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Immunohistochemical Quantification of the Tumor Necrosis Factor (TNF) Receptor II Expression in the Hepatic Tissue after Systemic Administration of the DNA-SWCNT

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Abstract. The Tumor Necrosis Factor is a key mediator in hepatic inflammatory response during acute exposure to xenobiotics. The cellular effects of TNF are mediated via two cell surface receptors, TNF receptor 1 and TNF receptor 2. The purpose of this study was to examine the expression and to identify the cellular localization of TNFR2 in hepatic tissue by immunohistochemistry after systemic administration of the SWCNT. In the same time the cellular infiltration and the weight of the liver was correlated with the TNF alpha receptor 2 expression. Mice were exposed intraperitoneally (ip) to either vehicle, phosphate-buffered saline (PBS), or SWCNT-DNA (1.5ml, 2.925 mg/kg) for 48 h. The hepatic response associated with SWCNT systemic administration was characterized by increased expression of the TNF receptor II especially in the hepatocytes from the centrilobular and midzonal areas of hepatic lobule. This induced elevation of the TNF receptor II is not followed by hepatic necrosis, inflammatory infiltration or significant changes in liver weight.

Keywords: carbon nanotubes, toxicity, liver, tumor necrosis factor receptor II.

INTRODUCTION

The Tumor Necrosis Factor (TNF) is a pleiotropic, potent proinflammatory cytokine that induces cellular responses such as proliferation, production of inflammatory mediators, and cell death. In conjunction with interleukin 6 (IL6) TNF regulates the acute-phase response, adhesion molecule activation, and expression of the antioxidant genes, being perhaps the most critical and important mediator of cellular injury, inflammation, cell death, apoptosis, and tissue healing process (1). TNF is released primarily from stimulated macrophages, lymphoid cells, mast cells, endothelial cells, fibroblasts, and neuronal cells (17), being a key mediator in hepatic inflammatory response during acute chemical exposure. The induction of toxic effects and inflammation on the liver tissues depends on specific TNFR signaling, intimately the molecular response and the role of TNFα in regulating hepatic inflammatory cytokine and receptors and apoptotic gene expression being unique for various xenobiotics (14, 9).

The cellular effects of TNF are mediated via two cell surface receptors, p55 (TNFα receptor type I, CD120a, TNFRSF1a) and p75 (TNFα receptor type II, CD120b, TNFRSF1b), a 55 kD and 70-80 kD glycoproteins with a single membrane spanning hydrophobic segment. These two receptors are structurally related, but functionally distinct and are coexpressed on the surface of most cells, although in different amounts (8). TNFα receptor type I is rather constitutively expressed on a broad spectrum of different cell types and has been shown to mediate most of the known biologic effects of TNF. In contrast, expression of TNFα receptor type II seems to be modulated by various stimuli, having an increased expression in the some tumoral cells after treatment, after IL-1b or biomechanical stimulation, hypoxia or in the cases of rejection of the tissues after organ transplant (3,5). The interaction between these two receptors is complex; both are additionally proteolytically released as soluble molecules capable of binding TNF, being possible that the ratio TNFR1/TNFR2 could control TNF-a responses under inflammatory conditions (7, 17).

The receptor II of the TNF is supposed to have an antiapoptotic role, acting through the nuclear factor kappa B (NF-KB) pathway, and also initiates cell survival by induction of antiapoptotic molecules and inhibition of proapoptotic proteins (cIAP-1/clAP-2) (10, 13).

We previously shown that SWCNT are rapidly accumulating in the liver after systemic administration, and also that the administration of these molecules is correlated with the increased expression of the oxidative stress markers (6, 12). One of the known pathway of the CNT toxicity is linked to the oxidative processes, the activation of the TNF expression being a possible mediator of the CNT pathogenesis since this molecule mediates the toxicity of many xenobiotics with metabolic pro oxidative effects. The studies of Hatice et al. prove that the oxidative damage on the cells structures is mediated by TNFα, since oxidative stress promotes TNFR receptor self-interaction and ligand-independent and enhanced ligand-dependent TNF signaling (2).

MATERIALS AND METHODS

The synthesis and the functionalisation technique of the SWCNT used in this experiment follow the protocol previously described by Simon et al. (11).

The experimental animal model was represented by 24 young male albino rats (Wistar strain) divided in two equal lots (SWCNT group and reference group). The SWCNT administration was done intraperitoneally in a volume of 1.5 ml per animal (SWCNT concentration of the solution was 390 mg/L). The reference lot was injected intraperitoneally with the vehicle saline solution (1.5 ml PBS). The animals were sacrificed at 48 hours from the administration point of the solutions. The detailed necropsy examination of the animals was followed by the measurement of the weight of the body and the measurement of the weight of the liver (absolute/relative). The measurement and gathering of the tissue samples was carried out following the recommendation of the INHAND (International Harmonization of Nomenclature and Diagnostic Criteria for Lesions in Rats and Mice).

Histology

The necropsy specimens were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5-7 µm with a microtome Leica RM 2125 RT, and stained routinely by Hematoxylin-Eosin (HE) method. The slides were examined under a microscope Olympus BX 51 and the images were taken with Olympus SP 350 digital camera and processed by a special acquisition and image processing program, Olympus Cell B.

Immunohistochemistry

The immunohistochemistry was performed on 5-7 µm using a polyclonal rabbit anti-rat and human TNF receptor II (abcam ab15563) as primary antibody, following a protocol previously described by Hoffman et al. (4). For the detection we used the LSAB system(LSAB+System HRP-DakoCytomation, K0679). The secondary antibodies, biotin polyvalent, streptavidine-HRP, sub layer DAB+, cromogene DAB+ and hematoxiline Meyer were included in the LSAB kit.

Quantification of the lesions and TNFα receptor II expression

The quantification and grading of the expression of the TNF alpha receptor II was achieved following the protocol previously described by Hoffmann et al. (4). The quantification was carried out visually within 10 high power field/slide at the 40x objective amplification, following the next semi quantitative scale: -score 0 ("basically no staining") was given for positive immunohistochemical staining for less than 5% of the cells; -score 1 for 5–25% ("weak") immunohistochemical positive staining; -score 2 ("moderate") for 26–50% positive staining and score 3 ("strong") for more than 50% positive staining. Mean values were calculated and used for comparison of the different expression of the TNFα receptor 2.

The extent of hepatic inflammatory cellular infiltration was examined following the gradation protocol previously described by Horn et all (14). The quantification was carried out within 10 high power field/slide at the 20x objective amplification, following the next scale: grade 0, no inflammatory cell influx; 1 no more than 1–3 cells/field; 2, few inflammatory cells (3 to5 cells/field); 3, moderate inflammatory cell infiltration (5 to15 cells/field); 4, marked inflammatory cell infiltration (greater than 15 cells/field); and 5, severe inflammatory cell infiltration(more than 15 cells/field).

RESULTS AND DISCUSSIONS

The hepatic tissue from the vehicle group (classified as PBS group) contained no or only weak TNFR2 positive staining, localized randomly within hepatic lobules, with a slide preference to the hepatocytes from the periportal areas.

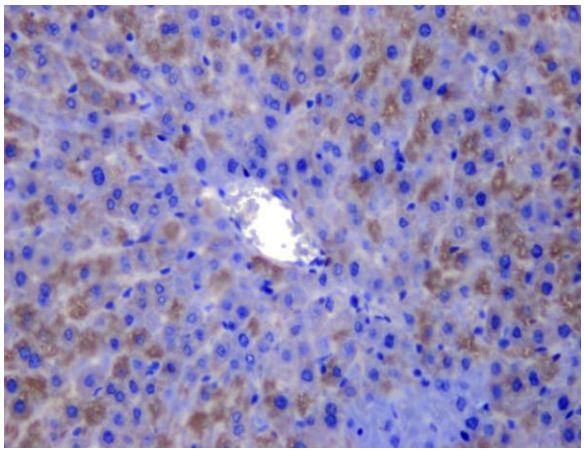

Fig.1. Liver, SWCNT group, immunohistochemical expression of the TNFR2, moderate expression (26–50% of the hepatocytes); Ob x20

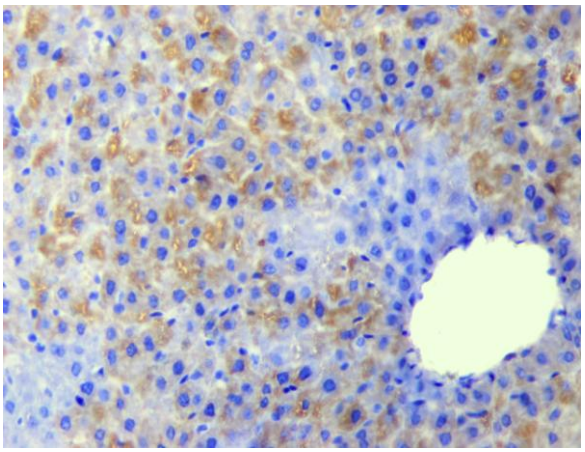

Fig.2. Liver, SWCNT group, immunohistochemical expression of the TNFR2, high expression (more than 50% of the hepatocytes). Obx20

Negative controls of the immunohistochemical staining by replacing the primary antibody with irrelevant IgG did not demonstrate positive staining (fig. 4).

The mean of the hepatic TNFR2- positive staining was significantly higher in the liver samples from the SWCNT group. A significant up regulation of TNFR2 was noticed especially in the hepatocytes from the centrilobular and midzonal areas of the hepatic lobules compared to PBS group samples. In the SWCNT group, the TNFR2 staining was restricted to the hepatocytes and to the few inflammatory cells found within the lobules. The endothelial cells of the arteries and veins stained poorly positive for TNFR2 (fig. 1, 2 and 3).

This elevation of the TNF receptor II expression is not followed by, inflammatory infiltration or significant changes in liver weight, the values found in the PBS and SWCNT group having little differences.

The hepatocytes reaction is intense for the TNF alfa, the receptor II can be the follow up of the oxidative stress induced by the nanotubes on a hepatic level. The TNFR2 pathway of TNF cytokine promotes either in cooperation with or independently of TNFR1, cell proliferation and cell survival (15).

Experimental Group	Liver weight	Inflammatory infiltrate	TNFRII expression
PBS Group	6.93±0.47	0.41	1.666
SWCNT Group	7.34±0.24	0.58	2.416

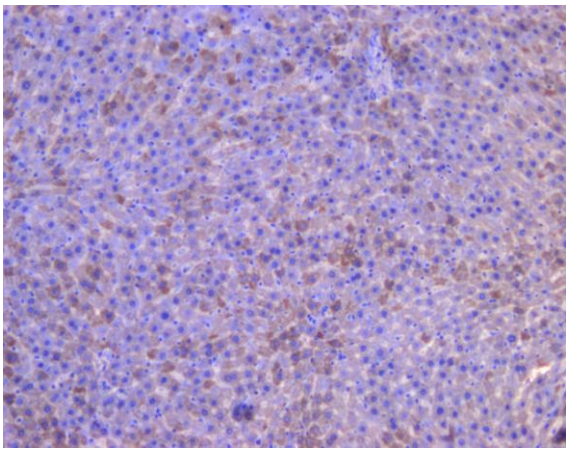

Fig. 3. Liver, PBS group, immunohistochemical expresion of the TNFR2, low expression (5–25% of the hepatocytes). Obx10

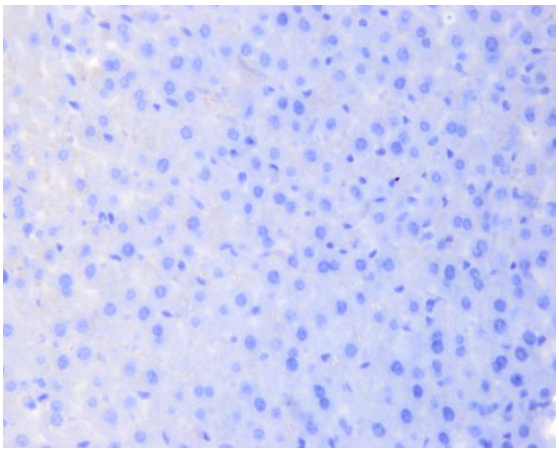

Fig. 4. Negative controls of the immunohistochemical staining. Lack of the signal for the TNF receptor II. Obx20

The contact between the hepatic cellular elements and single-walled carbon nanotubes does not induce the installment of a noticeable inflammatory response through the classical histological or cytological techniques at 48 hours from the administration of the SWCNT solution. This thing is due to the possible toxic mechanism of the carbon nanotubes that has as characteristic the late inflammatory response, the main histological alterations mentioned also in the field literatures being the proliferative inflammations, with a chronic character, around the accumulating areas of the nanotubes (18).

The absence of the inflammatory cell reaction and the hepatic tissue necrosis prior to the systemic administration and SWCNT accumulation denotes the lack of the significant acute hepatic toxicity. The same tissue response at the intraperitoneal administration of the nanotubes is found also by Pantarotto and Wang (16), authors which use the same administration path of SWCNT.

CONCLUSIONS

The hepatic response associated with SWCNT administration is characterized by increased expression of the TNF receptor II especially in the hepatocytes from the centrilobular and midzonal areas of the hepatic lobules. This elevation of the TNF receptor II is not followed by hepatic necrosis, inflammatory infiltration or significant changes in liver weight.

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