Adverse effects of cigarette smoke extract in human cells

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Health effects of tobacco smoking

- Tobacco is the single greatest cause of preventable death globally.
- Tobacco smoking was a definite cause cancer and cardiovascular and pulmonary diseases.
- Low levels of exposure, including exposures to second-hand tobacco smoke, lead to a rapid and sharp increase in endothelial dysfunction and inflammation.
- There are about 5 million smokers (20%) in Taiwan.
Cigarette smoke is a complex and dynamic mixture of more than 7,000 individual chemical constituents. >100 are toxic and at least 69 cause cancer.

Tobacco smoke is a known human carcinogen. The most damaging compounds in tobacco smoke include: Tar, Nicotine, 1,3-butadiene, Volatile chemical (Acrolein, Formaldehyde, Nitrosamines, Benzene), Heavy metal (Arsenic, Cadmium), Toxic gas (Ammonia, Carbon monoxide, Nitrogen oxide), Hydrogen cyanide.
How cigarette smoking causes diseases?

1. Oxidative stress [reactive oxygen (ROS) & reactive nitrogen species (RNS)]
2. DNA damage [DNA single-strand break, 8-oxo-2'-deoxyguanosine (8-oxodG), DNA-protein cross-links]
3. Inflammation
4. Cell death (apoptosis, necrosis, and necroapoptosis)
Whole cigarette smoke extract (100%) was prepared by bubbling smoke from 3 Marlboro Red cigarettes (5 cm/each) into 15 ml of PBS at a rate of 1 cm/min.

Su et al., 1998
CSE induced actin cytoskeleton reorganization in HUVEC

Exp Design:
1. Cells were exposed or not to various concentrations of CSE for 6 hrs.
2. After treatment, cells were fixed and stained for actin using rhodamine-labeled phalloidin.

Magnification for images at x400.

Chen et al., 2009
Cytochalasin D (CytD) partially inhibited the surface expression of ICAM-1 and E-selectin

Exp. Design:
1. HUVEC were pretreated with or without CytD (50 nM or 100 nM) for 30 min and were then washed with PBS.
2. Washed cells were incubated with 5% CSE for another 6 hr.
3. After treatment, cells were collected, and the surface expression of ICAM-1 & E-selectin was determined by flow cytometry.

① Control; ② 5% CSE for 6 hr; ③ 50 nM CytD + 5% CSE 6 hr; ④ 100 nM CytD + 5% CSE for 6 hrs.
Dose-dependent effect of CSE on cell morphology and actin cytoskeleton organization in EA.h926 cells
Time-dependent effect of CSE on cell morphology and actin cytoskeleton organization in EA.h926 cells

Cont  CSE, 1-h  CSE, 2-h  CSE, 4-h

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**Hours of 10% CSE treatment**

- 0
- 1
- 2
- 3
- 4

**Relative fraction of well-spread cells**

- 0.0
- 0.2
- 0.4
- 0.6
- 0.8
- 1.0
- 1.2

**Relative level of F-actin assembly**

- 0.0
- 0.2
- 0.4
- 0.6
- 0.8
- 1.0
- 1.2

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Actin cytoskeleton reorganization may be linked to CSE-induced pre-inflammation gene expression

- Cytoskeletal reorganization in skeletal muscle differentiation: from cell morphology to gene expression (Formigli et al., 2007).
- Actin has been shown to be an important regulator in RNA polymerase II transcription (Visa & Percipalle, 2010; Louvet E & Percipalle, 2009).
- The cytoplasmic dynamics of the actin cytoskeleton have been shown to regulate the subcellular localization of some transcription factors, such as MRTF-A and MRTF-B (Zheng et al., 2009).
Effects of thiol-containing antioxidants on CSE induced cell shrinkage in EA.hy926 cells

(A) Relative fraction of well-spread cells

(B) Relative fraction of well-spread cells

(C) Relative fraction of well-spread cells
Effects of non-thiol-containing antioxidants on CSE induced cell shrinkage in EA.hy926 cells
Effects of Ca$^{2+}$ chelators & Ca$^{2+}$ channel blocker on CSE induced cell shrinkage in EA.hy926 cells
CSE increases the free intracellular calcium level using Fluo-3/AM

- Control (con)
- 2.5% CSE
- 5% CSE
- 10% CSE

Fluo-3 intensity per cell

CSE (%)

- 0
- 2.5
- 5
- 10

Fluo-3 intensity per cell

- 0
- 100,000
- 200,000
- 300,000
- 400,000
- 500,000
- 600,000
- 700,000

* Significant difference compared to control
Cell-permeable Ca$^{2+}$ chelator blocks CSE-induced intracellular Ca$^{2+}$ increase in Ca$^{2+}$-free medium
A non-permeable Ca$^{2+}$ chelator blocks CSE-induced intracellular Ca$^{2+}$ increase in normal medium.
Transient receptor potential canonical (TRPC) channels inhibitor blocks CSE-induced intracellular Ca$^{2+}$ increase in normal medium.
Inflammation-/oxidative-related genes up-regulation
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