Original Paper

Effect of Nicotine on the Glutathione, Glutathione-s-transferase and Glucose-6-phosphate Dehydrogenase Activities in the Skeletal Muscle Fibres of Albino Rat

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ABSTRACT

Nicotine, a major constituent of tobacco, plays a critical role in smoking addiction. Nicotine has been reported to induce oxidative stress by producing Reactive Oxygen Species (ROS).

The present study was undertaken to examine the effects of nicotine on the antioxidant defense systems in the skeletal muscle fibre types (Type I, Type II) of albino rats. The animals were divided into three groups of six animals each, i.e., control, experimental-1 and 2. The animals received subcutaneous injection of nicotine in physiological saline at a dose of 0.5 mg/kg (Exptl. 1), and 1 mg/kg (Exptl. 2), 5 days a week, for 8 weeks. The animals were sacrificed at 20 hrs after the last treatment, and the following muscle fibres were isolated and analyzed - soleus (Type I), red vastus, and white vastus (Type II).

Nicotine was found to significantly deplete the rat muscle fibres GSH (glutathione) content, and the activity of GST (glutathione-s-transferase) and G-6-PD (glucose-6-phosphate dehydrogenase). This indicates that nicotine induces oxidative injury in the muscle fibres of rat.

Key Words: Albino rat; Nicotine; Glutathione; Glutathione-s-transferase; Glucose-6-phosphate dehydrogenase; Oxidative stress

Introduction

Nicotine, a major toxic component of cigarette smoke, is generally regarded to be a primary risk factor in the development of cardiovascular disorders, pulmonary disease and lung cancer.[1] Many researchers have determined that nicotine contributes to ROS (reactive oxygen species) production.[2] The formation of the ROS in cells leads to the formation of free radicals in metabolic processes. These harmful species cause damage to many molecules such as lipids, proteins and nucleic acids. The harmful effects are countered by the antioxidant defence system in cells. The most important free radical chain-breaking molecule in the antioxidant defence system in various tissues of the body is glutathione.[3,4] Furthermore, enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione reductase (GR) and glucose-6-phosphate dehydrogenase (G-6PD) are necessary to remove these radicals and keep the cells stable.

Under normal conditions, the reductive and oxidative capacity of the cell (redox state) is in favour of the oxidation.[5] However, the generated ROS in oxidative stress are removed by the antioxidant defence system. A number of drugs and chemicals increase the ROS production in specific organs of the body. Cigarette smoking is common in many societies. Two thirds of American adults are addicted to alcohol and 30% of them are addicted to both cigarettes and alcohol.[6,7] Nicotine, the major toxic component of cigarette smoke,[8,9] is a risk factor for various cardiovascular diseases and cancer. Kessler et al (1996) have determined a marked increase in nicotine content in all kinds of cigarettes in the last decade in the United States.[10] Shaw et al (2000) reported that one cigarette decreases lifespan by 11 minutes.[11] Half life

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of nicotine and its metabolite continine are 1.3-2.7 and 15-19 hours respectively.[12]

Histochemical myosine ATPase fibre typing is used to classify muscle fibres as Type I and Type II, which are known to correspond to slow and fast muscle fibres, respectively.[13] The enzymes that are analyzed reflect metabolic pathways that are either aerobic/oxidative or anaerobic/glycolytic.[14] This classification leads to 3 fibre types: Slow-twich oxidative (SO) soleus (Type I); fast-twitch oxidative glyoglytic (FOG) red vastus (Type II A); and Fast-twich glycolytic (FG) white vastus (Type II B).[13-15]

This study was carried out to determine the toxicity of nicotine in muscle fibres. We report on Type I and Type II A fibres which are rich in mitochondria and myoglobin giving them a red colour, with continuous activity and highly resistant to fatigue, hence low level damage was observed, but red vastus was more damaged than the Type I soleus and control. Type II B fibres are large cells, with limited capillary supply, slow delivery of oxygen and removal of waste products, and have few mitochondria, little myoglobin, resulting in a high level of damage than red vastus, soleus over control.

Materials and Methods

Animals: Male pathogen-free Wistar albino rats were obtained from the Department of Zoology, Animal House, S.V. University, Tirupati, Andhra Pradesh, India. The animals were housed six to a polypropylene cage, and provided with food and water *libitum*. The animals were maintained under standard conditions of temperature and humidity with an alternating 12 hr light/dark. Animals were fed standard pellet diet (Agro Corporation Pvt. Ltd., Bangalore, India) and maintained in accordance with the guidelines of the National Institute of Nutrition and Indian Council of Medical Research, Hyderabad, India.

Chemicals: Nicotine and other fine chemicals were obtained from Sigma Chemical Company, St. Louis, USA. All other chemicals and reagents used were of analytical grade.

Experiment Schedule: The animals were randomized into experimental 1, 2 and control groups of six animals each. Rats in experimental 1 and experimental 2 received subcutaneous injection of nicotine in physiological saline at a dose of 0.5 mg/kg body weight (experimental 1), 1 mg/kg body weight (experimental 2) (5 days a week for 8 weeks). The dilution was done in such a way that 1 ml of physiological saline contained the required dose of nicotine.

The experiment was terminated at the end of 8 weeks, and all animals were sacrificed by cervical dislocation after an overnight fast. Type I (soleus), Type II A & II B (red vastus, white vastus) muscle were removed, cleared of blood, and immediately transferred to ice-cold containers, containing 0.9% sodium chloride for various estimations.

Biochemical Investigations: For the analysis of enzymes such as GSH, GST and G-6-PD in the presence of nicotine toxicity, the following methods were used. Glutathione (GSH) content was assayed by the method of Theodorus et al (1981).[16] The glutathione content was expressed in nanomoles/gram wet weight of the tissue. Glutathione-s-transferase activity was measured with its conventional substrate 1-chloro-2,4-dinitrobenzene (CDNB) at 340 nm as per the method of Habig et al (1974), and activity was expressed as moles of thioether formed/mg protein/min.[17] Glucose-6-phosphate dehydrogenase activity was assayed by the method of Lohr and Waller (1965) as modified by Mastanaiah et al (1978).[18,19] The activity was expressed as moles of formazan formed/mg protein/hr.

Statistical Analysis: Statistical analysis was carried out using INSTAT software. The data was analyzed for the significance, and the results were presented with the P-value.

Results and Discussion

Knowledge of the toxicity of nicotine is important to understand tobacco-induced human diseases, as well as to assess the potential risks associated with the therapeutic use of nicotine as an aid to assist smoking cessation. Many drugs and chemicals have been shown to induce toxic side effects, and adverse or beneficial effects on multiple enzymes and metabolic processes. For example, a diuretic drug, acetazolamide inhibits carbonic anhydrase. Inhibition of specific enzymes due to drug use may exert pathological states in experimental models, for example, chronic nicotine administration inhibits cytochrome P450 (CYP2Abin liver, CYP1A1 in lungs) as well as generates free radicals, and exerts oxidative tissue injury.[20,21] The generation of oxygen free radicals can be prevented or scavenged by host antioxidant defense mechanism.[2]

Glutathione (GSH) is a major non-enzymatic antioxidant, and the most abundant non-protein thiol source in the cell. It has several important functions related to free radical metabolism.[22,23] Husain et al (2001) reported that chronic administration of nicotine significantly depletes GSH content in liver and testes.[24] In our study, a significant decrease in the GSH levels was demonstrated in all muscle types of albino rat, and more decrease was observed in white vastus tissue (**Table 1**). The depletion of GSH seems to be associated with an increase of lipid peroxidation and this ratio was reported by earlier workers also.[25] Nicotine is oxidized to its main metabolite continine in liver and causes the formation of free radicals in tissues. The formation of these radicals causes oxidative damage. The decrease in GSH in tissues leads to oxidative tissue damage.[24]

Glutathione-s-transferases are drug metabolizing and detoxifying enzymes involved in the intracellular transport and metabolism of steroid hormones. GSTs are multifunctional proteins present in many organs and show tissue specific expression. They have the capacity to detoxify electrophilic xenobiotics including cytotoxic drugs and carcinogens by catalyzing their conjugation with reduced glutathione (GSH).[26] Suleyman et al (2002) reported that nicotine also inhibits brain GST activity.[27] Our results also indicate that there is a significant decrease in nicotine treated experimental 1 and 2 rats, and more decrease in white vastus.

G-6-PD plays an important role in metabolism, and has a vital function in various tissues. The enzyme catalyzes the first step of the pentose phosphate metabolic pathway, which is the unique source of NADPH synthesis in various cells.[28-30] Metabolic diseases such as diabetes mellitus affect some enzyme activities, and it has been shown that some chemicals and drugs also inhibit G-6-PDH activity.[31] For example, incubation of the major product of lipid peroxidtion 4-hydroxy 2-nonenal with G-6-PD inhibits enzyme activity rapidly.[32] In addition, epiandrosterone inhibits erythrocyte G-6-PD uncompetitively, and suppresses hexose monophosphate shunt activity by more than 95%.[33] In this study, nicotine inhibited the G-6-PD activity in the skeletal muscle fibre tissues *in vivo* when compared with the control groups.

In the present study, the lower activity of GSH, GST and G-6-PD in all skeletal muscle fibres, and lower levels of antioxidant enzymes due to nicotine exposure may have caused the accumulation of free radicals generated by nicotine toxicity. This decrease in enzyme activity, most probably reflects the increased oxidative stress through nicotine toxicity by producing free radicals. Oxidative damage was mainly observed in the white vastus muscle fibres due to low mitochondrial concentration. It is suggested that prolonged exposure to nicotine produces significant oxidative tissue injuries in animals.

Table 1 Changes in glutathione (GSH) content in the muscle fibres of control and nicotine-treated experimental rats

S.No.	Name of the Tissue	Control	Experimental 1	Experimental 2
1	SOL	38.11 +/- 1.63	35.18* +/- 1.05 (- 7.67)	33.36* +/- 2.05 (- 12.46)
2	RV	34.37 +/- 2.16	31.46* +/- 2.17 (- 8.45)	32.57** +/- 1.92 (- 5.22)
3.	WV	32.86 +/- 1.18	27.28* +/- 2.12 (- 16.97)	24.23* +/- 3.15 (- 26.24)

The glutathione content is expressed in nanomoles/gram wet weight of the tissue.
Values in parentheses denote percent change over control/All values are mean +/-SD of 6 individual observations.
*All the values are significant at P<0.001; **All the values are significant at P<0.01

Table 2 Changes in glutathione-s-transferase (GST) activity in the muscle fibres of control and nicotine-treated experimental rats

S.No.	Name of the tissue	Control	Experimental 1	Experimental 2
1	SOL	0.09 +/- 0.03	0.07* +/- 0.01 (- 21.50)	0.05* +/- 0.01 (- 38.70)
2	RV	0.056 +/- 0.01	0.04** +/- 0.02 (- 23.21)	0.03* +/- 0.01 (- 33.92)
3	WV	0.04 +/- 0.011	0.02* +/- 0.01 (- 39.53)	0.01* +/- 0.001 (- 72.09)

Values are expressed in moles of thioether formed/mg protein/min.
Values in parentheses denote percent change over control/All values are mean +/-SD of 6 individual observations.
*All the values are significant at P<0.001; **All the values are significant at P<0.01

Table 3 Changes in glucose-6-phosphate dehydrogenase (G6PD) activity in the muscle fibres of control and nicotine-treated experimental rats

S.No.	Name of the tissue	Control	Experimental 1	Experimental 2
1	SOL	1.78 +/- 0.95	1.58* +/- 0.22 (- 11.29)	1.38* +/- 0.02 (- 22.67)
2	RV	1.52 +/- 0.11	1.44** +/- 0.16 (- 5.19)	1.34* +/- 0.31 (- 11.83)
3	WV	1.33 +/- 0.16	1.15** +/- 0.14 (- 13.56)	1.08** +/- 0.20 (- 18.51)

Values are expressed in moles of thioether formed/mg protein/min.
Values in parentheses denote percent change over control/All values are mean +/-SD of 6 individual observations.
*All the values are significant at P<0.001; **All the values are significant at P<0.01

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