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INVITED REVIEW

Metabolic correlations of glucocorticoids and polyamines in inflammation and apoptosis

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Abstract Glucocorticoid hormones (GC) are essential in all aspects of human health and disease. Their anti-inflammatory and immunosuppressive properties are reasons for therapeutic application in several diseases. GC suppress immune activation and uncontrolled overproduction and release of cytokines. GC inhibit the release of pro-inflammatory cytokines and stimulate the production of anti-inflammatory zcytokines. Investigation of GC's mechanism of action, suggested that polyamines (PA) may act as mediators or messengers of their effects. Beside glucocorticoids, spermine (Spm) is one of endogenous inhibitors of cytokine production. There are many similarities in the metabolic actions of GC and PA. The major mechanism of GC effects involves the regulation of gene expression. PA are essential for maintaining higher order organization of chromatin in vivo. Spermidine and Spm stabilize chromatin and nuclear enzymes, due to their ability to form complexes with negatively charged groups on DNA, RNA and proteins. Also, there is an increasing body of evidence that GC and PA change the chromatin structure especially through acetylation and deacetylation of histones. GC display potent immunomodulatory activities, including the ability to induce T and B lymphocyte apoptosis, mediated via production of reactive oxygen species (ROS) in the mitochondrial pathway. The by-products of PA catabolic pathways (hydrogen peroxide, amino aldehydes, acrolein) produce ROS, well-known cytotoxic agents involved in programmed cell death (PCD) or apoptosis. This review is an attempt in the better understanding of relation between GC and PA, naturally occurring compounds of all eukaryotic cells, anti-inflammatory and apoptotic agents in physiological and pathological conditions connected to oxidative stress or PCD.

Keywords Glucocorticoids · Polyamines · Inflammation · Mitochondria · ROS · Apoptosis

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Glucocorticoid hormones in medicine

Glucocorticoid hormones (GC) are essential in all aspects of human health and disease. Their anti-inflammatory and immunosuppressive properties are reasons for therapeutic applications in numerous diseases (Barnes 2006; Cosío et al. 2005; Goulding 2004; Baxter and Rousseau 1979). Endogenous GC exert a wide range of immunomodulatory activities, including lymphocyte apoptosis and the control of T cell homeostasis, while synthetic GC are in widespread use to treat autoimmune and inflammatory diseases (Lim et al. 2007).

Their anti-inflammatory properties were first discovered in the treatment of rheumatoid arthritis in 1948 (Schäcke et al. 2002). The molecular mechanisms responsible for their anti-inflammatory activity are still under investigation. However, recently, thanks to the development of new molecular biology techniques, there was a progress in the understanding of the mechanism of action of these drugs. Since extended GC treatment can cause unwanted side effects (growth retardation in children, osteoporosis, myopathy, diabetes mellitus and cardiovascular disorders,

such as hypertension), GC therapy is often limited (Schäcke et al. 2002; Herold et al. 2006).

Metabolic actions of GC at cellular level

GC are pleiotropic hormones that at pharmacological doses prevent or suppress inflammation and other immunologically mediated processes, most notably leukemias (Ploner et al. 2005). The effectiveness of this approach is based on the ability of GC to induce apoptosis of leukemic cells. Yet, the mechanisms by which GC cause apoptosis remain obscure (Sionov et al. 2006a, b).

GC exert the most of their effects through binding to the cytoplasmic GC receptor (GR), a member of the nuclear receptor superfamily, discovered in the 1970s, (Karin 1998). Although GR is predominantly cytosolic, a plasma membrane GR (mGR) has been detected in some lymphoid cells. Beside, there are mitochondrial GRs (mitGRs), mediators in reactive oxygen species (ROS) production (Sionov et al. 2006b).

The classic mode of GC action occurs through the direct regulation of gene transcription. Between 10 and 100 genes are thought to be directly regulated by GC. In the absence of ligand, GR resides predominantly in cytoplasm in a large poly-protein complex consisting of several proteins including hsp90, hsp70 and immunophilins (Duma et al. 2006). Upon binding of GC to GR, the HSP chaperone molecules are released and the hormone–receptor complex enters the nucleus where it binds to specific DNA sequences called *GC-responsive elements* (GREs), leading to the transcription rate change. GRs are ligand-activated transcription factors which activate the transcription of GC-responsive genes either directly, i.e. by binding to specific regulatory areas of these genes, so-called GREs, or indirectly, i.e. by interfering with the binding or function of other transcription factors (*trans*-repression mechanisms), (Duma et al. 2006; Lu and Cidlowski 2004; Cosío et al. 2005).

Beside the direct gene transactivation, there is a cross-talk between the GRs and activation factors, especially activator protein 1 (AP)-1 and nuclear factor kappa B (NF-κB), which controls several survival pathways. The interaction of GRs with these factors is believed to play a major role in GC-mediated apoptosis (Barnes 1998; Schäcke et al., 2002). Inhibition of NF-κB may occur through a recently described mechanism which involves the transcriptional activation of its cytoplasmic inhibitor, IκBa (Auphan et al. 1995; Boumpas 1972; Cosío et al. 2005).

The interaction of GRs with another family of transcription factors, like signal transducers and activators of transcription (STATs), may enhance the effects of certain cytokines (Tuckermann et al. 2005).

In the last few years, increasing number of human GC receptor (hGR) isoforms have been reported. Multiple hGR isoforms are generated from one single hGR gene via mutations and/or polymorphisms, transcript alternative splicing, and alternative translation initiation. Each hGR protein, in turn, is subject to a variety of posttranslational modifications, and the nature and degree of posttranslational modification alter receptor function. Specific nitration of the tyrosine residues of GRs by NCX-1015 results in the enhancement of GR-mediated events (Paul-Clark et al. 2003). Correctly folded GRs present "pockets" for cognate hormone recognition and motifs for recognizing specific GREs on target genes in DNA (Lu and Cidlowski 2004).

All of GC mechanisms mediated by GRs affect gene transcription directly or indirectly, referred as "genomic" mechanisms. In addition to them, there is an alternative action of GC, independent from modulating gene expression and for this reason defined as "non-genomic". These GC effects are mediated by plasma membrane and mitochondrial GRs. The non-genomic effects could be due to direct physicochemical interactions with cell membrane constituents, including ion channels and membrane-associated proteins. The non-genomic mechanisms are operative in the regulation of adhesion phenomena by GC-induced inhibition of lymphocyte cell-adhesion molecules (CAMs) synthesis. Non-genomic mechanisms are also involved in the activation of endothelial nitric oxide synthase (eNOS), generation of ROS activation of phosphatidylinositol-specific phospholipase C (PI-PLC) with subsequent transient calcium mobilization, activation of acidic sphingomyelinase leading to increased ceramide generation and lysosomal release of cathepsin B (Sionov et al. 2006b).

The mechanisms of GC at molecular levels may lead to the development of novel 'dissociated' steroids, more active in transrepression (interaction with transcription factors) than transactivation mechanism, with lesser risk of side effects. Some of the transcription factors that are inhibited by GC, such as NF-κB, which activates many immunological regulator genes, are also targets for novel GC-mediated anti-inflammatory therapies (Barnes 1998; Rhen and Cildowski 2005). The immunological modulator properties of GC in the first stadium of inflammation are in correlation with ability of GC to suppress the endothelium-mediated micro-vascular vasodilatation by: (1) down regulation of eNOS, (2) down regulation of cationic amino acid transporter-1 (CAT-1), and (3) suppression of ROS. The current scientific knowledge indicates that the GC inhibit inflammation through all three mechanisms: direct genomic, indirect genomic and no-genomic effects (Elenkov and Chrousos 2002; Iuchi et al. 2003; Schäfer et al. 2005; Rhen and Cildowski 2005).

The anti-inflammatory and immunosuppressive effects of GC

GC have important effects on the development and homeostasis of the immune system. Although GC have been widely used since the late 1940s, the molecular mechanisms responsible for their anti-inflammatory activity are still under investigation (Boumpas et al. 1993; Barnes and Adcock 1993; Elenkov and Chrousos 2002; Rhen and Cildowski 2005; Pitzalis et al. 2002). GC exert both negative and positive effects on various components of the immune response. The desired anti-inflammatory and immunosuppressive effects of GC are mainly mediated through repression or induction of gene transcription (De Bosscher et al. 2003; Rhen and Cildowski 2005). All GC actions are associated with increased expression of inflammatory genes. The expression of inflammatory genes is regulated by pro-inflammatory transcription factors (Fig. 1), such as NF-κB and AP-1, representing obvious targets for immunosuppressive therapies (De Bosscher et al. 2003; Cosío et al. 2005).

These factors modulate genes involved in the priming of the innate immune response, while their actions on the adaptive immune response are to suppress cellular (Th1) immunity and promote humoral (Th2) immunity (De Bosscher et al. 2003; Rhen and Cildowski 2005; Lim et al. 2007). GC inhibit the production of pro-inflammatory cytokines, such as IL-12, IL-6, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ, whereas they stimulate the production of anti-inflammatory cytokines such as IL-10, IL-4, and TGF-β (Elenkov and Chrousos 2002). These GC effects are associated with the decrease of the transcription rates of the genes for these interleukins and decrease of the stability of their messenger RNAs (Rhen and Cildowski 2005).

NF-κB binds DNA sequences called NF-κB elements and stimulates the transcription of cytokines, chemokines, cell-adhesion molecules, complement factors, and receptors for these molecules (Maiuri et al. 2004). Recently, NF-κB was suggested to be a target for GC-mediated immunosuppression. Direct interactions between the GRs and NF-κB probably account for most of the inhibitory effects of GC on NF-κB signal (McKay and Cidlowski 1998). GC could inhibit NF-κB by inducing the synthesis of its natural inhibitor, IκB (Baldwin 1996; Deroo and Archer 2001a, b). In its inactive state, NF-κB is sequestered in cytoplasm by IκB. Cytosolic factors IκB-α and IκB-β isoforms are specific regulators of NF-κB complex activation (Díaz-Guerra et al. 1997). Many inflammatory signals such as microbial pathogens, viral infections, TNF-α, interleukin-1 and others activate IκB kinases. Phosphorylation of IκB leads to its ubiquitination and degradation by proteasomes (Rhen and Cildowski 2005).

It is known that GRs have cysteine residues critical for steroid binding in their hormone-binding and DNA-binding

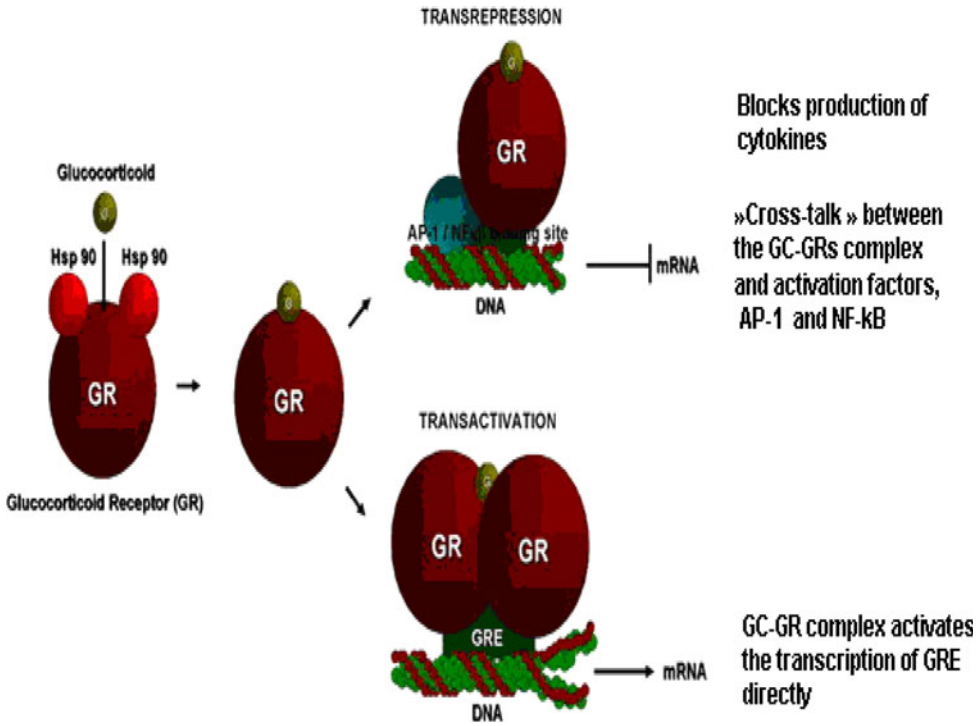

Fig. 1 GC act through the GC receptor which may remain as a monomer and thereby interacts with transcription factors to inhibit transcription of cytokine genes (*transrepression*) or they can dimerize and thereby interacts with GC-response elements (GRE) to induce transcription of genes (*transactivation*) (Intracellular signaling section, Michael A. Beaven, http://www.dir.nhlbi.nih.gov/labs/lmi/ics/ with modifications)

domains. It has been reported that nitric oxide (NO) modulates the activity of some enzymes and proteins through nitrosylation of cysteines forming *S*-nitrosothiols (Dash et al. 2003). In sepsis, the inducible form of iNOS activity, increases followed by release of high amounts of NO. GC have potent anti-inflammatory properties and are very effective in inhibition of the induction of this enzyme if administered before the shock onset. Therefore, *S*-nitrosylation of critical -SH groups in GRs by NO with consequent decrease in binding and affinity of GRs to GREs in DNA may be the mechanism which explains the failure of GC to exert their anti-inflammatory effects in septic shock.

The expression of inflammatory genes is regulated by pro-inflammatory transcription factors, such as NF-κB and AP-1, representing obvious targets for immunosuppressive therapies (De Bosscher et al. 2003; Cosío et al. 2005).

Evidence indicates that GC inhibit inflammation through all three mechanisms: direct and indirect genomic and non-genomic effects (Elenkov and Chrousos 2002; Rhen and Cildowski 2005; Herold et al. 2006).

GC and PA affect chromatin structure

The basic mechanisms of GC actions is modification of chromatin structure (Kishimoto et al. 2006). GRs "cross-talk" with transcriptional activators by various mechanisms. In these processes chromatin remodeling and modification of histones, the main components of chromatin, play a crucial role in gene transcription. The transcription factors NF-κB and AP-1 influence the acetylation of core histones resulting in elevated gene transcription (Deroo and Archer 2001b; Kishimoto et al. 2006).

The unique structural element of chromatin is the nucleosome (Deroo and Archer 2001a, b). GC may lead to deacetylation of histones at the site of inflammatory gene, resulting in tighter coiling of DNA and reduced access of transcription factors to their binding sites, thereby suppressing gene expression (Barnes 1998). GC reverse histone acetylation at the site of inflammatory gene transcription, either by direct binding of the activated GR to NF-κB-associated co-activators or by recruitment of histone deacetylases to the activated transcription complex (Kagoshima et al. 2003).

Lately, GC have been shown to induce the synthesis of a leucine zipper protein termed GILZ (GC-induced leucine zipper), which interacts and inhibits several transcription factors including NF-κB and AP-1. However, most studies tend to favor the so-called tethering model, where the GR interferes with DNA-bound transcription factors, preventing recruitment of co-activators or interaction with RNA polymerase (Tuckermann et al. 2005).

PA are essential for organization of chromatin structure and function

Chromatin structure plays a crucial role in the regulation of eukaryotic gene expression. Spd and Spm stabilize chromatin and nuclear enzymes due to their ability to form complexes with negatively charged groups on DNA, RNA and proteins (Heby 1981; Pegg 1986; Tabor and Tabor 1984; Heby and Persson 1990; Panagiotidis et al. 1995; Pelta et al. 1996; Thomas and Thomas 2001; Bachrach 2004; Johnson 2005; Moinard et al. 2005). As polycations, PA bind the specific locations in nucleosomes and can change the helical twist of DNA. Sp preferentially binds to the specific 'TATA' sequence element on DNA, suggesting that PA may be involved in the chromatin structure stabilization (Jänne et al. 2004; Wallace et al. 2003; Moinard et al. 2005).

Structural changes in chromatin are modulated in part through acetylation of nucleosomal core histones. Histones' acetylation could result in increased transcriptional activity in vivo (Ientile et al. 1988; Struhl 1998). Steady-state levels of histone acetylation are determined by the equilibrium established between histone acetyltransferases (HATs) and deacetylases (HDACs) (Hobbs and Gilmour 2000). Acetylation of core histones occurs on specific lysine residues contained within the N-terminal tail domains. These tail domains lie toward the outside of the nucleosome and interact directly with regulatory factors. Hyperacetylation of the histone tail domains renders them more accessible to transcription factors.

PA are repressors of transcription in vivo. Histone hyperacetylation antagonizes the ability of PA to stabilize highly condensed states of chromosomal fibers. It is possible that PA control expression of a small subset of genes (Pollard et al. 1999). Recent studies document effects of elevated intracellular PA levels on histone acetylation in proliferating cells, suggesting a mechanism by which altered PA biosynthesis contributes to aberrant expression of genes, facilitating tumor growth (Hobbs and Gilmour 2000). Acetylation of PA reduces their affinity for DNA and nucleosomes, thus the helical twist of DNA in nucleosomes could be regulated by cells through acetylation. Acetylation reduces the net positive charge of histones and weakens their interactions with DNA. Wu (1997) suggests that histone and PA acetylation act synergistically to modulate chromatin structure.

PA influence the histone acetyltransferase and deacetylase activities. Overall histone acetylation is increased in cells containing high levels of ornithine decarboxylase (ODC) and PA. The nuclear enzyme Spd N^8-acetyltransferase, which acetylates Spd at N-8 (Wallace and Fraser 2004) may act synergistically with histone acetyltransferase to destabilize inactive chromatin structure (Desiderio

et al. 1993). Lately, it was demonstrated that Spd-facilitated condensation of nucleosomes requires intact N-termini of core histones, and is impeded by histone acetylation (Pollard et al. 1999).

The nuclear aggregate of PA (NAPs), rich in Sp and Spd, interacts with DNA phosphate groups and influence, more efficaciously than single PA, both the conformation and the protection of the DNA (Pelta et al. 1996; D'Agostino and Di Luccia 2002; Matthews 1993).

Recently, some studies deal with genetic engineering of PA metabolism revealing their cellular functions (Janne et al. 1991, 2004). The first reports of human diseases apparently caused by mutations or rearrangements of the genes involved in PA metabolism have appeared (Pegg 1988; Pegg and Feith 2007; Jänne et al. 2004; Wallace et al. 2003; Moinard et al. 2005).

Apoptosis

The main reason for all unwanted side effects of GC is that desamethasone induces the overproduction of ROS, causing dysregulation of physiological processes. Generation of ROS, such as superoxide, hydrogen peroxide, and hydroxyl radicals that damage the cell, results in conditions known as *oxidative stress* as well as apoptosis.

Apoptosis, (Greek "falling") is a process by which specific cells are killed and removed for the benefit of the organism (Kerr et al. 1972). Physiologic cell death is part of the design of a multicellular organism, and such as is also called programmed cell death (PCD) (Bursch et al. 2000; Dash 2005; Cohen 1993; Green and Reed 1998). Apoptosis can be induced by many different stimuli, especially by ROS. The sources of oxidants are numerous. Most of them derive from enzymatic reactions, like degradation of purine bases, oxidative deamination of amino acids, degradation of PA and in mitochondrial respiratory chain, producing superoxide anion, hydrogen peroxide (H_2O_2), or nitric oxide (NO). Once produced, these species undergo conversion to secondary highly ROS and reactive nitrogen species (RNS), such as hydroxyl radical (OH^-) and peroxynitrite ($ONOO^-$). ROS and RNS, at basal levels, regulate signal transduction and protein function. However, elevated levels of ROS or RNS can damage critical cellular components such as membrane lipids, structural and regulatory proteins and DNA (Liu et al. 2002; Ott et al. 2007; Moylan and Reid 2007; Bernardi et al. 1999).

There are two major apoptotic pathways in mammalian cells: the death receptor (extrinsic) pathway, exemplified by FasL binding to an extracellular receptor and the mitochondrial (intrinsic) pathway. The mitochondrial pathway, activated by most cellular stresses, involves a change in mitochondrial transmembrane potential resulting in the release of cytochrome *c* from mitochondria into the cytosol. Cytochrome *c* then binds to apoptosis activating factor 1 (Apaf-1) and procaspase-9 forming apoptosome and activating caspases (Fig. 2).

"Cross-talk" between the death receptor and the mitochondrial pathways is mediated by the BID protein, a member of the Bcl-2/Bcl-xL family which increases the complexity of apoptosis activation. During apoptosis, chromatin condensation, DNA and nuclear fragmentation occur, and cells may detach from neighboring cells and separate into intact membrane-bound fragments called apoptotic bodies (Fadeel et al. 1999). Inflammation does not verify because the apoptotic bodies are rapidly phagocytosed by other cells, and no intracellular constituents are released (Que and Gores 1996; Thornberry and Lazebnik 1998).

NO, derived from L-arginine in a reaction catalyzed by NOS, has a specific place in apoptosis. NO prevents apoptosis by direct inhibition of caspase-3-like activity through protein *S*-nitrosylation (Kim et al. 1997). It has been suggested that NO may be able to inhibit lipid peroxidation by interfering with the propagation stage of the peroxidation chain reaction (Dash et al. 2003; Hogg et al. 1993; Kelley et al. 1999).

The activity of the transcription factor NF-κB is known to be redox-modulated (Baldwin 1996). Oxidative stress, mediated by peroxide, induces histones' hyperacetylation and enhanced accessibility of the restriction enzyme to this NF-κB region. The structural changes of chromatin activate NF-κB site and increase interleukin-1β-stimulated iNOS expression in the presence of oxidative stress.

Mitochondria are the primary site of GC and PA actions

In cell physiology mitochondria exert their function in energy production and programmed cell death or apoptosis (Roussel et al. 2004; Dash 2005; Psarra et al. 2006; Sionov et al. 2006a). The mitochondrial respiratory chain has been recognized as one of the major sources of intracellular ROS generation and an important target for the damaging effects of ROS (Demonacos et al. 1995; Liu et al. 2002; Ott et al. 2007). The accumulation of Ca^{2+} in mitochondria can trigger a phenomenon called mitochondrial permeability transition (MPT), characterized by the increased permeability of the inner membrane. This phenomenon leads to a bioenergetic collapse, and triggers the proapoptotic pathway. PA are known to enhance Ca^{2+} accumulation in mammalian mitochondria, buffering extra-mitochondrial Ca^{2+} concentrations to levels similar to those in cytosol of resting cells. This is the reason that PA induce the modulation of mitochondrial permeability transition, with

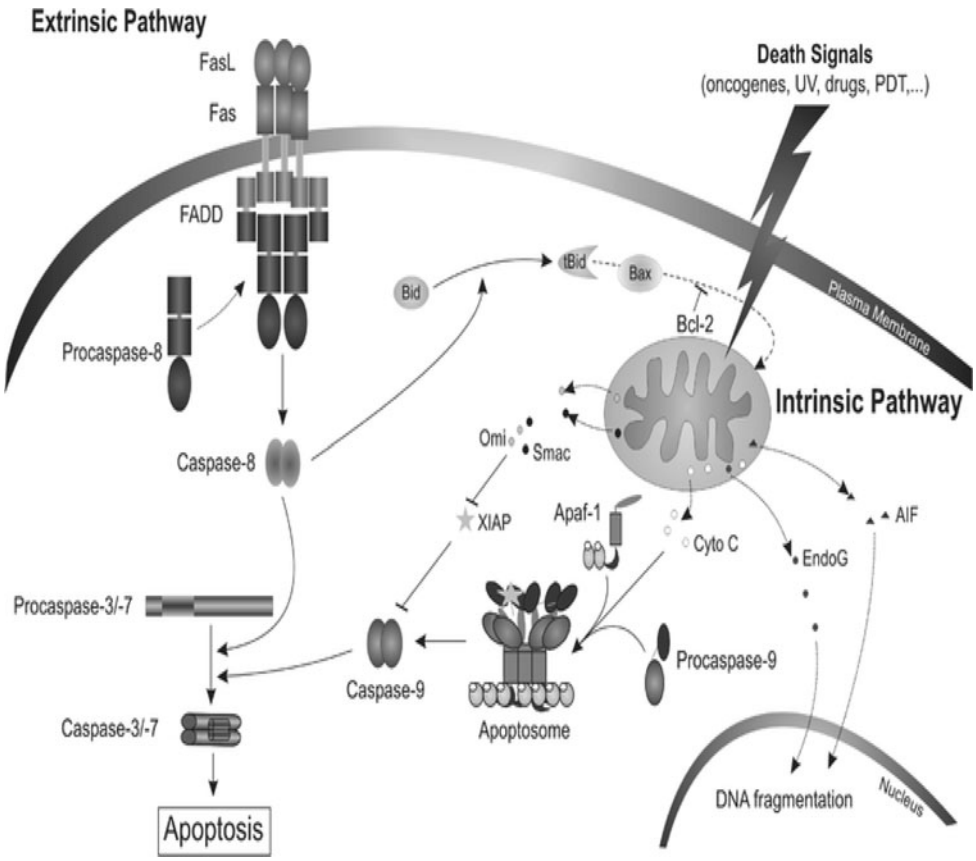

Fig. 2 The death receptor and mitochondrial pathway of apoptosis. There are two major apoptotic pathways in mammalian cells: the death receptor (extrinsic) pathway, exemplified by FasL binding to an extracellular receptor and the mitochondrial (intrinsic) pathway. The mitochondrial pathway, activated by most cellular stresses, involves a change in mitochondrial transmembrane potential resulting in the release of cytochrome *c* from mitochondria into the cytosol. Cytochrome *c* then binds to apoptosis activating factor 1 (Apaf-1) and procaspase-9 forming apoptosome and activating caspases (http://www.rsc.org)

triggering the apoptotic pathway (Stefanelli et al. 1998, 2000; Salvi and Toninello 2004).

GSs exert their capability in various immunomodulatory disease producing ROS at the mitochondrial level with the consequent T and B lymphocyte apoptosis (Cifone et al. 1999; Barnes 1998; Wang et al. 2006; Franchimont 2004).

The induction of apoptosis in thymocytes by GC is one of the earliest recognized forms of apoptosis (Distelhorst 2002; Lill-Elghanian et al. 2002). Yet, the mechanisms by which GC cause apoptosis remain obscure. GC show potent anti-inflammatory and immunosuppressive activities including the ability to induce T and B lymphocyte apoptosis (Cifone et al. 1999; Barnes 1998; Wang et al. 2006; Franchimont 2004). GC-induced apoptosis is mediated by the mitochondrial pathway (Sionov et al. 2006a). Glucocorticosteroid-induced apoptosis results in the production of ROS at the complex III of the ubiquinone cycle which can induce severe oxidative stress with negative implications on the stability of mitochondrial membrane (Tonomura et al. 2003). The literature data suggest that chronic GC treatment increases proton leakage across the mitochondrial inner membrane, thereby decreasing the efficiency of mitochondrial energy conversion in liver (Roussel et al. 2004). All the cell death events mediated at the mitochondria are regulated by proteasome (Pickle 2005).

There is a correlation between mitochondrial GR translocation and sensitivity of cells to GC-induced apoptosis. Interestingly, mitochondrial translocation of GR was observed only in cells sensitive to GC-induced apoptosis, but not in GC-resistant ones. In contrast, nuclear translocation occurs in both cell types (Sionov et al. 2006b). The presence of nucleotide sequences in the mitochondrial genome with homology to GREs, and with capacity to bind steroid receptors is supportive of a direct effects of these hormones on mitochondrial transcription (Demonacos et al. 1995; Psarra et al. 2006). Also, nuclear transcription factors NF-κB, AP-1, CREB and p53 have been found in mitochondria (Sionov et al. 2006b). Cross-talk between the GRs and activation factors, especially AP-1 and NF-κB, controls several survival pathways. The interaction of GR with these factors is believed to play a major role in GC-mediated apoptosis (Sionov et al. 2006b; Psarra et al. 2006).

It is believed that FasL, similar to other cytokines, is repressed by GC via GR interaction with other transcription factors, interfering with their transactivation ability. Human FasL is directly regulated by GR in a DNA binding dependent manner. GR downregulates FasL promoter by competing with NF-κB for binding to the common response element. Thus, FasL is the first gene described

whose repression by GR is mediated by sterical occlusion of NF-κB DNA binding. This type of repression represents an additional mechanism for the GR-NF-κB mutual antagonism (Novac et al. 2006).

GC induce apoptosis in lymphocytes. The process by which GC induce apoptosis is divided into three stages: an initiation stage that involves GC receptor-mediated gene regulation, a decision stage that involves the counterbalancing influence of prosurvival and proapoptotic factors, and the execution stage which involves caspase and endonuclease activation. Mitochondrial dysfunction and caspase activation are important steps in many forms of apoptosis induced with GC (Distelhorst 2002; Lill-Elghanian et al. 2002).

Live imaging by confocal microscopy revealed that lysosomal cathepsin B, an unrecognized component of this pathway to date, becomes rapidly activated in thymocytes after GC exposure (Wang et al. 2006). There is a mystery between the initiation and the execution stage of cell death. How do transcriptional changes mediated by corticosteroid receptor lead to programmed cell death? GC appear to oppose the action of growth factors. This concept is described as the 'Yin and Yang' of corticosteroid-induced apoptosis. It may be the balance between the prosurvival action of growth factors and the proapoptotic action of GC that ultimately determines a cell's fate. Growth factors increase the activity of a number of transcription factors that mediate expression of genes involved in cell proliferation (Papavassiliou 1995). GC decrease the activity of these transcription factors. All of them are targets of the multicatalytic proteasome. Thus, it is possible that proteasome-mediated degradation of prosurvival factors may be a central, Bcl-2 regulated step in GC-induced apoptosis (Distelhorst 2002).

Polyamine metabolism

Intracellular PA concentrations are maintained through the coordinated metabolic pathways of biosynthesis, transport, and catabolism. Biosynthesis of PA is predominantly regulated by the activities of ODC and *S*-adenosylmethionine decarboxylase (AdoMetDC). In intracellular PA catabolism three distinct polyamine oxidases are involved: a relatively constitutively expressed, peroxisomal N^1-acetylpolyamine oxidase (PAO), the rate-limiting and inducible cytosolic, Spd/Spm N^1-acetyltransferase (SSAT) and an inducible Spm oxidase (SMO/PAOh1) (Fig. 3) (Wang and Casero 2006). The catabolism of acetylated Spd and Spm by polyamine oxidases produces hydrogen peroxide, amino aldehydes, acrolein, cytotoxic agents found to induce programmed cell death (PCD) or apoptosis (Cohen 1998; Hölttä 1977; Schuber 1989; Seiler 1995; Murrray-Stewart et al. 2002; Vujcic et al. 2002, 2003; Wang et al. 2003, 2006; Pledgie et al. 2005; Seiler and Raul 2005).

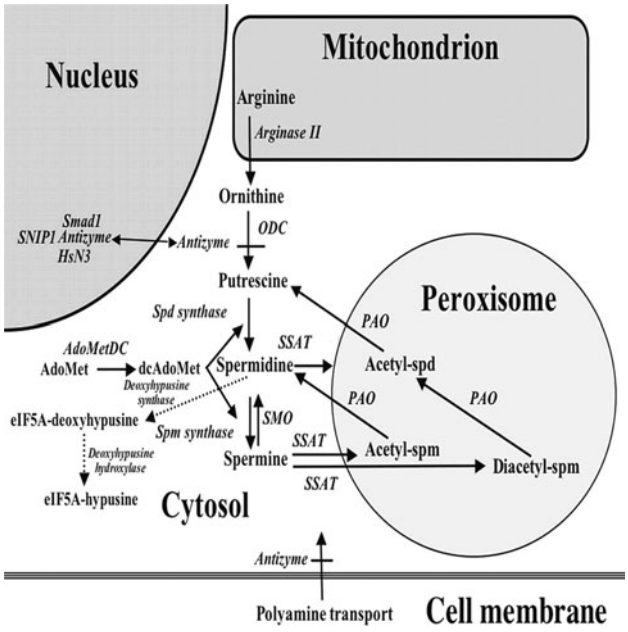

Fig. 3 The metabolism of PA: *ODC* ornithine decarboxylase, *Spd* spermidine, *AdoMetDC* *S*-adenosylmethionine decarboxylase, *dcAdoMet* decarboxylated AdoMet, *Spm* spermine, *SSAT* Spd/Spm N^1-acetyltransferase, *PAO* polyamine oxidase, *SMO* Spm oxidase, *eIF5A* eukaryotic initiation factor 5 A. [Jänne et al. (2004) with modifications]

PA, GC and inflammation

During the early immune response to infection or injury, macrophages synthesize pro-inflammatory cytokines, the most important compounds in the inflammatory reaction. One class of endogenous cytokine synthesis inhibitors are the GC hormones, which are produced during the stress response, and suppress immune activation and cytokine synthesis. Inflammatory cytokines and NO can regulate both polyamine biosynthesis and transport.

Also, a large body of evidence implicates Spm as an inhibitor of immune responses (Zhang et al. 1997). Some 55 years ago, Hirsch and Dubos (1952) discovered that Spm was the natural product in animal tissues capable of suppressing the growth of tubercle bacilli (Zhang et al. 1997). It is already known that Spm concentrations significantly elevates in tissues during inflammatory diseases, immune response, and neoplastic process, (e.g., tuberculosis, pneumonia, cancer) suggesting a direct role of Spm in limiting the growth or spread of an infectious agent or tumor (Zhang et al. 1997, 2000; Bjelakovic et al. 2006). The increase of Spm levels in tissues following injury, inflammation, and antigen stimulation, derived in part from the release of intracellular Spm from dying and injured cells, and in part by its stimulated biosynthesis (Seiler and Atanassov 1994; Zhang et al. 2000; Bjelakovic et al. 2006). It has been suggested that the accumulation of Spm, and

the products of its oxidative metabolism via polyamine oxidases, mediate the anti-inflammatory activity found in inflammatory exudates, human pregnancy serum, plasma from arthritic rats, and human rheumatoid synovial fluid (Colombatto et al. 1988; Seiler and Raul 2005). Infectious agents and mediators of inflammation can up-regulate polyamine catabolism by induction of SSAT. The catabolism of acetylated Spd and Spm by polyamine oxidase produces H_2O_2, which has been found to induce programmed cell death (PCD) (Babbar et al. 2007).

Spm oxidation exerts potent immunosuppressive effects in animal cells. Phosphatidylserine exposure, a potent engulfment signal for phagocytes, might contribute to the immunosuppressive effects of plasma PA through a controlled and rapid necrotic process involving Spm oxidation (Bonneau and Poulin 2000). Since Spm effectively inhibits cytokine synthesis in serum-free conditions, and in the presence of polyamine oxidase inhibitor aminoguanidine, oxidative metabolism of Spm is not required for the molecular mechanism of cytokine counter-regulation (Zhang et al. 1997). Spm suppresses the synthesis of pro-inflammatory cytokines in human PBMCs (Zhang et al. 2003; Southan et al. 1994) (Fig. 4).

Spm downregulates human neutrophil locomotion (Ferrante 1985), prevents NO production in macrophages activated by bacterial endotoxin (Szabo et al. 1994a, b), and is immunosuppressive to T cells (Seiler and Atanassov 1994). The in vivo application of Spm protected mice against the development of carrageenan-induced edema, giving evidence that Spm accumulation in tissues can counter-regulate the acute inflammatory response (Oyanagui 1984; Liao et al. 2006). During immune insults PA have a major impact on the neuronal integrity and cerebral homeostasis (Soulet and Rivest 2003).

PA may stimulate the binding of NF-κB to its specific response elements on DNA. However, PA depletion activates NF-κB in IEC6 cells, suggesting that there may be differences in response to PA between normal and transformed cells (Pfeffer et al. 2001).

Spm may influence TNF production. TNF, mainly produced by activated macrophages, is a cytokine with a broad spectrum of biological activities (from defense against

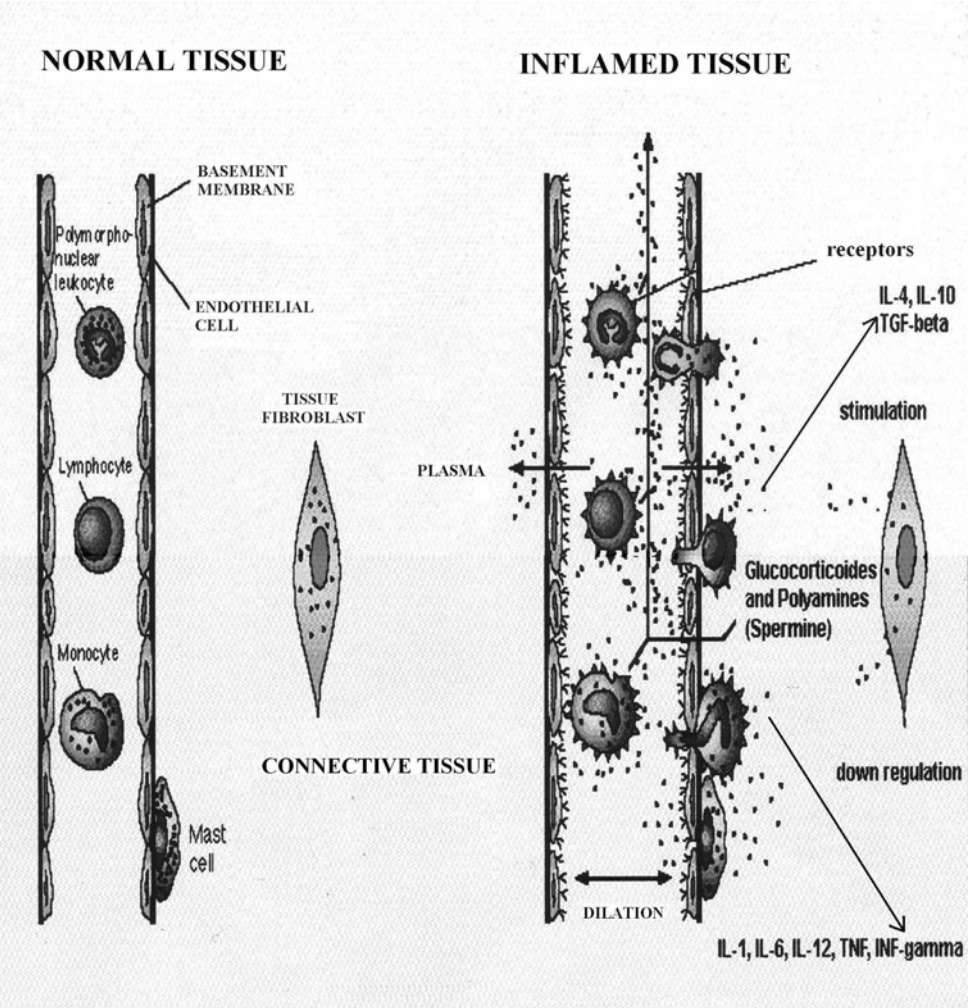

Fig. 4 Models of the pathogenesis of inflammation and immune injury. The recruitment of leukocytes at sites of inflammation, their subsequent activation, and generation of secretory products contribute to tissue damage. GC inhibit the production of pro-inflammatory cytokines, such as IL-1, IL-6, IL-12, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ, whereas they stimulate the production of anti-inflammatory cytokines such as IL-10, IL-4, and TGF-β. Spm inhibits the synthesis of IL-1, IL-6, TNF. (Boumpas et al. 1993; Chrousos 1995) with modifications

viral, bacterial and parasitic infections to immunoregulatory responses and the induction of cell death). This pleiotropic character is due to the fact that virtually all cells express receptors for TNF. TNF-induced cytokine production is mediated by a decrease in Spm (Penning et al. 1998; Pfeffer et al. 2001; Babbar et al. 2006a, b).

Inflammatory cytokines and NO have capacity to regulate PA biosynthesis and transport. NO may modulate the proliferative response in the early phase of inflammation by suppressing ODC, the initial and rate-limiting enzyme in the polyamine biosynthetic pathway (Bauer et al. 1999; Dash et al. 2003). ODC requires a cysteine in its active center for full enzymatic activity (Satriano et al. 1999; Bauer et al. 2001). The data of Bauer et al. (2001) support the hypothesis that NO inhibits ODC activity via *S*-nitrosylation of a critical cysteine residue(s) on ODC.

Spm and Spd induce apoptosis in extravillous trophoblasts following their oxidation and the production of hydrogen peroxide. NO is able to inhibit these effects (Dash et al. 2003). NO exerts the inhibiting effect on polyamine synthesis. On the other hand, Spd has the inhibitory effect on the expression of inducible NOS (Satriano et al. 1999). Shearer et al. (1997) have shown modifications in the expression of nitric oxide synthases (NOS) and PA synthesis at inflammation sites. The activation of NOS at the point of injury may favor the local accumulation of phagocytic cells. The activation and subsequent death of macrophages may be responsible for the release of arginase, leading locally to the breakdown of extracellular arginine to ornithine, the precursor of PA synthesis (Kepka-Lenhart et al. 2000; Shearer et al. 1997; Moinard et al. 2005).

PA, GC and apoptosis

The role of PA in apoptosis is unclear because both increased and decreased levels of PA have been observed in conjunction with apoptosis (Thomas and Thomas 2001; Seiler and Raul 2005). PA are involved in the mitochondrial pathway of apoptosis. Spm and Spd interact with mitochondrial membranes at two specific binding sites. This binding represents the first step of PA transport into mitochondria (Dalla Via et al. 1999; Toninello et al. 1990; Toninello et al. 1992, 2004).

Under normal conditions cytochrome *c* is found only in the mitochondrial inner membrane space. Cytochrome *c* release from mitochondria to the cytosol represents a critical step in apoptosis. It has been reported that PA can directly promote cytochrome *c* release from the mitochondria (Stefanelli et al. 2000). The antizyme (AZ) and glycosaminoglycans have specific roles in Spm and Spd uptake by mammalian cells and mitochondria. Spm uptake by mitochondria caused a release of cytochrome *c*, an enhancer of apoptosis (Hoshino et al. 2005).

High levels of PA induce apoptosis in a variety of cell types (Pouline et al. 1995). The accumulation of Spm in L1210 cells overexpressing ODC induces apoptosis (Penning et al. 1998). High levels of intracellular PA promote histone acetyltransferase activity resulting in chromatin hyperacetylation, suggesting a mechanism by which altered PA biosynthesis contributes to aberrant expression of genes, facilitating oxidative stress and tumor growth (Hobbs and Gilmour 2000). The inhibition of cell growth by overaccumulation of PA was mainly due to the inactivation of ribosomes through replacement of Mg^{2+} on magnesium-binding sites by PA (Datta et al. 1969; Stefanelli et al. 2000; Igarashi and Kashiwagi 2000, 2006).

An increase in PA levels has been found in hepatocytes growth factor-induced apoptosis (Yanagawa et al. 1998). Formation of hypusine, in which the butylamine moiety of Spd is transferred to the lysine residue in eukaryotic initiation factor (eIF-5A), appears to be an important part of the normal function of Spd. In the presence of excess putrescine, there was an inhibition of hypusine formation and a decrease in modified eIF-5A. The lack of hypusine caused by accumulation of Put led to apoptosis in a hepatoma cell line (Thomas and Thomas 2001; Igarashi and Kashiwagi 2000, 2006).

In some cell types induction of cell death by PA is due to their oxidation products rather than through direct effects (Szabo et al. 1994b; Maccarrone et al. 2001). Increased PA catabolism can result in substantial increase in intracellular ROS through the production of H_2O_2, aminoaldehydes and acrolein as by-products of all three polyamine oxidases activities (Vujcic et al. 2002; Wang et al. 2003; Dash et al. 2003; Pledgie et al. 2005; Seiler and Raul 2005). Excessive formation of ROS leads to chromatin degradation with DNA damage (Seiler and Raul 2005; Babbar et al. 2007). PA activate caspases (Stefanelli et al. 1998; Ray et al. 2000). All of these are reason that Spm treatment results in mitochondria-mediated apoptosis (Schiller et al. 2005).

A variety of non-steroid inflammatory drugs (NSAIDs), including aspirin, at physiological concentrations, can induce SSAT mRNA through transcriptional initiation mechanisms in Caco-2 cells. Promoter deletion analysis of the 5′ SSAT promoter-flanking region led to the identification of two NF-κB response elements. Aspirin treatment led to the activation of NF-κB signaling and increased binding at these NF-κB sites in the SSAT promoter, providing a potential mechanism for the induction of SSAT by aspirin in these cells. Aspirin-induced SSAT ultimately leads to a decrease in cellular PA content, which has been associated with decreased carcinogenesis (Babbar et al. 2006a).

Tumor necrosis factor α (TNF-α) can lead to the induction of NF-κB signaling with a concomitant increase in Spd/Spm N^1-acetyltransferase (SSAT) expression in A549 and H157 non-small cell lung cancer cells. Induction of SSAT leads to apoptosis (Babbar et al. 2006b). The inhibition of Iκ-B and activation of NF-κB activate Spd/Spm *N*(1)-acetyltransferase (Choi et al. 2006).

The induction of cell death was correlated with a dramatic increase in cellular Put levels (Takao et al. 2006). Accumulation of Put in mouse myeloma ODC overproducing cells line causes oxidative stress that leads to apoptotic cell death (Erez et al. 2002). Overexpression of a metabolically unchanged ODC in CHO cells induced a massive cell death unless the cells were grown in the presence of the ODC inhibitor alpha-difluoromethylornithine (DFMO) (Takao et al. 2006). Exposure of human leukemia cells to PA triggers caspase activation. Excessive intracellular level of Spm triggers caspase activation that is not mediated by oxidative mechanisms, and suggests a model where elevated free cytosolic PA may act as transducers of a death message (Stefanelli et al. 1998).

Fragmentation of DNA and formation of apoptosis bodies characterized apoptosis.

PA stabilize DNA by protecting them from oxidative stress or by inhibiting the endonucleases. PA depletion causes significant changes in chromatin and DNA structure. The destabilization of chromatin could be an important requirement for the apoptotic DNA fragmentation. Spm has been shown to prevent endonuclease-mediated DNA fragmentation and protect thymocytes from apoptotic cell death (Fraser et al. 2002; Thomas and Thomas 2001; Hegardt et al. 2001). In nucleus it is normally found in millimolar concentrations directly acting as a free radical scavenger (Ha et al. 1998; Sava et al. 2006). Spm is a major natural intracellular compound capable of protecting DNA from free radical attack (Ha et al. 1998).

The effect of PA depletion on apoptosis depends on the cell type and the nature of apoptotic stimuli. The intracellular depletion of PA in chondrocytes can inhibit both the death receptor pathway and the mitochondrial pathway (Stanic et al. 2006). The characteristics of the camptothecin-induced apoptosis (increases in caspase activity, release of cytochrome *c* from mitochondria, translocation of Bax to mitochondria, and cleavage of Bid) were inhibited in cells depleted of PA (Yuan et al. 2002). Reduction PA levels, is one of all functional mediators of apoptosis caused by 5-FU in colon carcinoma cells (Zhang et al. 2003).

Experimental results demonstrate that PA depletion inhibits γ-irradiation-induced apoptosis in vitro and in vivo. Intestinal epithelial cells (IEC-6) pretreated with 5 mM α-DFMO for 4 days show PA depletion and a significantly reduced radiation-induced caspase-3 activity and DNA fragmentation. This protective effect was prevented by the addition of 10 μM exogenous Put (Deng et al. 2005). These results indicate that depletion of PA may be involved in the caspase activating signal cascade (Ray et al. 2000). DFMO depletes H9c2 cardiomyoblasts of PA and protects the cells against ischemia-induced apoptosis (Tantini et al. 2006). The PA depletion increases expression of the inhibitors of apoptosis (IAP) family of proteins, c-IAP2 and XIAP by activating NF-κB in intestinal epithelial cells (Zou et al. 2004; Wei et al. 2004).

Spm can prevent swelling and MPT induction by acting as a free radical scavenger. Spm inhibits H_2O_2 production in rat liver mitochondria and maintains glutathione and sulphydryl groups at normal reduced level (Sava et al. 2006). By acting as a free radical scavenger, Spm, in the absence of Ca^{2+}, inhibits H_2O_2 production and maintains glutathione and sulphydryl groups at normal reduced level, so that the critical thiols responsible for pore opening are also consequently prevented from being oxidized (Pavlovic et al. 1992; Ha et al. 1998; Salvi and Toninello 2004; Sava et al. 2006).

PA are GC-type anti-inflammatory drugs, and may be mediators of GC actions (Oyanagui 1984). GC are well-known proapoptotic agents in certain classes of lymphoid cells (Abbott and Bird 1983). GC exert catabolic effects on lymphoid tissues in which they cause programmed cell death by depletion of PA, probably as a consequence of the modulation of PAO activity (Bjelakovic and Pavlovic 1987; Bjelakovic et al. 2006; Ferioli et al. 1999; Miller et al. 2002; Tomitori et al. 2005).

The treatment of mouse thymus in vivo with dexamethasone, a synthetic GC, induces apoptosis with the changes of PA metabolism: the biosynthetic enzymes activities, ODC and *S*-adenosylmethionine decarboxylase were decreased, while the activity of the rate-limiting catabolic enzyme Spd/Spm N^1-acetyltransferase (SSAT) was increased (Desiderio et al. 1995, 1998). The reduction in PA levels was only evident through a few hours after the first signs of apoptosis. The decrease in PA levels was also observed in dexamethasone-induced apoptosis in mouse thymocytes in vitro and in Fas-induced apoptosis of human leukemic T cells (Webb et al. 2003). The decrease of intracellular levels of PA seems to be important for the progression of the apoptotic process and the exogenous addition of Spm protected mouse thymocytes from apoptosis induced by dexamethasone (Desiderio et al. 1995; Hegardt 2000). Spm has a protective effect on mitochondria of dexamethasone-treated thymocytes. GC are well known as proapoptotic agents (Abbott and Bird 1983).

GC induce apoptosis in some classes of lymphoid cells by inter-nucleosomal DNA cleavage. Spm prevents DNA fragmentation as well as the activation of caspase-9, -8 and -3 (Ferioli et al. 1999, 2000; Hegardt et al. 2001, 2003; Miller et al. 2002).

The total inhibition of caspase-9 activity was due to Spm-mediated inhibition of cytochrome *c* release from the mitochondria into the cytosol (Brune et al. 1991; Hegardt et al. 2000, 2003). Spm inhibits dexamethasone-induced apoptosis upstream of caspase-9 activation in mouse thymocytes and in the human leukemic T cell line Jurkat (Hegardt et al. 2001). The application of dexamethasone during immunogenesis caused the decrease of PA concentrations in liver and spleen. At the same time dexamethasone decreased PAO activity in liver and spleen of sensitized guinea pigs, increasing PAO activity in tissues of unsensitized animals (Bjelakovic et al. 2006). Diminished PA level in liver and spleen is in agreement with literature data, which point out the inhibitory effect of dexamethasone on ODC by the induction of ODC-antizyme synthesis (Bishop et al. 1985).

Because of their importance in many cellular functions, PA have long been the focus of research as potential chemotherapeutic agents. By testing the effects of dexamethasone, and two polyamine inhibitors, DFMO and methyl glyoxal bisguanylhydrazone (MGBG), on the clonal line of human acute lymphoblastic leukemia cells, CEM-C7-14. Miller et al. (2002) found that dexamethasone alone killed these cells, though only after a delay of at least 24 h. Combination of pretreatment with sublethal concentrations of both DFMO, the inhibitor of the ornithine decarboxylase, and MGBG, the inhibitor of *S*-adenosylmethionine decarboxylase, followed by addition of dexamethasone, results in strong synergistic killing of cells. They have shown that transcriptional downregulation of the proto-oncogene c-myc is a critical signal in the pathway of the GC-evoked apoptosis of CEM cells. These studies suggest that PA are protective against dexamethasone, and preliminary administration of DFMO and MGBG renders cells more sensitive to the lethal effect of GC (Miller et al. 2002).

All these observations show that more studies are needed to clarify the role of PA and GC in apoptosis.

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