

Supplementary information

Figure S1: Histological characterization (H&E staining) of rat (a, f) lung, (b, g) brain, (c, h) kidney, (d, i) liver and (e, j) spleen after Gd-SWCNTs or Fe-SWCNT treatment (0.5mg/kg b.wt, i.v) at day 5. The Gd-SWCNTs and Fe-SWCNT were present as aggregates (red arrows) in the alveolar epithelial lining of the lungs, in the cerebral cortex of brain, in the proximal tubules of the kidney, in the hepatocytes of liver, red pulp of spleen. The histological specimens do not show any signs of inflammation or tissue architectural damage.

Ultrastructural localization of Gd-SWCNTs in liver by transmission electron microscopy (TEM)

TEM (Figure S2) was performed to characterize the ultra-structural accumulation of the Gd-SWCNTs in the liver cells. Their accumulation was further corroborated by confocal Raman imaging (Figure S3). The TEM analysis of the histological specimens at day 1 (Figure S2a), and day 5 (Figure S2b,c) did not reveal any ultra-structural changes to the liver cells. The images show normal cellular morphology with numerous mitochondria (characteristic feature of liver), nucleus, and endoplasmic reticulum. Day 1 post injection, the Gd-SWCNT are present as small (green arrows), or large (red arrow) aggregates mainly in the endosomes (brown arrows) (Figure 2a). The sub-cellular localization of Gd-SWCNTs is similar to other previous *in vitro* reports with SWCNTs.^{1,2} Day 5 post injection, the Gd-SWCNTs aggregates were also surrounded by a large number of mitochondria (Figure 2b,c). Mitochondria play a major role in metabolism of xenobiotic compounds by induction of cytochrome p450 enzymes, majority of which are also present in the endosomes.^{3,4}

Confocal Raman microscopy was performed to confirm, and corroborate the presence of Gd-SWCNTs in the liver tissue samples (Figure S3). Figure S3A shows an optical image of liver tissue section after day 1 treatment with Gd-SWCNTs. Raman spectra were obtained from a few selected points (red dots in Figure S3B) in the region of Gd-SWCNTs aggregates (red cross in Figure S3A). This region shows the characteristic G-band (1596 cm^{-1}) Raman peak of SWCNTs (Figure S3C). Figure S3D shows a Raman G-band intensity map of SWCNTs in the liver tissue section. Figure S3E shows the overlay of the Raman G-band intensity map (Figure S3D) on the optical image confirming the presence of Gd-SWCNTs. Other prominent peaks observed in the spectra were from compounds found in normal liver tissue and paraffin (Suppl info: Table S2).

Figure S2: Ultrastructural localization of Gd-SWCNTs (0.5 mg/kg b.wt, i.v) in liver tissue before and after treatment at day 1 and day 5. (a) Representative TEM image histological specimen of Gd-SWCNT at day 1, (b) Gd-SWCNT at day 5, and (c) magnified region of image b. The image (a) shows many mitochondria (M), a characteristic feature of hepatic tissue, and normal nucleus (N) of a hepatocyte. The Gd-SWCNTs are clearly internalized in the endosomes/vacuoles (brown arrows) of the hepatocyte (red arrows). The regions of Gd-SWCNT internalized vacuoles appear to be surrounded by many mitochondria.

Figure S3: Raman spectra and G band intensity map of Gd-SWCNTs in rat liver. A) bright field image of the unstained liver tissue after 1 day treatment of Gd-SWCNT (0.5mg/kg b.wt, i.v). B) In liver tissue, Raman G-band intensity was acquired at different spots (marked as red points) for the nanotubes for their localization. (C) Raman spectra of Gd-SWCNTs uptaken by the liver

cells. (D) Area map of Raman G-band intensity of Gd-SWCNTs in liver cells. (E & F) Area map of the Raman G-band intensity of Gd-SWCNTs in liver cells overlaid on optical micrograph of the same region.

Supplementary Table S1: Characteristic Raman peaks of Gd-SWCNT aggregate in the liver tissue

748 cm ⁻¹	DNA
1000 cm ⁻¹	Phenylalanine bound & free NADH
1117 cm ⁻¹	Glucose
1155 cm ⁻¹	Glycogen
1300 cm ⁻¹	Phospholipids
1378 cm ⁻¹	Paraffin
1596 cm ⁻¹	Gd-SWCNTs
1618 cm ⁻¹	Tryptophan

Supplementary Table S2: Expressed gene categories in liver associated with 0.5mg/kg.b.wt of (a) Gd-SWCNTs and (b) Fe-SWCNTs administration for one day as compared to control. The expressed genes having more than 2 fold change as compared to control ($p < 0.05$) are selected and represented as either up-regulated or down-regulated.

(a) A total of 42 genes got effected after day 1 treatment with Gd-SWCNTs, of which 30 genes are up-regulated (9 of which are not yet characterized) and 12 genes down-regulated (3 of which are not yet characterized). The data shown in table represents the genes that characterized.

Ref Seq Transcript ID	Gene Symbol	Description	Expression
NM_053774	Usp2	ubiquitin specific peptidase 2, Usp2	Up-regulated
NM_052798	Zfp354a	zinc finger protein 354A, Zfp354a	Up-regulated
NM_134383 /// XM_001075165	Elov16	ELOVL family member 6, elongation of long chain fatty acids (yeast), Elov16	Up-regulated
NM_145677	Slc25a25	solute carrier family 25 (mitochondrial carrier, phosphate carrier), member 25, Slc25a25	Up-regulated
NM_053962	Sds	serine dehydratase, Sds	Up-regulated
NM_031678	Per2	period homolog 2 (Drosophila), Per2	Up-regulated
NM_001106683 /// XM_001054640 /// XM_216525	Mfsd2	major facilitator superfamily domain containing 2, Mfsd2	Up-regulated
NM_017332	Fasn	fatty acid synthase, Fasn	Up-regulated
NM_012543	Dbp	D site albumin promoter binding protein, Dbp	Up-regulated
NM_139192	Scd1	stearoyl-Coenzyme A desaturase 1, Scd1	Up-regulated
NM_134383 /// XM_001075165	Elov16	ELOVL family member 6, elongation of long chain fatty acids (yeast), Elov16	Up-regulated
NM_022671	Onecut1	one cut domain, family member 1, Onecut1	Up-regulated
NM_001108180 /// XM_001071908 /// XM_236571	Ky	kyphoscoliosis peptidase, Ky	Up-regulated
NM_134449	Prkcdpb	protein kinase C, delta binding protein, Prkcdpb	Up-regulated
NM_001009671	Coq10b	coenzyme Q10 homolog B (S. cerevisiae), Coq10b	Up-regulated
NM_017332	Fasn	fatty acid synthase, Fasn	Up-regulated
NM_058209	Zfp37	zinc finger protein 37, Zfp37	Up-regulated
NM_031741 /// XM_001075869	Slc2a5	solute carrier family 2 (facilitated glucose/fructose transporter), member 5, Slc2a5	Up-regulated
NM_001108797 ///	Fam117b	amyotrophic lateral sclerosis 2 (juvenile) chromosome	Up-regulated

XM_001069873 /// XM_343576		region, candidate 13, Als2cr13	
NM_019242	Ifrd1	Interferon-related developmental regulator 1, Ifrd1	Up-regulated
NM_001106983 /// XM_001056587 /// XM_220209	Prss32	Protease, serine, 32 (predicted), Prss32_predicted	Up-regulated
NM_012551	Egr1	early growth response 1, Egr1	Down-regulated
NM_017206	Slc6a6	solute carrier family 6 (neurotransmitter transporter, taurine), member 6, Slc6a6	Down-regulated
NM_138504	Osgin1	oxidative stress induced growth inhibitor 1, Osgin1	Down-regulated
NM_173322	Pnrc1	proline-rich nuclear receptor coactivator 1, Pnrc1	Down-regulated
NM_024362	Arntl	aryl hydrocarbon receptor nuclear translocator-like, Arntl	Down-regulated
NM_030845	Cxcl1	chemokine (C-X-C motif) ligand 1, Cxcl1	Down-regulated
NM_001134844 /// XM_001062335 /// XM_001070917 /// XM_001070953 /// XM_341808	Cyp2b1 /// Cyp2b2	cytochrome P450, family 2, subfamily b, polypeptide 2 /// similar to Cytochrome P450 2B12 (CYPIIB12), Cyp2b2 /// LOC687222	Down-regulated
NM_001107084 /// XM_001055978 /// XM_221333	Bcl6	B-cell leukemia/lymphoma 6 (predicted), Bcl6_predicted	Down-regulated
NM_001106834 /// XM_001056334 /// XM_001056400 /// XM_001056458 /// XM_217192	Rora	RAR-related orphan receptor alpha (predicted), Rora_predicted	Down-regulated

(b) A total of 15 genes got effected after day 1 treatment with Fe-SWCNTs, of which 10 genes are up-regulated (2 of which are not yet characterized) and 5 genes down-regulated (3 of which are not yet characterized). The data shown in table represents the genes that characterized.

RefSeq Transcript ID	Gene Symbol	Gene Title	Expression
NM_022671	Onecut1	one cut homeobox 1	Up-regulated
NM_001013187	Slc25a30	Solute carrier family 25, member 30	Up-regulated
NM_145677	Slc25a25	solute carrier family 25 (mitochondrial carrier, phosphate carrier), member 25	Up-regulated
NM_139192	Scd1	stearoyl-Coenzyme A desaturase 1	Up-regulated
NM_144755	Trib3	tribbles homolog 3 (Drosophila)	Up-regulated
NM_053962	Sds	serine dehydratase	Up-regulated
NM_001107084 /// XM_001055978 /// XM_221333	Bcl6	B-cell CLL/lymphoma 6	Down-regulated
NM_030845	Cxcl1	chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)	Down-regulated

References

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- (3) Omura, T. *Chem Biol Interact* **2006**, *163*, 86.
- (4) Anandatheerthavarada, H. K.; Addya, S.; Dwivedi, R. S.; Biswas, G.; Mullick, J.; Avadhani, N. G. *Arch Biochem Biophys* **1997**, *339*, 136.









