行政院國家科學委員會專題研究計畫成果報告

計畫題目: **氧自由基參與黃麴毒素造成動物細胞基因毒性機制之探討**

Research Title: The involvement of oxygen radicals in the aflatoxin-induced genotoxicity in animal macrophages

計畫編號: NSC 86-2313-B-002-036
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This is a short report of the research project funded by National Science Council (NSC). The execution of this research project has been extended for one year after the approval of the funding by National Science Council. This delay was due to several problems concerning the required materials and equipments that have to be purchased from foreign country. Especially for aflatoxin originally ordered from Sigma Co., a special license must be issued by the U.S. government before the shipment, which caused a serious delay for the progress of the research.

Nevertheless, in our laboratory, we have established several ways to improve the techniques for the analysis of DNA strand breaks induced by the oxidant by using the single cell gel electrophoresis (SCGE) assay. First of all, we established the original assay technique in our laboratory by using hydrogen peroxide as the oxidant and applied it to the fresh collected rat peritoneal macrophages. A significant increase in the DNA strand breaks was observed comparing the control group. The pattern of dose-response was also noted. We later tried the different ways to prepare the slides for the electrophoresis. We found different layers of both regular and low-melting-temperature (LMT) agarose that sandwiched or embedded the oxidant-challenged-cells, could also as effective as those way described previously. Different type of glass slides form various suppliers showed various extent of attachment for both regular agarose and LMT agarose gel to these slide. Fully frosted slide apparent provides better attachment for agarose gel than that by the smooth-surfaced slide when the slide were immersed in the lysis solution. These slides, however, somewhat hindered the visibility of the DNA comet under UV microscope when stained with ethedium bromide. These problems might be solved by increasing the concentration of the agarose or the number of the gel layers on the smooth-surfaced slides. The modification of the SCGE assay, which took less time and efforts to perform the

SCGE assay than others, makes the assay more valuable for the detection of DNA strand breaks.

For the project, we have also trained graduate students to become familiar with the electronic microscopy which can be used in the future study of the morphological changes of apoptosis that might be induced by the treatment of aflatoxin. It is a time consuming effort to employ the electronic microscopy and dark room technology in our research. Since that the chemical has arrived recently, this part of experiment could be started in the very near future. We expect to find the morphological changes in the mitochondrion that might be correlated with apoptosis, which will be further confirmed by other assays such TUNEL assay, apoptotic laddering formation, and etc.

Our evaluation for the project, beside the delay, we are confident that our original design were proper and the reasonable and publishable experimental results can be expected in the future. In addition, this funding also provide the invaluable opportunity for the training of both graduate and undergraduate students to obtain the basic laboratory skills, and knowledge as well, in the field of molecular biology.

Upon the completion of the study, we will re-submit a complete report in full length according to the “report preparation guidance” specified by NSC.

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