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THESIS ABSTRACT

The development of targeted anti-inflammatory therapies using nanotransporters of drugs

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CONTENTS

Keywords: inflammation, acute inflammation, chronic inflammation, cytokines, chemokines, endothelial cells, human vascular smooth muscle cells, monocytes / macrophages, cell adhesion molecules, apoE-deficient mice, adhesion, transmigration, nanotherapy, nanocarriers, lipid nanoparticles, liposomes , chemokine receptor antagonists, resistin, MAPKinases, clodronate, apoptosis.

Inflammation is a mechanism of the innate immune system as part of a complex biological response of vascular tissue to harmful stimuli (pathogens, damaged cells, irritants). The end result of the inflammatory process is to protect the body by infection and to restore the structure and function of damaged tissues. Inflammation may be un acute reaction, resulting in the elimination of inflammatory stimuli and tissue recovery in physiological conditions, or may be chronic reaction leading to exacerbation and the developing of various pathologies, depending on the reaction severity. Chronic inflammation is involved in numerous pathologies including atherosclerosis, cancer, rheumatoid arthritis, multiple sclerosis, inflammatory bowel disease, Alzheimer's, lupus, allergies, etc..

Activated endothelium has an important role in the inflammatory. Under normal conditions, the vascular endothelium is non-thrombogenic and non-sticky. Changes in the microenvironment affects endothelial cells that will express adhesion molecules (VCAM-1, ICAM-1, E-and P-selectin, fractalkina), which determine leucocytes and platelets recruitement and adhesion by their corresponding receptors. In addition to adhesion molecules, chemokines such as MCP-1 (CCL2), RANTES (CCL5), fractalkina (CX3CL1) express by endothelial cells bind to receptors on the surface of leukocytes and with adhesion molecules contribute to their adhesion and transmigration. Chemokines bind to glycosaminoglycans present on the endothelial cell membrane and guide the entry of leukocytes into arterial wall. Monocytes are attracted by CCL2, CCL5 and CX3CL1 that activate their receptors CCR2, CCR5 and CX3CR1 on monocytes.

Due to the involvement of cellular adhesion molecules and chemokines in the development of chronic inflammatory diseases, therapeutic intervention lead to new opportunities in prevention and treatment of these diseases. Functional manipulation of chemokine system, such as inhibition of chemokine / chemokine receptors interaction using chemokine antagonists (CA) or chemokine receptor antagonists (CRA), could be an important therapeutic option to prevent the inflammatory cells accumulation and diseases progress (atherogenesis, metastasis). An approach to modulate drug targeting to a specific site is to design transport vehicles able to "deliver" the drug in the cell and to recognize targeted

molecules. The most promising for targeting to specific sites of nanotransporters is the attachment of ligands on their surface able to carry the active substance in the cell or to recognize specific target molecules.

This thesis has proposed the introduction of different therapeutic strategies based on nanoparticles to control inflammation and to interfere in the steps of recruitment leukocytes due to the association between chronic inflammation and various diseases and the impoatant role of leukocyte adhesion and subendothelial migration . The first part will present current issues on the use of nanoparticles for the development of targeted anti-inflammatory therapies. The original part of the paper is focused on (1) the development of therapeutic agents nanocarriers to achieve a targeted transport to activated vascular endothelium, the purpose beeing to interfere selectively with endothelial dysfunction or inhibit chemokine-receptor interaction and to prevent accumulation of pro-inflammatory cells in the vessel wall, and (2) the use of nanoparticles to modulate the monocytes / macrophages functions in order to reduce atherosclerotic plaque inflammation.

The first part of the thesis shows the current state of knowledge and is organized in two chapters.

Chapter 1 presents general concepts about inflammatory process. Penetration of pathogenic microorganisms or shel altered molecules, lead to onset of an acute inflammatory response characterized by infiltration of neutrophils in the first 6-24 hours and then are replaced by monocytes / macrophages. Proinflammatory agent is removed and reversible collateral tissue injury occurs, cellular debris removal by macrophages and restoration of tissue homeostasis with complete restoration of the original tissue architecture. If it is not properly induced an inflammatory response, the stimulus persists, there is infiltration of macrophages and lymphocytes that synthesize cytokines and chemokines and amplify the inflammatory response, causing irreversible tissue damage. It produces fibrosis - initial tissue is replaced by connective tissue. Chronic inflammation is involved in numerous pathologies such as atherosclerosis, chronic obstructive pulmonary disease, rheumatoid arthritis, multiple sclerosis, cancer, obesity, asthma, inflammatory bowel disease, and neurodegenerative diseases.

Chapter 2 presents some introductory concepts about nanotherapy in decreasing the inflammatory process. Many cells and mediators involved in inflammation, can be used as markers in the target or in the disease stage detection .

Patients presentation in late stages of the disease, the organisms failure to adapt at current therapy models, and improving the biological activity of pharmacological agents / drugs by encapsulation in nanoparticles led to the development of a new therapy and diagnostic strategies - nanoteragnostic that it assume both patient diagnosis and treatment.

Because unincorporated drugs can easily diffuse from /in blood vessel, leading to a decrease of the active substance in the affected area, was performed their encapsulation in the nanoparticles. If nanoparticles have left the blood vessel, they can not coming back because of their large size and has place a progressive accumulation in the affected area. coupling of peptides or antibodies to the nanocarriers surface, able to recognize molecules expressed by affected cells may lead to a targeted transport of active substances in the affected tissue.

In Part II - "Original Contributions" are presented the results of experiments aimed at controlling inflammation and able to interfere in the steps of leukocyte recruitment. The present work was centralized to achieve three major objectives:

I. targeted transport in activated vascular endothelium using nanocarriers for therapeutic agents to selectively interfere with endothelial dysfunction

II. development of a specific transport nanosistem compounds to activated endothelial cells to inhibit chemokine-receptor interaction, in order to prevent accumulation of proinflammatory cells in the vascular wall

III. using nanotechnology to modulate functions of monocytes / macrophages to reduce atherosclerotic plaque inflammation.

In Chapter I I presented targeted transport of therapeutic agents in nanocarriers to activated vascular endothelium. In inflammation, the endothelium plays an important role. Excessive and sustained exposure to pro-inflammatory mediators lead to endothelial dysfunction translated, in part, by expressing of cell surface adhesion molecules (eg E-and P-selectin, VCAM-1 and ICAM-1) that mediate leukocyte recruitment and adhesion to endothelium.

Induction or increased expression of specific molecules provides opportunities for the transport of selective therapeutic agents to an certain affected endothelial vascular segment.

To achieve a selective transport to activated endothelium they were used various nanosystems (liposomes, lipid nanoparticles) and was evaluated the targeting ability of nanocarriers to activated endothelium compared with normal endothelium, internalization efficiency of nanoparticles by endothelial cells, and therapeutic effects of compound incorporated into nanoparticles.

In this purpose, were performed studies in vitro, on cultured endothelial cells and also was followed targeted efficiency of vascular endothelium with liposomes using animal models using transgenic mice that develop atherosclerosis (ApoE-deficient mice). In vitro and in vivo results indicate a specific binding of liposomes directed to VCAM-1 and the liposomes obtained can be further used as carriers of drugs to endothelium covering atheromatous plaques.

Also, preliminary results show that AG490,free and encapsulated in nanoparticles contribute to decreasing of monocytes adhesion to activated endothelium by inhibiting Jak2/STAT3 pathway, and the effect of the inhibitor is increased by encapsulation in nanocarriers.

Chapter II is focused to obtein a specific transport nanosistem of therapeutic agents, able to destabilize and controlled release of compound encapsulated on the target cell surface. These nanocarriers could be efficient means of trasnsport that allow a controlled release of the chemokine receptor antagonists on the surface of the activated endothelial cells that block the chemokine / chemokine receptors interaction and prevent the transmigration of leukocytes into the subendothelial space.

Leukocyte infiltration is strongly regulated by chemokines. Chemokines are small polypeptides, highly specialized, which function as regulators of cell trafficking through interactions with G protein-coupled receptors on the surface of leukocytes. In our research we studied the effect of chemokine receptor antagonists on monocytes adhesion and infiltration through endothelial monolayer mimicking inflammatory conditions. Using Xcelligence system, I followed in real time: 1) endothelial cells monolayer formation, 2) the effect of proinflammatory cytokine TNF-α on endothelial cells, 3) adhesion and infiltration of monocytes and 4) the protective effect of chemokine receptor antagonists on the monolayer integrity. The data indicate that blocking the chemokine receptor CCR2 and CCR5 can significantly reduce transmigration through the endothelium, this being achieved through a decrease in the accumulation of monocytes in the blood vessel wall and reduce the inflammatory process. However, experimental and clinical testing of potential inhibitors of chemokines and their receptors for the treatment of inflammatory and immunological diseases have side effects. Local transport of chemokine receptors antagonists, in certain areas may be a better strategy to slow down or interrupt the inflammatory process. Design of transport vehicles to be able to "deliver" the drug at the affected site is a promising way to treat inflammation. Therefore, to obtain a therapeutic benefit by specific targeting of activated endothelial, we designed target-sensitive liposomes capable to release the compund

incapsulated-in after binding to target. They can be efficient transport vehicles for a controlled release of the chemokine receptor antagonists to activated endothelial surface capable to block chemokine / chemokine receptors interaction and to prevent transmigration of leukocytes into the subendothelial space.

In Chapter III I watched the modulation functions of monocytes / macrophages using nanotechnology in order to reduce atherosclerotic plaque inflammation.

Macrophages are essential in the inflammatory process that accompanies atherogenesis, from fatty streaks formation until to the processes leading to plaque rupture and acute coronary syndromes. One of the objectives was to follow the production of a recently discovered proinflammatory cytokine, resistin, by human monocytes as a result of their exposure to high glucose. We also investigated the mechanisms involved in the administration effect of clodronate liposomes (treatment leads to depletion of monocytes / macrophages) on the viability of vascular wall cells (endothelial cells - EC, smooth muscle cells - SMC), monocytes and macrophages. We studied the effects of apoptotic monocyte interaction with vascular wall cells and we looked at monocytes / macrophages adhesion to endothelial cells, smooth muscle cells and changes occurring in proliferation (PCNA) and intracellular signaling pathways (MAPKinazele: p38 and ERK) in smooth muscle cells.

The results show that: (1) significant levels of glucose induce resistin expression in human monocytic U937 (mRNA and protein), (2) p38, ERK1 / 2 and JNK MAPKinases and NF-kB transcription factor are involved in this mechanism, (3) insulin significantly reduced resistin expression induced by high glucose independent of signaling pathways MAPKinases: PI3K, ERK1 / 2 and p38.

Experiments designed to study the administration effects of clodronate liposomes on vascular wall cells and monocytes / macrophages show that small unilamellar liposomes are taken predilection by endothelial cells and smooth muscle cells, whereas multilamellar liposomes are internalized by human monocytes U937, murine macrophages Raw 264.7 and peritoneal macrophages isolated from mice. cell viability studies have shown that endothelial cells and smooth muscle cells viability is not significantly affected by MLV and SUV encapsulated clodronate, whereas, the biological activity of clodronate encapsulated in MLV on macrophages is higher than free clodronate with a significant decrease in their viability; the type of cell death induced is apoptosis.

Induction of apoptosis in monocytes determine changes in their interactions with vascular wall cells. Communication between apoptotic cells and smooth muscle cells causes a decrease in proliferation 35% and phosphorylation of p38 (~ 35%) and ERK (70%)

MAPkinases in smooth muscle cells. Also, the monocyte apoptosis was induced by clodronate liposomes show increased adhesion to blood wall cells compared with normal monocytes. Following the results obtained in different experimental models with inflammation and by using different types of nanotransport, we can draw the following conclusions: 1) liposomes directed to VCAM-1 are able to transport drugs to activated endothelial cells , 2) lipid nanoparticles can be used for intracellular transport in the endothelial cells of hydrophobic substances, 3) the nanosystem of target-sensitive transport developed can provide local therapeutic agents in the vicinity of activated endothelium and 4) the liposomes may be able to transport active substances to modulate the functions of monocytes / macrophages for reducing the inflammatory process.

Studies in this paper are the applicative nature, the ultimate goal being to control and reduce the inflammatory process using different therapeutic strategies based on nanocarriers.

Number of figures in the first part - 17

Number of figures in the original (Part II) - 40

Indications bibliography – 177

Papers published in international journals ISI - 10

Papers published in national journals CNCSIS – 2

Abstracts of posters presented at international scientific meetings - 48

Abstracts of posters presented at national scientific meetings - 6

Trainings and courses – 4

Participation in research projects - 4 national, 2 international.