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Chronic ethanol consumption-induced oxidative stress, vascular inflammation and hypertension: involvement of TNF-α R1 receptor and perivascular adipose tissue

Introduction: Chronic ethanol consumption induces increase in blood pressure, oxidative stress and vascular inflammation with increased production of TNF-α (1,2). It is known that activation of the TNFR1 receptor induces activation of several cellular pathways that leads to oxidative stress (3). Moreover, inflammation may involve participation of PVAT (*Perivascular adipose tissue* - PVAT), known as a major source of adipokines and pro-inflammatory cytokines (Szasz &Webb, 2012). However, the role of TNF-α in the induction of oxidative stress associated with chronic ethanol consumption and the involvement of PVAT in such response remains elusive.

Objectives: The aim of this study was evaluate the role of TNF-α in the induction of oxidative stress and increases blood pressure caused by chronic ethanol consumption and the involvement of PVAT.

Methods: Wild type (wt) C57/BL6 and knockout mice for TNFR1 receptor ($TNFR1^{-/-}$) were treated with ethanol 20% (v/v) for 9 weeks. Systolic blood pressure (SBP) was measured. The thoracic aorta with ($PVAT^+$) and without PVAT ($PVAT^-$) and plasma was used for determination of levels of: O_2^-, thiobarbituric acid reactive species (TBARS), nitrate/nitrite (NOx), H_2O_2 and cytokines. Superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) were evaluated (Ethical committee: 12.1.1654.53.9). Groups were compared using two-way analysis of variance (ANOVA), *post hoc* comparisons were performed using Bonferroni test. A *p* value below 0.05 was considered significant.

Results: The ethanol treatment increased the SBP in wt animals (control:115.7±5.1, n=9/ethanol:130.7±1.8, n=9) and this increase was less pronounced in $TNFR1^{-/-}$. The ethanol treatment increased the levels of (pg/mg protein) TNF-α ($PVAT^-$,control:12.9±2.6,n=8/ ethanol:27.7±4.8,n=8 / $PVAT^+$, control:32.1±1.2,n=8 / ethanol:41.8±5.4,n=8) and IL-6 in wt ($PVAT^-$, control:22.0±1.4, n=6 / ethanol: 35.7±1.5, n=8; $PVAT^+$, control:40.7±3.2, n=7/ ethanol:48.7±4.9, n=7). Ethanol increased levels of O_2^- (RLU/mg protein) in wt aorta ($PVAT^-$,control:149±6.7, n=7/ ethanol: 250±13.8,n=7; $PVAT^+$, control:409 ± 48.3, n=6 / ethanol: 592±51.5,n=7) but not in $TNFR1^{-/-}$. The levels of TBARS (nmol/mg protein) increased in wt aorta ($PVAT^-$, control: 9,9±1,3, n=9 /ethanol:15±1.9, n=10; $PVAT^+$, control: 8.5±0.9, n=8 /ethanol: 16±2.3, n=8) and plasma (nmol/ml) after treatment ethanol but not in $TNFR1^{-/-}$. The tissue levels of H_2O_2 (nmol/mg protein) decreased in wt aorta after treatment with ethanol but not in $TNFR1^{-/-}$. The levels of NOx (nmol/mg protein) were reduced after treatment with ethanol in wt aorta (PVAT,control:23±4.7,n=7/ethanol:9±1.6,n=7; $PVAT^+$,control:20±4.6, n=5/ ethanol:3±0.5,n=6) and this decrease was not observed in $TNFR1^{-/-}$. Treatment with ethanol increased the activity of SOD in wt aorta ($PVAT^-$, control:64±4.3, n=7/ethanol: 83±4.0, n=6; $PVAT^+$,control:80 ±2.6, n=7/ ethanol:93±4.2, n=7) and CAT ($PVAT^-$, control:118±12.5,n=6/ ethanol:163±14.8, n=7;$PVAT^+$, control:87±7.5, =7/ ethanol:106±14.4,n=7). The treatment reduced plasma levels GSH in wt animals. Such changes were not observed in $TNFR1^{-/-}$ animals.

Conclusions: The TNF-α is an important mediator of vascular oxidative stress and increased blood pressure induced by ethanol and PVAT does not display a beneficial/protective action in reducing this damage caused by ethanol.

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3. Zhang *et al.* (2009). *Clin Sci* 116(3): 219-230;
4. Szasz and Webb (2012). *Clin Sci* 122: 1–12.