Cigarette smoke condensate reduces the detoxifying capabilities of rat lens

Sir,

Epidemiological studies indicate that cigarette smoking increases the risk of developing cataract while cessation of smoking reduces the risk.[1] It is postulated that the complex mixture of trace metals, polycyclic aromatic hydrocarbons and nitro compounds in cigarette smoke act as pro-oxidants that exert oxidative damage to lens and possibly initiate cataractogenesis.[2] Thus, this may be a crucial mechanism of cataractogenesis in smokers. However, very few studies are available on the effects of cigarette smoking on the endogenous antioxidant mechanisms. Therefore, we conducted a preliminary study to record the effect of cigarette smoke on the detoxifying mechanisms of organ-cultured lens and validate a quick in vitro screening model for potential anti-cataract agents.

Adult Wistar rats (150-200 g) of either sex, were used in accordance with institutional ethical guidelines. They were sacrificed using anaesthetic ether and lens dissected (weight range of 0.02-0.04 g) for the present study. Cigarette smoke condensate (CSC) was prepared according to the method of Shalini et al.[2] A leak proof system was improvised that trapped by bubbling through distilled water (24 ml) containing dimethyl sulfoxide (DMSO, 50 µl). This was filtered through glasswool and centrifuged at 9000 rpm for 5 min to give CSC. The smoke content from six cigarettes (Wills, ITC Ltd) was trapped by bubbling through distilled water (24 ml) containing dimethyl sulfoxide (DMSO, 50 µl). This was filtered through glasswool and centrifuged at 9000 rpm for 5 min to give CSC. The smoke content was standardized by ensuring that the optical density (OD) was 0.6 at an absorption maximum of 270 nm (Beckman Spectrophotometer). A 1:1 dilution of CSC was standardized by ensuring that the optical density (OD) was 0.6 at an absorption maximum of 270 nm (Beckman Spectrophotometer). A 1:1 dilution of CSC was standardized by ensuring that the optical density (OD) was 0.6 at an absorption maximum of 270 nm (Beckman Spectrophotometer).

The lens was homogenized in phosphate buffered saline (PBS) to prepare a 10% homogenate. Glutathione-S-transferase (GST) activity in the homogenate was estimated according to the method of Habig, et al.[3] The kinetic profile was read at 340 nm for every 30 s up to 180 s. To elucidate the effect of various concentrations of CSC on GST activity in homogenate, increasing volume of CSC (10, 20, 40 and 80 µl) were added to normal. The enzyme catalyzes the conjugation of 1-chloro-2, 4-dinitrobenzene (CDNB) with GSH to form a complex and the product is measured spectrophotometrically. The result is expressed as nmol of CDNB conjugated/min/mg protein. The protein levels of rat lens were estimated by the standard Lowry’s method.[4]

Two tailed unpaired t-test was applied to compare the level of significance between the means of treated and control group for corresponding time points. In GST estimation each concentration of CSC was compared to normal levels and P<0.05 was considered statistically significant.

There was a significant fall in normal GSH level in rat lens (5.98 ± 0.41 µmol) as the duration of CSC exposure was increased from 0.5 to 2 h (0.49 ± 0.10 µmol) (Figure 1). In comparison to normal activity of GST in rat lens, there was a significant fall in its activity with 20, 40 and 80 µl of CSC (Figure 2).

Epidemiological studies, in vivo studies along with in vitro studies, provide a correlation between cigarette smoking and cataractogenesis.[5] As the lens is an immunologically isolated

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Figure 1: GSH levels in normal and CSC exposed rat lens. Data represented as mean±SEM. *P<0.05, ***P<0.0001 significantly different from control group; n=6

Figure 2: GST activity in normal and CSC exposed rat lens. Each point is the representation of mean±SEM. *P<0.05, **P<0.01 significantly different from the normal value; n=6
organ, the cataractogenic action of smoke due to direct topical incidence is less likely. The possible route could be systemic absorption of the inhaled smoke. We chose to study the activity of GST in presence of CSC, since it is actively involved in detoxification of toxic agents by catalyzing their conjugation to GSH and thereby eliminating them from circulation. Significant amount of GST is known to be present in ocular tissue where it plays a pivotal role in protecting lens clarity. Studies indicate that deletion of GST gene was significantly higher in cataract patients than in controls and that cataract patients lacking the GST gene were significantly younger than cataract patients possessing the GST gene. On the other hand, GSH is also implicated in imparting non-enzymic antioxidant defense to the lens. It is required to maintain protein sulfhydryls in the reduced form. It prevents their oxidation by reacting with potential oxidants and electrophilic compounds. Thus depletion of GSH and GST has been linked to various toxic effects to different organs, including cataracts by X-ray and naphthalene.

In this study it was noted that within half-an-hour there was a rapid depletion of GSH in lens when exposed to CSC. A maximum fall is observed when the lenses are exposed for 2 h. Concomitantly there was a fall in GST activity also. This suggests that chronic exposure to CSC depletes the endogenous defense system. The present in vitro study provides evidence that CSC exhibits oxidative stress and negatively affects the detoxifying mechanisms of the lens.

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**References**


