Differentical Effects of PAHs and PAHs-Quinone on Oxidative Stress Damage in A549 Cells

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A variety of chemical compounds present in the environment getting more threaten human health, such as cancer, asthma, allergic, and skin disease. Especially, several environmental pollutants can cause adverse health effects through the generation of oxidative stress. Among chemical compounds, polycyclic aromatic hydrocarbons (PAHs), generated through the burning of fossil fuels and incomplete combustion of organic matters, are widespread in the environment and known as carcinogens/endocrine disruptors.

Quinones represent a class of toxicological intermediates which can create a variety of hazardous effects *in vivo*, including acute cytotoxicity, immunotoxicity, and carcinogenesis. Alternatively, quinones are highly redox active molecules which can redox cycle with their semiquinone radicals, leading to formation of reactive oxygen species (ROS). Oxidative stress, caused by ROS, is reported to activate the transcription factor nuclear factor kappa B (NF-κB) in cell line. The redox-sensitive transcription factor NF-κB plays an important role in the expression of a variety of genes participate in regulating the immune response, cell survival, inflammation, and cancer. In addition, activation of the MAPKs, which include ERK, JNK and p38 pathways, contribute to induction of NF-κB activity in response to an array of extracellular stimuli.

This study shows that PAHs and PAHs-quinone have effects through the generation of ROS. To determine whether oxidative damage caused by PAHs-quinone by way of production ROS, we incubated A549 cells with 5 μM of PAHs or PAHs-quinone for 1 hr and measured the generation of ROS and depletion of GSH and transcription factor NF-κB. To examine the intracellular ROS, several PAHs-quinone enhanced generation of ROS significantly. In contrasts, PAHs failed to exert oxidative damage effects rather than PAHs-quinone. Especially, enhanced intracellular ROS level was observed Acenaphthene (ANT) treated cell in the highest. Indeed, oxygenated derivatives PAH, Acenaphthenequinone (AQ), generated ROS higher than ANT. These enhancements showed PAHs-quinone involved an inducing of oxidative damage. A similar degree of generation was observed in Phenanthrene (PA) and 9,10-Phenanthrenequinone (PQ).

Table 1 ROS induction during treatment PAHs and PAHs-Quinone

	ROS level	PAHs (5μM)	ROS level	PAHs-Quinone (5μM)	ROS level
Control	100.00 ± 3.14	Phenanthrene	100.86 ± 0.17	9,10-Phenanthrenequinone	156.68 ± 1.16
H_2O_2	146.73 ± 1.76			Phenanthrene-1,4-quinone	92.95 ± 1.22
NAC	34.54 ± 0.33	Naphthalene	93.31 ± 1.50	1,2-Naphthoquinone	65.98 ± 0.03
		Acenaphthene	120.48 ± 0.74	**Acenaphthenequinone**	**147.64 ± 0.63**
		Benz(a)anthracene	99.47 ± 2.12	1,2-Benzanthraquinone	96.02 ± 3.17
		Anthracene	96.83 ± 2.11	Anthraquinone	89.85 ± 0.77
				1,4-Anthraquinone	86.44 ± 3.54
		Chrysene	106.96 ± 4.52	1,4-Chrysenequinone	135.38 ± 1.79

To test whether PAHs and PAHs-quinone are able to induce NF-κB in A549 cells, cells were

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transfected with NF-κB luciferase reporter gene. And then I treated higher activity compounds PA, PQ, ANT and AQ 5uM for 1hr. Results showed that only AQ could induce marked increases of NF-κB transcriptional activity and increased in NF-κB activation and degradated I-κB by dose responsible. Also PAHs-quinone may play an important role in the cellular defense mechanism as well as endocrine disruption.

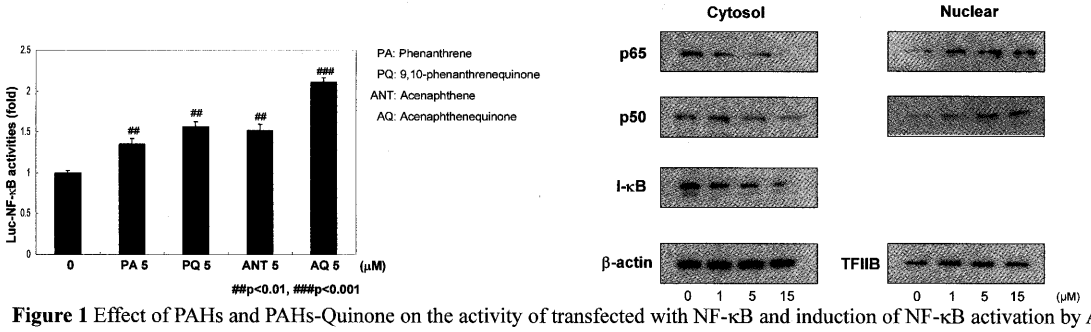

Figure 1 Effect of PAHs and PAHs-Quinone on the activity of transfected with NF-κB and induction of NF-κB activation by AQ

To confirm whether ROS is involved in activation of NF-κB or not, the scavenger of ROS, N-acetylcysteine (NAC) was used and then treated AQ. NF-κB luciferase activity increased about 2-fold compared to that of a transfected cell exposure to AQ. In contrast, NAC suppressed increasing NF-κB activity on NAC pre-incubated cells. These results demonstrated that antioxidant and glutathione precursor, NAC inhibits NF-κB luciferase activity by AQ.

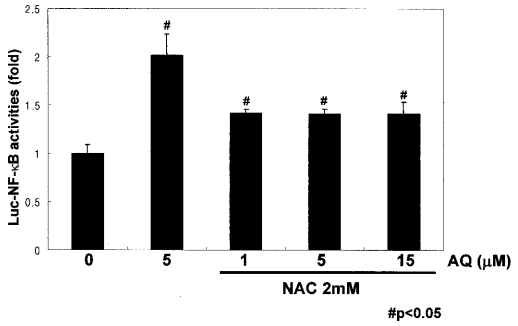

Figure 2 Role of ROS generation on cells treated AQ

To examine the possibility that phosphorylation of ERK and p38 MAPK is also involved in AQ-induced NF-κB activation. We measured protein levels using anti-active ERK antibody and phosphorylation JNK and p38 were examined. These results indicated that exposing AQ resulted in increases of p-JNK and p38. These effects showed that AQ led to a strongly induction of phosphorylation of MAPK family, p-JNK and p38, suggesting that MAPK family are related in the induction of NF-κB activation on exposing AQ. We also tested whether p38 MAPK inhibitor could block AQ-induced NF-κB activation or not. As shown this graph, p38 MAPK inhibitor blocks activation of NF-κB by AQ by NF-κB luciferase activity. These results further strengthen our postulate that the effect of AQ would be related the generation of ROS via MAPKs pathway.

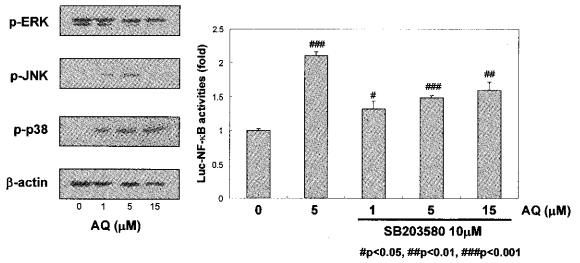

Figure 3 Induction of phosphorylation of MAPKs by AQ

From the above results, our findings suggest that NF-κB activation induced by AQ due in part to ROS generated and was mediated MAPKs phosphorylation, which AQ was higher activity than other quinoid PAHs in our study.