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Dimethylbenz[a]anthracene induces oxidative stress and reduces the binding capacity of the mitochondrial 18-kDa translocator protein in rat aorta

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ABSTRACT

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In this study, rats surviving 18 weeks after 7,12-dimethylbenz[a]anthracene (DMBA) exposure showed robust pathological changes in the aorta. This correlated well with decreases in 18-kDa translocator protein (TSPO) binding capacity in this tissue. As expected, markers for oxidative stress, including thiobarbituric-acid-reactive substances, and advanced oxidation protein products, showed that the applied DMBA exposure increased oxidative stress in the aorta. Our study suggests that TSPO may be involved in toxic DMBA effects in the aorta, including inflammatory responses and reactive oxygen species generation.

Introduction

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Atherosclerosis is a degenerative inflammatory process characterized by fibroid, lipid-containing plaques in the arterial walls. In most cases, the plaque is associated with dysfunctional endothelial barrier lining, activation of adhesion molecules, proliferation of smooth muscle cells, and impaired vasorelaxation and anticoagulant/antiplatelet properties (Ross, 1999; Costopoulos et al., 2008). A number of identified systemic risk factors are thought to contribute to this disease, including hypertension and hyperlipidemia. Further, the vascular endothelium is known to be susceptible to physiological insult due to circulating xenobiotics and inflammatory mediators. For example, chemical injury provoked by polycyclic aromatic hydrocarbons (PAHs), such as benzo[a]pyrene (B[a]P), 3-methylcholanthrene (MC), and 7,12 dimethylbenz(a)anthracene (DMBA), may damage endothelial cells and exacerbate the effects of aging and shear stress (Wakabayashi, 1990; Izzotti et al., 2001; Iwano et al., 2005). It has been shown that DMBA can accelerate the development of atherosclerotic plaques in various experimental animal models that spontaneously develop atherosclerotic plaques (Albert et al., 1977; Paigen et al., 1986; Izzotti et al., 2001). In the present study, we applied DMBA to otherwise healthy animals to be able to better study the effects of DMBA per se.

It has been suggested that hydrocarbons, such as PAH, can be metabolically transformed into more toxic products, such as epoxides and quinines, that can initiate atherosclerosis by injuring endothelial and muscle cells (Huberman et al., 1979; Moorthy et al., 2002; Barros et al., 2004). It is unclear to which extent the above-mentioned events are mediated by changes in the plasma-lipid profile or by increased oxidative-stress injury (Curfs et al., 2004; Raa et al., 2007). Increased reactive oxygen species (ROS) production under these conditions can provoke inflammatory responses in the cardiovascular system, including damage to endothelial cells and cellular macromolecules (Esterbauer and Chessman, 1990; Limón-Pacheco and Gosebatt, 2008).

Mitochondria are particularly vulnerable to oxidative stress (Netto et al., 2002; Oliveira et al., 2006; Szeto, 2006). Recent studies by us and others suggest that the mitochondrial 18-kDa translocator protein (TSPO) may be involved in many components of cardiovascular disease (Veenman and Gavish, 2006; Papadopoulos et al., 2006). TSPO is abundant in the cardiovascular system and is also known as peripheral-type benzodiazepine receptor (PBR). In the cardiovascular lumen, TSPOs are present in platelets, erythrocytes, lymphocytes, and mononuclear cells (Canat et al., 1993; Maeda et al., 1998; Veenman and Gavish, 2006). 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; French and Matlib, 1988; Stoebner et al., 1999; Veenman and Gavish, 2006). TSPO's primary intracellular location is the outer mitochondrial membrane (O'Beirne et al., 1990; Krueger and Papadopoulos, 1990; Gavish et al., 1999; Veenman et al., 2007). Regarding cardiovascular diseases, TSPO has been found to be involved in ischemic processes, including oxidative stress and apoptosis (Demerle-Pallardy et al., 1991; Myers et al., 1991; Kunduzova et al., 2004). Recently, we found that 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; Veenman et al., 2008, 2009; Zeno et al., 2009). In turn, ROS levels also affect TSPO function (Delavoie et al., 2003).

The present study was undertaken to provide more data relating changes in TSPO binding characteristics to cardiovascular damage, such as can be found in the aorta. Bearing in mind that different tissues and organs have different rates of metabolic activity and oxygen consumption due to distinct antioxidant systems (Limón-

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Pacheco and Gonsebatt, 2008), we applied various doses and times of DMBA exposure. In addition to our TSPO-binding assays, we assayed the effects of DMBA aortic histology in correlation with oxidative stress parameters in the aorta wall and with the plasma-lipid and stress profile. We used outbred rats (Wistar), which typically do not show atherosclerosis within the time frame we used, to ensure that the effects we studied were primarily due to DMBA application.

Materials and methods

Materials

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Wistar rats were obtained from the Military Medical Academy (VMA, Belgrade, Republic of Serbia) and maintained and bred in our own animal facilities at the Department of Physiology and Biochemistry of the Faculty of Natural Sciences and Mathematics (Ss Cyril and Methodius University, Skopje, Republic of Macedonia). Rat feed was obtained from Filpaso (Skopje, Republic of Macedonia). [3 H]PK 11195 was obtained from New England Nuclear (Boston, Massachusetts, USA) and PK 11195 from Sigma-Aldridge (Rehovot, Israel). DMBA was purchased from Sigma-Aldridge GmbH (Steinheim, Germany). Enzyme-colorimetric test kits were obtained from Human Diagnostics (Wiesbaden, Germany). Trichloroacetic acid (TCA), 2-thiobarbituric acid (TBA), trifluoroacetic acid (TFA), 1,1,3,3-tetraethoxypropane (TEP), T-chloramine, and phosphate buffer solution (PBS; 1 M) were from Sigma-Aldrich. 2,4-dinitrophenylhydrazine (DNPH), Folin-Ciocalteu reagent, and guanidine chloride, were obtained from Merck (Darmstadt, Germany). Various chemicals and consumables were obtained from standard commercial sources.

Research subjects

Male white Wistar rats, 20 weeks old at the start of the experiment, were used. The rats were kept on a regular dark-light cycle and received water and regular chow *ad libitum*. All procedures with the animals were in accord with the National Institutes of Health (Bethesda, Maryland, USA) guidelines for the care and use of experimental animals (NIH publication No. 85-23, revised 1996), and the experimental protocol was reviewed and approved by the ethics committee of the Ss Cyril and Methodius University, Skopje, Republic of Macedonia.

Treatment and tissue handling

Administration of DMBA

For each experiment, DMBA was dissolved in sesame oil, as described previously (Carter and Carter, 1988; Whitsett et al., 2008). Animals were administered DMBA by intragastral intubation, as described previously (Löscher et al., 2001; Motoyama et al., 2008). The animals received different doses of DMBA and were sacrificed at various time periods after DMBA application, as listed below:

1. The control group (C) was exposed to vehicle (i.e., 1 mL of sesame oil) administered once by intragastral intubation and sacrificed 18 weeks after DMBA administration ($n = 16$).
2. The DS10 group ($n = 6$) received a single dose of DMBA (10 mg in 1 mL of sesame oil). Animals of the DS-10 group were sacrificed 10 weeks after DMBA administration.
3. The DS20 group ($n = 18$) was exposed to a single dose of DMBA (20 mg in 1 mL of sesame oil) and sacrificed 18 weeks after DMBA administration.
4. The DR20 group ($n = 24$) was exposed to DMBA four times at weekly intervals (i.e., 4x 5 mg of DMBA in 0.5 mL of sesame oil) and sacrificed 18 weeks after the first dose of DMBA administration.

One of the aims of this experiment was to correlate the stoichiometry of dose, frequency of administration, and duration of time after DMBA initiation with oxidative stress, cardiovascular damage, and TSPO-binding characteristics, in the aorta.

Tissue collection

The animals were anesthetized with a ketamine-xylazine mixture [90 mg/kg, intraperitoneally (i.p.) and 10 mg/kg, i.p., respectively], applied after an overnight fast of 12 hours, followed by exsanguination. Samples of blood and aorta were harvested between 10:00 a.m. and 12 noon. Separated heparinized plasma samples obtained after the exsanguination were stored at -80°C for 6–8 weeks until further analysis. All other tissue samples were also removed immediately, flash frozen, measured, and stored at -80°C until further analysis, or processed immediately for histological assays.

As described further below, for assays of the oxidative stress markers, thiobarbituric-acid-reactive substances (TBARS), protein carbonyls (PCs), and tissue homogenates were prepared in 1.12% KCl at $+4^{\circ}\text{C}$. For advanced oxidation protein products (AOPPs), tissue homogenates were prepared in 50 mM of PBS at $+4^{\circ}\text{C}$. For these oxidative stress assays, we used an Ultrasonic Homogenizer (Cole-Parmer Instrument Co., Chicago, IL, USA). For TSPO-binding assays, tissue homogenates were prepared in 50 mM of PBS on ice, using a Kinematika Polytron (Luzerne, Switzerland), as described previously (Danovich et al., 2008).

Histology

Aortas of the four experimental groups were subjected to histological analysis. The aortas were dissected transversely into 5-mm segments, beginning at the aortic arch and continuing into the abdominal aorta in all animals. After removal and dissection, the aorta segments were immediately fixed in 10% buffered formalin and processed for embedding in paraffin. Five sections (3–5 μm) of each aorta segment were randomly chosen for histological analysis. In this way, 20 aortic slice samples along the length of the aorta were microscopically investigated and quantified, utilizing a light microscope connected to a video camera (Nikon-Eclipse E600, Program Lucia 4.21; Nikon, Düsseldorf, Germany). Histological sections of all cases were stained with hematoxylin and eosin.

Plasma assays

The blood samples from the exsanguination were collected into tubes and centrifuged ($1450 \times g$, 4°C , 10 min). At day 0 of the experiment in question, and at the end of the experiments (18 weeks for the C, DS20, and DR20 groups and 10 weeks for the DS10 group), standard stress parameters were measured in plasma of rats: albumin (Alb), total proteins (Tot proteins), creatinine (Creat), urea, creatine kinase (CK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and uric acid (UA). Measurements were done with a clinical chemistry analyzer system (AU 400; Olympus, Tokyo, Japan), using routine clinical chemical assays. Standard kits were used to measure total plasma cholesterol, triacylglycerides, and high-density (HDL) cholesterol (enzyme-colorimetric test; Human Diagnostics).

Protein quantification

General protein content of aorta homogenates for carbonylization assays was measured according to Levine et al. (1990). For [3 H]PK 11195 binding assays and specific assays for oxidative stress, general protein content in the aorta were measured according to Lowry et al. (1951).

Lipid peroxidation assays

Lipid peroxidation (LPO) products from the aorta were measured as TBARs in 2.5% (w/v in 1.12% KCl) of tissue homogenates, according to the method of Okhawa et al. (1978), as modified by Draper and Hadley (1990). The results of the TBAR assays were presented as nanomoles of TBARs per milligram of protein, using 1,1,3,3-tetraethoxypropane (TEP) as the external standard, measured at 535 nm with a spectrophotometer (Cintra, UV/Vis Spectrometer; GBC, Melbourne, Australia).

PC assays

Levels of PCs were determined in aorta homogenates (2.5% w/v in 1.12% KCl), according to Levine et al. (1990). The carbonyl content was calculated from the absorbance measurement spectrophotometrically at 340 nm (Cintra) with the use of a molar absorption coefficient of 22,000 M $^{-1}$ cm $^{-1}$.

AOPP assays

Levels of AOPPs in aorta homogenates were determined spectrophotometrically at 340 nm (Cintra) and calibrated with chloramine-T solutions that, in the presence of potassium iodide, absorb at 340 nm (Witko-Sarsat et al., 1996). Briefly, 200 μ L of 2.5% tissue homogenate diluted 1:5 in 50 mM of PBS (containing butylated hydroxytoluene, 100 μ M, and EDTA, 1 mM), was placed in wells of a 96-well microtiter plate and 10 μ L of 1.16-M potassium iodide was added, followed by 40 μ L of acetic acid after 2 minutes. For blanks, 10 μ L of 1.16-M potassium iodide was added to 200 μ L of PBS, followed by 20 μ L of acetic acid. AOPP concentrations were expressed as nanomoles per milligram of protein of analyzed tissue.

TSPO-binding characteristics

Maximal binding capacity (B_{max}) and equilibrium dissociation constant (K_d) of the binding of the TSPO-specific ligand, [3 H]PK 11195, were determined in whole-cell-membrane homogenates from the aorta, according to previously described methods (Awad and Gavish, 1987; Veenman et al., 2002; Danovich et al., 2008). Radioactivity was counted with a 1600CA Tri-Carb liquid scintillation analyzer (Packard, Meriden, Connecticut, USA). Scatchard analysis of [3 H]PK 11195 binding was done to determine the B_{max} and K_d values.

Statistical analysis

Data are presented as means \pm standard deviation ($n > 5$). Significance was determined by using analysis of variance and post-hoc analysis, as appropriate, while for dependent groups, the paired-samples *t*-test was used. The significance of association was determined by the two-tailed *t*-test. STATISTICA 5.0 (Stat Soft, Inc., Tulsa, Oklahoma, USA) was used. $P < 0.05$ was considered indicative of significant differences.

Results

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The animals of this study were observed daily, and the vast majority appeared healthy throughout the course of the experiment, with the exception of the death of 1 rat of the DS20 group (5.6%) and 2 rats of the DR20 group (8.3%). In the DS10 group, no mortality was registered. No morphologically visible abnormal outgrowths appeared in any of the rats. At sacrifice, gross examination of internal organs generally revealed no abnormalities. Thus, no obvious tumorigenic effects were observed in the DMBA-exposed male rats with the gross anatomical investigation of the present study. However, in some rats treated with DMBA, reductions in testis size of approximately 30% were observed (in 2 DS20 rats, and in 3 DR20 rats).

To detect potential general effects of the DMBA treatment, we also measured a number of stress parameters in blood plasma that typically are used to determine the potential occurrence of shock or injury (Table 1). UA was significantly increased in all groups with different DMBA exposures applied, as compared to the control. Creatinine levels were significantly decreased in all three DMBA-treated groups, compared to the control. DMBA administration also caused significant decreases in enzyme activity of ALT in all three experimental groups, compared to vehicle control (Table 1). Also, compared to vehicle control, CK activity and LDH were significantly increased in groups with a single-dose application of DMBA (DS10 and DS20), while in the group with repeated DMBA application (DR20), the above parameters were also increased, but with high within group variability (Table 1). Levels of AST, Creat, Tot protein, and Alb were significantly decreased in the DS20 and DR20 groups in comparison to the control (Table 1). The most significant changes regarding the stress parameters were observed in the DS20 group (Table 1). In contrast to the biochemical parameters measuring stress level, plasma lipids remained unchanged in all groups, relative to the start of the experiment (Table 1).

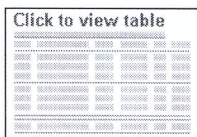


Table 1. Effects of exposure of rats to DMBA on stress-related parameters measured in blood plasma.

We observed histological changes in the endothelium of aorta from rats exposed to DMBA for 10 and 18 weeks (Figure 1; Table 2). In the endothelium of the aorta wall of rats treated with single doses of DMBA, we observed the appearance of foamy cells (20–25% of the lumen circumference) in 3 of 6 analyzed DS10 rats (50%) and in 8 of 12 analyzed DS20 rats (67%) (Figure 1B; Table 2). Almost half of the DS20 animals showed disruption of the endothelial layer (5 animals; 42%), together with the foamy appearance of the cells, which was visible among 8 animals (Figure 1C; Table 2). In the remainder of the analyzed DS10 and DS20 groups (3 and 5 animals, respectively), no signs of pathology in the aortas were observed. In the group with repeated application of DMBA (DR20), foamy cells were visible among 11 animals (67%; Table 2), of which 3 showed disruption of the endothelial layer, which represents 20% of the total analyzed number. Larger abnormalities, visible as proliferatory and migratory activity of medial layer of the aorta wall, as well inflammatory cells and thrombus, appeared in 6 of the 11 animals with foamy cell appearance, which is 40% of all analyzed animals in this group, and 54% of animals with pathologies in this group (Figure 1D and 1E; Table 2). In the remainder of the analyzed DR20 group (4 animals; 27%), no signs of pathology in the aortas were observed. The aortas of the analyzed 12 vehicle control animals remained negative for pathologies (Figure 1A; Table 2).

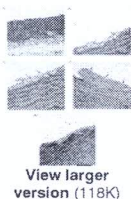


Figure 1. Representative photomicrographs of healthy and damaged aorta wall: (A) Aorta of the vehicle control group, free of pathological changes. (B) Focal infiltration of foamy cells (arrows) in the endothelium of the aorta of a rat exposed to DMBA; DS10, in this case. (C) Disruption of endothelial layer (arrows); DR20, in this case. (D) Parietal thrombus (arrows) attached to the endothelium, with the presence of necrotic nuclei and erythrocyte remnants without further reaction of the underlying layer, as observed only in the DR20 group. (E) Proliferative and migratory activity (encircled) of the medial layer observed in the DR20 group. Hematoxylin and eosin staining for all micrographs. DS10, group with a single dose of 10 mg of DMBA per rat; DS20, group with a single dose of 20 mg of DMBA per rat; DR20, group with 4 x 5 mg of DMBA per rat (one dose each week). Scale bars are 100 μm as indicated.

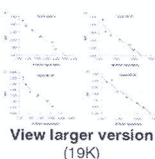


Figure 2. Representative Scatchard plots of saturation curves of [³H]PK11195 binding to membrane homogenates of the aorta, respectively, of vehicle control rats (A) and of rats exposed to DMBA (B–D). B, bound; B/F, bound over free. DS 10, DS 20, and DR 20 are described in the Materials and Methods.

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Group	Parameter	Value	Significance
Vehicle Control	B _{max}	4078 ± 1192 fmol/mg	
	K _d	1.8 ± 1.1 nM	
	B/F		
	Other		
DS10	B _{max}		
	K _d		
	B/F		
	Other		
DS20	B _{max}		
	K _d		
	B/F		
	Other		
DR20	B _{max}		
	K _d		
	B/F		
	Other		

Table 2. Histological abnormalities in aorta wall in all groups after DMBA exposure.

Regarding protein oxidation and LPO in the aorta (Table 3), in the DMBA-treated groups, increases in TBARS and AOPP levels could be observed in several instances, compared to vehicle control. In detail, AOPP levels were significantly higher in groups DS10 and DR20 (156 and 100%, respectively) and also higher in group DS20, but not significantly (90%). However, no significant changes were observed in protein carbonyl formation in all three experimental groups. Regarding LPO, TBAR production was also significantly increased in groups with 20 mg of DMBA applied (61% for DS20, 59% for DR20).

Click to view table

Group	Parameter	Value	Significance
Vehicle Control	TBARS		
	AOPP		
	Protein Carbonyl		
	LPO		
DS10	TBARS		
	AOPP		
	Protein Carbonyl		
	LPO		
DS20	TBARS		
	AOPP		
	Protein Carbonyl		
	LPO		
DR20	TBARS		
	AOPP		
	Protein Carbonyl		
	LPO		

Table 3. Aorta oxidative-stress parameters in rats exposed to DMBA and vehicle control.

Binding assays with the TSPO-specific ligand, [³H]PK 11195, were done to determine the potential effects of DMBA administration on the TSPO-binding characteristics in rat aorta (Figure 2). In the present study, TSPO-binding characteristics of the aorta of untreated vehicle rats were as follows: B_{max} = 4078 ± 1192 fmol/mg; K_d = 1.8 ± 1.1 nM (Table 4). Regarding the effect of DMBA, we found significantly reduced TSPO-binding capacity in the aorta in groups DS10 (37%) and DR20 (40%), compared to the vehicle control (Table 4). No significant change with [³H]PK 11195 binding assays was observed in the rat aorta of the DS20 group (Table 4). No significant differences were observed between experimental and control groups regarding the K_d.

Click to view table

Group	Parameter	Value	Significance
Vehicle Control	B _{max}	4078 ± 1192 fmol/mg	
	K _d	1.8 ± 1.1 nM	
DS10	B _{max}		
	K _d		
DS20	B _{max}		
	K _d		
DR20	B _{max}		
	K _d		

Table 4. Effect of DMBA exposure on TSPO-binding characteristics in the aorta.

Discussion

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With this study, we investigated the potential effects of DMBA on atherosclerosis and oxidative stress in correlation with TSPO-binding characteristics in the aorta. In the plasma, we assayed general stress parameters and the plasma-lipid profile. Further, as it is known that TSPO takes part in oxidative stress and, potentially, may be involved in cardiovascular disorders (Veenman and Gavish, 2006; Veenman et al., 2008, 2009; Zeno et al., 2009), we also determined the effects of DMBA treatment on TSPO-binding characteristics in the aorta.

We also investigated the potential consequences of dose, frequency of administration, and term from initial DMBA application until sacrifice of the animals. The repeated-dose paradigm (i.e., fragmenting one large dose of DMBA to smaller, nonlethal fractions) is considered the optimal application model to induce malignant tumors (Huggins et al., 1964; Löscher et al., 1993; Mevissen et al., 1996; Gao et al., 2007). Also, regarding the etiology of atherosclerosis, multiple injections of PAHs have been applied (Albert et al., 1977; Curfs et al., 2004, 2005; Catanzaro et al., 2007). Nonetheless, most of the toxic PAH effects on the aorta and other tissues have been shown with animal models applying one dose (Harrigan et al., 2004; Hakkak et al., 2005;

Motoyama et al., 2008). Depending on the particular report, time from administration of PAH to sacrifice ranges from 24 hours to 270 days. Regarding DMBA, both single and multiple doses were applied to accelerate plaque formation in atherosclerosis-prone animal models (Batastini and Penn, 1984; Majesky et al., 1985; Curfs et al., 2003). We used three regimens of DMBA application to outbred Wistar rats (DS10, DS20, and DR20) and times of 70 (10 weeks) and 126 days (18 weeks) to determine the effect of DMBA per se. The different effects of the three regimens are discussed below.

Morphologically and histologically, treatment with DMBA of the DS20 and DR20 groups resulted in moderate damage of the aorta cardiovascular wall, which appeared most pronounced in the DR20 group. This type of damage in the aorta was comparable to that observed by others after DMBA exposure in animal models [e.g., apolipoprotein E-knockout (ApoE-KO) mice, cockerels, and SC-strain chickens] that spontaneously develop plaques (Revis et al., 1984; Paigen et al., 1986; Gairola et al., 2001; Curfs et al., 2005). The DS10 group in our study remained relatively unaffected in this respect, with only focal infiltration of foamy cells into the endothelium. Thus, our study shows that also in outbred rats, otherwise not atherosclerosis prone, DMBA can also induce atherosclerotic effects, in particular with terms of 18 weeks and a total DMBA dose of 20 mg per rat. As the DR20 group showed the most pronounced aorta damage, this group also most consistently showed an elevation of oxidative-stress parameters (i.e., TBARs and AOPP).

Of the plasma-stress parameters investigated in this study, ALT, Creat, and UA showed significantly increased levels in all groups exposed to DMBA. CK and LDH, which may be indicators of toxic effects in the liver (Meffert et al., 2005; Yousef et al., 2006), showed increased levels in the DS10 and DS20 groups. In parallel, we observed decreased levels of AST, ALT, Tot proteins, and Alb in the plasma of the DS20 and DR20 groups, which may be either related to impaired protein synthesis in the liver or due to increased protein catabolism in this organ (Harrison and Levitt, 1987; Baker et al., 2001). Decline in the activities of aminotransferases also serve as an indicator of altered cell-membrane permeability. The significant enhancement in UA levels observed in all DMBA-exposed groups may have had direct injurious effects on the aortic endothelium (Meisinger et al., 2002). Of the three DMBA-exposed groups, the DS20 group presented the most indications of general stress. In contrast to the above-mentioned stress parameters, plasma lipids remained unchanged in all groups (Table 2).

Several studies have suggested that TSPO may be involved in cardiovascular pathology, including inflammatory and immune responses (Drugan et al., 1988; Gavish et al., 1992; Salvetti et al., 2000; Veenman and Gavish, 2006; Veenman et al., 2007). We hypothesized that TSPO can be involved locally in the development of atherosclerosis. Further, several studies have suggested an involvement of TSPO in oxidative stress, including ROS affecting TSPO functions, such as apoptosis and cholesterol transport (Delavoie et al., 2003; Kunduzova et al., 2004; Veenman et al., 2007; Zeno et al., 2009). Further, recent studies by us indicate that TSPO participates in ROS generation at mitochondrial levels (Veenman et al., 2008, 2009; Zeno et al., 2009). Thus, since DMBA causes injury as well as oxidative stress in the aorta, we expected to see TSPO responses in this organ. In the present study, in applying DMBA to induce chemical irritation to the aorta, we found decreased TSPO-binding capacity in the aorta of groups DS10 and DR20. These effects were in correlation with increased oxidative stress parameters in the aorta in both groups. In particular, TSPO decreases correlated well with AOPP increases in the aorta. As TSPO-binding levels are typically reduced in the aorta, this may be a compensatory response directed to reduce damaging effects by DMBA, a mechanism that has also been proposed with *in vitro* studies with the toxin, CoCl₂ (Zeno et al., 2009). We also found an enhancement of TSPO-binding capacity in the heart of the groups with a single DMBA application, DS10 and DS20 (unpublished results).

Apart from the aorta, TSPO-binding capacity was also reduced in the liver (unpublished results), potentially indicative of DMBA's toxic effects. The brain did not show changes in TSPO-binding characteristics (unpublished results) suggesting that this organ may remain relatively unharmed with DMBA exposure. Since TSPO appears to control cholesterol transport and modulate steroidogenesis in endocrine tissues (Papadopoulos et al., 1997; Veenman et al., 2007), we were also interested in TSPO binding in the testes of DMBA-treated rats. A significant enhancement in TSPO-binding capacity was observed in the testes of DS20 and DR20 rats (unpublished results). As the size of the testes can be affected by DMBA exposure, the enhanced TSPO-binding levels present a further indication of DMBA's damaging effects on the male reproductive system in rats.

Regarding the lack of enhanced plasma-lipid levels in the present study, in another study, we found that enhanced plasma-lipid levels due to a chronic high-fat, high-cholesterol diet of rats also enhanced oxidative stress in the aorta, which was also associated with reduced TSPO density in the aorta (unpublished results). Vascular damage, however, was minimal to moderate in that study. It may be hypothesized that a reduction in aortic TSPO levels may present a protective mechanism against atherosclerosis. Possibly, TSPO responses in the aorta may be effective against chronic responses to lipid overload, while TSPO may not be able to cope with acute toxic challenges, such as those presented by DMBA.

Conclusions

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In conclusion, exposure of rats to DMBA caused oxidative stress in the aorta, as well as pathological changes. The pathological changes appeared to be related to dose or duration of DMBA exposure. As we used outbred Wistar rats, apparently genetic disposition is not a precondition for cardiovascular damage induced by DMBA. Pathological signs and oxidative-stress markers in the aorta were most consistently elevated upon repeated doses of DMBA totaling 20 mg. A single dose of 20 mg of DMBA most effectively induced effects on plasma-stress indicators. Long-term changes in blood-lipid profiles due to DMBA treatment were not observed. We suggest that reductions in TSPO-binding capacity may present protective mechanisms (Zeno et al., 2009). The potential involvement of TSPO in cardiovascular damage and enhanced oxidative stress due to DMBA treatment may have implication for future therapeutic applications.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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