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Chapter- 13

Evaluation of Biochemical Changes of Benzo (A) Pyrene Induced Lung Carcinogenesis in Vivo and its Prevention by Vesicular Drug Targeting

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Keywords: Benzo(a)pyrene, ROS, Biochemical changes, lung carcinogenesis, prevention by liposomal Curcumin

Abstract:

Lung cancer is the leading cause of cancer mortality world-wide. Cigarette smoking is the most established risk factor for lung carcinogenesis; however, the effects of benzo (a) pyrene [B(a)P], one of the key carcinogens in smoke, on the progression of lung cancer are obscure. The identification of key regulatory and molecular mechanisms involved in lung carcinogenesis is, therefore, critical to understanding this disease and could ultimately lead to targeted therapies to improve prevention and treatment. In an earlier study, I observed the effect of curcumin on the changes in the activities of endogenous antioxidants and lipohydroperoxide in rat lung injury by the administration of B(a)P. In the present study, I am interested in investigating whether B(a)P produces ROS, which activates inflammatory mediators and Wnt/β-catenin signaling to produce a lot of transcriptional genes and biochemical changes involved in lung carcinogenesis and its mechanistic prevention by the targeting of liposomal curcumin in rat.

Introduction:

The incidence of lung cancer is strongly correlated with cigarette smoking, with about 90% of lung cancers arising as a result of tobacco use (Biesalski et al., 1998; Peto et al., 2006; Maiti & Samanta, 2018; Dey & Guha, 2020). It is the single best-documented risk factor for all lung cancer types (Wynder et al., 1994). Each cigarette contains a mixture of carcinogens, including the tobacco-specific nitrosamine NNK and polycyclic aromatic hydrocarbons (PAHs) such as benzo(a)pyrene (B(a)P), among others, along with tumor promoters and co-carcinogens (Hoffmann et al.,1997; Hoffmann et al., 1990; Boga & Bisgin, 2022). The complex of PAHs and the tobacco-specific nitrosamine NNK in cigarette smoke is the mixture that is most likely to be involved in the induction of human lung cancer (Saha & Yadav, 2023; Mehta et al., 2023). Passive smoking, or the inhalation of tobacco smoke from other smokers sharing living or working quarters, is also an established development of lung cancer.

In my previous study, ROS were generated in BEAS-2B cells exposed by metals and induced and activated inflammatory mediators, Wnt/β-catenin signal transduction pathway, the key molecules responsible for carcinogenesis. Whether the same role of B(a) P-induced key

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factors may happen in lung carcinogenesis and inhibition of it on the basis of the mechanism of drug action will be examined.

Oxidative stress refers to the phenomenon of the production of ROS, namely superoxide (O_2), hydroxyl (OH) and peroxyl (ROO) radicals and hydrogen peroxide (H_2O_2), which exert a threat to lung cells. Lung cells, when exposed to ROS, trigger their self-antioxidant protection mechanism to counter the oxidative attack. But in the diseased state, ROS rises above the tolerance level and lung cells are not able to counter the ROS and thus succumb to irreversible lung cell damage. Antioxidant compounds were tested to protect the lung against those reactive oxygen intermediates. But, simple antioxidant therapy is not an effective approach to counter oxidative damage and combat lung cancer, as the concentrations of antioxidants to interact with lung cells become diluted. Hence, it is essential to develop a delivery system for vectoring antioxidants to lung cells. Liposome mediated drug delivery to lung cells is potentially significant among other delivery devices not only for its nontoxic nature, biodegradability, and non-immunogenetics but also for vectoring the encapsulated drug to a particular target organ. Furthermore, owing to the presence of mannosyl-fucosyl receptors on the surface of lung cells, mannose coated liposomes are effective in site-specific drug delivery to lung tissues.

Curcumin, a polyphenol compound, the principal curcuminoid of the Indian curry Spice turmeric, is known for its antitumor, antioxidant, anti-proliferative, anti-mutagenic, antiarthritic, anti-amyloid and anti-inflammatory properties (Mukherjee Nee Chakraborty et al., 2007). It is widely used as a dietary spice and coloring in cooking and as a herb in traditional Indian medicine.

The aim of this study is to optimize an antioxidant and anti-carcinogenic curcumin in mannosylated liposome formulation and to evaluate its mechanism of action in combating lung carcinogenesis by using an in vivo rat model of B(a)P-induced lung cell injury.

Methodology:

A. Animal experiment:

Adult male Swiss Albino Rats, each weighing approximately 100-120gm, were acclimatized to conditions in the laboratory (26-28˚C, 60-80% relative humidity, 12h light/dark cycle) for 7 days prior to the commencement of the treatment during which they were receiving food (purchased from Hindustan Lever Limited, Maharashtra, India) and drinking water. Sixty rats were divided into ten groups of six animals. For the 1st set of five groups, normal group was kept by injecting olive oil twice in a week for consecutive six weeks and normal food for 18 weeks. One experimental group was injected B(a)P (i.p. 50mg/kg b wt in 0.5ml olive oil) twice in a week for consecutive six weeks. Out of remaining three experimental groups, one group was injected (i.v.) with free Curcumin (0.5ml suspension of 0.2% tween 80 aqueous solution containing 0.33mg Curcumin) twice in a week for 16 weeks after four weeks of B(a)P treatment. Other two groups were provided (i.v.) liposomal and mannosylated liposomal Curcumin (each 0.5ml suspension containing 0.33mg liposomal and mannosylated liposomal

Curcumin respectively) twice in a week for 16 weeks after four weeks of B(a)P exposure. The 2nd set of five groups was repeated for another study. At the end of 18th week, rats in normal, P(a)P-control and other experimental groups were sacrificed and their lung tissues were removed and washed with cold physiological saline and either used for experiments or kept at -70˚C. The animal experiment has been approved by Institutional Animal Ethical committee.

B. Estimation of endogenous antioxidants level in normal, B(a)P and Curcumin treated rat lungs

For the determination of endogenous anti-oxidants defense such as GSH, SOD, GPx, GR, GST, catalase, lungs of normal and experimental animals were homogenized separately in 50mM chilled phosphate buffer pH 7.4 containing 1mM EDTA.

C. Estimation of GSH level

Glutathione level in tissue homogenate was determined by using tetrachloroacetic acid with EDTA as protein precipitating agent. The mixture was allowed to stand for 5 minutes prior to centrifugation for 10 minutes at 200g. The mixture was then transferred to a new set of test tubes and 0.3M phosphate buffer and Ellmen reagent (5, 5^1 dithiobis- 2 nitrobenzoic acid in 1% Na-citrate) were added. After completion of the total reaction, solutions were read at 412nm. Absorbance values were compared with a standard curve generated from known GSH concentration to evaluate liver homogenate GSH levels.

D. Estimation of SOD activity

The assay of superoxide dismutase for liver homogenate was performed with 10mM ferricytochrome c.

E. Estimation of GPx activity

To measure the GPx activity, homogenate containing the enzyme source was mixed with 0.25M potassium phosphate buffer, 25mM EDTA, glutathione reductase, 40mM glutathione(GSH), 20mM NADPH. The mixture was mixed and then incubated for 2 minutes at 37^0C. The reaction was initiated by adding t-butyl hydroperoxide at the final concentration of 0.3mM. The mixture was stirred and the absorbance was read immediately at 340nm at 1-minute intervals for 4 minutes. The absorbance change during the 2 to 4-minute interval was used to calculate enzyme activity. The activity was determined and expressed as µmol NADPH oxidized/min/mg protein.

F. Estimation of GR activity

GR was assayed. A 3ml reaction mixture contained 100mM phosphate buffer (pH 7.0), 1mM GSSG, 1mM EDTA, 0.1mM NADPH, and 25 to 50 µl enzyme extract. The reaction was started by adding the enzyme extract. The rate of NADPH oxidation was followed by monitoring the decrease in absorbance at 340nm with a recording spectrophotometer. The activity was expressed as µmol of NADPH oxidation/min/mg protein.

G. Estimation of GST activity

GST activity was determined in a total volume of 1.0ml, containing 100mM potassium phosphate buffer (pH 6.5) and 2mM each of GSH and 1-chloro-2-4-dinitrobenzene (final concentration). The rate of formation of S-2-4-dinitropgenylglutathione (a GSH-1-chloro-2,4-dinitrobenzene conjugate) by enzyme extract was quantified at 340nm using the extinction coefficient of 9.6L $mmol^{-1}$ cm^{-1} (Maiti and Chatterjee, 2000) and the activity was expressed as n mol/min/mg protein.

H. Estimation of catalase activity

The rat lung homogenate was used for catalase activities. The reaction mixture contained sodium phosphate buffer (0.05M, pH 7.0), 50mmol/L^{-1} H_2O_2 and 50µl of enzyme extract in a 3ml volume. The activity was assayed by monitoring the decrease in absorbance at 240nm as a consequence of H_2O_2 consumption and enzyme activity expressed as amount of H_2O_2 decomposed per minute per mg. of protein.

I. Lipid peroxidation assay

Lipid peroxidation in the lung homogenate was determined by measuring the amount of lipohydroperoxides. The lung cell membrane was extracted twice in a chloroform-methanol mixture (2:1, v/v). The pooled extract was evaporated to dryness under a nitrogen atmosphere at 25˚C and redissolved in cyclohexane. Lipids in cyclohexane solvent were assayed at 234nm and the results were expressed as µmol of lipohydroperoxide/mg protein by using an €m of 2.53 $x10^4$ L mol^{-1} cm^{-1}.

Results:

GSH level in the lungs decreased by the exposure of B(a)P, where that level increased by the treatment of curcumin (Table-1). SOD activity in the lungs reduced by the exposure of B(a)P, but this activity is found to increase by the treatment of curcumin (Table-1). GPx activity decreased by the exposure of B(a)P, but it increased by the treatment of curcumin (Table-1). GR activity of lung homogenate was reduced by the exposure of B(a)P, whereas this activity increased by the injection of curcumin to rats (Table-1). GST activity of rat lung homogenate decreased by the treatment of B(a)P but it increased by the treatment of curcumin (Table-1). Catalase activity of rat lung decreased with the treatment of B(a)P, whereas this activity increased with the treatment of curcumin (Table-1).

Table 1. Effect of curcumin on the changes in GSH, SOD, GPx, GR, GST and catalase activities in rat lung by the induction of B(a)P.

100xGSSG/GSH		SOD (Units/mg protein)	GPx µmole NADPH oxidation/min/mg protein	GR µmole of NADPH oxidation/min/mg protein	GST nmole produced/ mg protein	Catalase µmole H_2O_2 reduced/min/mg protein
Normal	1.50±0.15	4.23±0.34	40.46±4.24	3.02±0.38	230±16.5	241±15.3
B(a)P treated	0.95±0.09*	2.52±0.19*	20.21±2.04*	1.51±0.13*	132±10.4*	111±9.2*
Curcumin treated	1.12±0.11 #	3.82±0.21 #	32.53±6.28 #	2.23±0.21 #	196±14.4 #	201±12.4 #

Each value was expressed as mean ± S.D. for 5 rats in each group. Statistical significance: *p<0.05, where B(a)P treated group compared with normal and #p<0.05, where curcumin-treated group compared with B(a)P treated group.

In my study, the lipohydroperoxides increased by the treatment of B(a)P where it decreased by the treatment of curcumin (Table-2).

Table 2. Effect of curcumin on lipid peroxidation in the lung of normal and experimental rats.

	Lipohydroperoxides (µmol/mg protein)
Normal	0.7±0.06
B(a)P treated (A)	1.3±0.11*
(A)+Curcumin treated	1.0±0.09#
Values are mean ± SD for 5 rats. *p<0.05 significantly different from normal. #p<0.05 significantly different from B(a)P treated.	

Discussion & Conclusion:

Lung cancer, the most common cause of cancer-related death in men and women, is responsible for 1.3 million deaths world-wide annually (WHO February 2006 Cancer), representing 18% of global cancer deaths (Field et al., 2006) where about 90% of lung cancer mortality is due to cigarette smoking (Chyou et al., 1992). There is a great variation in the prevalence of lung cancer in different geographical areas. Nearly 70% of all the new cases of lung cancer in the world occur in developed countries (Parkin et al., 1988). B(a)P is a significant pro-carcinogenic substance, which requires metabolic activation to electrophilic reactive metabolites for its carcinogenic activity (Gelboin, 1980). It is well established that B(a)P, after sequential metabolic activation principally by cytochrome P450, generates 7,8- diol – 9, 10- epoxide-benzo(a)pyrene, which is believed to be the ultimate carcinogenic metabolite of B(a)P (Su et al., 2006) that leads to the formation of DNA adducts and initiates mutations responsible for tumor development. The toxic manifestations of the carcinogenic intermediates of B(a)P are being considered caused primarily due to the imbalance between pro-oxidant and anti-oxidant homeostasis and also due to its ability to bind to sulphydryl groups of proteins and cellular non-protein thiols such as glutathione (GSH) and to inhibit energy production which may be exerted through excess production of ROS. This study will report on one of the mechanisms of lung carcinogenesis exposed by B(a)P in rat model. It will also focus on the mechanistic regulation of lung carcinogenesis by the exposure of curcumin. Mannosylated liposomal Curcumin may be a therapeutic tool because of its efficacy in combating lung carcinogenesis. Although progress has been made in reducing incidence and mortality rates and improving survival, lung cancer still accounts for more deaths. Moreover, progress can be accelerated by applying existing cancer control knowledge across all segments of the population and by applying new discoveries in cancer prevention, early detection and treatment (Ahmedin et al., 2009; Madhu et al., 2022; 2023).

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