Dual-modality, fluorescent, PLGA encapsulated bismuth nanoparticles for molecular and cellular fluorescence imaging and computed tomography

Supplemental data

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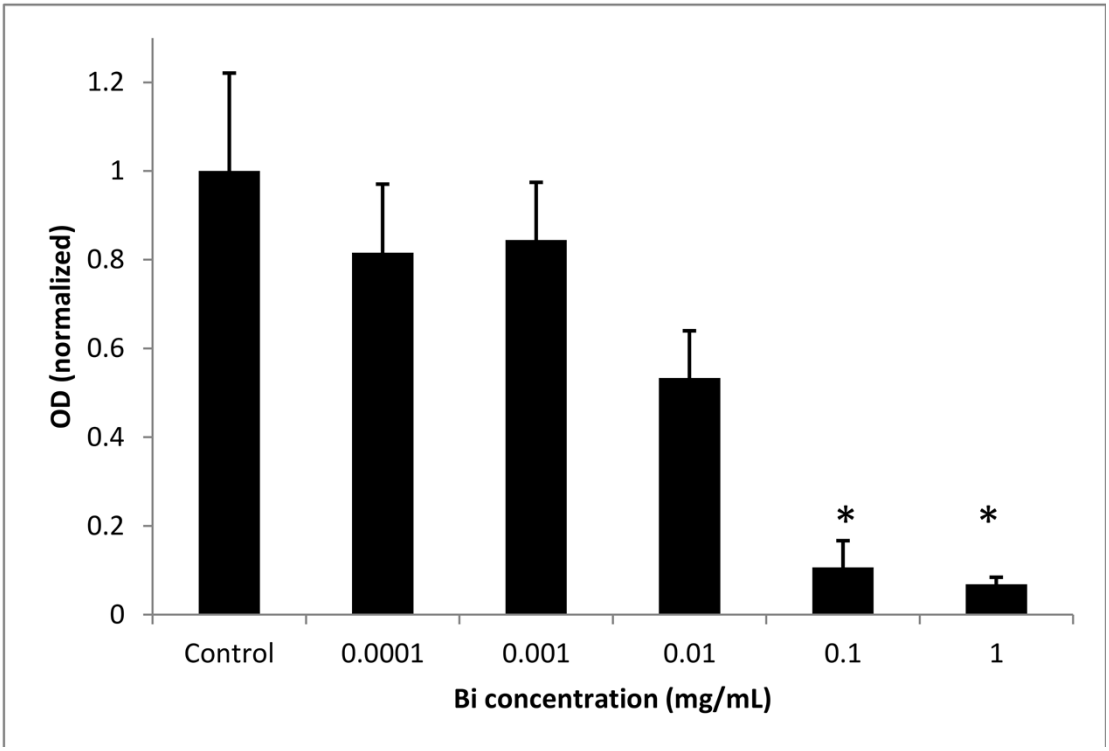
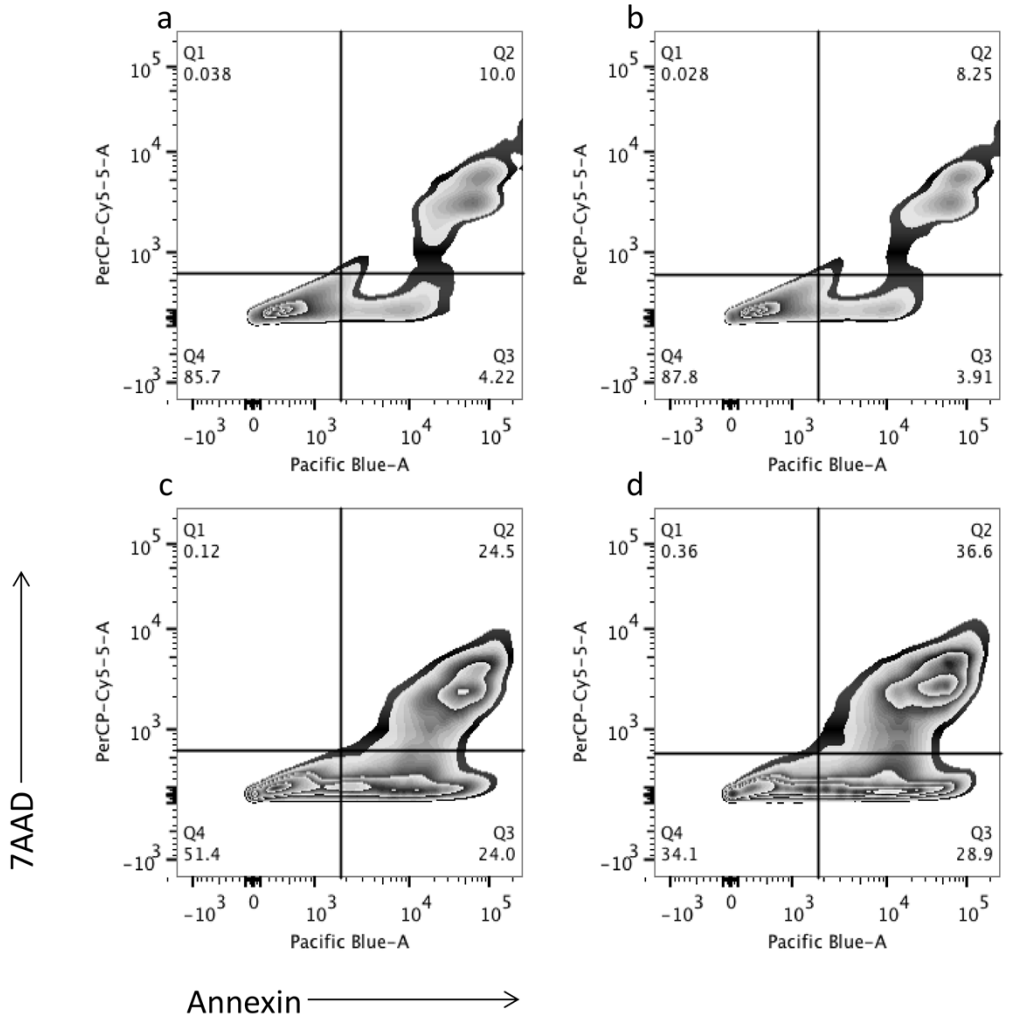
Results:



Supplemental Figure 1: Effect of bismuth concentration on cell proliferation. STO cells were co-incubated with Bi nanoparticles for 2 days, then an MTT assay was performed. * indicates significant difference from control (t-test, $p<0.0083$).



Supplemental Figure 2: Effect of bismuth on cellular viability.
Cells were incubated with particles for 24 hours then analyzed for viability by flow cytometry. Low concentrations of bismuth did not affect viability, but concentrations of 50 pg/cell and 150 pg/cell had substantial effects on viability. a) no bismuth, b) 5 pg/cell, c) 50 pg/cell, d) 150 pg/cell. Horizontal axis is annexin V (apotosis), vertical axis is 7AAD (necrosis). Quadrants were set using the unstained control as reference.

Detailed results from in vivo toxicity study

Group	Sex	Animal No.	Bismuth-PLGA Nanoparticles	
			mg/kg	Route
1	M	1 - 3	vehicle	IV
2	M	4 - 6	vehicle	IP
3	M	7 - 9	2	IV
4	M	10 - 12	2	IP
5	M	13 - 15	20	IP
6	M	16 - 18	20	IV

Clinical observations:

No animals displayed any apparent clinical abnormalities, although loose stools were present from two animals from groups 3 and 6 at 24 hours post injection and were found in the cage of group 2 at day 8. A transient, statistically significant decline in body weight of rats from group 6 was identified on day 2 but was not noted on day 8 of the study.

Serum chemistry values:

At 24 hours post injection of the particles, a statistically significant increase in serum glucose was noted in animals from group 3 (245.5±22.5 mg/dL) compared to vehicle-injected animals (156.3±3.4 mg/dL). Significant increase of ALP was also noted in group 6 (389±4 vs 208±33 U/I). Other clinical chemistry parameters were not significantly different between the groups, although some values at 24 hours post injection were not quantifiable due to low concentration of these parameters in some samples.

At 7 day time-point statistically significant changes were observed in groups 3 and 6, and in group 5. These included increase in globulin concentration (group 3 - 2.5 ±0.1 g/dL, group 6 - 2.8 ±0.1 g/dL; compared to 2.3 ±0.1 g/dL in the vehicle group) and decrease in creatine kinase concentration (group

3 – 579.0 ±40.7 mg/dL, group 6 – 168.0 ±4.0 mg/dL; compared to 875.0 ±76.5 mg/dL in the vehicle group). Magnesium was statistically decreased in group 6 (1.9 ±0.1 mg/dL) compared to vehicle control (2.4 ±0.1 mg/dL). Total Protein was statistically elevated in group 6 (5.63 ±0.12 mg/dL) compared to vehicle control (5.23 ±0.03 mg/dL). Chloride was statistically elevated in group 5 (99.7 ±0.3 mmol/L) compared to vehicle control (98.0 ±0.1 mmol/L). The transient increases in liver enzymes were resolved at this time point.

Hematology:

At 24-hours post-injection, mean platelet volume (MPV) was statistically increased in the group of animals receiving 20mg/kg IV (11.9 ±0.1 fL) compared to vehicle control (9.4 ±0.1 fL). Statistical analyses for hematology could not be performed on the samples from 5 animals (1, 7, 8, 15, and 18) at 24 hours post-dosing, due to clotting of the samples post collection.

At 7-day time-point again MPV was significantly increased in the group receiving 20mg/kg IV (9.8 ±0.3 fL) compared to vehicle control (8.4 ±0.1 fL). The same group had a statistically significant reduction in hematocrit (38.0 ±0.7 %) compared to vehicle control (44.0 ±1.2 %) and hemoglobin (11.97 ±0.39 g/dL vs 13.43 ±0.12 g/dL in control animals), while increased white blood cell counts (8.73 ±0.38 $x10^3$/µl vs 6.47 ±0.56 $x10^3$/µl in control animals) and lymphocyte count (7.57 ±0.32 $x10^3$/µl vs 5.03 ±0.38 $x10^3$/µl in control animals).

Pathological and histopathological evaluation:

No statistically significant changes in organ weights were noted and no gross changes were identified on necropsy. Histopathological evaluation of a single animal per group was performed on lungs, spleen, liver and kidneys. Examinations revealed that in all groups, but group number 2, animals had mild

changes in the lung encompassing mild to moderate BALT hyperplasia and/or polymorphonuclear infiltrates within subepithelial bronchial stroma. BALT hyperplasia was moderate in animals receiving IV injection of particles and these animals also had peribronchial eosinophilic infiltrates, which were not identified in other animals. In an animal from group 6, focal regions of alveolar macrophages within alveoli were identified. Evaluation of the spleen in group number 4 revealed a few clusters of monomorphic perivascular infiltrates in small regions were omentum was attached, and focally extensive region of low cuboidal mesothelial cells of splenic capsule. Focal periportal lymphocytic infiltrates were identified in the liver of an animal from group 6. In the same animal changes in the kidneys were also identified consisting of individualized and rafts of sloughed necrotic epithelial cells with pyknotic nuclei within the deep cortical tubules along corticomedullary junction. Tubules were also lined by low cuboidal epithelium and the tubular epithelium was pale and basophilic.

Report conclusions

20 mg/kg IV dose of test compound resulted in several statistically significant clinical chemistry and hematology changes compared to vehicle control. These changes are suggestive of a low-grade inflammatory or immune response and potentially low grade organ damage. Both inflammatory responses and organ damage have been confirmed in histopathological evaluation of organs. Further exploration of the pharmacodynamics of PLGA encapsulated bismuth nanoparticles is warranted to clarify these results.