110% specified in the European Commission Directive 96/23/EC [1]. The RSD% for intra-day precision of the identified flavonols ranged from 0.59 to 1.43%, and the RSD% for inter-day precision ranged from 0.64 to 1.86%. The validation parameters of the methodology met the requirements of the normative documents and confirm the suitability of the methodology for application.

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EFFECTS OF NICOTINE ON THE SENESCENCE

Seniuk I.V., El-Asri Abdeladim, Harrouch Hamza
National University of Pharmacy, Kharkiv, Ukraine

Introduction. Senescence is defined as an irreversible state of cell cycle arrest categorized as telomere-dependent or intrinsic/replicative senescence and telomere-independent or extrinsic/stress-induced senescence. Telomeres cap the ends of chromosomes in post-mitotic cells, preventing chromosome fusion. Telomere shortening depends on the species and they shorten by 50 to 200 base pairs (bp) with each cell division in diploid cells. For example, in human fibroblasts, telomeres are shortened by 50 bp [1], while in human lymphocytes, in culture, the rate is 120 bp per cell division. Hayflick and others observed a limit, which later become known as the Hayflick limit, for the number of cell divisions that post-mitotic cells can undergo (around 50–70 divisions) before becoming quiescent. This limit defines the basis of replicative senescence because it involves the shortening of telomeres over time. This type of senescence is central to the 'telomere attrition' theory of aging, which states that the shortening of telomeres over time inevitably leads to decline and death [2].

Materials and methods. The scientific literature on the experimental study of the effects of nicotine on human and animal senescence was used.

Results and discussion. Aging and smoking are two major risk factors in cardiovascular disease (CVD) development. Although recent reports have shown that smoking stimulates senescence in the lung, it is unknown whether smoking also accelerates senescence of the cardiovascular (CV) system. ECs isolated from atherosclerotic lesions in patients going through artery bypass graft surgery showed increased markers of inflammation, oxidative stress, and accelerated senescence,

compared with non-smokers. Interestingly, smokers going through this procedure were an average of 10 years younger than non-smokers, suggestive of premature atherosclerosis induced by cigarette smoke. The observation that senescence markers are upregulated by cigarette smoke in human plaques indicates that senescence could be relevant to vascular dysfunction induced by tobacco.

In VSMCs, senescence is induced by Ang II, which is the key effector of the rennin angiotensin system (RAS). Circulating Ang II induces hypertension, while the local and intracellular production of Ang II results in inflammation, cell proliferation, fibrosis, and atherosclerosis. Ang II exerts its pathological effects by binding to AT1R, leading to Nox1 upregulation; ROS production; and phosphorylation of the kinases p38MAPK, Akt, and ERK. Ang II also induces inflammation by activating the transcriptional factor NF-κB. Once activated, NF-κB translocates to the nucleus, increasing the expression of proinflammatory cytokines, including IL-6, IL-1β, and TNF-α; chemokines, such as monocyte chemoattractant protein-1 (MCP-1); and cell adhesion molecules, such as vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1). An increased production of these pro-inflammatory molecules contributes to vascular injury and atherosclerosis. Furthermore, the binding of Ang II to AT1R induces vascular senescence *in vivo* and *in vitro* in VSMCs [3].

Our previous work demonstrated that Ang II and zinc overload induce the senescence of VSMCs by an ERK-dependent mechanism and that Nox1 mediates these effects. Additionally, Nox4, an inducible enzyme that produces hydrogen peroxide, shows the opposite effects on senescence. In VSMCs, Nox4 downregulation induces senescence, while in ECs, reduced Nox4 expression inhibits senescence. Nox2 downregulation was also associated with reduced EC senescence. A more comprehensive review of the role of NADPH oxidases in vascular senescence can be found in our recent review.

Senescence is an ROS-dependent mechanism, so the upregulation of ROS generation systems like Nox1, or downregulation of antioxidant enzymes, such as catalase, or transcription regulators involved in ROS metabolism, such as FOXO1, the sirtuin Sirt1, or PGC-1α, which is a master regulator of mitochondria biogenesis, promotes senescence. Our group also shed light on the mechanism by which PGC-1α modulates senescence by reporting that a reduced expression of sequestosome in PGC-1α VSMCs mediates senescence by increasing ROS and reducing autophagy [4].

Nox1 increases ROS levels in response to tobacco smoke in VSMCs and increases the expression of MMP-9, which is also upregulated by nicotine. An increased expression of MMP-9 is associated with a poor prognosis in lung cancer and breast cancer, and with increased inflammation associated with senescence. Therefore, Ang II and nicotine share common signaling pathway cascades by activating p38MAPK and ERK signaling and inflammation, and by increasing the expression of Nox1 and MMPs. However, whether Nox1 also mediates cigarette smoke and nicotine effects in senescence remains to be elucidated [5].

Cigarette smoke is a known exacerbator of age-related pathologies, such as CVD, atherosclerosis, and cellular aging (senescence). However, the role of nicotine and its major metabolite cotinine is yet to be elucidated. Considering the growing

amount of nicotine-containing aerosol use in recent years, the role of nicotine is a relevant public health concern. A number of recent studies and health education sites have focused on nicotine aerosol-induced adverse lung function, and neglected CV impairments and diseases. A critical review of the present scientific literature leads to the hypothesis that nicotine mediates the effects of cigarette smoke in the CV system by increasing MAPK signaling, inflammation, and oxidative stress through NADPH oxidase 1 (Nox1), to induce vascular smooth muscle cell (VSMC) senescence. The accumulation of senescent VSMCs in the lesion cap is detrimental as it increases the pathogenesis of atherosclerosis by promoting an unstable plaque phenotype.

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THE EFFECTS OF MEALS AND AGE ON NICOTINE METABOLISM

Seniuk I.V., El Mehdi Tolbi, Benzid Yassine
National University of Pharmacy, Kharkiv, Ukraine

Introduction. Nicotine underlies tobacco addiction, influences tobacco use patterns, and is used as a pharmacological aid to smoking cessation. The absorption, distribution and disposition characteristics of nicotine from tobacco and medicinal products are reviewed. Nicotine is metabolized primarily by the liver enzymes CYP2A6, UDP-glucuronosyltransfease (UGT), and flavin-containing monooxygenase (FMO). In addition to genetic factors, nicotine metabolism is influenced by diet and meals, age, sex, use of estrogen-containing hormone preparations, pregnancy and kidney disease, other medications, and smoking itself. Substantial racial/ethnic differences are observed in nicotine metabolism, which are likely influenced by both genetic and environmental factors.

Materials and methods. Analysis of scientific literature and results of clinical diagnostic parameters of medical organizations.

Results and discussion. An implication of the high degree of hepatic extraction is that clearance of nicotine should be dependent on liver blood flow. Thus, physiological events, such as meals, posture, exercise, or drugs perturbing hepatic blood flow, are predicted to affect the rate of nicotine metabolism. Meals consumed during a steady state