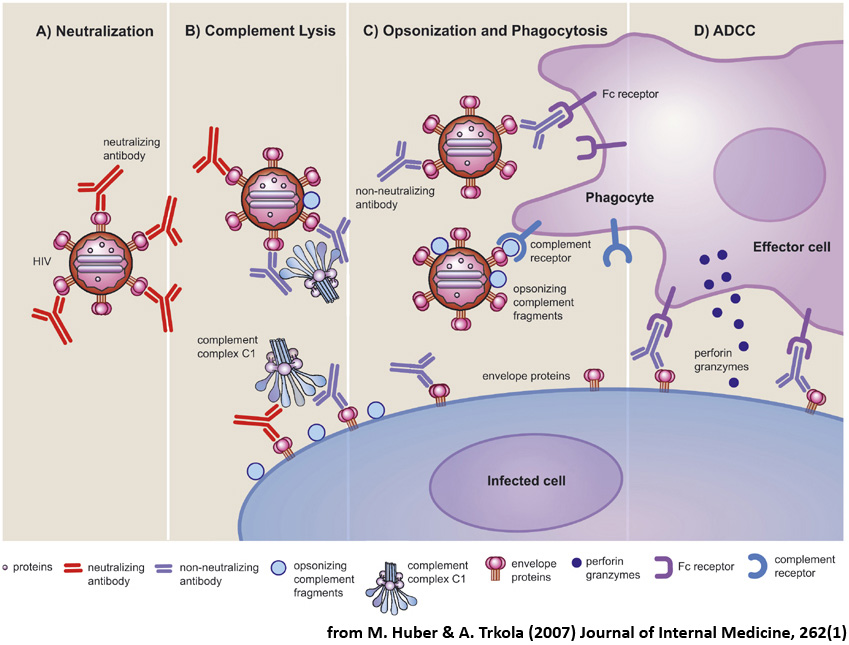
Intravenous immunoglobulins exert numerous therapeutical effects through its Fab or Fc regions. A plethora of Fab- or Fc region-mediated mechanisms action of has been described that might act separately or in concert, depending on pathogenesis or stage of clinical condition.

**Fab-mediated effects of IVIGs**

Major functions of IVIGs that are exert via **Fab region** are neutralization, namely:

***Neutralization of microorganisms and its toxins***

Important functions of the Fab region of IVIG are neutralization of microorganisms (by interfering with microorganisms attachment to host cell receptor or by targeting various steps in their lifecycle) and non-specific opsonization by binding to the surface of microorganisms leading to phagocytosis (Figure 2). It is most often used to treat immunodeficiency disease. Thus, this therapy can help people with weakened immune systems fight of infections.



**Слика 2.**

***Neutralization of С3а and C5a anaphylotoxins***

It has been experimentally shown that the administration of IVIG can neutralize anaphylatoxins C3a and C5a. The mechanism of action of IVIG is achieved through the Fab region. The results of a favorable therapeutic effect were recorded only in animal models: the experimental model of asthma (mouse) and the experimental model of shock (pig), but in clinical studies of asthma therapy, the use of IVIG did not show satisfactory results.

However, it has been reported that Fab region of IVIGs neutralizes C3a and C5a anaphylatoxins that mediate inflammation in autoimmune rheumatic conditions; IVIG binds to complement proteins and sequesters them away from auto-antibodies, which helps prevent the formation of membrane attack complex in dermatomyositis.

**Fc-mediated effects of IVIGs**

Functions of IVIGs that are exert via **Fc region** are anti-inflammatory, namely:

***Modulation of B lymphocyte maturation and function***

This mechanism of action of IVIG is achieved through the Fc region of the immunoglobulin that binds to FcγRIIB (CD32) expressed on B lymphocytes. In this way, inhibitory signals are triggered by the activation of SH2-containing inositide phosphatase (SHIP), a phosphatase that hydrolyzes phosphatiphyllinositol and disturbs the function of intracellular signaling that takes place through BTK (Bruton's tyrosine kinase) and PLCγ (phospholipase Cγ). Inhibition of BTK and PLCγ disturbs the maturation and activation of B lymphocytes.

***Competitive inhibition of of IgG binding to FcRn receptor***

With their Fc region, immunoglobulins bind to the neonatal Fc receptors (FcRns) which are expressed on endothelial cells and enterocytes. Binding antibodies are internalized into the cell and in this way their proteolytic degradation is prevented, which results in an extended lifespan of the antibody.

One of the mechanisms of action of IVIG is competitive inhibition of autoantibodies binding to FcRn. After the administration of IVIG, a number of antibodies present in the IVIG "cocktail" bind to FcRn, which prevents the binding of antibodies present in the patient's blood. In this way, it is possible to treat autoimmune diseases mediated by autoantibodies.

***Inhibition of C3b and C4b deposition***

The Fc region of antibody is responsible for this effect of IVIG. IVIGs bind to complement fragments C3b and C4b thus preventing the deposition of immune complexes in the tissue and consequently inflammation. It is still only used in preclinical research and it is debatable whether this mechanism of action can be applied in clinical studies.

***Inhibition of macrophage activation***

The Fc region of IVIG is responsible for this effect. Inhibition of macrophage activation is realized by:

1. blocking the activating Fcγ receptors on the macrophages, which prevents their activation and production of pro-inflammatory cytokines such as TNF-α, IL-12 and IL-1

2. stimulating the expression of the inhibitory receptor FcγRIIB on the cell surface of macrophage.

It has been shown that the administration of sialic acid-enriched IVIG (SA-IVIG) can inhibits macrophage activation by increasing the expression of the inhibitory FcγRIIB receptor on their membrane.

***Modulation of dendritic cell function***

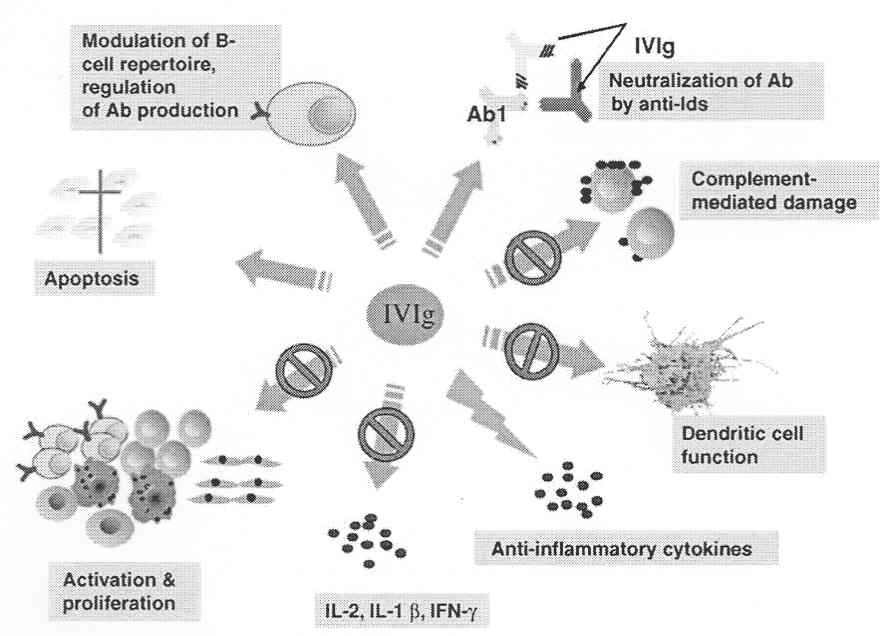
IVIGs exert immunomodulatory effect on antigen-presenting cells, especially on dendritic cells. A key step in the immunopathogenesis of autoimmune diseases is antigen presentation to autoreactive T helper CD4+ lymphocytes. *In vitro* treatment of dendritic cells with the IVIG has been shown to inhibit the differentiation and maturation of these cells. In addition, IVIGs have been reported to reduce the capacity of mature dendritic cells to secrete proinflammatory cytokines upon stimulation, while increasing the production of the immunosuppressive cytokine such as IL-10. These immunomodulatory effects of IVIGs are potentially important in controlling the acquired immune response in autoimmune diseases and organ transplantation.

***Expansion of regulatory T lymphocytes***

CD4+CD25+ FoxP3+ regulatory T cells (Tregs) play a key role in the dominant control of self-reactive T cells, contributing to the maintenance of immunologic self-tolerance. Their repertoire of antigen specificities is as broad, and they are capable of recognizing both self and nonself antigens, thus enabling them to control various immune responses.

It appear that one of major therapeutic effect of immunoglobulin is the expansion of regulatory CD4+CD25+FoxP3+ T lymphocytes. It has been reported that IVIGs increases both the number and the suppressive capacity of regulatory T cells. Naimely, IVIG alters dendritic cell function, cytokine and chemokine networks, and T lymphocytes, leading to development of regulatory T cells.

Taking all these data together, it can be concluded that the therapeutic effects of IVIG are complex and include several mechanisms that work together, namely: neutralization of autoantibodies, modulation of antibody production, modulation of cytokine expression and function, modulation of maturation and function of dendritic cells, inhibition of complement, enhancement of autoantibody clearance by blocking FcRn, and modulation of Fc receptor expression and function (Figure 3).

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**Figure 3.**

**CLINICAL USES OF INTRAVENOUS IMMUNOGLOBULINS**

Intravenous immunoglobulins are used as replacement therapy (passive immunization) to for the prevention and treatment of infections in patients with immunodeficiency disorder. The most common therapeutic application protocol is at a dose of 100-400 mg/kg body weight for 3-4 weeks. Due to their anti-inflammatory effect, IVIGs can be used in the treatment of autoimmune diseases in a dose of 1-2 g/kg body weight for five days, once a month for 3 to 6 months. After that, they are given for 3-4 weeks in a dose of 100-400 mg/kg body weight.

The most common clinical conditions and diseases where the use of intravenous immunoglobulins is indicated are:

- allogeneic bone marrow transplantation

- chronic lymphocytic leukemia

- idiopathic thrombocytopenic purpura

- HIV infection in children

- primary immunodeficiency

- Kawasaki syndrome

- chronic inflationary demyelinating polyneuropathy.

**ADVERSE EFFECTS OF IMMUNOGLOBULIN THERAPY**

Although immunoglobulin is well tolerated, adverse effects occur in less than 5% of patients. The majority of these adverse effects are mild and occur immediately after the infusion, in the form of redness of the cheeks, headache, chills, dizziness, increased sweating, cramps, pain and sensitivity at the injection site, fatigue, pain in the muscles and in the lower back, nausea and a drop in blood pressure. If side effects occur during treatment, the infusion should be slowed down or stopped. Some side effects are rare but serious, and include:

* **anaphylaxis** occurs in 1 in 500-1000 cases and is usually the result of sensitization to IgA in patients who are IgA deficient. It occurs either immediately after or during IVIG therapy. Prevention is the application of IVIG, which are "purified" of IgA;
* **aseptic meningitis** occurs rarely and is presented by headache, neck stiffness, nausea, vomiting and photophobia
* **cardiovascular disorders** occur rarely and are manifested by the appearance of extrasystoles, heart rhythm disorders and a drop in blood pressure
* **renal dysfunction** is more common in patients who already have a kidney disease. If swelling of the legs and ankles, anuria or oliguria occur after IVIG therapy, it is necessary to stop the therapy and consult a nephrologist.
* **other side effects:** postinfusion hyperproteinemia, pseudohyponatremia, thrombosis, vasculitis or eczema.

Antihistamines and/or intravenous hydrocortisone can be given for treatment of these conditions.

**CORTICOSTEROIDS**

Corticosteroids are a large group of steroid hormones that are synthesized during cholesterol metabolism in the cortex of the adrenal gland. They are involved in the regulation of numerous processes such as reaction to stress, immune response, inflammation, carbohydrate and protein metabolism as well as water and electrolyte balance. There are two groups of corticosteroids:

* glucocorticoids (cortisol), which have an anti-inflammatory effect and control the metabolism of carbohydrates, fats and proteins
* mineralocorticoids (aldosterone) that control water and electrolyte levels.

Corticosteroids are powerful anti-inflammatory drugs that alter the transcription of many genes.

**PHARMACOKINETICS OF CORTICOSTEROIDS**

Most corticosteroids are well absorbed after oral administration. Most synthetic corticosteroids, with the exception of prednisolone, have low affinity for corticosteroid-binding globulins and bind primarily to albumin. Only a small percentage of circulating corticosteroids which are not bound to protein and they are free to exert their biological functions, while corticosteroids bound to proteins are protected from metabolic degradation.

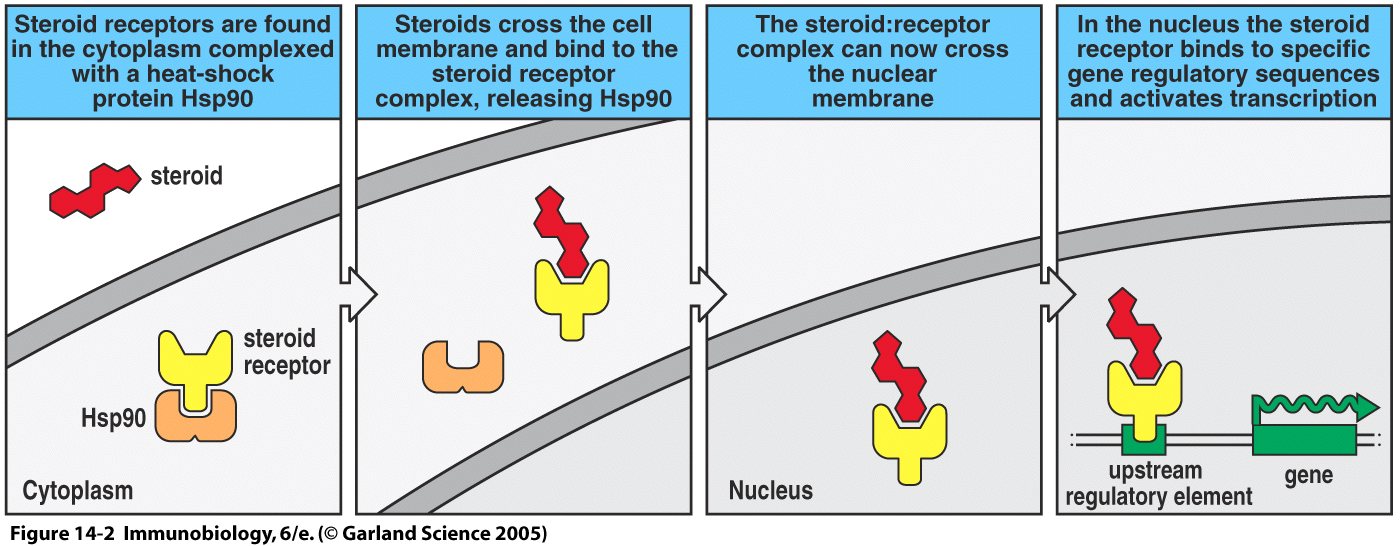
Corticosteroids are metabolized primarily in the liver into inactive metabolites that are excreted by the kidneys. 95% of corticosteroid metabolites are excreted through the kidneys, and the rest through the digestive system.

The two most commonly used corticosteroids are cortisone and prednisone, which are inactive until they are converted *in vivo* to the active metabolites cortisol and prednisolone, respectively.

**MECHANISMS OF CORTICOSTEROID ACTION**

The actions of glucocorticoids are predominantly mediated through the specific intracellular glucocorticoid receptor (GR). Glucocorticoids diffuse through the cell membrane and bind to GR in the cytoplasm, which results in a change in receptor conformation and separation from the heat shock protein (HSP) to which it was previously bound. A homodimer is formed and it enters the nucleus and binds to short DNA sequences and induces either activation or repression of transcription of a particular gene (Figure 3).

The glucocorticoid/GR complex causes repression of the transcription factors such as nuclear factor-kappa B (NF-kB) and activator protein -1 (AP-1), and thus prevents the induction of inflammatory processes. The action of corticosteroids can result in increased transcription of genes for anti-inflammatory cytokines, as well as suppression of transcription and translation of genes for pro-inflammatory cytokines. For example, they inhibit the synthesis of pro-inflammatory cytokines: IL-1β, IL-2, IL-3, IL-6, IL-11, TNF-α, GM-CSF and cytokines that are important in allergic inflammation (IL-4 and IL-5).



**Figure 3.** Being small, lipophilic substances, glucocorticoids readily pass the cell membrane by diffusion and enter the cytoplasm of the target cells, where most of their action is mediated by binding to the intra-cytoplasmic glucocorticoid receptors. The binding of the glucocorticoid to the glucocorticoid receptor results in the shedding of heat-shock proteins, which are otherwise bound to the glucocorticoid receptor, which results in the formation of the activated glucocorticoid receptor-glucocorticoid complex, which easily translocate to the nucleus. In the nucleus of the target cells, this complex reversibly binds to several specific DNA sites resulting in stimulation (transactivation) and suppression (transrepression) of a large variety of gene transcription. Repression of transcription factors NF-κB, AP-1, and interferon regulatory factor-3 (IRF3) results in suppression of synthesis of pro-inflammatory cytokines such as IL-1, IL-2, IL-6, IL-8, TNF, IFN-γ, Cox-2, VEGF, and prostaglandins. Transactivation of transcription factors, including glucocorticoid response elements (GREs), leads to activation of the synthesis of anti-inflammatory cytokines such as IL-10, NF-κB inhibitor, and lipocortin-1.

**Table 1. Immunosuppressive and anti-inflammatory effects of corticosteroids**

|  |  |
| --- | --- |
| **Corticosteroid therapy** | |
| **The effect of corticosteroids on:** | **Physiological effect** |
| **IL-1, TNF-α, GM-CSF, IL-3, IL-4, IL-5, CXCL8** | **Cytikine-mediated inflammation** |
| **INOS** | **NO** |
| **Phospholipase А2 (PLA2)**  **COX-2**  **Lipocortin-1** | **Prostaglandins, leukotrienes** |
| **Adhesive molecules** | **Decreased migration of leukocytes from blood vessels** |
| **Endonucleases** | **Induction of lymphocyte and eosinophil apoptosis** |

Under the influence of glucocorticoids, cells synthesize and release lipomodulin (a glycoprotein that inhibits the action of phospholipase A2). Inhibition of **phospholipase A2** results in the decreased arachidonic acid release, and thus slows down the production of pro-inflammatory metabolites such as prostaglandins and leukotrienes.

After only one administered dose of glucocorticoids, the **migration and accumulation of neutrophils** at the site of inflammation is reduced, which reduces the symptoms of acute inflammation.

Glucocorticoids can also directly suppress the activity of cells that participate in the inflammatory reaction: they **inhibit the phagocytic ability of neutrophils and monocytes**, the **production of collagenase enzymes**, as well as cytokines **IL-1** and **TNF-α**.

Glucocorticoids **inhibit the activity of NO synthetase**, which is responsible for vascular dilation in the inflammation.

Collectively, glucocorticoids **suppress the immune response** and **reduce or block the inflammatory process.**

Corticosteroids are one of the most powerful immunosuppressive agents. The suppression of inflammation by corticosteroids has therapeutic significance in many diseases such as allergic, autoimmune and inflammatory diseases. For the purpose of immunosuppression, they are used to prevent rejection of the transplanted organ.

**ADVERSE EFFECTS OF CORTICOSTEROIDS**

The two main forms of side effects of corticosteroids occur due to long-term use of high doses or due to sudden discontinuation of therapy. If corticosteroids are used continuously for one month, they should not be stopped suddenly due to the appearance of serious problems. The most common side effect of corticosteroids is increased susceptibility to infections. In addition, other frequent side effects are negative calcium balance and osteoporosis, increased appetite and centripetal obesity, impaired wound healing, and growth disorders in children. Other common consequences are myopathy, avascular necrosis, hypertension, purpura, hyperlipidemia, euphoria or depression, diabetes, and cataracts.

**NONSTEROIDAL ANTI-INFLAMMATORY DRUGS**

Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most commonly used drugs which have three main effects:

* anti-inflammatory: they reduce inflammation
* analgesic: they relieve pain
* antipyretic: reduces fever.

The most popular examples of drugs in this group are aspirin, ibuprofen and naproxen.

All the mentioned effects of these drugs are the result of blocking the specific enzyme called cyclooxygenase (COX), which results in a decrease in the production of prostaglandins and thromboxane:

* anti-inflammatory effect: they reduce vasodilatation and indirectly reduce edema, but do not reduce the accumulation of inflammatory cells
* analgesic effect: they reduce the sensitization of nociceptive nerve endings, which are under the influence of inflammatory mediators such as bradykinin and 5-hydroxytryptamine
* antipyretic effect: they reduce the synthesis of prostaglandins that affect the thermoregulatory center in the hypothalamus.

The enzyme COX has at least two different isoforms: COX-1 is the constitutive isoform, present in many tissues, and it converts arachidonic acid into classes of prostaglandins which stimulate physiological functions in metabolism. COX-2 is an inducible isoform, it is produced in inflammatory conditions, and it is induced by inflammatory mediators.

Most nonsteroidal anti-inflammatory drugs as nonselective inhibitors of the COX enzymes, so it is considered that the anti-inflammatory effect of non-steroidal anti-inflammatory drugs is the result of COX-2 inhibition, and that side effects are the result of inhibition of the constitutive COX-1 isoform of the enzyme.

Common side effects of NSAIDs are: dyspepsia, diarrhea/constipation, nausea and vomiting, and in some cases gastric bleeding and ulceration, rash, urticaria, photosensitivity reactions, acute renal failure, analgesic nephropathy (at chronic use).

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