Original Paper

Changes in the Oxidative Metabolism Due to Nicotine Toxicity in the Skeletal Muscle Fibres of Male Albino Rat

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ABSTRACT

Nicotine has been reported to induce oxidative stress both in vivo and in vitro. The present study was undertaken to examine the effect of nicotine on oxidative metabolism in skeletal muscle fibre types (Type I & Type II) of male albino rats. The animals were divided into two groups: Group-I (control), and Group-II (experimental). The latter received subcutaneous injections at a dose of 0.5 mg/kg body weight (Experiment-I), 1 mg/kg body weight (Experiment-II), 5 days/week for a period of 8 weeks. The animals were sacrificed after 24 hours of the last treatment, and skeletal muscle fibres such as soleus (SOL), red vastus (RV) and white vastus (WV) were isolated and analyzed.

The activity of lactate dehydrogenase (LDH) increased in nicotine-treated rats of both experiment-I, and experiment-II. Succinate dehydrogenase (SDH), isocitrate dehydrogenase (ICDH), malate dehydrogenase (MDH) and glucose-6-phosphate dehydrogenase (G-6-PDH) were decreased in soleus (SOL), red vastus (RV) and white vastus (WV) skeletal muscle fibres. These findings indicate nicotine-induced oxidative stress in the skeletal muscle fibres of male albino rats.

Key Words: Nicotine; Lactate dehydrogenase; Isocitrate dehydrogenase; Succinate dehydrogenase; Malate dehydrogenase; Glucose-6-phosphate dehydrogenase; Skeletal muscle; Albino rat

Introduction

Nicotine is a major toxic component of cigarette smoke.[1] A report by Kessler et al (1996) revealed a tremendous increase in the levels of nicotine observed in all brands of cigarettes sold in the United States during the past 10 years.[2] Shaw et al (2000) reported that one cigarette reduces one's life by 11 minutes.[3] Nicotine is oxidized into its metabolite cotinine, which has a long half-life[4] and may play a significant role in vascular diseases.[5] Chronic administration of nicotine to rats reportedly induces cytochrome P-450 IIE1,[6] as well as generates free radicals in tissues of rats and exerts oxidative tissue injury.[7,8] Nicotine is metabolized by various pathways, of which cotinine is the primary product of the C-oxidation pathway of nicotine biotransformation.[9,10] In rat liver, nicotine is metabolized by CYP1A2, CYP2B1, CYP2C11 and other CYP forms; CYP2B1 also being constitutively expressed in rat lung.[11]

The rapid elimination of nicotine has been attributed to its metabolism, as well as distribution to some tissues.[12] The predominant effects of nicotine in the whole intact animal or human consist of an increase in blood pressure, increase in plasma free fatty acids, and lung injury.[13-15] Nicotine has been extensively studied in separately designed *in vivo* and *in vitro* experimental systems using either nicotine or smokeless tobacco extract.[16-18] Some of the biological and physiological end points of tobacco consumption have been attributed to its major alkaloid, nicotine.[19,20] Cigarette smoking causes harmful cardiovascular and atherogenic effects resulting from changes in lipid metabolism.[21] There are numerous harmful substances found in tobacco smoke, and nicotine is one of the most toxic components.[22] Smoking has been

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identified as the second leading risk factor for death from any cause worldwide.[23,24]

Nicotine has been known to cause oxidative stress by inducing the generation of reactive oxygen species (ROS) in the tissues. These ROS in turn are capable of initiating and promoting oxidative damage in the form of lipid peroxidation (LPO). LPO is known to cause cellular injury by inactivation of membrane enzymes and receptors, depolymerisation of polysaccharides, as well as protein cross-linking and fragmentation. Nicotine is chemotactic for polymorphonuclear (PMN) leucocytes, and enhances the responsiveness of PMN leucocytes to activated complement C5a, thus generating ROS. It disrupts the mitochondrial respiratory chain leading to the increased generation of superoxide anions and hydrogen peroxide.[25] Nicotine has however been reported to have a positive effect in patients with some neurodegenerative diseases (such as Alzheimer´s and Parkinson´s diseases) and has been suggested for the treatment of these CNS disorders.[26]

For the present study, histochemical myosine ATPase fibre typing was used to classify muscle fibres as Type-I and Type-II, which are known to correspond to slow and fast muscle fibres, respectively.[27] The enzymes that were analyzed reflect metabolic pathways that are either aerobic/oxidative or anaerobic/glycolytic.[28] This classification leads to 3 fibre types: slow-twich oxidative (SO) soleus (Type-I); fast-twitch oxidative glycolytic (FOG) red vastus (Type-II A), and fast-twich glycolytic (FG) white vastus (Type-II B).[27,28] In the present study, nicotine-induced changes were studied in these skeletal muscle fibres of male albino rats.

Materials and Methods

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

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.

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

Biochemical Investigations: 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) muscles were removed, cleared of blood, and immediately transferred to ice-cold containers containing 0.9% sodium chloride for various estimations. Lactate dehydrogenase activity was determined by the method of Nachlas et al (1960)[30] as suggested by Prameelamma & Swami (1975),[31] isocitrate dehydrogenase by Korenberg & Pricer (1951)[32] as modified by Mastanaiah et al (1978),[33] succinate dehydrogenase and malate dehydrogenase by Nachlas et al (1960)[30] as suggested by Prameelamma & Swami (1975),[31] and glucose-6-phosphate dehydrogenase activity by Lohr & Waller (1965),[34] as modified by Mastanaiah et al (1978).[33]

Statistical Analysis: Statistical analysis was carried out using INSTAT software. The data were analyzed for significance: the results were presented with the P-value experimental-I (all the values are significant at $P<0.01$), and experimental-II (all the values are significant at $P<0.001$).

Results and Discussion

Nicotine affects a variety of cellular processes ranging from induction of gene expression to secretion of hormones and modulation of enzymatic activity. Lactate dehydrogenase is a key enzyme of anaerobic glycolysis and catalyses the reversible oxidation of lactate to pyruvate in the terminal step of glycolysis. Any alteration in LDH activity indicates change in the production of pyruvate to lactate under anaerobic conditions favouring the reoxidation of NADH. Reduction in LDH activity towards the formation of pyruvate implies that the NAD-dependent LDH activity is not favouring the formation of pyruvate and consequent low feeding of lactate into gluconeogenic pathway or into Krebs citric acid cycle for further oxidation.[35]

In the present study, LDH activity was increased as compared to the control in both experimental–I and II (**Table 1**). Increased LDH activity in the media demonstrated that cellular membrane integrity was disturbed in nicotine treated cells.[36] The high activity of LDH demonstrated that cellular membrane integrity was disturbed, as shown by Watanabe et al (1995)[37] and Yildiz et al (1998, 1999)[36,38] following nicotine administration. It has been suggested that chronic nicotine treatment affects cardiac function (Hu et al 2002)[39] by modulating the expression of genes involved in energy metabolism and signal transudation. Turegano et al (2001)[40] demonstrated that chronic as well as acute administration of nicotine produced strong increases in different dehydrogenase activity. Moreover, Mall et al (1985)[41] concluded that smoking may aggravate the course of acute ischaemic heart disease with high CPK values.

Isocitrate dehydrogenase (ICDH) is an important enzyme in Krebs cycle which catalyses the reversible oxidation of isocitrate to oxalosuccinate, followed by decarboxylation leading to the formation of alpha-ketoglutarate. The rate of dehydrogenation observed in the absence of divalent ions is lower than the rate of the overall reaction. Approximately 75-90% of NADP-specific enzyme is located in non-particulate fraction of the cytoplasm and the remainder in the mitochondria.[42] The intra- and extra- mitochondrial enzymes differ from each other in electrophoretic mobility and in immunological properties.[43] In the present study, ICDH activity was decreased as compared to the control in both experimental–I and II (**Table 2**). Decreased NADP-ICDH activity as a consequence of nicotine toxicity results in the reduced production of NADPH which plays a crucial role in the detoxification processes. The effect of nicotine on ICDH

Table 1 Changes in LDH Activity in Skeletal Muscle of Control, Nicotine-treated Experimental-I, and Experimental-II Groups of Male Albino Rats

S.No	Name of the Tissue	Control	Experimental-I	Experimental-II
1	SOL	1.683 ± 0.055	2.125 ** ± 0.077 (+26.26)	3.201* ± 0.038 (+90.19)
2	RV	0.876 ± 0.037	1.261** ± 0.031 (+43.94)	2.496* ± 0.132 (+18.49)
3	WV	0.775 ± 0.038	0.958** ± 0.025 (+23.61)	1.670* ±0.216 (+11.54)

Values are expressed in micromoles of formozan formed/mg protein/hour. Values in parentheses denote percent change over control. *All the values are significant at P<0.001. **All the values are significant at P<0.01

Table 2 Changes in ICDH Activity in Skeletal Muscle of Control, Nicotine-treated Experimental-I, and Experimental-II Groups of Male Albino Rats

S.No.	Name of the Tissue	Control	Experimental-I	Experimental-II
1	SOL	1.870 ± 0.045	1.186** ± 0.048 (-36.57)	0.938* ± 0.019 (-49.83)
2	RV	1.540 ± 0.048	1.158** ± 0.028 (-24.80)	0.848* ± 0.038 (-44.93)
3	WV	1.245 ± 0.026	0.948** ± 0.031 (-23.85)	0.544* ± 0.028 (-56.30)

Values are expressed in micromoles of formozan formed/mg protein/hour. Values in parentheses denote percent change over control. *All the values are significant at P<0.001. **All the values are significant at P<0.01

in skeletal muscle fibres has not been reported so far, so this report may be the first in that regard.

Succinate dehydrogenase (SDH) is a key enzyme of Krebs cycle which catalyses the reversible oxidation of succinate to fumarate. It is tightly bound to inner mitochondrial membrane, and serves as a link between electron transport system and oxidative phosphorylation. The oxidation of succinate and malate and their interplay in metabolic processes have been widely established in biological oxidations.[44,45] Decreased SDH activity was observed in all skeletal muscle fibres (**Table 3**) of rats treated with nicotine, indicating depressed oxidative metabolism in mitochondria. Since the activity of SDH is reduced, it is evident that this might affect the conversion of malate to oxaloacetate by MDH because of low succinate oxidation.

Malate dehydrogenase (MDH) catalyses the oxidation of L-malate to oxaloacetic acid using NAD as cofactor and the reaction is reversible. Although the reaction is endergonic, it goes in the forward direction very rapidly in the cell, because of the rapid removal of reaction products, oxaloacetate and NADH in subsequent steps. The MDH reaction is strictly stereospecific for the L-stereoisomer of malate. The cells of higher animals contain two forms of MDH: one is in the mitochondria and the other in the extra-mitochondrial cytoplasm.[46] The enzymes from these two sources differ in their electrophoretic mobility, molecular weight, amino acid composition and kinetic properties indicating a differential protein nature of the enzyme. As a member of the Krebs cycle, it participates in terminal oxidation and interlinks the electron transport system and oxidative phosphorylation system.[45]

A decrease in specific activity of MDH was observed in both experimental–I and II, in all the muscle fibres of rats treated with nicotine, suggesting decreased utilization of malate (**Table 4**). The decreased MDH activity could be attributed to low availability of substrate, lesser conversion of succinate-fumarate-malate, and the changes in the structural integrity of mitochondria.

Glucose-6-phosphate dehydrogenase (G-6-PDH) is an important enzyme in HMP shunt pathway, which occurs in the cytosol of the cell and is an alternative pathway for glucose oxidation. This pathway provides a major portion of the cell's NADPH which functions as biochemical reductant. G-6-PDH is known to occur in two distinct forms: one located in cytosol which is specific to NADP (Meizer et al 1977),[47] and the other located in the microsomes utilizes either NADP or NAD.[48,49] G-6-PDH plays a critical role in cell growth by providing NADPH for redox regulation.[50] A major role of NADPH in erythrocytes is regeneration of reduced glutathione, which prevents haemoglobin denaturation, preserves the integrity of red blood cell membrane sulphydril groups, and detoxifies hydrogen peroxide and oxygen radicals in and on the red blood cells.[51]

The G-6-PDH activity was decreased in both the experimental–I and II, in all the skeletal muscle fibres (**Table 5**). Gumustekin et al (2005)[52] reported that administration of nicotine inhibited the G-6-PDH in rat tissues, including muscle.

Table 3 Changes in SDH Activity in Skeletal Muscle of Control, Nicotine-treated Experimental-I, and Experimental-II Groups of Male Albino Rats

S.No	Name of the Tissue	Control	Experimental-I	Experimental-II
1	SOL	1.945 ± 0.031	1.770** ± 0.029 (-18.97)	1.565* ± 0.038 (-19.53)
2	RV	1.480 ± 0.052	1.153** ± 0.031 (-22.09)	0.794* ± 0.038 (-46.35)
3	WV	1.197 ± 0.027	0.952** ± 0.031 (-20.46)	0.453* ± 0.064 (-62.15)

Values are expressed in micromoles of formozan formed/mg protein/hour. Values in parentheses denote percent change over control. *All the values are significant at P<0.001. **All the values are significant at P<0.01

Table 4 Changes in MDH Activity in Skeletal Muscle of Control, Nicotine-treated Experimental-I, and Experimental-II Groups of Male Albino Rats

S.No.	Name of the Tissue	Control	Experimental-I	Experimental-II
1	SOL	0.918 ± 0.044	0.871** ± 0.033 (-51.19)	0.823* ± 0.046 (-10.34)
2	RV	1.840 ± 0.025	1.598** ± 0.050 (-13.15)	1.126* ± 0.027 (-38.80)
3	WV	1.410 ± 0.047	1.185** ±0.039 (-15.95)	0.928* ± 0.034 (-34.18)

Values are expressed in micromoles of formozan formed/mg protein/hour. Values in parentheses denote percent change over control. *All the values are significant at P<0.001. **All the values are significant at P<0.01

Table 5 Changes in G-6-PDH Activity in Skeletal Muscle of Control, Nicotine-treated Experimental-I, and Experimental-II Groups of Male Albino Rats

S.No	Name of the Tissue	Control	Experimental-I	Experimental-II
1	SOL	2.660 ± 0.031	0.668** ± 0.050 (-74.88)	0.341* ± 0.078 (-87.18)
2	RV	0.721 ±0.034	0.40** ± 0.031 (-44.52)	0.251* ± 0.039 (-65.18)
3	WV	0.540 ± 0.031	0.286** ± 0.041 (-47.03)	0.165* ± 0.032 (-69.44)

Values are expressed in micromoles of formozan formed/mg protein/hour. Values in parentheses denote percent change over control. *All the values are significant at P<0.001. **All the values are significant at P<0.01

Conclusion

The results from this study reveal higher activity of LDH, and lower activity of ICDH, SDH, MDH, G-6-PDH in all the muscle fibres. This type of increased/decreased activity due to nicotine toxicity in skeletal muscle fibres produces oxidative damage, mainly in the white vastus, due to low mitochondrial concentration. Therefore, this work indicates that oxidative damage produces nicotine toxicity in the skeletal muscle fibres of albino rats.

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