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**Toxicological Screening of
Short-Term Exposure of Sidestream
Cigarette Smoke on Angiogenesis**

Sohail Ejaz, Irina Chekarova, Chae Woong Lim*

Biosafety Research Institute, Department of Pathology, College of Veterinary Medicine, Chonbuk National University, Jeonju, Republic of Korea

Angiogenesis is a fundamental process that ensures adequate metabolic supply to tissues in numerous biological and pathological states, including tumorigenesis. Cigarette smoking causes numerous adverse effects, some of which are associated with disruption of normal process of angiogenesis. It is believed that sidestream cigarette smoke severely affects the normal process of angiogenesis by effecting different components that help in normal process of angiogenesis. Sidestream cigarette smoke is composed primarily of smoke that emanates from the burning end of cigarette, smoke that the smoker exhales, and contaminants that diffuse through the cigarette paper. The aim of the present study was to ascertain the toxicological effects of different sidestream cigarette smoke solution (SSCSS) on angiogenesis by using chicken chorioallantoic membrane (CAM) assay. Decrease in total vascular area, diameter of secondary and tertiary blood vessels, cell proliferation, migration of blood vessels towards ectoderm and number of capillary plexuses formation was observed by application of SSCSS.

Scanning microscopy also revealed deviation in pattern formed by the major capillary plexuses and the fibrillar elements of the mesoderm in treated CAMs. It is concluded that SSCSS inhibit processes that may hinder normal process of angiogenesis resulting in abnormal blood supply to tissues, decreased repair and remodeling, which are common problems among smoke-exposed individuals. Further study is required to delineate the effects of different chemicals in SSCSS on angiogenesis.

Key words: Angiogenesis, SSCSS, CAM

Corresponding author : Chae-Woong Lim
(063-270-3788, E-mail lcw@chonbuk.ac.kr)

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**Impaired Wound Healing by
Exposure of Different Mainstream
Whole Smoke Solutions of
Commercial Cigarettes**

Sohail Ejaz, Irina Chekarova and Chae Woong Lim*

**Biosafety Research Institute, Department of Pathology, College of Veterinary Medicine, Chonbuk National University, Jeonju, Republic of Korea*

Cigarette smoke has been shown to potentiate wound damage and delayed ulcer healing. The chicken dorsum excisional wound assay was used to elucidate the deleterious effects of different mainstream whole smoke solutions (MSWSS) on the fundamental

processes of wound healing. Gross, histopathology, SEM and computer based 3D image probing techniques were utilized to quantify different toxic effects of MSWSS on wound healing. A total of 160 chicks, aged 1 week, divided in eight groups were exposed to MSWSS with different nicotine concentration; 0.2 mg (group A), 0.3 mg (group B), 0.5 mg (group C), 0.6 mg (group D), 0.7 mg (group E) and 1mg (group F). A very highly significant reduction ($P < 0.001$) in wound closure was observed among all MSWSS treated groups at day 8 post-wounding. Histological investigations revealed a significant impede outcome in the re-epithelialization of all MSWSS exposed wounds. Delayed dermal matrix regeneration and maturation of collagen bundles were observed among all MSWSS treated wounds. Similar results were achieved through SEM of treated wounds. Histological and image probing analysis unveiled the scanty neovascularization among MSWSS treated wounds. Abbot curve, angular spectrum and different other parameters of 3D surface topographies of wounds revealed a very highly significant reduction ($P < 0.001$) in angiogenesis among all MSWSS treated groups. These annotations validate the damaging effects of MSWSS on the healing of wounds.

Keywords Mainstream smoke, Cigarette, Chicken, Wound, Angiogenesis

Corresponding author : Chae-Woong Lim
(063-270-3788, E-mail : lcw@chonbuk.ac.kr)

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Functionally Active *Helicobacter Pylori* Vacuolating Cytotoxin in *Escherichia Coli*

Mi-Ran Ki⁽¹⁾, Sun-Hee Do⁽¹⁾, Il-Hwa Hong⁽¹⁾, Da-Hee Jeong⁽¹⁾, Hai-Jie Yang⁽¹⁾, Dong-Wei Yuan⁽¹⁾, Hye-Lim Lee⁽²⁾, Dong-Hwan Kim⁽¹⁾, and Kyu-Shik Jeong^{(1)*}

⁽¹⁾Department of Veterinary Pathology, College of Veterinary Medicine, Kyungpook National University, 702-701, Daegu, Republic of Korea and ⁽²⁾Department of Biotechnology and Bioindustry, College of Natural Sciences, Catholic University, 712-702, Daegu, Republic Korea

The vacuolating cytotoxin (VacA) which is a major pathogenic factor of *Helicobacter pylori* induces cellular vacuolation and apoptosis. Although the expression of this toxin in *Escherichia coli* has been attempted, the production of a functionally active recombinant VacA has been rare. In this study, we attempted to produce the active recombinant VacA with methionine substituted for alanine at N-terminal and 8X histidine tag conjugated at C-terminal in *E. coli* by inducing soluble expression at low temperature. We produced the 90-kDa VacA which was able to induce vacuolation and apoptosis in AGS cells and HeLa cells. In addition, anti-sera raised in a rabbit by intradermal injections of this recombinant proteins reacted in a immunoblot with a 88-kDa protein in supernatants from ATCC 49503 and ATCC 43504, *vacA* positive-strains with the *vacA* genotype s1/m1. Immunoglobulins purified by using protein G agarose neutralized the cytotoxic activity