Inflammation as a defensive and autoaggressive process
Inflammation

- Inflammation is defined as a complex system of unspecific defensive responses of vascularized tissues to their damage.
- The term inflammation is not identical with the term infection, because inflammation can be caused by any tissue damage (not necessarily bacteria, or viruses etc.).
- Inflammation is a process of high importance, it is defensive process beneficial for the body.
- The main role of inflammation is to remove damaged tissue, cells, and to prepare optimal microenvironment for tissue repair and also to induce changes in the body, which will enhance immune processes and contribute to the homeostasis (e.g. reaction of acute phase).
- Dysregulated inflammatory process can become auto-aggressive process, and it can cause a damage to the distant organs, it can cause MODS or even death - dysregulated delocalized inflammation (SIRS).
Differences in the literature related to the understanding of the term SIRS

1. SIRS (Systemic inflammatory response syndrome (1992))
   - Systemic response to the localized inflammation in the body (e.g. pancreatitis, tonsillitis etc.)
     - Not necessarily auto-aggressive
     - Inflammatory process is already delocalized, but not dysregulated – after dysregulation occurs, then it will turn into auto-aggressive SIRS

2. SIRS
   - Delocalized and dysregulated inflammatory process of high intensity leading to the disturbances in microcirculation and organ perfusion, with potential transition to the MODS
   - It is usually secondary dysfunction of an organ (lungs, kidneys), which is not caused by a primary damage to the organ, but as a consequence of extremely exaggerated and dysregulated inflammatory response of the body to the primary insult
   - Untreated SIRS leads to the MODS and death
Autoaggressive inflammation leading to MODS

Defensive inflammation

Intensity of the inflammation

Intensity of the insult

# - normal reactivity
*
- decreased reactivity
+ - increased reactivity
‡ - autoaggressive systemic inflammation
Essential difference between inflammation and aggressive SIRS

- Inflammation as a **defensive process** is localized and regulated
- Inflammation as an **auto-aggressive process** is delocalized and dysregulated
Different perspectives in classification of inflammation

- **Duration**
  - Acute (acute pharyngitis)
  - Peracute (peracute epiglottitis)
  - Chronic (chronic pancreatitis)

- **Aetiology**
  - Infectious (viruses, bacteria, parasites)
  - Non-infectious (trauma, ischemia, atherosclerosis)

- **Major morphological findings (subject of pathological anatomy)**
  - Alterative
  - Exudative
  - Proliferative

- **Extent of damage**
  - Superficial
  - Deep
  - Limited
  - Diffuse
Systems participating in inflammation

- Endothelium
- Leukocytes
- Platelets
- Complement
- Coagulation system
Endothelium

- **Physiologic conditions**
  - Antithrombotic surface
  - Local regulation of the vascular lumen
  - Selective permeability of the vessel wall

- **During inflammation**
  - Changes of the lumen towards vasodilation
  - Expression of adhesion molecules with adhesion of cells and proteins
  - Rather thrombogenic surface
  - Highest permeability of the vessel wall for fluids, molecules and cells
  - Regulation of adhesion of leukocytes and their migration to the interstitium
Platelets

- **Physiologic conditions:**
  - Ready to provide a primary haemostatic plug after the vessel wall damage
  - Platelet surface and their mediators communicate and react with the plasma coagulation system in the process of formation of secondary plug (fixed by fibrin)

- **During inflammation**
  - Change of the shape – multiple pseudopodia
  - Adhesion and aggregation
  - Release of many pro-inflammatory and pro-thrombogenic mediators
Leukocytes

- **Mononuclear phagocytes** (monocytes and macrophages), APC, both types are capable of phagocytose, main producers of TNFα a IL1β

- **Polymorphonuclear leukocytes**
  - Phagocytose
  - Cytotoxic potential (ROS, RNS, hydrolytic enzymes, antibacterial proteins)
  - Histiocytes, mast cells, basophils
  - Eosinophil
  - Lymphocytes T a B, NK bells etc.
  - Role on the inflammation: effector, signal, regulator
Coagulation system

- it provides fibrin (stabilisation of the primary platelet plug) also fibrinolytic system is important in these processes because it destroys fibrin and its degradation products as soon as the vessel integrity is restored.

During inflammation:

- Activated macrophages, monocytes and endothelial cells produce TF
- Activation of F XII, prekalikrein and F XI
- Thrombin facilitates transformation of fibrinogen to fibrin
- Body produces tPA normally – plasminogen activator which lead to the production of plasmin
- During inflammation PAI is released to block the conversion of plasminogen to plasmin
Complement

- The main tasks of complement in the defence response are pro-inflammatory (C3a, C5a), opsonisation (C3b, C4b) a cytolytic function (C5b)

- In case of enhanced activation:
  - Vasodilation with increased vascular permeability
  - Haemolysis
Inflammation step by step
Step 1 – tissue damage

Pathogenic factor: NOXA
- Biological (microorganisms)
- Physical (trauma, radiation)
- Chemical (poisons, toxins)
- Metabolic (hypoxia)
- Immunologic (autoantibodies)
- Neurohumoral dysregulation (stress gastric ulcer)

Inflammation – complex system of defensive reactions on vascularized tissues – mostly stereotypic

- Removal of the noxa/damaged tissue
- Dilution of chemicals
- Restriction of the noxa
- Reparation, regeneration of the defect
Step 2 – recognition of the damage

- APC (antigen presenting cells) – macrophages, histiocytes, dendritic cells and others express PRR which make them able them to recognize ,,strangers“ – strange patterns of proteins via **PRR – pattern recognition receptors**

- PRR (TLRs) are expressed on the membranes of all immune cells

### Pathogen associated molecular patterns (PAMPs)
A part of the structure of microorganisms

- LPS, peptidoglycan, sources are viable or already killed microbes

### Damage associated molecular patterns (DAMPs)
Endogenous signal molecules released from damaged cells

**alarmins**
Step 3 – activation of immune cells in the tissue

- Cells after the recognition of a stranger by PRR start to release of pro-inflammatory signal molecules named cytokine (chemokines) IL6, TNFα, IFNγ.
- Cytokines have following effects:

  **Autocrine**
  Regulation the function of the very cell that has released them – mainly stimulation to production of more cytokines

  **Paracrine**
  Impact on the cells in the neighbourhood – immune cells, endothelial cells

  **Endocrine**
  Impact on distant organs where they travel by blood stream – liver, lymphatic nodes, bone marrow, hypothalamus
Activation of immune cells via NFκB

- Activation of PRR after the tissue damage or after a detection of strange proteins leads to the initiation of the intracellular signalling leading to the release of transcription factor NFκB (and other transcription factors) from its inhibitory subunit and its migration to the nucleus, where NFκB initiates transcription of other cytokines.
- Tissue macrophages, monocytes, mast cells, platelets, and endothelial cells after exposure to an antigen or PAMP, DAMP produce cytokines. TNF-α & IL-1 are released first and initiate several cascades.

Activation of nuclear factor-κ B (NF-κ B) inhibitor. Once the inhibitor is removed, NF-κ B initiates the production of mRNA, which induces the production other proinflammatory cytokines.

- IL-6, IL-8, and interferon gamma are the primary proinflammatory mediators induced by NF-κ B
Step 4 – destruction of damaged tissue

- Activated PMN and other cells are releasing enzymes, which have cytotoxic – destructive potential
- The main role of such process is removal of damaged tissue thus providing optimal space for regeneration or repair
- This process must be localized – otherwise the organs out of the inflammatory site would be damaged too
- Body has defensive anti-systems which can neutralize these aggressive factors when needed

- Oxidants (ROS, RNS, NO)
- Activated complement
- Proteases (collagenase, elastase, proteinase 3, cathepsin G)
- Hydrolases
- Antiproteases (α1-antitrypsin)
- Antioxidants
Inflammatory response

- **vasodilatation** – increase blood supply into the target tissue and enhance the supply of the inflammatory cells, mediators, factors necessary for the optimal course of inflammation

- **increased permeability of the capillary wall** – transfer of the cells and molecules to the tissue where they operate

- **transmigration of the cells (macrophages, lymphocytes, neutrophils) into the target tissue** – infiltration, it is caused and regulated based on chemotaxic stimuli

- **changes of biosynthetic & metabolic profiles of many organs** /liver, spleen, lymphatic tissue/

- **activation of immune system** – including cells, enzymatic system in the plasma /complement, coagulation/, this activation is equal to the extent of tissue damage
Macroscopic signs of inflammation

- **CALOR** – inflamed focus is warm (bc of vasodilation, which brings a heat to the tissue)
- **RUBOR** – inflamed focus is red (identical reason)
- **TUMOR** – inflamed focus is swollen – the cause is increase of the vascular permeability for fluids and cells
- **DOLOR** – pain is an important signal of the tissue damage, inflammatory mediators and cytokines can either directly activate nociceptive nerve endings or can decrease their threshold for activation (sensitisation)
- **FUNCTIO LAESA** – tissue/organ is not functioning properly
Step 5 – regeneration or reparation

- **Regeneration** – some tissues have ability to regenerate fully mainly if some of the extracellular components were not damaged by inflammation (e.g. basal membrane, or retikulin network in the liver acinus) – regeneration ad integrum

- **Reparation/repair** – the defect is healed by a scar
- Immune cells themselves (mainly macrophages) produce factors which initiate repair process – bFGF, TGFβ

- These factors influence *fibroblasts, endothelial cells and epithelial cells* which proliferate and migrate to „repair“ damaged tissue

- Repair/regeneration consist of processes such as: **epithelisation, angiogenesis and fibroproduction**, which are regulated by a plethora of growth factors and are out of the scope of this lecture
Processes at systemic level
Reaction of acute phase

- defensive and adaptive mechanism necessary for the maintenance of homeostasis

- The acute phase reaction is innate uniform adaptive response to damage of the integrity of organism. Acute phase is a group of reactions elicited by humoral factors, especially pro-inflammatory cytokines (IL-1, IL-2, IL-6, TNF α) and axis hypothalamus- pituitary gland-adrenal cortex- stress reaction.

APR is time limited – pro-inflammatory cytokines are cleaved from plasma within several hours, APP are present in the serum for at least 48 hours).

- maintenance of water, ion and temperature homeostasis
- anti-infectious processes
- perception of pain as a signal of tissue damage
- removal of irreversibly damaged tissue
- optimal energy supply
- optimal pool of structural molecules, mainly amino acids for the production of antibodies, hormones, regeneration and repair
The acute-phase reaction

Expert Reviews in Molecular Medicine © 2006 Cambridge University Press
**Local acute inflammatory response**

- **IL-1, TNF-α, IL-6**
- **IL-6, TNF-α**

**Hypothalamus**

- (via pituitary)
- **ACTH**
  - **Adrenal cortex**
    - **Corticosteroids**

**Liver**

- **Acute-phase proteins:**
  - C-reactive protein (CRP)
  - Serum amyloid A (SAA)
  - Fibrinogen
  - Mannose-binding protein
  - Complement components

**Bone marrow**

- (↑ CSF by stromal cells and macrophages)

**Leukocytosis**

(↑ white blood cells)

**Prostaglandins**

**Fever**
IL-1, IL-6, cortisol, epinephrine, thrombin

Acute phase proteins:
- CRP
- Haptoglobin
- Hemopexin
- Ceruloplasmin
- a1 glycoprotein (LPB)

Protease inhibitors:
- a1 – antitrypsin,
- a1 – antichymotripsin,
- a2 – macroglobulin

Plasminogen activator inhibitor (PAI)
- Heparin factor II

Coagulation factors:
- Fibrinogen
- Components of complement
- C3
Regulation of inflammation at local level

- Inflammatory response must be actively terminated as soon as it becomes undesirable to avoid so called "bystander" damage of distant organs.

- If mechanisms regulating inflammation failed, the inflammation can continue as "chronic inflammation" or a MODS develops.
Resolution of inflammation is achieved by these mechanisms

- Short life-time of inflammatory mediators in vivo
- Production and release of (TGF \(\beta\)) by macrophages
- Production of anti-inflammatory cytokines IL4 and IL10
- Production of specialized anti-inflammatory mediators (resolvin, mresin, neuroprotectin)
- Downregulation of leukotrienes
- Production of antagonists of receptors for IL1 and soluble receptor for TNF\(\alpha\)
- Apoptosis of immune cells in the tissue after their task was done
- Desensitization of receptors caused by high concentration of their ligands (e.g. cytokines)
- Destruction of cytokines and chemokines by MMP
SIRS

- Syndrome of systemic inflammatory response is generalized acute inflammatory reaction, which has spread to the entire body and it usually has auto-aggressive potential.

- It is intense and dysregulated immune response to the local, multiple or combined tissue damage.

- Inflammation in SIRS has lost its protective and defensive features and turns into the delocalized and dysregulated process, leading to the secondary damage of distant organs.

- Intensity if SIRS is proportional to the extent of tissue damage (to the intensity of the primary insult).
SIRS is defined as presence of 2 or more of the following signs:

- **Fever of more than 38°C or less than 36°C**
- **Heart rate of > mean + 2SD for given age without external stimulation, treatment or painful stimuli, or bradycardia less than 10th percentile for given age without evidence of prolonged stimulation of the vagus nerve**
- **Respiratory rate > mean + 2SD for given age or mechanical ventilation support PaCO2 level of less than 32 mm Hg**
- **Abnormal white blood cell count (>12,000/µL or <4,000/µL or >10% bands)**
| **SIRS** | Two or more of:  
Fever of more than 38°C or less than 36°C  
Heart rate of > mean + 2SD for given age without external stimulation, treatment or painful stimuli, or bradycardia less than 10th percentile for given age without evidence of prolonged stimulation of the vagus nerve  
Respiratory rate > mean + 2SD for given age or mechanical ventilation support PaCO2 level of less than 32 mm Hg  
Abnormal white blood cell count (>12,000/µL or <4,000/µL or >10% bands) |
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sepsis</strong></td>
<td>SIRS due to severe infection, in case of confirmed or just suspected source (blood cultures not necessary)</td>
</tr>
<tr>
<td><strong>Bacteraemia</strong></td>
<td>Presence of viable bacteria within the bloodstream, not necessarily causing problems</td>
</tr>
<tr>
<td><strong>Sepsis induced hypotension</strong></td>
<td>Presence of a systolic blood pressure of less than 90 mm Hg or a reduction of more than 40 mm Hg from baseline in the absence of other causes of hypotension, resistant to treatment by vasopressors and fluid resuscitation</td>
</tr>
<tr>
<td><strong>Septic shock</strong></td>
<td>Clinical syndrome caused by a sepsis, characterized by persistent hypotension and perfusion abnormalities (mostly in microcirculation), despite optimal fluid and vasopressor resuscitation</td>
</tr>
<tr>
<td><strong>MODS</strong></td>
<td>Multiple organ dysfunction syndrome – dysfunction of kidneys, liver, lungs, heart, GUT caused by a severe hypo perfusion, and other complications of SIRS – physiological derangements in which organ function is not capable of maintaining homeostasis</td>
</tr>
</tbody>
</table>
Most common causes of SIRS

The most common causes of SIRS related to infectious disease are as follows:

- bacterial infection, wound infection (burns, surgical wounds, diabetic foot and other infectious complications), cholecystitis, cholangitis, other abdominal infections, pneumonia both nosocomial or community acquired, urogenital infections, meningitis and other less frequent conditions.

Non-infectious causes as underlying conditions for SIRS:

- acute intestinal ischemia, pancreatitis, GUT bleeding, autoimmune diseases, burns, aspiration, cirrhosis, inadequate reaction to drugs, cocaine, amphetamines, theophylline in high dose, myocardial infarction, trauma and other causes.
Stages of SIRS

- **Stage I:** Following an insult, *local cytokines are produced to initiate the inflammatory response*, thereby promoting wound repair and recruitment of the reticular endothelial system.

- **Stage II:** *Small amount of local cytokines is released to the circulation to improve the boost the local response.* This leads to growth factor stimulation and the recruitment of macrophages and platelets. This acute phase response is typically well controlled by a decrease in the pro-inflammatory mediators and by the release of endogenous antagonists. **The goal is to maintain homeostasis**

- **Stage III:** If homeostasis is not restored, a significant systemic reaction occurs. The *cytokine release leads to destruction rather than protection.* A consequence of this is the activation of numerous humoral cascades and the activation of the reticular endothelial system and subsequent loss of circulatory integrity. **This leads to organ dysfunction MODS**
CARS represent the system of negative feedbacks in cytokine and endocrine network (including hypothalamus-pituitary-adrenal axis) and limits extend and duration of systemic inflammation.

The balance between the SIRS and CARS course represents the balance between optimal inflammatory response and the extent of immunosuppression.

cytokines with anti-inflammatory action IL-4 a IL-10 - responsible for the decrease of TNF-α, IL-1, IL-6, and IL-8
production of receptor site antagonists for TNF-α, and IL-1
activation of the axis hypothalamus-pituitary gland-adrenal cortex with overproduction of CS

The disturbed balance might result into two extreme situations:

- excessive activity of proinflammatory cytokines leads to severe SIRS with potential risk of organ dysfunction and death
- excessive anti-inflammatory response leads to immunosuppression and risk of increased mortality later phases of clinical course
• Balance
• SIRS ↔ CARS
• Defensive inflammatory reaction ↔ immunosuppression

SIRS dominance – massive uncontrolled release of pro-inflammatory cytokines leading to the aggressive SIRS with MODS (early mortality)

CARS dominance – massive uncontrolled release of anti-inflammatory cytokines leading to the suppression of the immune system (late mortality)
SIRS: Systemic Inflammatory Response syndrome

Pro-inflammatory cytokines

Anti-inflammatory cytokines

Compensatory anti-Inflammatory Response syndrome

aggressive SIRS

normal inflammation

CARS
S.I.R.S.

- inflammatory diseases (lupus, vasculitis...)
- massive tissue injury/ischemia
- bacterial fungal viral protozoal
- multiple transfusions
- burns neoplasia
- other

M.O.D.S

M.O.F.S & SHOCK
SIRS

- Endo/exotoxins of microbes
- Complexes Ag – Ab
- Complement

**Activation**

- Monocytes, macrophages, mast cells, endothelium, platelets

**Cytokines**

- IL 1, TNFα.

**Cytokines**

- IL-6, IL-8, IFNγ

**Proteins of acute phase**

- Coagulation cascade
- Complement
- Production of PAF, PG, LT

**Proinflammatory interleukins**

**Local and systemic effects**

- Fever
- Release of stress hormones (NA, vasopressin, RAA)
- Lung damage, treatment resistant hypotension

**Cortisol has inhibitory effect, holds the system of NFkB and its inhibitor together**

**Extracellular stimuli**

- NFkB
- LPS
- Ubiquitination
- Proteolysis

**DNA gene promoter**
Pathogenesis of SIRS signs

- Fever
  - The effect of pyrogens on the hypothalamus
  - The central thermostatic set point elevates to the higher set point
  - Mechanism responsible for the heat production are enhanced, but heat loss is inhibited to conserve heat
  - Production and loss of heat are regulated at this increased point until the level of pyrogens is decreased
Pathogenesis of SIRS signs

- hypotension – is a consequence of the drop of peripheral vascular resistance due to vasodilatation induced by cytokines and other proinflammatory agents, and partially is a consequence of the cardio inhibitory effect of proinflammatory molecules

- tachykardia
  - drop of blood pressure inhibits the firing activity of baroreceptors in the arcus aortae
  - natural high activity of sympathetic centre in the brainstem becomes dominant
  - the effects of sympathetic system on the heart involve increased heart rate – tachycardia and increased strength of myocardial contractions
  - tachycardia is one of the powerful compensatory mechanisms capable to increase oxygen tissue supply ...

We need more oxygen due to fever, or due to stress – both speed up metabolic rate
increased breathing rate and hypocapnia
- stimulation of breathing is a complex process
- example – fever – increased oxygen consumption therefore it is necessary to increase ventilation to take more oxygen, stress also contributes to this

changes of the leucocytes (WBC)
- The action of proinflammatory cytokines on the white blood cell population in the bone marrow - they are stimulated to proliferate thus providing optimal immune defence
- if stimulation of the WBC is strong, then younger „un maturated“ cells are released from the bone marrow into the blood stream – bands

decreased count – mechanisms of rolling, adhesion and migration into the tissue are responsible for the progressive decreasing of WBC count in the blood
- this blood pool is not adequately completed from the marrow pool – proliferation and maturation might be not enough to stabilize normal WBC count
Organ dysfunction MODS

Mechanisms responsible for the development of organ dysfunction

- **vasodilatation** – abnormal distribution of circulating volume - tissue hypoperfusion
- **increased vascular permeability** - impairment of Starling’s mechanisms – displacement of fluids into the interstitial space
- **endothelial damage with expression of CAM and small thrombi in microcirculation** - disseminated intravascular coagulation
- **production of reactive oxygen species** by neutrophils
- **production of proteases** by neutrophils
- **production of NO by inductive NO synthase** – refractory hypotension
The correlation between inflammation and coagulation is critical to understand the progress of SIRS.

- IL-1 and TNF-α affect endothelial surface, leading to the expression of tissue factor. Tissue factor initiates the production of thrombin, thereby promoting coagulation, and is a pro-inflammatory mediator itself.

- Fibrinolysis is impaired by IL-1 and TNF-α via production of plasminogen activator inhibitor-1 (PAI-1).

- Cytokines disrupt naturally occurring anti-coagulating factors antithrombin III and protein-C (APC) dysbalance may lead to microvascular thrombosis, including organ dysfunction.
Coagulation cascade

IL - 1, TNFα

- Endothelial damage
- Release of tissue factor
- Production of thrombin
- ↑ PAI 1
- Disturbances in fibrinolysis

DIC

Pro-coagulation

Anti-coagulation

Complement

C3a, C5a

- vasodilation
- ↑ vascular permeability
- endothelial damage

MODS
Respiratory Dysfunction

- very common - tachypnoe, hypoxaemia and respiratory alkalosis. May progress to ALI acute lung injury & ARDS
- primary problem is pulmonary capillary endothelial dysfunction - interstitial and alveolar oedema of protein and phagocytic immune cell rich exudative fluid.
- destruction of pneumoctyes type II, destruction of surfactant molecules, and onset of microatelectatic areas in lungs.
Cardiovascular Dysfunction

Heart and vessels are sensitive to inflammatory mediators - mainly NO – drop of BP is very refractory to volume support, inotropic or conventional vasoconstrictors

- response to the fall in BP is an increase in CO
- baroreceptors mediate a tachycardia and stroke volume increases due to decreased afterload but hypovolaemia may decrease preload and thus cardiac output
- intrinsic myocardial depression is present within 24 hours of the onset of SIRS due to endotoxin and pro-inflammatory cytokines
- constitutive NO in the heart is responsible for leucitropy, the ability of the myocardium to relax, thus maximizing end diastolic filling and coronary artery perfusion.
- tachycardia reduces coronary perfusion
Renal Dysfunction

- kidney maintains renal blood flow and glomerular filtration through autoregulation dependent on the tone of the afferent and efferent arterioles, an auto-regulation is disturbed in SIRS.

**cytokine-induced systemic vasodilatation** and relative **hypovolaemia** in SIRS are responsible for renal hypoperfusion.

Kidney produces intrinsic vasoconstrictors in response to cytokines and the renin-angiotensin-aldosterone system.

In common with other tissues, the kidney is susceptible to **leucocyte mediated tissue injury** with neutrophil aggregation in response to chemokines and production of proteases and ROSs.
GIT hypoperfusion - barrier function of the intestinal wall is compromised

After that a translocation of intraluminal bacteria or their endotoxin might occur – worsening the homodynamic parameter primarily disturbed due to SIRS.
Haematological Dysfunction

- (DIC) produces both bleeding and microvascular thrombi which have been proposed as mechanisms of multiorgan dysfunction.

- The cytokine-mediated activation of coagulation in SIRS occurs via the tissue factor dependent extrinsic pathway.

- Attenuation of the anticoagulant systems furthers the procoagulant state.

- Antithrombin III (ATIII) is an inhibitor of the serine proteases responsible for coagulation clotting factors IXa, Xa, XIa and XIIa and thrombin.

- Thrombomodulin is an endothelial cell derived inhibitor of clotting and activator of fibrinolysis. It acts as a thrombin binding protein, reducing the effects of thrombin.

- The thrombin-thrombomodulin complex has further anti-coagulant properties as an activator of protein C which, with cofactor protein S, inactivates factors V and VIII. In sepsis, the production of thrombomodulin by endothelial cells is downregulated by pro-inflammatory cytokines and circulating free levels of protein S are reduced.
Metabolic complications

- Tissue hypoxia
- Dysbalance of haemodynamics - inadequate perfusion distribution – peripheral tissues \( \downarrow \text{O}_2 \) and necessary substrates based on metabolic demands – anaerobic metabolism – lactic acid
- Mitochondria
  - Block of respiratory chain \( \downarrow \text{energy of cells} \)
  - Production of reactive oxygen species