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| 学位授与の題目 | Studies of environmental quinoid PAHs on their potential human adverse health effects in priority oxidative stress (環境中多環芳香族炭化水素類キノン化合物による酸化ストレスを介した生体影響に関する研究) |
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ABSTRACT

Human and other living organisms are constantly exposed to a large number of potentially toxic environmental chemicals, including ubiquitous polycyclic aromatic hydrocarbons (PAHs). PAHs, represent a major class of organic compounds, are generally formed in the environments relating to production, refining, and application of coal, mineral oil, smoking, and oil shale. PAHs are highly lipid-soluble and thus readily absorbed from the gastro-intestinal tract and alveolus of mammals. They are rapidly distributed in a wide variety of tissues with a marked tendency for localization in body fat. Numerous epidemiological studies have shown that the exposure to PAHs can increase risk of cancer, respiratory diseases, arteriosclerosis, bronchitis, asthma, and other respiratory diseases as well as changes in heart rate variability. Although we are closer to achieving the goal since realizing that environmental pollutants can be altered through metabolism in living organisms and photolysis in the atmosphere, there is still a long way to go. Among their derivatives, quinoid PAHs are toxicologically important air pollutants. Quinone and their products, semiquinones and hydroquinones, are toxicological interest because of their ability to generate reactive oxygen species (ROS) and to form covalent bonds with tissue macromolecules. A given quinone may process the ability to a) redox cycle, wherein the cyclical reduction and reoxidation of the quinone result in generation of ROS and depletion of reducing equivalents, and/or b) directly arylate nucleophiles. Therefore, to assess the degree to which redox cycling and direct arylation contribute to the toxicity of quinones is an obligate step.

Once activated reaction of quinone, their reduced products activate ROS-generating pathways. ROS are important signaling molecules regulating a wide range of cellular responses. Depending on the amount and the site of generation, ROS may be involved in processes such as apoptosis/necrosis, cellular adaptation to an oxidative environment, the induction of antioxidant genes, and signal transduction. It is therefore important to know how and where quinone-driven ROS production takes place when a quinone-induced cellular response occurs. Oxidative stress is described generally as a

condition under which increased production of free radicals, reactive species (including singlet oxygen and reactive lipid peroxidation products, such as reactive aldehydes and peroxides), and oxidant-related reactions increase to cause damage. Owing to the dimension of quinone, many researches focus on elucidating the molecular reaction mechanism of quinoid PAHs in oxidative injurious property. Nevertheless, many questions still surround the causality and the disease mechanism of observed associations.

In this research, I concentrated on the adverse effects of quinoid PAHs. Especially, I examined whether quinoid PAHs, having 2 to 4 rings, all of which are normally present in the environment, have potential effects as inducers on oxidative stress, to see if they lead to several human diseases. My findings suggested a mechanism by which certain quinoid PAHs have potential oxidative activity and thus an important role in human diseases.

INTRODUCTION

Quinoid PAHs, quantified in the environment, are consequential components of air pollutants. They are present as the components of diesel exhaust particles (DEP), airborne particulate matter (PM) and wood smoke particles and have been widely documented to have the propensity for oxidative damage which produces significant amounts of ROS undergoing either enzymatic or nonenzymatic redox cycling with their semiquinone radical. ROS, generated in normal cellular biological reactions and metabolic processes, may either be beneficial role and/or inflict damage activities in the biological system. However, incessant exposure to quinone is able to increase the mortality and morbidity under accumulated the production of high reactive free radicals and oxidant-related reactions. These high reactive radicals are well known to be a cause of adverse health effects in cells such as proteins, lipids, and DNA oxidation, resulting irreversible biological damages. According to this oxidative stress, a condition where the redox balance between oxidant and antioxidant is disrupted, resulting toward a pathological deterioration. Although yet to be further established, recent epidemiological researches have represented in conjunction between the exposure to quinoid PAHs and the adverse health effects in-vivo or in-vitro tests. Despite injurious health effects of quinoid PAHs in the biological system, their toxicological interests have been poorly elucidated.

In this study, I examined whether quinoid PAHs, having 2 to 4 rings, all of which are normally present in the environment, have potential effects to destroy thiol group, and to enhance the generation of ROS, and/or nuclear factor (NF)- κ B-modulated COX-2 induction might involve environmental quinoid PAHs. These findings suggest that environmental quinoid PAHs might play a role in human adverse health effects through the increase of oxidative stress.

RESULTS AND DISCUSSION

Constantly exposed to environmental air pollutants, including PAHs and their various derivatives have threatened humans and other living organisms. The adverse

effects of environmental chemicals and compounds are often due to intracellular reactions and/or free radical metabolites. Of these compounds, kinds of oxygen containing substituents, quinoid PAHs have been extensively researched. Most quinones have the ability to generate ROS undergoing enzymatic, and/or nonenzymatic redox cycling. However, there is a paucity of data about the mechanisms by which quinoid PAHs induces human adverse health effects. To clarify several adverse effects of quinoid PAHs, the interplay of ROS and quinones was investigated in the present study. The focus of this study is the role of quinoid PAHs in the cytotoxicity and inflammatory processing. From this study, at least four fruitful distinct parts were suggested as follows:

- 1) *Para*-quinoid PAHs, including 1,4-NQ, -PQ, -AQ, and -CQ can easily generate ROS in NADPH-cytochrome p450 reductase system than *ortho*-quinoid PAHs and as a results, trigger cell death, apoptosis in cellular system.

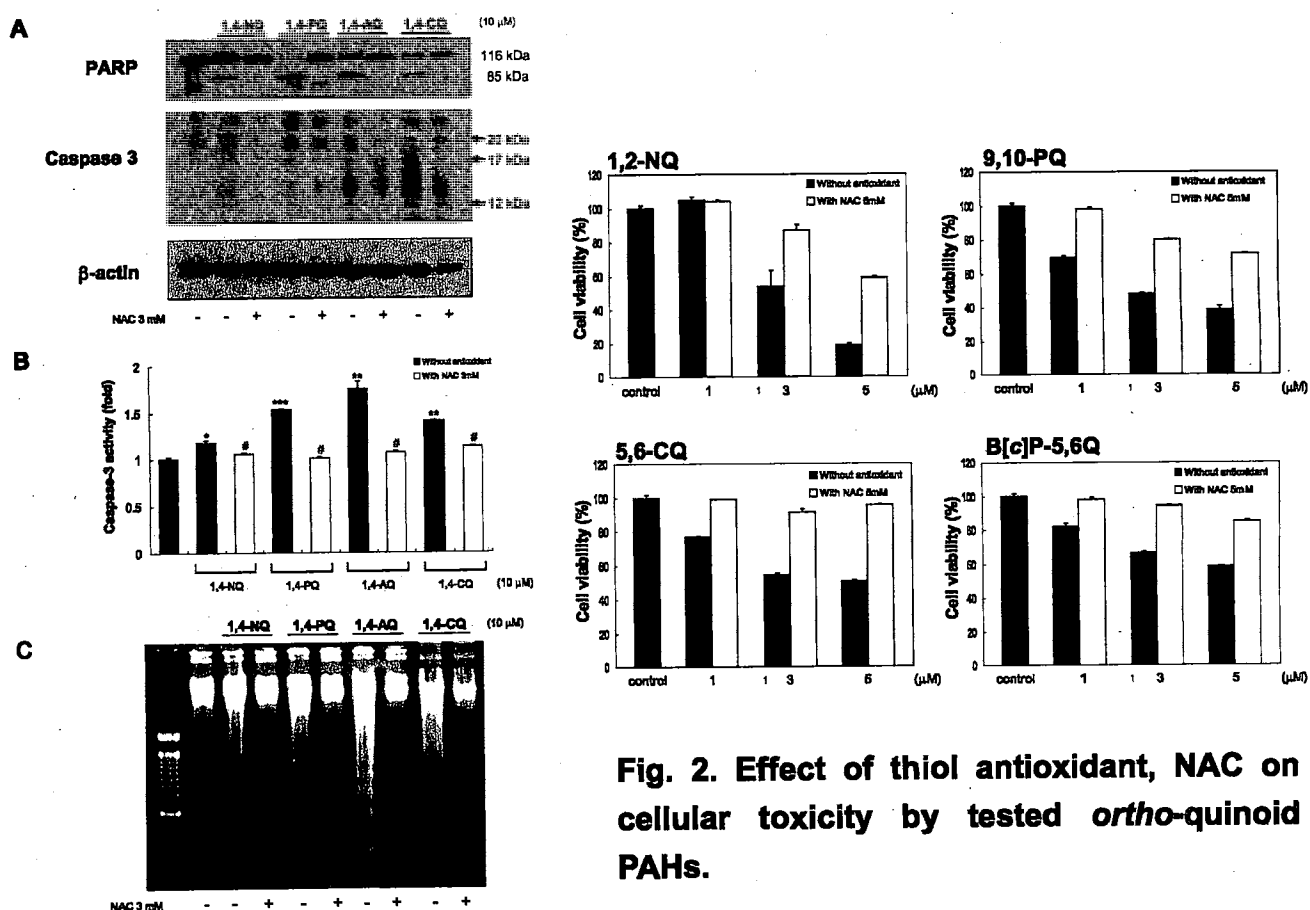


Fig. 1. Effect of NAC on *para*-quinoid PAHs-induced apoptosis

- 2) Particularly *ortho*-type, might serve an important oxidant function, such as a loss of thiol contents, resulting in the modification of protein and the effect for contribution of cytotoxicity.
- 3) From the result of intracellular ROS, acenaphthequinone (AcQ) contribute to NF-(B-modulated COX-2 expression through the ROS generation other than quinoid PAHs tested.

4) From the result of the response of an A549 cells to AcQ could contribute to the inflammatory process in human pulmonary lung diseases as the NF-(B activation and LTB4 formation by 5-LO-derived ROS generation.

These experimental evidences suggest that the role of quinoid PAHs in mediating the adverse health effects is correspond with the highly redox active properties, leading to induction of oxidatively damages. Significance of the current finding not only confirms the role of quinoid PAHs in cytotoxicity, but also reveals the activation mechanism of NF-(B and COX-2 by ROS generation in the exposure of quinoid PAHs for the first time.

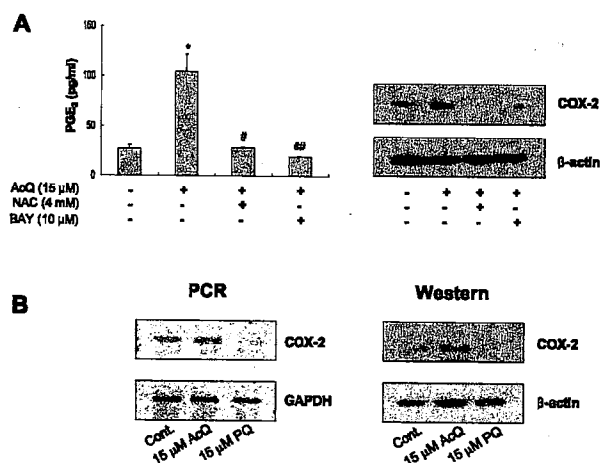


Fig. 3. Induction of COX-2 protein by AcQ was abrogated by NAC preincubation.

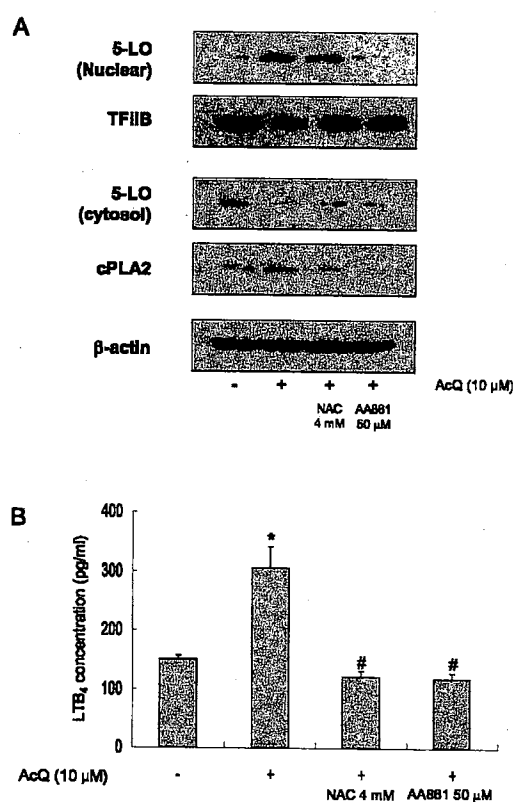


Fig. 4. NAC and AA861 inhibits AcQ-induced 5-LO constitutive expression.

学位論文審査結果の要旨

[審査経過] 審査方針従い、基礎学力を確認し、各委員による面接と諮問を行った。1月30日に口頭発表(最終試験)を行い、終了後に開催した最終審査委員会において協議の結果、次のように判定した。

[審査結果] 最近、都市大気汚染の原因の一つである自動車排ガス粉塵と循環器系疾患との関連が疑われている。本研究は、その発症機序の一つとして、自動車排ガス粉塵に含まれる多環芳香族炭化水素 (PAH) 類のキノン化合物が、生体内で活性酸素を生成して酸化ストレスを引き起こすためではないかとの仮設に立って研究を行った。その結果、PAH 類キノン化合物が活性酸素を生成してヒト細胞をアポトーシスに至らしめる、PAH 類キノン化合物がタンパク質のチオール基を酸化してその結果細胞毒性を生じる、PAH 類キノン化合物が活性酸素を生成することにより転写因子である NF- κ B を活性化させて炎症反応を誘発する、ことを明らかにした。これらの研究結果は、これまでに知られている PAH 類の発ガン性や内分泌かく乱作用以外の新しい毒性を明らかにした成果に留まらず、大気汚染と心筋梗塞などの循環器系疾患との関連を解明できる糸口を提供した意義も大きい。よって、審査委員会は博士(薬学)に値すると判定した。