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Chapter 3

Thrombosis, Atherosclerosis and Atherothrombosis – New Insights and Experimental Protocols

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Additional information is available at the end of the chapter

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Abstract

Previous studies have shown that TSPO as well as apolipoprotein E (Apo E) can be associated with processes such as cholesterol metabolism, oxidative stress, apoptosis, glial activation, inflammation, and immune responses. As a ligand for cell-surface lipoprotein receptors, apolipoprotein E can prevent atherosclerosis by clearing cholesterol-rich lipoproteins from plasma. Furthermore, TSPO takes part in the regulation of gene expression for proteins involved in adhesion, which potentially may play a role in platelet aggregation. There are indications that the Apo E protein is involve in platelet aggregation, while TSPO platelet levels have been found to be increased with various neurological disorders, in particular, in stress-related disorders. The role of platelets in atherogenesis and the potential therapeutic impact of TSPO ligands on disease prevention are of great interest. To determine TSPO binding characteristics in this paradigm, we applied binding assays with [^{3}H]PK 11195 on isolated platelets and erythrocyte membranes. The in vivo findings in Apo E knockout mice revealed that TSPO levels appear to be enhanced in platelets and erythrocytes of Apo E knockout mice, and thus suggest that TSPO and Apo E expression may be interconnected in relation to some aspect of the host defense response. Other organs tested, such as liver, testis, brain, heart, aorta, lung, kidney and spleen, did not show a difference in TSPO binding levels between Apo E knockout mice and wild-type mice. This suggests that TSPO levels may be part of a feedback control system for steroid production (responding to alterations in steroid levels), rather than being regulated by a feed-forward signal provided by cholesterol (i.e. TSPO levels in relation to steroidogenesis are not being regulated by cholesterol levels in vivo).

Keywords: Dyslipidemia, Platelets, Translocator protein, Erythrocytes, Apo E-KO mice

1. Introduction

1.1. The connection of apolipopoprotein E and platelet activation to inflammation and atherosclerosis

Atherosclerosis is a chronic inflammatory disease of the arteries of multifactorial etiology that involves a complex interaction among oxidized lipids, plasma proteins, vascular endothelial and smooth muscle cells and platelets [1–3]. Hypercholesterolemia and especially high concentrations of low-density lipoproteins (LDL) are significant factors for the premature development of atherosclerotic plaque. In parallel, elevated LDL levels exert prothrombotic effects via platelet activation [4].

Dysfunction or low levels of platelets predispose to bleeding, while high levels, although usually asymptomatic, may increase the risk of thrombosis, thus relating to atherosclerosis and Apo E malfunction [5]. Studies from Kolodgie et al. demonstrated that platelets and erythrocytes can penetrate the plaque through angiogenic capillary breakages [6]. Furthermore, platelets with their pro-atherogenic potential are regarded as discrete immune cells [7, 8]. They can accumulate lipids in the hypercholesterolemic environment and are involved in the early phase of atheromatous formation [9]. Oxidized low-density lipoproteins (ox-LDLs) are suggested to be implicated in the early phase of atherosclerosis by recruiting inflammatory cells in the subendothelium that elucidate the thrombotic process [10]. Several cells including endothelial cells, macrophages and smooth muscle cells determine ox-LDL formation. *In vitro* studies have shown that ox-LDL binds to platelets via CD36 and lectin-like endothelial receptor for ox-LDL (LOX-1 receptors), promoting platelet activation [11, 12]. Experimental studies demonstrated a marked reduction of thrombus formation in animals with the deletion of ox-LDL receptor CD36 from platelets [13]. In accordance with a number of *in vitro* results, *in vivo* LOX-1 expression, was not only upregulated under the influence of proinflammatory stimulus but also induced in adipose tissue after high-fat diet feeding, suggesting that increased LOX-1 expression may promote atherosclerosis [14, 15].

Apolipoprotein E functions as an important carrier protein in the redistribution of lipids among cells, by incorporating into high-density lipoproteins (as HDL-E), and plays a prominent role in the transport (by incorporating into intestinally synthesized chylomicrons) and metabolism of plasma cholesterol and triglyceride through its ability to interact with the LDLR and the receptor binding of Apo E lipoproteins (apoER) [16, 17]. The metabolic activity of Apo E is sensitive to its lipid environment; purified Apo E does not interact with the LDLR [18]. Apo E at the surfaces of VLDL and chylomicrons is also inactive unless these lipoproteins are from hypertriglyceridemic subjects [19] or have undergone substantial lipolysis to form remnant particles [20]. Apo E is part of different metabolic pathways in the body. One of these pathways is endocrine-like, and involves the redistribution of lipids among cells of different organs. It takes lipids from the areas where the lipid is synthesized and distributes them to other areas where the lipids are used or stored. Another pathway is paracrine-like, where the lipids are transported among cells in the same organ or tissue. Since Apo E is involved directly in the uptake and distribution of plasma lipids, it is natural that it has several implications for

cardiovascular disease. Interestingly, oxLDL as a ligand for LOX-1 is highly present in circulation and lesion formation in atherosclerotic prone animals, such as Apo E KO mice.

The Apo E is also involved in various pathways that are unrelated to lipid transport, such as the stimulation of lymphocytes and macrophage secretion [21]. This appears important for facilitating local cholesterol redistribution and for reverse cholesterol transport [22]. As a ligand for cell surface lipoprotein receptors, apolipoprotein E can prevent atherosclerosis by clearing cholesterol-rich lipoproteins from plasma. Indeed, atherosclerosis in Apo E-deficient (Apo E2/2) mice can be prevented by transplantation of normal murine bone marrow cells [23], by macrophage-specific expression of the human Apo E transgene [24], or by adenovirus-mediated gene replacement [25]. It was also found that altered Apo E expression leads to enhanced inflammation responses [26–28].

Defects in Apo E sometimes result in its inability to bind to the LDL receptors, which leads to an increase in a person's blood cholesterol. Recently, it has been suggested that high cholesterol levels due to Apo E malfunction may constitute a risk factor for Alzheimer's disease [29, 30]. According to the expressed isoforms, Apo E might be involved (i) in maintaining the integrity of the aging CNS [31]; (ii) in repair, growth and maintenance of myelin and axonal membranes during development and after an injury [32]; (iii) in neurite outgrowth [33]; (iv) in neurotoxicity [34]; and (v) in pathological processes in general, including Alzheimer's disease [35].

Riddell's study found that HDL-E was a powerful inhibitor of agonist-induced platelet aggregation, through interaction with saturable binding sites in the platelet surface membrane, which further suggested that Apo E exerts its antiplatelet aggregation effect via L-arginine: nitric oxide pathway by enhancing the production of endogenous nitric oxide (NO) [36]. Calcium is central to the control of platelet reactivity, interacting with diverse second messengers through a myriad of complex but tightly regulated, signalling pathways [37]. Two important control elements for suppression of platelet activation are the cyclic nucleotides, cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP), and agents that increase their intraplatelet levels exert antiaggregatory effects both *in vitro* and *in vivo* [38]. Although Apo E induced increases in both cAMP and cGMP, additional experiments implicated a specific stimulation of guanylate cyclase activity and a rise in cGMP as prerequisites for the antiplatelet action of Apo E. Using the L-arginine:nitric oxide pathway, the vascular endothelium synthesizes NO from the terminal guanidine nitrogen atoms of L-arginine using a soluble enzyme called NO synthase (NOS). NO then binds to soluble guanylate cyclase to produce cGMP, which has inhibitory effects on platelet aggregation. The increased levels of cGMP also decrease the amount of cAMP phosphodiesterase, the enzyme that converts cAMP to AMP. The decrease in cAMP phosphodiesterase causes an increase in cAMP, and cAMP also has an inhibitory effect on platelet aggregation [10, 36].

1.2. Potential functional commonalities between apolipoprotein E and 18 kDa translocator protein

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 [36]. 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 [22, 39–42].

The TSPO was previously known as peripheral type benzodiazepine receptor (PBR), since it is capable of binding benzodiazepines and is found in most if not all peripheral tissues [43]. Mitochondrial membranes form the primary location for TSPO [44]. It is an integral membrane protein that interacts with a wide variety of endogenous ligands, such as cholesterol and porphyrins, and is also a target for several small molecules with substantial *in vivo* efficacy. When complexed with the TSPO-specific radioligand PK11195, TSPO folds into a rigid five-helix bundle.

TSPO are present in platelets, lymphocytes and mononuclear cells and are also found in the endothelium, the striated cardiac muscle, the vascular smooth muscles and the mast cells of the cardiovascular system. As TSPO is known to regulate heme metabolism, TSPO may play essential roles in erythrocyte function [45]. TSPO in the cardiovascular system also 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 [46]. Mast cells are considered to be important for immune response to pathogens [47], and they have also been implicated in the regulation of thrombosis and inflammation and cardiovascular disease processes such as atherosclerosis as well as in neoplastic conditions [48]. Benzodiazepines have been found to bind to specific receptors on macrophages and to modulate *in vitro* their metabolic oxidative responsiveness [49]. Haemin, isolated from human erythrocytes, competitively inhibits mitochondrial benzodiazepine binding with a Ki of 41 nM [50]. The TSPO might present a therapeutic target for arrhythmia, myocardial infarction and cardiac hypertrophy by reducing ROS. For example, the suppression of TSPO can prevent caspase cascade activation and cytochrome *c* release, thus inhibiting ROS overload [5, 51–60]. The results from several *in vitro* studies have implicated TSPO in cholesterol transport into mitochondria, the late-limiting step in the steroid biosynthesis. Therefore, TSPO has been considered as a critical factor in steroidogenesis. Moreover, the ubiquitous expression and evolutionary conservation of TSPO from bacteria to mammals strongly suggested its essential role in cellular processes. This presumption was further supported by the results of an earlier study that claimed the embryonic lethality of TSPO whole-body knockout (KO) mice, although the details of the methods for the design and generation of the KO mice were not provided [52, 53].

A scheme of the involvement of mitochondrial TSPO in various cellular functions and disease is given in Figure 1. For detailed discussions regarding these functional pathways, see refs. [49] and [58].

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. Various synthetic ligands that bind to TSPO have been developed. Endogenous ligands that bind to TSPO include: protoporphyrin IX, DBI and its fragment TTN and PLA2. The TSPO is involved in various functions as indicated towards the bottom of the figure. Abbreviations: ANT, adenine nucleotide transporter; ATP, adenosine triphosphate; DBI, diazepam-binding

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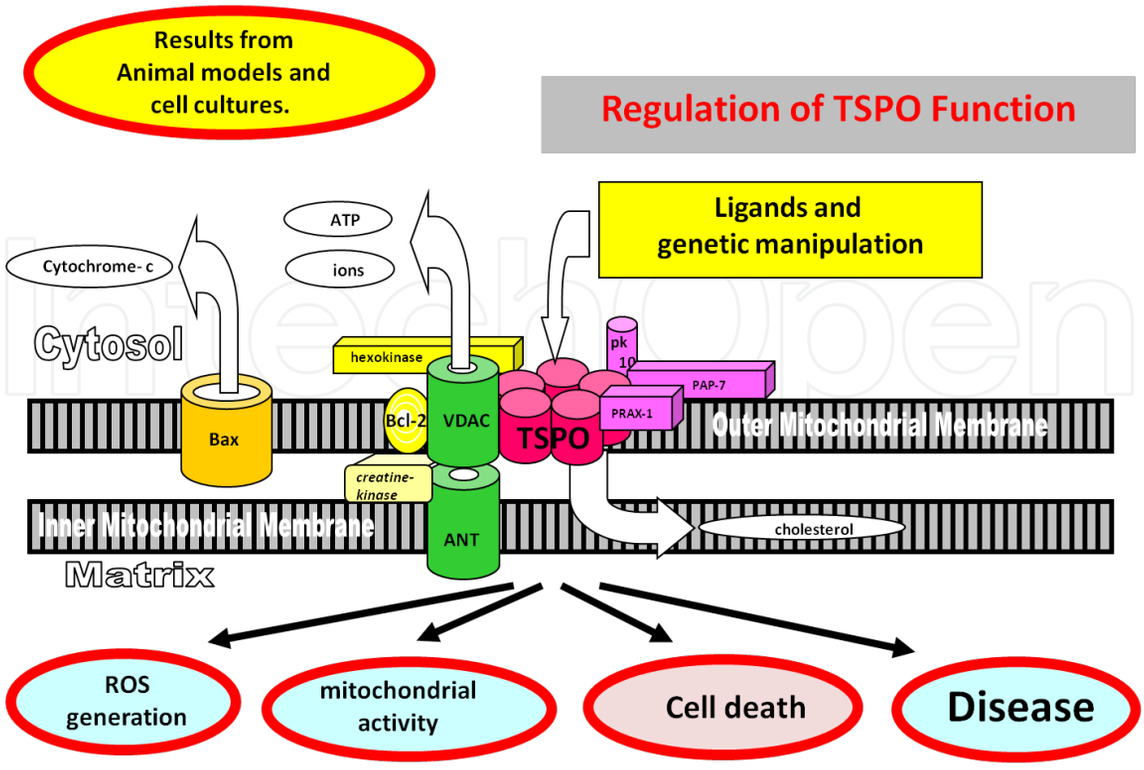

Figure 1. The 18 kDa mitochondrial translocator protein (TSPO) in relation to cellular function and disease.

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, triakontatetraneuropeptide; VDAC, voltage-dependent anion channel.

Recently, it was shown that TSPO ligands specifically designed for this purpose can increase lifespan in animal models for human disease [61]. For example, the occurrence of cardiac arrest, the typical cause of death in R6-2 mice, is delayed considerably in this animal model for Huntington disease [62]. In numbers, average lifespan is increased from ±12 weeks to ±15 weeks. Translated to the human situation, this would mean in simple terms that life expectancy of Huntington disease patients can potentially be increased from an average of 60 years to an average of 85 years following treatments with the appropriate TSPO ligands. Such TSPO ligands were also shown to prevent and counteract brain edema associated with seizures developed after systemic injections of kainic acid in rats [61, 62].

Anti-inflammatory properties of TSPO ligands have also been demonstrated in nonneuronal tissues [63]. TSPO ligands have been shown to reduce inflammation in animal models of rheumatoid arthritis [59], carrageenan-induced pleurisy [64] and pulmonary inflammation [65]. A summary of the involvement of TSPO and apoE in functions common to both as suggested by previous studies discussed above is presented in Figure 2. Therefore, we studied whether knockout of apolipoprotein E in mice may have an effect on TSPO expression in platelets and erythrocytes. This could suggest that TSPO is involved in the regulation of functions under the control of ApoE as mentioned in Figure 2.

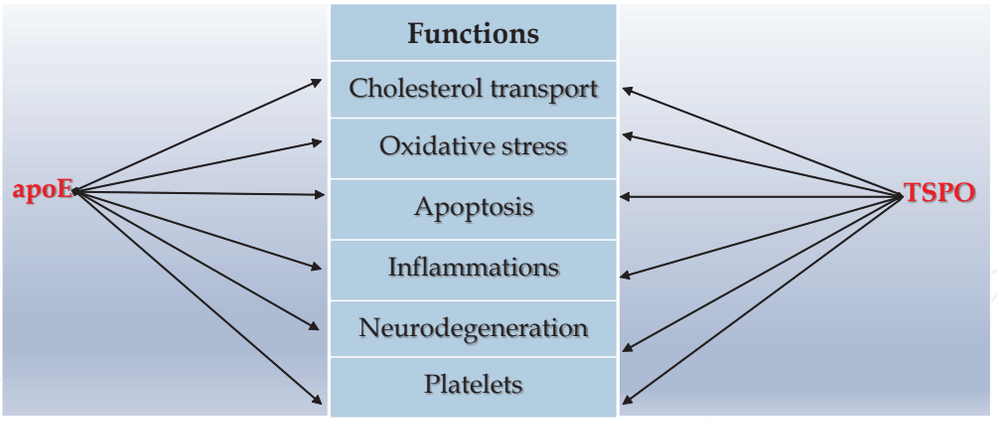

Figure 2. Possible correlations between Apo E and TSPO due to potential participation in various functions.

2. Materials and methods

Apolipoprotein E–deficient mice (Apo E KO mice) present a well-established model to study atherogenesis. In these mice, reverse cholesterol transport is dysfunctional, increasing the blood cholesterol level up to five times higher than in normal mice [66]. As Apo E deficiency causes increased cholesterol levels, which in turn may modulate TSPO function, we were interested to study whether TSPO binding characteristics may be affected in erythrocyte and platelet membranes. For this purpose, we used 12- to 14-week-old healthy male C57BL/6 mice (i.e. wild type, WT) and 14-week-old Apo E knockout (KO) mice. The mice were housed in polycarbonate cages in a pathogen-free facility set on a 12-h light–dark cycle and given *ad libitum* access to water and standard laboratory feed. All experimental procedures were carried out following the guidelines of the International European ethical standards for the care and use of laboratory animals (Community Council Directive 86-609). All protocols were approved and reviewed by the local ethics committee.

Platelets and erythrocytes preparation. Up to 1 mL of mouse blood was drawn by cardiac puncture of deeply anesthetized fasting mice in a terminal procedure and collected in plastic tubes containing trisodium citrate [3/8%, 1/10 (v:v)].

Platelet preparation

i. To obtain platelet-rich plasma (PRP), blood was centrifuged at 200×*g* for 20 min

ii. Then, 3/4 of the supernatant was collected to a new tube and centrifuged at 1000×*g* for 30 min

iii. The pellet was washed with Tyrode's buffer (Sigma-Aldrich, T1788) in a volume identical to the volume of the original fluid and centrifuged at 1000×*g* for 15 min and the pellet that contained only platelets was used for binding assay

Erythrocyte preparation

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i. The pellet obtained from the first centrifugation (protocol i) at 200×*g*, was washed three times in isotonic solution of sodium chloride and used for isolation of erythrocyte membranes

ii. Purified erythrocytes were suspended in a 30-fold volume of 20 mM PBS containing 1 mM MgCl2, pH 7.5, allowed to stand for one hour prior to centrifugation at 13,000×*g*, 40 min at 4°C, in order to obtain erythrocyte membrane as pellet [67]

iii. Protein concentration of membrane preparations were determined by the Bradford assay [68, 69]

Assays of TSPO binding characteristics by [^{3}H]PK 11195 binding measurements. Maximal binding capacity (B_{max}) and equilibrium dissociation constant (K_d) of the binding of the TSPO-specific ligand, [^{3}H]PK 11195, in whole cell membrane preparations from the platelets and erythrocytes were assayed as described previously [40, 70, 71]. [^{3}H]PK 11195 (1-(2-chlorphenyl)-*N*-methyl-*N*-(1-methypropyl)-3-isoquinolinecarboxamide) was obtained from New England Nuclear (Boston, MA). Unlabeled PK 11195 was purchased from Sigma-Aldrich, Israel (Rehovot, Israel).

Procedure

- For binding assays of the B_{max} and the K_d, the reaction mixtures contained 400 μL of the membrane preparation in question (≈100 μg protein) and 25 μL of [^{3}H]PK 11195 solution (0.2 to 6 nM final concentrations) in the absence (total binding) or presence (nonspecific binding) of 75 μL unlabeled PK 11195 (10 μM final concentration)
- After incubation for 90 min in ice-water bath, the samples were vacuum filtered through Whatman GF/C filters, washed three times with 4 mL of 50 mM phosphate buffer and placed in vials containing 4 mL of Opti-Fluor (Waltham, MA)
- Radioactivity was counted after 12 h with a 1600CA Tri-Carb liquid scintillation analyser (Packard, Meriden, CT). Scatchard analysis of [^{3}H]PK 11195 binding was done to determine the B_{max} and K_d values
- For one point binding analysis, the concentration of 6 nM [^{3}H] PK 11195 was applied

The Bradford assay is based on the equilibrium between three forms of Coomassie blue G dye. Under strong acidic conditions, the dye is stable as a doubly protonated red form. Upon binding to protein, it is most stable as an unprotonated, blue form. Determination of microgram quantities of protein in the Bradford Coomassie brilliant blue assay is established by measurement of absorbance at 590 nm [69]. For this protein assay, Bradford solution (comprising Coomassie Brilliant Blue G-250 and an acid with a pKa of 1–2) was obtained from Bio-Rad (Munich, Germany).

Procedure

- 10 μL from each sample was mixed with 200 μL of Bradford solution in 96-well plates, and incubated for 10 min in the dark at room temperature

- Absorption was measured using an ELISA Reader or "ULTRASPECTRO 2000" at 595 nm. The protein concentration was determined by comparing the absorption values of the protein samples to the appropriate BSA standard curve, prepared from the BSA volumes: 0, 2, 4, 8, 10, 15, 20, 25, 50, 100 and 150 μL, completed to a final volume of 800 μL by DDW

3. Results with discussion

Atherosclerosis is characterized by inflammatory infiltration into the arterial wall of macrophages, dendritic cells, platelets and activated T cells, resulting in plaque formation. This process is initiated by inappropriate lipid metabolism, calcium signaling and increased burden of secretory pathways [3]. Macrophages, especially in the atherosclerotic plaque, contribute to the local inflammatory responses by secreting proinflammatory cytokines [72]. Activated macrophages have been demonstrated with high TSPO expression levels [73, 74]. TSPO has been identified in human leukocytes and erythrocytes and appears to play roles in several aspects of the immune response and the regulation of the host defense response in general [43, 46]. In addition, TSPO was detected in the plasma membrane of neutrophils, where it was shown to stimulate adhesion and motility [75].

Apolipoprotein E may also be involved in the immune response, as suggested by the impaired immune response in Apo E-deficient mice [76]. TSPO levels in platelets of rats and humans have been demonstrated to respond to a wide variety of pathological conditions [46, 77, 78]. It has been suggested that TSPO density in platelets can be used as a promising biological marker of stressful conditions [79–81].

To determine TSPO binding characteristics in Apo E-KO mice, we applied binding assays with the TSPO-specific ligand [^{3}H] PK 11195. Since cholesterol plays an important role in steroidogenesis, atherosclerosis and Alzheimer's disease, which have been correlated with TSPO and Apo E expression, we expected to find changes in TSPO binding levels in platelets and erythrocytes of Apo E-knockout mice compared to their WT strain. Indeed, regarding the platelets and erythrocytes, a significant increase ($p < 0.05$) in TSPO binding was noticed in Apo E knockout mice compared to WT (Table 1). In addition, our results showed no major difference in TSPO B_{max} between WT and Apo E-KO mice in other tissues (data not shown); suggesting that the effects seen on platelets and erythrocytes may be quite specific in relation to atherosclerosis.

Scatchard analysis demonstrates [^{3}H]PK 11195 binding of 1923 ± 1010 fmol/mg protein in erythrocyte membranes of wild-type mice vs. 3142 ± 1761 fmol/mg measured in erythrocytes membranes in the Apo E KO group (Figure 2). The obtained results from erythrocytes' membranes in our study are in the range of those in human membranes as measured by Olson et al. [67]. In these human erythrocytes, the B_{max} was 1120 fmol/mg protein and the K_d 3.9 ± 0.4 nM. It has been suggested that TSPO present in the plasma membrane of erythrocytes is involved in redistribution of intracellular cholesterol to change the nuclear membrane rigidity prior to erythrocyte maturation [82]. Recent studies by us have shown that TSPO is involved in the regulation of gene expression as well as heme metabolism [45, 83, 84]. More studies may

provide deeper insights into how TSPO is involved in molecular biological mechanisms and biological functions of erythrocytes.

	Wild type mice			Apo E KO mice		
Cells	**B/ 6nM** (fmoles/mg)	**Kd** (nM)	**n**	**B/ 6nM** (fmoles/mg)	**Kd** (nM)	**n**
Platelets	1445 ± 736	0.8 ± 0.3	6	**6201 ± 2681****	1.3 ± 0.5	6
Erythrocytes	1923 ± 1010	2.6 ± 0.8	7	**3142 ± 1761***	4.2 ± 1.8	6

Table 1. [^{3}H] PK 11195 binding parameters in mice erythrocyte and platelet membranes

Average B values fmoles/mg protein and K_d values (nM) of 6 nM [^{3}H]PK 11195 binding to TSPO in erythrocyte and platelets membranes of WT (Bb-Control) and Apo E KO mice, fed with standard feed. One-way analysis of variance ANOVA was used, with Mann–Whitney as the post hoc, nonparametric test. Data are expressed as mean ± SD; *, $p < 0.05$; **, $p < 0.01$, vs. control.

Activated platelets are detected in increased numbers in the circulation of patients with atherosclerosis, coronary artery disease, and hypercholesterolemia. In the advanced atherosclerosis model of Apo E KO mice, subendothelial infiltration of monocytes/macrophages and platelets was observed, suggesting intimate interactions of platelets and macrophages in early atherosclerosis [85]. A recent paper by Nishikawa et al. demonstrated the role of hydrogen sulfide (H_2S), a gasotransmitter, in inhibiting platelet aggregation by interfering the cytosolic Ca^{2+} mobilization in a cAMP-dependent manner, comparable to what is observed for nitric oxide (NO) [86]. Studies by us have suggested that functions regulated by NO require the presence and activation of TSPO [87]. A study from Hamilton et al. indicated that activation of platelets with thrombin and other agents can promote atherogenesis [2]. Activation of human platelets is inhibited by two intracellular pathways regulated by either cyclic AMP or cyclic GMP. The importance of cyclic second messengers in modulating platelet reactivity is well established, elaborating the lipid-lowering effects of simvastatin therapy on inhibition of platelet aggregation through increasing the levels of both cAMP and cGMP [4]. Although Apo E induced increases in both cAMP and cGMP, additional experiments implicated a specific stimulation of guanylate cyclase activity and a rise in cGMP as prerequisites for the inhibitory effects on platelet aggregation of Apo E. Nonetheless, the mechanisms by which platelets promote atherogenesis need further observations.

Both TSPO and Apo E have been found to be involved in neurodegeneration. Both molecules have been associated with particular common factors being part of neurodegenerative diseases, such as cholesterol metabolism, oxidative stress, apoptosis and inflammation (Figure 1). Furthermore, Apo E was found to be involved in platelet aggregation, while TSPO expression in platelets has been associated with various pathological conditions, including neurological disorders [46]. Some authors have reported the protective effects of TSPO agonists in

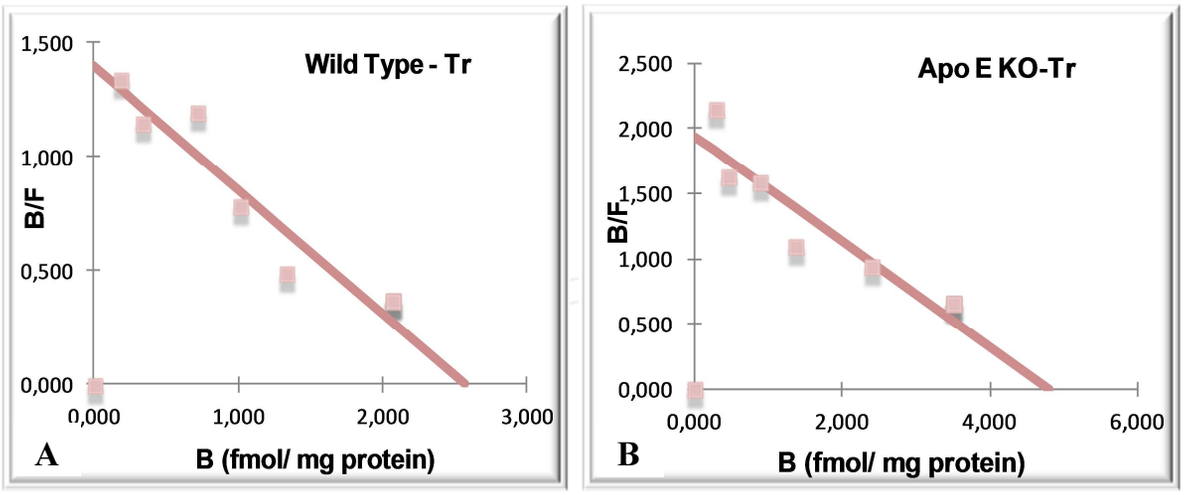

Figure 3. Representative examples of Scatchard plots of saturation curves of [^{3}H] PK 11195 binding to membrane preparations of platelets of wild-type mice (A) and apo E-KO mice (B). Abbreviations: B, bound; B/F, bound over free.

experimental neuropathy, suggesting reparative actions of brain TSPO in such a disease [88]. A recent study from Giannaccini et al. demonstrated upregulated TSPO expression in the brain region of leptin-deficient obese mice (ob/ob) [89]. In addition, the induction of TSPO expression under neuropathological conditions suggests that this molecule may be involved in the response of the neural tissue to inflammation [90, 91]. Indeed, application of TSPO ligands can dramatically decrease neuropathological symptoms of various diseases and injuries [61, 62]. However, the precise role of TSPO in the injured neural tissue needs to be investigated further.

In conclusion, the platelets' and erythrocytes' constant exposure to enhanced levels of cholesterol in the bloodstream may be associated with increased TSPO levels. One mechanism may be that apoE's modulation of NO levels may affect TSPO expression in the platelets and erythrocytes. Our study indicates that the Apo E mouse presents a promising animal model to elucidate the exact role of TSPO in platelets and erythrocytes in a cholesterol-dependent atherosclerosis. Thus, the TSPO represent a novel therapeutic target and diagnostic tool for cardiovascular disease and its complications.

Explanation of abbreviations and symbols: ANOVA, analysis of variance; ApoE$^{-/-}$ KO, apolipoprotein E knockout mice; ApoER, apolipoprotein E receptor; BSA, bovine serum albumin; cAMP, adenosine 3,5-cyclic monophosphate; DBI, diazepam binding inhibitor; CAM, cell adhesion molecule; Er, erythrocytes; H_2S, hydrogen sulfide; HDL, high-density lipoprotein; kDa, kilodalton; K_d, 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; LDLR, low-density lipoprotein receptor; LOX-1, the lectin-like endothelial receptor for ox-LDL; mPTP, mitochondrial permeability transition pore; MCP-1, monocyte chemoattractant protein-1; NADP, nicotinamide adenine dinucleotide phosphate; NADH, reduced nicotinamide adenine dinucleotide; NO, nitric oxide; NOS, nitric oxide synthase; ox-LDL, oxidized low-density lipoproteins; PBR, peripheral-type benzodiazepine receptor; PK 11195, 1-(2-

chlorophenyl)-N-methyl-N-(1-methyl-prop1)-3 isoquinoline carboxamide; ROS, reactive oxygen species; TSPO, 18 kDa translocator protein.

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