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The 18 kDa Translocator Protein as a Potential Participant in Atherosclerosis

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1. Introduction

1.1 Inflammation in atherosclerosis

Inflammation is a process integral to atherosclerosis, a concept that dates back from the studies by Ross (1999). Since then, circulating markers have been established as predictive to atherosclerosis and its clinical events (Hansson et al., 2005; Packard & Libby, 2008). Accumulated subendothelial lipid, particularly if oxidized, exacerbates the local inflammatory reaction and maintains activation of the overlying endothelium (Tiwari et al., 2008). Atheroma formation involves expression of selectins and adhesion molecules and also expression of chemokines, in particular monocyte chemoatractant proteins-1 (MCP-1). Chemokines are proinflammatory cytokines that function in leukocyte chemoattraction and activation. Atheroma prone mice lacking MCP-1 develop smaller atherosclerotic lesions than those expressing MCP-1. Once captured at the vascular wall, inflammatory cells migrate into the subendothelial space where, under the influence of local chemokines, they become activated. There the monocytes mature into macrophages and express the necessary scavenger receptors to ingest modified lipids and become macrophage foamy cells. The predominant role of the macrophages in atherosclerosis is to ingest and dispose of atherogenic lipids. However, activated macrophages and T cells also express a variety of proinflammatory cytokines and growth factors that may contribute to atherosclerotic plaque formation. The progression of an atherosclerotic plaque is best understood in terms of dynamic interaction between a subendothelial inflammatory stimulus and the local reactive "wound healing" response of surrounding vascular smooth muscle cells (VSMCs) (Clarke & Bennett, 2006).

Inflammation produces reactive oxygen species (ROS) as a by-product, and antioxidant therapeutic strategies may have proved disappointing possibly because oxidative events are a consequence, rather than a cause of atherosclerosis. In this scenario, ROS scavenging would have little impact on the disease process. This notion is consistent with the observation that the relationship between the risk factors of atherosclerosis and inflammation is tight, in that all of the established cardiovascular disease risk factors are predictive of circulating inflammation markers. Also, modification of atherosclerotic risk factors by lipid lowering therapies, cessation of smoking, weight loss, and improved glucose control reduces circulating markers

of inflammation. These and other findings suggest that inflammation is a primary process and oxidative stress is only secondary one in relation to atherosclerosis (Rodriguez-Moran et al., 2003). Nevertheless, traditional anti-inflammatory therapies do not add to the recovery process, moreover, they may even slightly exacerbate atherosclerotic events (Libby et al., 2011).

1.2 Therapeutic strategies in atherosclerosis

The presence and biological consequences of DNA damage in atherosclerosis imply that both prevention and reversal of damage are therapeutic aims. *In vitro,* antioxidants can ameliorate ROS-induced DNA damage, even though antioxidant trials in humans have been disappointing so far. Whereas high dietary intake of vitamin E and C is associated with reduced risk of cardiovascular disease (CVD), well powered clinical trials in atherosclerosis-related CVD have indicated that supplements with vitamin C or vitamin E alone do not provide sufficient benefit, in comparison to, for example, statins (Kunitomo et al., 2009). Specific antioxidants scavenge or metabolize some, but not all of the relevant oxidized species. For example, radical scavengers will limit lipid peroxidation, but will have no effects on protein modification by peroxinitrite ($ONOO^-$), cell signalling by H_2O_2, or HOCl mediated oxidation reaction. Thus, whenever a physiological process goes unchecked in case of disease, strategies that rely simply on scavenging the offending species must be employed with extreme caution (Stocker and Keaney, 2005).

In contrast, cholesterol lowering by diet is associated with a reduction in DNA damage, at least in animal models (Singh et al., 2009). Drugs that have been proven to alter plaque progression have also been shown to alter vascular oxidative stress. In particular, 3-hydroxy-3-methylglutaryl coenzyme A (HMGCoA) reductase inhibitors (Statins) reduce NAD(P)H oxidase activation and superoxide production *in vitro*, in part because of their capability to inhibit membrane translocation (and thus activity) of the small GTP-binding protein Rac-1, which is a regulatory component of vascular NAD(P)H oxidase activation (Cosotpoulos et al., 2008). Another trial, which investigated the use of niacin combined with a prostaglandin D2 receptor antagonist, intended to reduce cutaneous flushing, and has shown durability of benefit (Insull, 2009; Libby et al., 2011). Namely, together with lipid lowering therapy acute clinical benefits were cumulative, but tolerability issues has limited its use. Understanding the non-lipid associated events in atherogenesis raises the prospect of developing drugs targeted at specific events in its pathogenesis which might act synergistically with lipid lowering drugs to enhance plaque stability (Singh et al., 2009).

1.3 Translocator Protein 18 kDa (TSPO) as a potential participant in atherosclerosis

It was shown previously that the 18 kDa translocator protein (TSPO) is present throughout the cardiovascular system and may be involved in cardiovascular disorders such as ischemia. At cellular levels TSPO is present in virtually all of the cells of the cardiovascular system, where they appear to take part in responses to various challenges that an organism and its cardiovascular system face (Veenman & Gavish, 2006), including atherosclerosis and accompanying symptoms (Onyimba et al., 2011; Bird et al., 2010; Dimitrova-Shumkovska et al., 2010a,b,c).

1.3.1 TSPO - Structure and localization

TSPO can be found in various tissues (Gavish et al., 1992, 1999). The TSPO is also known as peripheral type benzodiazepine receptor (PBR), since it is capable of binding benzodiazepines and is found in most if not all peripheral tissues (Veenman et al., 2007).

Mitochondrial membranes form the primary location for TSPO (Anholt et al., 1985). The present name of the TSPO, translocator protein, was chosen because of the TSPO's capability to transport molecules over the outer the mitochondrial membrane (Papadopoulos et al., 2006). For example, TSPO is known to transport cholesterol over the outer the mitochondrial membrane. At the mitochondria, TSPO are closely associated with the 32 kDa voltage-dependent anion channel (VDAC) and the 30-kDa adenine nucleotide translocator (ANT) (Mc Enery et al., 1992; Veenman et al., 2007; **Figure 1**). VDAC and ANT are considered to form the core components of the mitochondrial permeability transition pore (mPTP) (Galiegue et al., 2003). The ratio of TSPO to VDAC and ANT appears to be tissue- and treatment-dependent (Golani et al., 2001; Veenman et al., 2002). The mitochondrial location of the TSPO is interesting in relation to cardiovascular diseases, as it is well known that mitochondria are a main source of cellular ROS (Lenaz, 1998). Furthermore, several studies have shown that the TSPO appears to be a participant in ROS generation at mitochondrial levels (Veenman et al., 2008,2010a; Zeno et al., 2009; Choi et al., 2011). As discussed above, ROS may play a role in cardiovascular diseases.

Furthermore, by its interactions with the VDAC and the ANT, the TSPO is able to modulate the flow of electrolytes over the outer and inner mitochondrial membranes and participate in the collapse of the mitochondrial membrane potential (Kugler et al., 2008; Zeno et al., 2009). In this context, TSPO is a participant in the initiation of mitochondrial apoptosis cascade, including release of cytochrome c from the mitochondrial membrane potential (Levin et al., 2005; Kugler et al., 2008; Veenman et al., 2010). It has been suggested that the role of TSPO in importing proteins and cholesterol into the mitochondria may partake in mitochondrial membrane biogenesis, required for cell growth and proliferation (Papadopoulos et al., 2006; Veenman et al., 2007). Even though many functions were attributed to the TSPO, its primary roles are still discussed and the mechanisms whereby TSPO takes part in many of these functions still need further clarification (Veenman & Gavish, 2006; Veenman et al., 2010b). Because TSPO appear to be involved in a large variety of physical diseases, mental disorders and responses to stress, clinical benefit may be attainable by increasing knowledge regarding the TSPO. Including its involvement in cardiovascular disorders.

TSPO are found throughout the animal kingdom, including insects, mollusks, pisces, amphibians, aves and mammals (Peterson et al., 1988; Veenman et al., 2007), yet have not been found in reptiles as to date (Bolger et al., 1986). Widely expressed throughout the body, TSPO exhibit different patterns of tissue specific expression (Golani et al., 2001; Veenman et al., 2002). *In vivo* studies showed the rank order of TSPO binding density in rats to be adrenal >> kidney ~ heart ~ testis ~ ovary >> liver ~ brain (Awad & Gavish, 1987; Gavish et al., 1999). In humans and dogs, the heart appears to be one of the organs with high TSPO density (Veenman & Gavish, 2006).

The rat TSPO protein consists of 169 amino-acids, is highly hydrophobic, and is rich in tryptophan. TSPO appears to constitute a five α-helical transmembrane structure that stretches the outer mitochondrial membrane. The human homologous TSPO also consists of 169 amino-acids, including two cysteine residues that may allow for S-nitrosylation of the protein. (Gavish et al., 1999; Babbage, A. Direct Submission, GenBank: CAB55884.1, 2009). The TSPO gene is conserved from prokaryotes to plants and animals, including humans and appears to have the hallmarks of a typical housekeeping gene, suggesting that this gene's product has a basic cellular function (Gavish et al., 1999).

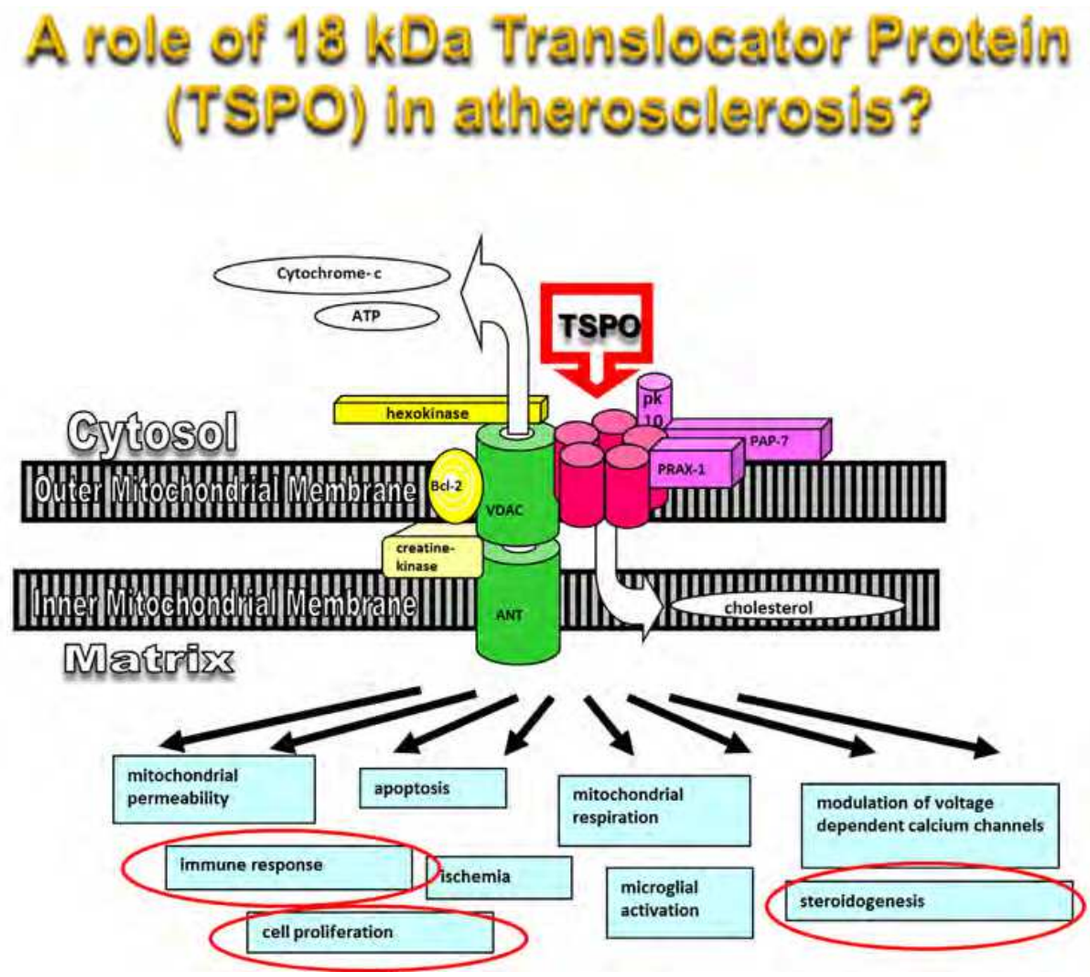

Fig. 1. TSPO structure, localization and functions. TSPO molecules are often found in groups and in conjugation with VDAC and ANT. As indicated in the figure, pk10, PRAX-1, and PAP7 face the cytosol. Furthermore, molecules of the Bcl-2 family and creatine kinase and hexokinase can be attached to VDAC and ANT. The TSPO is involved in various functions some of which are indicated towards the bottom of the figure. The encircled functions may relate to an association of the TSPO with cardiovascular pathology. Abbreviations: ANT, adenine nucleotide transporter; ATP, adenosine triphosphate; DBI, diazepam binding inhibitor; PAP7, PBR associated protein 7; PBR, peripheral-type benzodiazepine receptor; pk10, protein of 10 kiloDalton; PLA2, phospholipase A2; PRAX-1, PBR associated protein 1; TSPO, translocator protein (18-kDa); TTN, triakontatetra-neuropeptide; VDAC, voltage dependent anion channel (Veenman et al., 2007).

Recently, TSPO has been found to occur not only in the 18 kDa form, but also as 36-, 54-, and 72 kDa TSPO polymers (Delavoie et al., 2003). This topic has arisen from the electron microscopic observation that the 18 kDa TSPO protein was organized in clusters of 2-7 molecules on Leydig cell mitochondrial membranes (Papadopoulos et al., 1997). It was also suggested that free TSPO (meaning not in complex with VDAC and ANT) may be present in mitochondrial membranes (Veenman et al., 2002; Liu et al., 2003). This claim further takes into consideration that modulation of steroidogenesis only requires a mitochondrial channel that is

formed by the mitochondrial TSPO, without participation of VDAC and ANT (Papadopoulos et al., 2006). It should be noted that, apart from mitochondria, TSPO can be found in various other subcellular locations (Veenman & Gavish, 2006). Nuclear/perinuclear-located TSPO, for example, is considered to play a part in cell proliferation (Brown et al., 2000). Other studies detected TSPO binding in plasma membrane and in mature human red blood cells, which lack mitochondria (Oke et al., 1992). It has been suggested that intracellular locations of the TSPO in human lymphocytes might be correlated with its capacity to bind to various endogenous TSPO ligands at these locations, and that this capability might be related to the ratio of TSPO to VDAC and ANT in such locations (Gavish et al., 1999).

1.3.2 General TSPO functions

TSPO have been implicated in various functions **(Figure 1)**, including apoptosis, steroidogenesis, oxidative stress, mitochondrial respiration, modulation of voltage dependent calcium channels, effects on the immune and phagocyte host-defence response, microglial activation related to brain damage, ischemia, regulation of the mitochondrial membrane potential, inflammation, cell growth and differentiation, and cancer cell proliferation (For reviews, see: Gavish et al., 1999; Veenman & Gavish, 2006, 2011; Veenman et al., 2007, 2008, 2010b, 2011). TSPO may potentially be involved in the regulation of several major stress systems, such as the Hypothalamic-Pituitary-Adrenal (HPA) axis, the sympathetic nervous system, the renin-angiotensin axis and the neuroendocrine-immune axis (Gavish et al., 1999; Veenman and Gavish, 2000, 2006). Thus, TSPO possibly plays a role in the mediation of organisms' various adaptations to stress and anxiety disorders.

In the endocrine system, TSPO is well known to participate in steroid production and may also play a role in the host-defence response (Papadopoulos et al., 2006). The presence of TSPO in glia of the central nervous system (CNS) has suggested that they might also be involved in glial functions in the brain. In neurodegenerative disorders, including Alzheimer's disease, TSPO ligand binding density is increased in the affected brain regions. Thus, it has been suggested that TSPO in glia may play a role in neurodegenerative processes and brain damage. In animal studies, both PK 11195 and Ro5-4864 presented neuroprotective effects against brain injury, which have been suggested to involve neurosteroid activation (Veenman et al., 2002; Veenman & Gavish, 2006, 2011; Soustiel et al., 2008). Alternatively, it has been suggested that enhanced levels of TSPO in neural cells (due to damage, disease, etc.) are inductive for apoptosis. In particular, activation of the TSPO may lead to a decrease of the mitochondrial membrane potential, mitochondrial dysfunction, and subsequent release of mitochondrial cytochrome c, followed by the activation of a caspase cascade leading to apoptosis (Levin et al., 2005).

Interestingly, as ROS generation accompanies in cardiovascular diseases, it has also been shown that oxidative stress modulates TSPO structure and function (Delavoie et al., 2003). Vice versa, TSPO appears to be an essential participant in ROS generation at mitochondrial levels induced by various agents (Veenman et al., 2008, 2010a; Zeno et al., 2009; Choi et al., 2011). In addition, Carayon et al. (1996) demonstrated that Jurkat cells transfected with human TSPO cDNA exhibited increased resistance to H_2O_2 toxicity, suggesting a function of these sites and their ligands in protecting cells against the toxicity of ROS produced during inflammatory processes. In the liver, the TSPO was found in co-localization with a ROS scavenger, the mitochondrial manganese-dependent superoxide dismutase (SOD) (Fischer et al., 2001). At present, no conclusive model is available to incorporate the various interactions between the TSPO and ROS.

1.3.3 Cholesterol translocation and steroidogenesis in relation to TSPO

TSPO has been reported to take part in the translocation of cholesterol from the outer to the inner mitochondrial membrane, which is the rate-limiting step in steroidogenesis (Papadopoulos et al., 1990). The fact that TSPO is abundant in steroidogenic endocrine organs (Benavides et al., 1983; Gavish et al., 1999; Papadopoulos et al., 2006), such as the adrenal gland and male and female gonads in rats, has been the first suggestion that TSPO may play a role in steroidogenesis.

The biosynthesis of steroids in all steroidogenic tissues begins with the enzymatic conversion of the precursor cholesterol to form pregnenolone. This reaction is catalyzed by the enzyme cholesterol side-chain-cleavage (P-450scc), which is located on the matrix side of the inner mitochondrial membrane and is dependent on an electron transport system comprised of adrenodoxin and adrenodoxin reductase. Pregnenolone leaves the mitochondrion to undergo enzymatic transformation in the endoplasmic reticulum, giving rise to the final steroid products (Papadopoulos et al., 2006; Veenman et al., 2007). The rate-limiting step in this process, is the transport of cholesterol from cellular stores across the aqueous intermembrane space of the mitochondria to the inner mitochondrial membrane and P-450.

It has been suggested that TSPO and StAR (steroidogenic acute regulatory protein), the latter which is involved in the acute trophic hormone regulation of steroid synthesis, work together in the cholesterol transport into the mitochondria (Stocco & Clark, 1996). Steroids have also been shown to be able to affect TSPO ligand-binding characteristics (Veenman et al., 2007), for example, as demonstrated by a 10-day estradiol treatment of rats that resulted in a marked reduction in TSPO binding in the rat testis, and up regulation of these sites in the kidney (Gavish & Weizman, 1997). Other observations were provided by the removal of the testes, which caused a significant decrease in TSPO density in Cowper's glands and the adrenal gland, while administration of testosterone acetate prevented this castration induced TSPO depletion (Weizman et al., 1992). Furthermore, removal of the pituitary gland, which resulted in the elimination of corticotrophin (ACTH) secretion, caused a significant reduction in the adrenal TSPO density (Anholt et al., 1985). Recently, it has been shown that steroid treatment can regulate gene expression of the TSPO (Mazurika et al., 2009).

Three-dimensional models of the channel formed by the five α-helices of the TSPO indicated that it would be able to accommodate a cholesterol molecule in the space delineated by the five helices. According to these models, the inner surface of the channel formed by the TSPO molecule would present a hydrophilic but uncharged pathway, allowing amphiphilic cholesterol molecules to cross the outer mitochondrial membrane (Papadopoulos et al., 2006; Veenman et al., 2007).

TSPO ligands are reported to induce TSPO-mediated translocation of cholesterol from the outer mitochondrial membrane to the inner mitochondrial membrane (Papadopoulos et al., 1997). Moreover, PK 11195 and Ro5-4864 (the classical specific TSPO ligands) increased cholesterol transport into the mitochondria and subsequent steroid synthesis in gonadal, adrenal, brain, and liver cells (Delavoie et al., 2003). Ro5-4864 directly stimulated the release of corticotrophin releasing hormone (CRH) in rats, whereas PK 11195 directly stimulated the secretion of ACTH. In various models of induced stress in rats, the increase in CRH and ACTH was attenuated by treatment with diazepam. It was reported that the presence of the endogenous ligand DBI was vital for steroidogenesis and stimulated cholesterol transport. DBI regulated steroidogenesis activated by ACTH and luteinizing hormone via binding to TSPO, and thus controlled mitochondrial cholesterol transport (Papadopoulos et al., 1991; Brown et al., 1992).

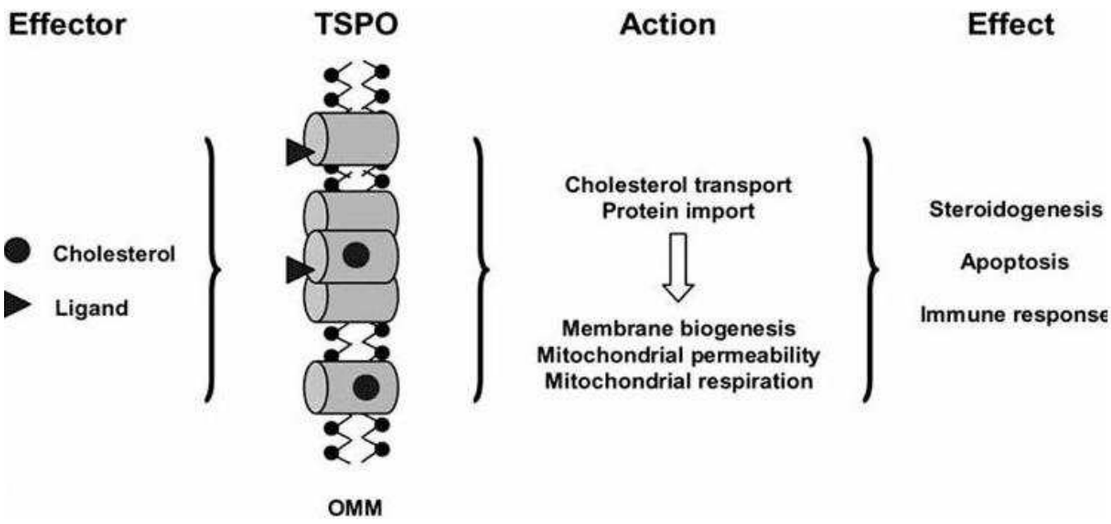

Fig. 2. Proposed model of cholesterol and ligand binding to TSPO. TSPO is found either as a monomer or polymer in the outer mitochondrial membrane. Binding of cholesterol and TSPO ligands to TSPO at this site may result in import of cholesterol or protein into the mitochondria. This may support cellular functions such as membrane biogenesis, mitochondrial permeability, and mitochondrial respiration. The final effects may include steroidogenesis, apoptosis, and immune responses. Abbreviation: OMM, outer mitochondrial membrane (Veenman et al., 2007).

The actual participation of TSPO in mitochondrial cholesterol translocation was demonstrated by disruption of the TSPO gene in Leydig cells, which resulted in the arrest of cholesterol transport into the mitochondria as well as steroid formation, while the reintroduction of TSPO by cDNA rescued steroidogenesis (Papadopoulos et al., 1997). The suggestion of TSPO involvement in steroidogenesis modulated by TSPO ligands was further supported by TSPO antisense knockdowns in MA-10 Leydig cells, which reduced steroid production (Hauet et al., 2005).

It has been proposed that TSPO polymerization modulates the function of this receptor in cholesterol transport, since polymer formation induced by ROS increased both TSPO ligand binding and cholesterol-binding capacities (Delavoie et al., 2003). The monomer binds cholesterol with high affinity but not so with TSPO ligands. The presence of cholesterol on the TSPO monomer prevents the ROS - induced polymer formation. The polymer binds TSPO ligands with high affinity and ligand binding induces rapid cholesterol binding. This process would allow a membrane that contains TSPO to import high levels of cholesterol in a time and ligand dependent manner. Apart from the significance of cholesterol transport by TSPO for steroidogenesis, it may also be relevant for membrane biogenesis and metabolic needs required for cell survival (Veenman et al., 2007). TSPO's interactions with cholesterol may be suggestive of a role of TSPO in atherosclerosis.

1.3.4 TSPO and responses to cardiovascular damage

In the cardiovascular lumen, TSPO are present in platelets, erythrocytes, lymphocytes, and mononuclear cells (Maeda et al., 1998). In the walls of the cardiovascular system, TSPO can be found in the endothelium, the striated cardiac muscle, the vascular smooth muscles, and the mast cells (Taniguchi et al., 1980; Veenman & Gavish, 2006). Regarding cardiovascular

diseases, the TSPO has been found to be involved in ischemic processes, including oxidative stress and apoptosis (Kunduzova et al., 2004). Furthermore, TSPO may be involved in aortic damage due to diet and toxins (Dimitrova-Shumkovska et al., 2010a,b,c).

TSPO in the cardiovascular system appears to play roles in several aspects of the immune response, such as phagocytosis and the secretion of interleukin-2, interleukin-3, and immunoglobulin A (Veenman & Gavish, 2006). Mast cells have been implicated in immune responses to pathogens, in the regulation of thrombosis and inflammation, and in cardiovascular disease processes such as atherosclerosis, as well as in neoplastic conditions (Wojta et al., 2003; Marshall, 2004). Studies have shown that the benzodiazepines' inhibition of serotonin release in mast cells could reduce the blood brain permeability and influence pain levels and decrease vascular smooth muscle contractions (Veenman et al., 2006). Benzodiazepines have been found to bind to specific receptors on macrophages and to modulate in vitro their metabolic oxidative responsiveness (Lenfant et al., 1985). Since these tissues and cells possess TSPO, but not CBR, it is most likely that these benzodiazepines cause their effects via the TSPO present in these tissues and cells.

Recently, we have established that the TSPO appears to be an active participant in the generation of ROS at mitochondrial levels and maintenance of the mitochondrial membrane potential, in relation to apoptosis (Kugler et al., 2008; Zeno et al., 2009). In turn, ROS levels also affect TSPO function (Delavoie et al., 2003). As a result of this, we suggest that the TSPO may be involved in oxidative stress related to cardiovascular disorders.

1.3.5 TSPO ligands

A wide variety of endogenous molecules with affinity for the TSPO have been identified, including Diazepam Binding Inhibitor (DBI), which is an 11 kDa polypeptide of 86 amino acids. As its name suggests, DBI was originally shown to inhibit the binding of [^{3}H] diazepam to brain membranes and gamma aminobutyric acid (GABA) activated Cl - channel activation. DBI has the same low (μM) affinity for both the TSPO and the CBR. Other putative endogenous ligands for TSPO include the porphyrins (protoporphyrin IX, mesoporphyrin IX, deuteroporphyrin IX and haemin), which are known to modulate enzymatic activity of several enzymes and are involved with several mitochondrial proteins (Gavish et al., 1999; Zeno et al., in press). These compounds exhibit a very high (nM) affinity for TSPO, and hence are considered as putative endogenous TSPO ligands (Verma et al., 1987). Regarding synthetic ligands, the TSPO exhibits nanomolar affinity to the benzodiazepine Ro5-4864 (4'- chlorodiazepam, **Figure 3**), but low affinity to most other benzodiazepines species (Le Fur et al., 1983). Furthermore, it has been reported that isoquinolines, such as 1-(2- chlorophenyl)-N-methyl-N-(1-methyl-prop 1)-3 isoquinoline-carboxamide (PK 11195, **Figure 3**) interact specifically with TSPO (Le Fur et al., 1983; Gavish, 2006Veenman &) and that PK 11195 is currently the most widely used TSPO ligand, in part due to its high affinity for TSPO for all of the studied species (Le Fur et al., 1983; Veenman & Gavish, 2006).

PK 11195 and Ro5-4864 compete with each other in binding experiments, suggesting overlapping but not necessarily identical binding sites. It has been suggested that they interact with two different conformations or domains of the mitochondrial TSPO (Awad & Gavish, 1987). Behavioral studies have demonstrated that Ro5-4864 possesses anxiogenic and convulsant properties, whereas PK 11195 has been found to be anxiolytic and anticonvulsant (Gavish et al., 1999). Other studies have shown that Ro5-4864 and PK 11195

have identical effects, for example inhibition of apoptosis (Kugler et al., 2008). These effects are similar to, albeit less strong than TSPO knockdown, implying that these TSPO ligands block the pro-apoptotic functions of the TSPO (Levin et al., 2005; Zeno et al., 2009).

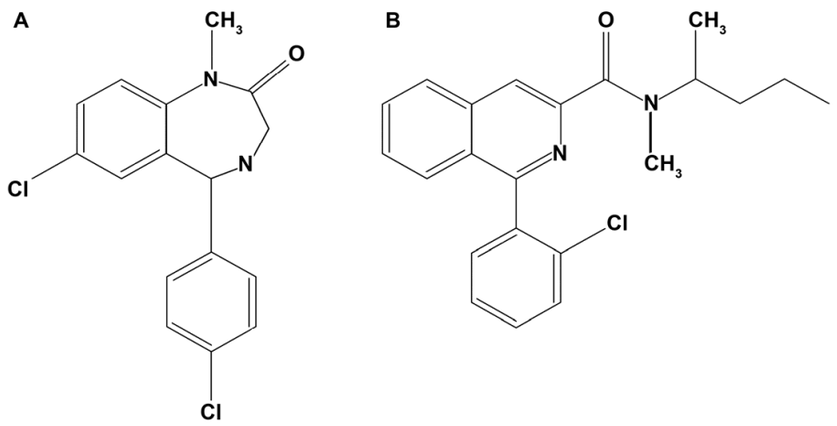

Fig. 3. Chemical structures of the two archetypical TSPO ligands: (A) the benzodiazepine, Ro5-4864; and (B) the isoquinoline carboxamide, PK 11195.

In some cases, various effects of TSPO ligands have been observed in TSPO deficient and TSPO knockdown cells (Hans et al., 2005), raising the issue of the possible presence of TSPO-independent mechanisms of action of these ligands (Falchi et al., 2007).

The effects induced by TSPO ligands have been widely investigated in steroidogenic cells (Gavish et al., 1999; Casellas et al., 2002). In addition, effects of TSPO ligands have been studied in non-steroidogenic cells, including cardiovascular tissues: 1) they were shown to modulate physiological mechanisms such as cellular respiration in heart, kidney, and liver (Moreno-Sanchez et al., 1991; Veenman & Gavish, 2006), 2) generation of ROS in neurons and HL60 human leukaemia cells (Fennell et al., 2001; Jayakumar et al., 2002), 3) anion transport in kidney (Basile et al., 1998), 4) mitochondrial permeability transition in cardiomyocytes (Chelli et al., 2001), 5) inhibition of cell proliferation in human fibroblasts (Kletsas et al., 2004), and 6) apoptosis in various cell lines (Decaudin et al., 2002; Chelli et al., 2004).

Numerous findings have suggested that TSPO ligands might act as potential therapeutic agents that may be useful for the treatment of a large spectrum of diseases. TSPO drug ligands are evaluated regarding their ability to regulate neurosteroid synthesis and brain function, to detect tumor cells *in vivo*, and to modulate apoptotic rates, with major potential therapeutic implications for cancer therapy (Galiegue et al., 2003). PK 11195 has been administrated safely to patients, and has been suggested to be included in clinical trials as a chemo sensitizing agent. In addition, alternative TSPO ligands with potential therapeutic effects are being developed in various laboratories.

2. TSPO binding density decreases in aorta due to atherogenic challenges

By its very nature, plaque rupture is difficult to study directly in humans. Therefore, animal models have been developed to study atherosclerosis, including plaque rupture and thrombus formation, and also how to take measures to prevent these from happening. However, all of the existing models (biological or mechanical triggering models, the Watanabe heritable hyperlipidemic (WHHL) rabbit model, the apolipoprotein E (ApoE)

mouse model, and the LDL-receptor mouse model) suffer the drawback of lacking an end-stage atherosclerosis that would show plaque rupture accompanied by platelet and fibrin-rich occlusive thrombus at the rupture site (Singh et al., 2009). This is a very important limitation. There are additional disadvantages of the existing models, such as long preparatory activities, complicated manipulation, high cost of development, low yield of triggering, and high mortality, which hamper the execution of large-scale studies. Also, the study of human tissue in *in vitro* cell systems is limited by the obvious fact that these are not whole organisms, accompanied by the inherent problem of drifting phenotypes.

For the present study, regarding the potential involvement of the TSPO in atherosclerosis, we used outbred rats (*Wistar*) to study dietary factors applied by us that contribute to cardiovascular damage. We chose these outbred rats since they are atherosclerotic non - prone animals and under normal circumstances they typically do not show cardiovascular damage within the time-frame we applied. Although the use of rodents as a model may have some limitations to achieve complete understanding of the diet - disease relations in humans, it presents a unique opportunity to simultaneously explore several underlying mechanisms *in vivo* in the whole organism which is otherwise difficult to achieve with other approaches (Dorfman et al., 2003).

Classes of risk factors for cardiovascular disorders (CVD), also named "cardiovascular toxins", are presented by environmental pollutants with very well known carcinogenic effects (Iwano et al., 2006). Not only may pollutants exacerbate and accelerate CVD, risk factors associated with CVD could predispose and sensitize for pollutant toxicity. Chronic hypercholesterolemia, for instance, could significantly affect xenobiotic metabolism and disposition by either altering the expression of detoxification enzymes in liver and peripheral tissues, or by providing additional circulating nucleophylic binding sites, e.g., lysine residues of apolipoprotein and ethanolamine phospholipids (Miyata et al., 2001; Miller and Ramos, 2001). Realizing the need to study cardiovascular toxicity as a significant consequence of exposure to environmental pollutants, one of the aims of our research was to highlight the effects and cell responses due to exposure to carcinogen and air pollutant 7, 12 dimethylbenz [α]anthracene (DMBA) on hyperlipidemic rats chronically exposed on high fat high cholesterol (HFHC) diet. In previous studies we showed that HFHC diet as well as DMBA exposure caused oxidative stress in the aorta, in association with damage to this organ, as well as reduced TSPO binding density in this organ (Dimitrova-Shumkovska et al., 2010a,b,c). To further illustrate these effects, we present here data from a recent study combining HFHC diet with DMBA exposure.

To determine the effects of an HFHC diet in combination with DMBA exposure in our paradigm of cardiovascular damage, rats received a custom tailored HFHC diet (Dimitrova-Shumkovska et al., 2010a). Before application of the HFHC diet, the rats were randomized into 2 general groups: 1) control rats **(C-rats)** receiving commercial standard pellet feed for a period of 18 weeks (n = 18); 2) experimental rats **(HFHC+DMBA)** (n = 12) receiving HFHC diet for a period of 18 weeks, and then a single administration of 10 mg DMBA / 1 mL sesame oil applied by gavage), followed by an additional 4 weeks with HFHC diet. After 22 weeks in total, animals were sacrificed by exsanguination, and procedures related to TSPO binding characteristics, ROS parameters in aorta and histopathology were done, as described in detail previously (Dimitrova-Shumkovska et al., 2010 a,b,c). Specimens of aorta and plasma were collected for lipid analysis and analysis of parameters of oxidative stress. The parameters of oxidative injury that we studied included lipid peroxidation (TBARs assay described by Okhawa et al., 1989, modified by Draper and Hadley, 1990);

protein carbonylation (Levine et al., 1990, adopted by Reznick et al., 1994 and modified by Shacter, 2000), and advanced oxidized protein products (AOPP, Witko-Sarsat et al., 1996). Furthermore, anatomical observation and histopathology of aorta were performed. To determine TSPO binding characteristics in this paradigm we applied binding assays with [^{3}H]PK 11195. The effects of HFHC diet and of DMBA application by themselves were analyzed previously (Dimitrova-Shumkovska et al., 2010 a,b,c). Building on these previous studies, the present study seeks to determine whether the toxin DMBA may exacerbate the oxidative stress due to atherogenic diet.

In the endothelium of the aorta wall of the **HFHC+DMBA** rats, we observed the appearance of foamy cells (20-25% of the lumen circumference, visible among 5 animals from 8 analyzed). Early mild fibrosis, was observed in 2 of the 8 rats. All the control aortas remained negative for these changed, as described before (Dimitrova-Shumkovska et al., 2010 a, b, c). These present results indicate that combining the HFHC diet with the DMBA exposure do not cause more damage to the aorta of rats than previously found with HFHC diet alone (Dimitrova-Shumkovska et al., 2010a,b,c). These previous studies also did show that HFHC diet was more damaging to the aorta than the administration of 10 mg of DMBA alone (Dimitrova-Shumkovska et al., 2010a, b).

Regarding protein oxidation and lipid peroxidation in the aorta (**Table 1**), in the HFHC + DMBA rats significant increases in TBARs, AOPP and PC levels could be observed, compared to vehicle control. In detail, regarding lipid peroxidation, TBARs production was significantly increased more than 2 fold (+129%, $p < 0.05$) in comparison to control (Table 1). Regarding protein oxidation, AOPP levels showed a significant more than 3 fold increase compared to control (+216%, $p < 0.001$). Protein carbonyls (PC) in the aorta showed an increase of 47% compared to control. The obtained results were similar to those of the HFHC diet only group (Dimitrova-Shumkovska et al., 2010a), but the observed effect of enhanced oxidative stress was higher than in DMBA only treated rats (Dimitrova-Shumkovska et al., 2010b). In the control tissue (kidney) such effects differences between the HFHC-DMBA and vehicle groups not observed **(data not shown)**.

Variables / Aorta	Control	HFHC + DMBA
TBARs nmol/mg	1.22 ± 0.2 (n=8)	2.8 ± 1.4* (n=7)
AOPP nmol/mg	12.5 ± 4.5 (n=8)	39.5 ± 13.0** (n=7)
PC pmol/mg	63.5 ± 24.0 (n=8)	93.5 ± 24.1* (n=7)

Table 1. Effects of HFHC diet combined with a DMBA exposure (HFHC + DMBA) on aorta oxidative stress parameters in rats. Mann Whitney non-parametric test, * $p < 0.05$, ** $p < 0.01$.

Binding assays of the aorta with the TSPO specific ligand [^{3}H]PK 11195 were done to determine potential effects on TSPO binding characteristics in HFHC + DMBA treated rats. For representative examples, see Figure 4. The kidney was used as a control tissue, where no changes were expected. The B_{max} and K_d values for TSPO in the aorta and kidney of control rats (**Table 2**) were in the range of previous described results (Gavish et al., 1999; Dimitrova-Shumkovska et al., 2010a,b,c). In the present study TSPO binding characteristics of the aorta of untreated vehicle rats were as follows: B_{max} = 4100 ± 1400 fmol/mg and K_d = 1.2 ± 0.4 nM (**Table 2**). Regarding the effect of HFHC+DMBA, we observed significantly reduced TSPO binding capacity in aorta by 49% compared to vehicle control (**Table 2, Figure 4**).

The TSPO B_{max} in aorta of the HFHC+DMBA group (Bmax = 2092 ± 670 fmol/mg) was not significantly different from those subjected to HFHC diet alone as reported previously (Dimitrova-Shumkovska et al., 2010a,b) or DMBA exposure alone. No significant differences were observed between experimental and control groups regarding the K_d (Table 2).
In contrast to aorta, a highly significant enhancement in the B_{max} of TSPO (+ 41%) determined with [^{3}H]PK 11195 binding was observed in testis tissue due to the HFHC + DMBA treatment, compared to control (**data not shown)**. This was similar to the effects seen with HFHC diet alone and DMBA treatment alone (Dimitrova-Shumkovska et al., 2010a,b,c). [^{3}H]PK 11195 binding levels in kidney appeared not to be significantly affected by the HFHC + DMBA treatment **(Table 2)**. For all tissues, both in the HFHC + DMBA group and control group, K_d values determined with [^{3}H]PK 11195 binding were in the nM range (0.8 – 1.9 nM, which is in the range typically observed for [3H]PK 11195 binding (Awad & Gavish, 1987; Dimitrova-Shumkovska et al., 2010a,b). This implies also that all of these K_d values were not affected by HFHC and DMBA exposure.

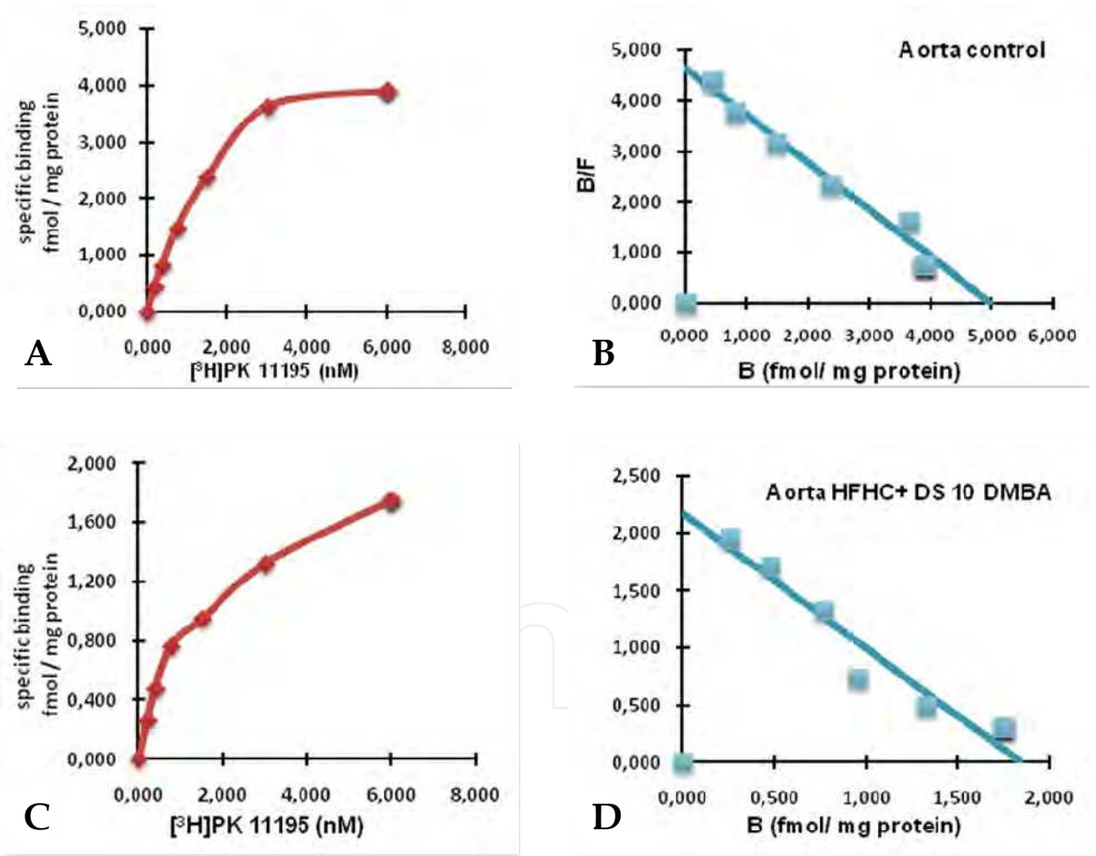

Fig. 4. Representative examples of Scatchard plots **(B, D)** and saturation curves **(A,C)** of [^{3}H]PK 11195 binding to membrane homogenates of aorta**,** respectively of vehicle control rats **(A,B)** and of rats exposed to HFHC and DMBA **(C,D).** Abbreviations: C = vehicle control; The experimental group of HFHC+DMBA treatment (HFHC + DS 10 DMBA) is as described in the text. B: bound; B/F: bound over free.

	C - Control				HFHC+DMBA			
Tissue	**n**	**B max (fmol / mg)**		**Kd (nmol)**	**n**	**B max (fmol / mg)**		**Kd (nmol)**
Aorta	**10**	**4100 ± 1400**		**1.2 ± 0.4**	**6**	**2092 ± 670 ***		**1.4 ± 0.8**
Kidney	**7**	**4270 ± 900**		**1.9 ± 0.9**	**7**	**4543 ± 870**		**2.8 ± 0.8**

Table 2. Average B_{max} values fmoles / mg protein and K_d values (nM) of [^{3}H]PK 11195 binding to TSPO of aorta and kidney of HFHC+DMBA exposed rats versus vehicle control (C-Control). Kruskal-Wallis non-parametric, one-way analysis of variance ANOVA was used, with Mann-Whitney as the post-hoc, non-parametric test, * $p < 0.05$.

3. Discussion

It is well known that the TSPO is involved in tumorigenicity and also appears to be involved in atherosclerosis (Veenman & Gavish, 2006; Veenman et al., 2008; Dimitrova-Shumkovska et al., 2010a,b,c). Research over the past 30 years has suggested striking similarities between the pathways leading to atherosclerosis and cancer (reviews: Ross et al., 2001; Ramos and Partridge, 2005). Benditt and Benditt (1973) proposed that atherosclerotic plaque could be seen as neoplasm of smooth muscle cell origin, thereby paving the way for further research on the parallels between atherosclerosis and cancer. In accordance to this hypothesis, there is a body of evidence showing that established mutagenic and carcinogenic polycyclic aromatic hydrocarbons (PAHs), including methylcholantrene, benzopyrene, and DMBA, cause DNA-adducts in atherosclerotic lesions in humans or atherosclerotic prone animals (Izzoti et al., 2001; Iwano et al., 2005; Knaapen et al., 2007). Furthermore, smoking represents major risk factors for both cardiovascular disease and cancer (Knaapen et al., 2007; Catanzaro et al., 2007; Chiang et al., 2009). In addition, several animal studies have shown that components in tobacco smoke accelerate atherosclerosis in atherosclerotic prone animals, because of an increase in inflammatory cell content in atherosclerotic plaques (Izotti et al., 2001; Curfs et al., 2005).

For this study a HFHC diet was used in order to determine relations between systemic hypercholesterolemia, atherogenic pathology and oxidative stress in the cardiovascular system, in correlation with modulations in TSPO binding characteristics in these organs (Dimitrova-Shumkovska, 2010a). To observe the development of atherosclerosis in various animal models, atherogenic diets containing cholesterol, saturated fat, cocoa butter, and chocolate have been applied to vertebrates, including rodents (Faggiotto et al., 1984; Dimitrova, 2002; Kitade et al., 2006). Since the rat presents a resistant animal model for provoking atherosclerosis, relatively long time-courses are required to induce even moderate hypercholesterolemia and triglyceridemia (Nakamura et al., 1989; Lorkowska et al., 2006). Previous studies applying 1 – 2% cholesterol diets did not affect the endothelium, even though increased density of lipid loading at the adventitial vasa vasorum could be observed (Pisulewski et al., 2006; Lorkowska et al., 2006). Generally, HDL cholesterol is the dominating form in rats. Interestingly, increased expression of inflammatory cytokines (TNF, IL-1, IL-8 and VCAM-1) and augmented foamy cell formation can be found during chronic infection induced by Chlamydia pneumonia in white rats (Aziz, 2006). Furthermore, rats show augmented thrombotic response under hypertensive and hyperlipidemic conditions (Singh et al., 2009). These previous studies suggested that rats could present a useful model for studying hypercholesterolemia along with hypertension, but not a suitable model for atherosclerosis. Our diet containing 3% cholesterol not only was able to

overcome at least in part the rats' resistance to elevation of plasma cholesterol levels but also initiated moderate cardiovascular damage (Dimitrova-Shumkovska et al., 2010a).

As a further indication of the validity of the HFHC diet (with 3% cholesterol) applied by us, the rats in our study showed obesity as well as hyperlipidemia and steatohepatitis (Dimitrova-Shumkovska et al., 2010a). Parameters for enhanced oxidative stress, as we detected in plasma of our HFHC rats may correlate with inflammatory processes, including atherogenic effects observed by us and others (Witko-Sarsat et al., 1998; Liu et al., 2006; Dimitrova-Shumkovska et al., 2010a). In particular the high AOPP levels observed in the liver, plasma, and aorta, as induced by the HFHC diet, may present factors reflecting liver pathology and atherogenesis (Watanabe et al., 2004; Oettl et al., 2008; Dimitrova-Shumkovska et al., 2010a).). In general, the involvement of oxidized proteins in atherosclerosis has been studied less than oxidized lipids. However, protein oxidation products have been found in the extracellular matrix of human and animal atherosclerotic plaques (Woods et al., 2003; Li et al., 2007). The study by Liu et al. (2006) was the first to our knowledge to provide *in vivo* evidence for a causal relationship between chronic AOPPs accumulation and atherosclerosis. This research suggests that increases in plasma AOPP, particularly in a hypercholesterolemic environment, accelerate atherosclerosis. Interestingly, a study from Wong et al. (2008) showed that protein carbonyls are not merely damaging, but can also serve as a second messenger for signal transduction in vascular smooth muscle cells.

Previous studies have shown that the TSPO is present throughout the cardiovascular system (Veenman & Gavish, 2006). Furthermore, the TSPO has been reported to be involved in oxidative stress and inflammation. In more detail, the TSPO is involved in various mechanisms that also have been found to play a role in atherosclerosis, including oxidative stress, ROS generation, inflammation, immune responses, apoptosis, and mitochondrial cholesterol transport (Papadopoulos et al., 1997, 2006). Most recent experimental data suggest that TSPO plays regulatory roles in adhesion to the extracellular matrix, vascularization, heme metabolism, and processes affected by nitrosylation of various proteins (Veenman and Gavish, 2011; Zeno et al., 2011; Bode et al., submitted). Thus, we assumed that the TSPO may be involved in cardiovascular disorders as induced in our paradigm.

Oxidative damage mediated by DMBA exposure and HFHC diet presents two examples of the plethora of risk factors in provoking atherosclerosis. Different forms of oxidative stress may give rise to different oxidation products, several of which were elevated in the aorta of our model induced by DMBA exposure and HFHC applied to the rats of this research (**Table 1**). As we used outbred Wistar rats, genetic disposition apparently is not a precondition for cardiovascular damage induced by DMBA. Histopathological analysis of the aorta showed that the HFHC + DMBA treatment induced foamy cells and fibrinoid connective tissue accumulation, as reported also for the separate treatments of HFHC and DMBA by themselves (Dimitrova-Shumkovska et al., 2010a,b,c). Furthermore, in the aorta the TSPO expression was inversely correlated with aggravated oxidative stress **(Table 2)**.

Previous *in vitro* studies of rat liver have shown as well that particular forms of oxidative stress can reduce TSPO binding density in this organ. For example, 0.001 mM Fe $[^{2+}]$ in combination with 1 mM ascorbate reduced TSPO binding density by half (Courtiere et al., 1995). Interestingly, it was also shown that in response to UV irradiation-induced ROS covalent TSPO polymers were formed in Leydig and breast cancer cells *in vitro* and *in vivo,* resulting in increased binding affinity of the TSPO (Delavoie et al., 2003). Other evidence

for TSPO's participation in oxidative processes has been indicated in a study by Carayon et al. (1996), where a correlation between the levels of TSPO expression and the resistance to H_2O_2 toxicity was demonstrated in hematopoietic cell lines. It may be considered that the reduction in TSPO binding density determined in the present research and other studies may impair such protective, anti-oxidative functions of the TSPO (Dimitrova-Shumkovska et al., 2010a,b,c). Alternatively, the reduced levels of TSPO may be a compensatory response to the challenges posed by the HFHC diet and DMBA exposure, i.e. reduced ROS generation otherwise due to TSPO activation and protection against the triggering of cell death, as for example was also found in various studies applying knockdown of TSPO by genetic manipulation *in vitro* (Levin et al., 2005; Zeno et al., 2009). Possibly, the enhanced levels of AOPP found in liver and aorta may be a contributing factor to the reduced binding density of TSPO in these organs (Dimitrova-Shumkovska et al., 2010a, b,c). As an alternative explanation, regulation of TSPO binding density can take place via modulations of gene expression (Giatzakis and Papadopoulos, 2004). With various studies we have demonstrated that steroids and stress are able to regulate TSPO binding density (for example, Veenman & Gavish, 2006; Veenman et al., 2007, 2009; Mazurika et al., 2009). As mentioned, growth hormone may take part in the effects of obesity. Similarly, testosterone levels may also be involved in the effects of atherogenic diet as discussed further below.

Our previous studies indicated the HFHC diet alone had more profound effects than DMBA exposure with a single dose of 10 mg in regard to aortic damage, histopathological changes in the liver, and oxidative stress measured in blood plasma and liver of rats (Dimitrova-Shumkovska et al., 2010a,b,c,). In the present study, also regarding oxidative stress in aorta, effects of HFHC combined with DMBA were more pronounced than of DMBA alone with a dose of 10 mg, but not more pronounced than of HFHC alone. The combination of HFHC diet and DMBA exposure did not appear to present a major synergetic effect on TSPO binding characteristics in the aorta. Possibly TSPO responses in the aorta may be part of a protective mechanism against lipid overload. Others have also suggested a role for TSPO in vascular inflammatory responses, for example in vascular permeability caused by carrageenin (Lazzarini et al., 2001). Presently, it is not known which components of the vascular wall, i.e. mast cells, smooth muscular, or dermal vascular endothelial cells, would be important for the potential correlation between TSPO expression and atherosclerosis (Morgan et al., 2004; Veenman & Gavish, 2006). Also, the importance of TSPO in relation to other mechanisms potentially associated with cardiovascular damage needs further research.

As TSPO may be modulated by ROS, and can modulate ROS generation itself (Courtiere et al., 1995; Papadopoulos et al., 1997, 2006; Delavoie et al., 2003; Veenman et al., 2007, 2008; Zeno et al., 2009), it can be postulated that the oxidative stress detected in aorta of our HFHC +DMBA rats may be associated directly with a reduction in TSPO binding density in these organs. While we did not see synergetic effects of HFHC and DMBA in rat aorta, preliminary data by us suggest that the combined effect of HFHC and DMBA in rat liver may lead to enhanced reductions in TSPO binding densities in this organ, compared to each treatment alone (unpublished results). At present it is not clear whether the TSPO levels modulate oxidative stress, or whether TSPO levels are only affected by oxidative stress. More studies are needed to resolve these questions.

As our studies showed an increase in the Bmax of TSPO binding in the testes, it would be interesting to study by which mechanisms this may occur. As discussed above, steroid hormones can have an effect on TSPO expression. Interestingly, several studies reported

important positive correlations between high fat saturated supplementation and the levels of urinary excretion of testosterone (Hammoud et al., 2006). This is an important point to consider in evaluating levels of testosterone bioactivity in the body (Hill et al., 1980). Another approach to increase bioavailable testosterone would be to decrease the levels of sex-hormone binding globulin (SHBG). Reed et al. (1987) noted that normal men fed with a high fat diet showed increased SHBG levels, whereas a diet low in fat resulted in decreased in SHBG levels. It has been reported that decreased SHBG levels result in elevated testosterone bioactivity (Longcope et al., 2000). As with previous studies, showing increased TSPO binding density in the testes after DMBA and HFHC exposure (Dimitrova-Shumkovska et al., 2010a,b,c), also the combination of DMBA and HFHC exposures increased TSPO binding density in the testes (unpublished results). Potentially, this may be due to changes in testosterone levels, as previous studies have shown that increased testosterone can increase TSPO binding levels (Weizman et al., 1992).

Regarding future studies, it would be interesting indeed to find out whether modulation of TSPO responses by TSPO ligands would be able to counteract or enhance the effects of HFHC alone or with DMBA exposure. Similarly, it would be interesting to study in this paradigm the effects of hormones, as they are known to affect TSPO expression. For example, it is known that testosterone levels are reduced in humans as well as in rats as a consequence of a fattening diet and obesity (MacDonald et al., 2010). Since reduced testosterone levels are correlated with reduced TSPO levels in various tissues (Weizman et al., 1992), this may very well present part of the mechanism whereby the HFHC diet and the obesity of the rats of this research may lead to reduced TSPO levels in the aorta and liver. Alternatively, if enhanced oxidative stress in our paradigm contributes to changes in TSPO levels in various types of tissue, it will be interesting to study TSPO homomer polymerization, a phenomenon that has been reported by Delavoie et al. (2003). The potential appearance of TSPO multimers would suggest whether changes in TSPO binding capacity in some of the tissues studied may be due to the oxidative stress caused by the HFHC diet.

4. Conclusions

Our studies have shown that HFHC diet as well as DMBA exposure of rats can lead to oxidative stress in the aorta, as well as a reduction of TSPO binding density. The combination of HFHC diet plus an exposure to DMBA of rats did not affect the studied parameters regarding histopathological damage, oxidative stress and TSPO binding characteristics in aorta more than the maximal effects achieved by HFHC diet (Dimitrova-Shumkovska et al., 2010a), although they were higher than with DMBA treatment alone (Dimitrova-Shumkovska et al., 2010b). As addition of DMBA to HFHC treatment does not enhance levels of oxidative stress or changes in TSPO binding density in the aorta elicited by HFHC alone, this may indicate that HFHC treatment by itself already elicit maximal response from the TSPO / oxidative stress “system” in the aorta. Potentially, as the effects are not further enhanced, this may mean that the TSPO responses in the aorta indeed are a physiological response and do not simply represent damage to the TSPO protein (due to oxidative stress or otherwise).

We consider that the TSPO responses in aorta may present compensatory functions to deal with the oxidative stress induced by HFHC diet and DMBA exposure. Alternatively, the TSPO response may either be part of the oxidative stress mechanisms, or result from it. As

discussed, TSPO function is not restricted to oxidative stress, but also encompasses adhesion to the extracellular matrix, angiogenesis, heme metabolism, protein nitrosylation, apoptosis, and immune responses.

Our research does show that exposure to irritants of the vascular endothelium (metabolical of chemical) decreases 18kDa TSPO binding capacity in the aorta. These decreases in TSPO binding capacity are potentially related to the oxidative stress in this organ. The data of this study suggest that TSPO may present a target for novel therapies designed to reduce the risk of atherosclerosis, including its component of oxidative stress.

5. Summary

The 18 kDa translocator protein (TSPO) is present throughout the cardiovascular system and may be involved in cardiovascular disorders. At cellular levels TSPO is present in virtually all of the cells of the cardiovascular system, where they appear to take part in the responses to various challenges that an organism and its cardiovascular system face, including atherosclerosis and accompanying symptoms. Several studies have shown that the TSPO appears to be a participant in reactive oxygen species (ROS) generation at mitochondrial levels. This may be part of oxidative stress challenges a cell may face. This potentially may play a role in cardiovascular diseases. In this context, TSPO modulates the initiation of mitochondrial apoptosis cascade. Furthermore, TSPO may be a participant in processes related to adhesion to the extracellular matrix, vascularisation, heme metabolism, and processes affected by nitrosylation of various proteins. Oxidative damage mediated by DMBA exposure and HFHC diet presents two examples of the plethora of risk factors in provoking atherosclerosis. Our studies have shown that a high fat, high cholesterol (HFHC) diet as well as 7, 12 dimethylbenz[α]anthracene (DMBA) exposure of rats can lead to oxidative stress in the aorta, in association with damage to the aorta wall, as well as a reduction of TSPO binding density. We consider that the TSPO responses in aorta may present compensatory functions to deal with the oxidative stress induced by HFHC diet and DMBA exposure. Alternatively, the TSPO response may either be part of the oxidative stress mechanisms, or result from it. The reviewed studies suggest that TSPO may present a target for novel therapies designed to reduce the risk of atherosclerosis, including its component of oxidative stress.

6. Explanation of abbreviations and symbols

ACTH, adrenocorticotropic hormone; ANOVA, analysis of variance; ANT, 30kDa adenine nucleotide translocator; (AOPPs), advanced oxidation protein products; Apo E-/- KO, apolipoprotein E knockout mice; cAMP, adenosine 3,5-cyclic monophosphate; CBR, central-type benzodiazepine receptor; DBI, Diazepam Binding Inhibitor CVD, cardiovascular disease; DMBA, 7, 12 Dimethylbenz[*a*]anthracene; DS 10 - single dose of 10 mg DMBA administered (10 mg/ 1ml of sesame oil); GABA, gamma-amino butyric acid; HDL, high-density lipoprotein; HFHC- high fat high cholesterol diet; HMGCoA, 3-hydroxy-3-methylglutaryl coenzyme A reductase; H_2O_2, hydrogen peroxide; Hb, hemoglobin; IL-1, interleukin-1 (IL-2, etc.); kDa, kilodalton; Kd, equilibrium dissociation constant; *K*m, equilibrium constant related to Michaelis-Menten kinetics (similarly, *K*d, *K*a, *K*eq, *K*s); LDL, low density lipoproteins; mPTP, mitochondrial permeability transition pore; MCP-1, monocyte chemoatractant proteins-1; NADP, nicotinamide adenine dinucleotide phosphate;

NADH, reduced nicotinamide adenine dinucleotide; PAHs, polycyclic aromatic hydrocarbons; PBR, peripheral-type benzodiazepine receptor; PC protein carbonyls; PK 11195, 1-(2- chlorophenyl)-N-methyl-N-(1-methyl-prop 1)-3 isoquinolinecarboxamide; $ONOO^-$, peroxinitrite ; Ro5-4864, (4'- chlorodiazepam); ROS , reactive oxygen species; SaβG, senescence-associated β galactosidase; SOD, superoxide dismutase activity; TBARs, thiobarbituric acid reactive substances; TNF, tumor necrosis factor; TSPO, 18 kDa translocator protein; VCAM, vascular cell adhesion molecule; VDAC, 32 kDa voltage-dependent anion channel; VSMCs, vascular smooth muscle cells.

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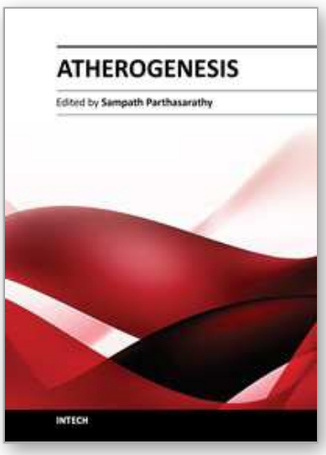

Atherogenesis

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This monograph will bring out the state-of-the-art advances in the dynamics of cholesterol transport and will address several important issues that pertain to oxidative stress and inflammation. The book is divided into three major sections. The book will offer insights into the roles of specific cytokines, inflammation, and oxidative stress in atherosclerosis and is intended for new researchers who are curious about atherosclerosis as well as for established senior researchers and clinicians who would be interested in novel findings that may link various aspects of the disease.

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