African Journal of Cellular Pathology 3:25-29 (2014)
The Official Journal of the Society for Cellular Pathology Scientists of Nigeria
www.ajcpath.com

EFFECT OF CIGARETTE SMOKE ON SOME ENZYMES OF THE DESCENDING AORTA OF ADULT WISTAR RATS (RATTUS NORVEGICUS)

Popoola, NA[1*], Enaibe, BU[2], Adekomi, DA[3], Raheem, SA[4], Olajide, OJ[2], Mustapha, T[4]

1. Department of Bioscience and Biotechnology, Kwara State University
2. Department of Anatomy, University of Ilorin
3. Department of Anatomy, Ekiti State University
4. Department of Anatomy, University of Lagos

Corresponding Author: Popoola NA
Email:abdulgafarp1@gmail.com, abdulgafar.popoola@kwasu.edu.ng

ABSTRACT

Aim: The effect of cigarette smoke on the aorta of Wistar rats was investigated for possible enzymatic destruction.

Methods: Thirty six Wistar rats 200±10g were randomly grouped into 3 of 12 rats in each group. One group was exposed to cigarette smoke (Benson and Hedges containing 1.1mg nicotine) once daily at 18.00hours. The second group was exposed to smoke from cotton wool of equal weight as of the cigarette. The third group was the control and was not exposed to smoke. The animals had feeds and water ad libitum. The rats were sacrificed on 14th and 28th days, the aortae excised, homogenized in 0.2M sucrose and assayed for Glucose 6 Phosphate Dehydrogenase (G6PDH), Lactase Dehydrogenase (LDH) and Alkaline Phosphatase (ALP).

Results: A significant decrease in ALP and LDH and an insignificant increase in G6PDH over a period of 14 days were observed. However, the activities of the three enzymes were significantly decreased over a period of 28 days.

Conclusion: Exposure to cigarette smoke can affect certain enzymes of the wall of the descending aorta.

Key words: Descending aorta, G6PDH, LDH, ALP

INTRODUCTION

The purpose of this work was to investigate the effects of cigarette smoke on carbohydrate metabolism considering some of the enzymes of carbohydrate metabolism like Glucose-6-Phosphate Dehydrogenase (G-6-PDH), Lactate Dehydrogenase (LDH) and Alkaline Phosphatase (ALP). Both active and passive smoking have the potentials to harm almost every organ in the body and are associated with the leading cause of mortality, cardiovascular disease, cancer, stroke and chronic pulmonary disease (Wigand, 2006). Passive exposure to tobacco smoke has been found to significantly increase the risk of ischemic heart disease and asthma (Titus et al., 2001).Cigarette smoke is a complex mixture that causes a variety of diseases, such as lung cancer, chronic obstructive pulmonary disease (COPD), and cardiovascular disease (U.S. Department of Health and Human Services 2004; International Agency for Research on Cancer, 2004). It has been hypothesized that the chemical composition of mainstream smoke (MS) and consequently its toxicological effects depend to a great extent on the burning temperature of the tobacco (Patskan and Reininghaus, 2003). Nicotine is the most active and the most toxic component of cigarette smoke (Mycek et al., 1997). In combination with coal tar and carbon monoxde found in cigarette smoke, nicotine represents a serious risk factor for lung and cardiovascular diseases, various cancers as well as other illnesses (Mycek et al., 1997).Cigarette use by pregnant women has also been shown to cause birth defects including mental retardation and physical disabilities (WHO, 2008).

Cigarette has been shown to contain about 599 additives and 4,000 chemicals in its smoke, more than 19 of which are carcinogenic (Myceket al., 1997). The use of nicotine containing agents such as cigarette is particularly harmful in hypertensive patients. Many patients with peripheral vascular disease experience an exacerbation of symptoms with smoking. For example, nicotine induced vasoconstriction can decrease coronary blood flow, adversely affecting the patient with angina (Myceket al., 1997). The aorta is the largest artery in the body. It is an elastic artery that conducts blood away from the heart. The aorta in quadrupeds, divides into ascending and descending trunks (Young et al., 2006).

MATERIALS AND METHOD

Care of Animals

A total of 36 adult male Wistar rats of weights ranging between 190 grams and 210grams were used for the investigation. The rats were procured presumably healthy from the Animal Holdings Unit of Department of Anatomy, University of Ilorin. They were kept under standard laboratory conditions of 24 hours light at room temperature. They were fed with growers marsh obtained from Bethel Agrofeeds, Sawmill Area, Ilorin, and were given water ad-libitum throughout the period of the administration.

Grouping of Animals/Exposure to Cigarette Smoke

The 36 adult Wistar rats were randomly grouped into three (3) of A, B and C, each group consisting of 12 rats (n=12). Each of the rats in the experimental group (A) was exposed to the smoke from a stick of cigarette once daily. This was done by burning completely a stick of Benson and Hedges brand of cigarette in a moderately aerated container where the rats were laying and allowed to inhale, at 18:00 Hrs.The animals in the first control group (B) were each exposed to smoke from completely burnt cotton wool of equal weight as cigarette (weighed with an Analytical Sensitive Weighing balance, Metler, England) at the same time. The weighed cotton wool burnt for 5 minutes in a moderately aerated container where the rats were laying and also allowed to inhale. The animals in the second control group (C) were exposed to atmospheric air only. The exposure lasted 14 days and 28 days on relatively short and long term bases respectively. The rats were weighed on daily basis and were closely observed each day for any physical changes.

Animal Sacrifice

On the 14th day after the exposure, 18 rats, (6 per group) were sacrificed by cervical dislocation, while the remaining 18 rats (6 per group) were also scarified on the 28th day by the same method at the same time. The thorax was immediately opened, the descending aorta identified, excised and weighed, 0.5g part of which was then homogenized in 2.5M cold sucrose solution and kept in refrigerator for enzyme estimation.

Biochemical Technique

Tissue preparation for Enzyme Estimation

The descending aortae for enzyme estimation were homogenized using porcelain mortar fitted with a porcelain pestle. The homogenate was poured in a test-tube and centrifuged at 5000rpm for 5 minutes using a centrifuge (model 90-1). The supernatants collected using Pasteur pipettes were immediately stored in the deep freezer (GC-R207WVQ) at -20^{o}C, and thereafter assayed within 48 hours. Using appropriate biochemical kits and a spectrophotometer, Alkaline Phosphatase (ALP), Lactate Dehydrogenase (LDH) and Glucose-6-phosphate dehydrogenase (G6PDH) were estimated. Statistical analysis of ALP, G6PDH and LDH activities in the descending aorta was done using SPSS (Statistical Package for Social Sciences; version 15) computer analysis program and the results were reported as mean ± standard error mean (M±S.E.M).

RESULTS

Table 1: ALP activity in the descending aorta (IU/g tissue) at $P < 0.05$ confidence interval. (Day 14)

Group	No of Rats	Mean± S.E.M
(A) Experimental	6	0.25 ± 0.27
(B) Control 1	6	0.39 ± 0.12
(C) Control 2	6	0.40 ± 0.14

Table 1 above shows a statistically significant decrease in the activity of ALP in the experimental group when compared with the control groups; and no significant difference between the two controls. The order of decrease is from group C to A.

Table 2: G6PDH activity in the descending aorta (IU/g tissue) at P < 0.05 confidence interval. (Day 14)

Group	N	Mean± S.E.M
(A) Experimental	6	0.82 ± 0.18
(B)Control 1	6	0.21 ± 0.35
(C)Control 2	6	0.17 ± 0.30

Table 2 above shows an increase (statistically insignificant) in the activity of G6PDH in the experimental group when compared with the control groups; and no statistically significant difference between the two controls. The order of increase is from group C to A.

Table 3: LDH activity in the descending aorta (IU/g tissue) at P < 0.05 confidence interval. (Day 14)

Group	N	Mean± S.E.M
(A)Experimental	6	0.29 ± 0.54
(B)Control 1	6	0.32 ± 0.43
(C)Control 2	6	0.53 ± 0.82

Table 3 above shows a statistically significant decrease in the activity of LDH in the experimental group when compared with the control groups; and no statistically significant difference between the two controls. The order of decrease is from group C to A.

Table 4: ALP activity in the Descending aorta (IU/g tissue) at P < 0.05 confidence interval. (Day 28)

Group	N	Mean± S.E.M
(A)Experimental (CG)	6	0.16 ± 0.30
(B)Control 1 (CW)	6	0.21 ± 0.59
(C)Control 2 (AA)	6	0.23 ± 0.67

Table 4 above shows statistically significant decrease in the activity of ALP in the experimental group when compared with the control groups; and no statistically significant difference between the two controls. The order of decrease is from group C to A.

Table 5: G6PDH activity in the Descending aorta (IU/g tissue) at P < 0.05 confidence interval. (Day 28)

Group	N	Mean± S.E.M
(A)Experimental	6	0.62 ± 0.24
(B)Control 1	6	0.86 ± 0.23
(C)Control 2	6	0.90 ± 0.40

Table 5 above shows a statistically significant decrease in the activity of G6PDH in the experimental group when compared with the control groups; and no statistically significant difference between the two controls. The order of decrease is from group C to A.

Table 6: LDH activity in the Descending aorta (IU/g tissue) at P < 0.05 confidence interval. (Day 28)

Group	N	Mean± S.E.M
(A)Experimental	6	0.03 ± 0.43
(B)Control 1	6	0.19 ± 0.46
(C)Control 2	6	0.20 ± 0.33

Table 6 above shows a statistically significant decrease in the activity of LDH in the experimental group when compared with any of the control groups; and no statistically significant difference between the two controls. The order of decrease is from group C to A.

Table 7: Comparative Means of the activities of ALP, G6PDH & LDH 14th day. (IU/g tissue)

Group	ALP	G6PDH	LDH
Experimental(CG)	0.25	0.82	0.29
Control 1 (CW)	0.39	0.21	0.32
Control 2 (AA)	0.40	0.17	0.53

Table 8: Comparative Means of the activities of ALP, G6PDH & LDH 28th day. (IU/g tissue)

Group	ALP	G6PDH	LDH
Experimental(CG)	0.16	0.62	0.02
Control 1 (CW)	0.21	0.86	0.19
Control 2 (AA)	0.23	0.90	0.20

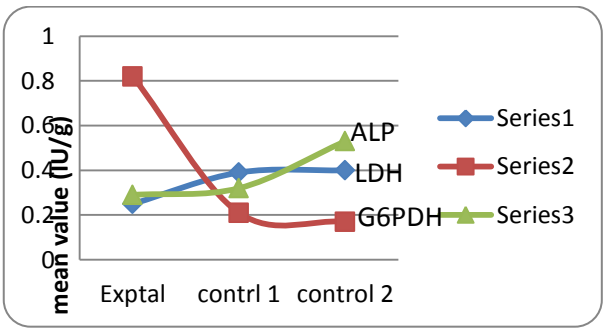

Fig 1: Chart representing the activities of the ALP, G6PDH & LDH for the 3 group (Day 14)

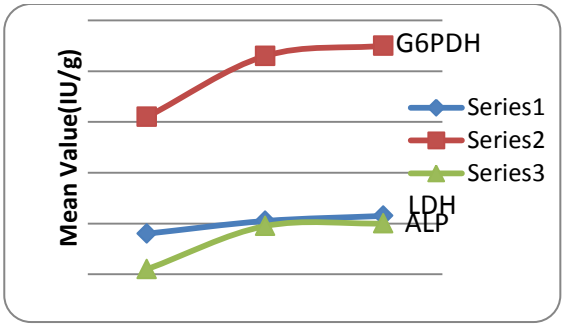

Fig 2: Chart representing the activities of the ALP, G6PDH & LDH for the 3 group (Day 28)

DISCUSSION

Alkaline Phosphatase (ALP) facilitates transport across membranes. It facilitates the breakdown of ATP to ADP and inorganic phosphate thereby making free energy available for metabolic processes (Murray et al., 2003). Its activity was found to be significantly reduced in the experimental group when compared to the control groups as at the 14^{th} and 28^{th} days. This results to a longer transient time of biomolecules in the aortic wall and a consequent decrease in transport of biomolecules across the layers of the descending aorta hence the low supply of nutrients in the experimental group. This result can be buttressed by the reported thickened of tunica intima and media layers of the wall of the descending aorta from the histological study performed by Popoola (2010). Glucose-6-phosphate Dehydrogenase (G6PDH) is an enzyme that catalyses carbohydrate metabolism through the glycolytic pathway or Hexose Monophosphate Shunt (HMS). It is important in the generation of the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) and the production of ribose (Sodeinde, 1992). The activity of G6PDH was found to be increased (although not statistically significant) in the experimental group compared with the control groups as at day 14. However, its activity was significantly reduced by day 28. This suggests initial increase in carbohydrate metabolism (generation of energy) through glycolytic pathway in the walls of the aorta which later slows down after a period of twenty eight days of exposure to cigarette smoke. This decrease may be said to result from oxidative stress caused by cigarette smoke; and consequently reduced nutrient flow across the layers of the descending aorta as explained by (Swann and Lessor-Schlaggar 2007).Lactate Dehydrogenase is an important enzyme of energy production in the cell, which serves as a marker enzyme for anaerobic carbohydrate metabolism, catalyzing the conversion of lactate to pyruvate. The large amount of NADH produced is oxidized by reducing pyruvate to lactate, a reaction catalyzed by LDH. The lactate produced during anaerobic glycolysis diffuses from the tissue and is transported out of the cell into circulation (Sodeinde 1992). However, the Embden -Meyerhof's pathway symbolized by LDH which is the alternative source of glucose metabolism during oxidative stress, as occurred above, is also slowed down because LDH activity is reduced significantly in the experimental group. The decrease in the level of LDH and G6PDH suggests reduction of carbohydrate metabolism/energy production, however more of carbohydrate metabolism occurred through the glycolytic pathway with comparatively higher enzyme activity. This can be explained in line with poor supply of nutrient through the endothelium to the tunica media and adventitia reported by (Sidney 2002) resulting from arterial constriction (on short term exposure to nicotine) and blood vessel blockage (on long term exposure to nicotine) (Myceketal., 1997).

CONCLUSION

Exposure to cigarette smoke can affect certain enzymes of the walls of the descending aorta.

AKNOWLEDGEMENTS

The researchers are sincerely grateful to Late Professor E.A Caxton-Martins for his great contributions and research guidance. We appreciate the efforts of the laboratory attendants of the Department of Anatomy, University of Ilorin.

REFERENCES

International Agency for Research on Cancer, 2004

Murray RK, Granner DK, Mayes PA, Rodwell VW (2003). Harpers Illustrated Biochemistry, McGraw Hill, New York, 109.

Mycek MJ, Harvey RA, Champe PC, and Fisher BD (1997). Pharmacology, Lippincott, William and Wilkins, Philadelphia: 20-36.

Patskan G., Reininghaus W (2003). Toxicological evaluation of an electrically heated cigarette. Part 1: Overview of technical concepts and summary of findings. J. Appl. Toxicol. 23:323–328.

Sidney S (2002).Cardiovascular Consequences of Marijuana Use.Journal of Clinical Pharmacology. 42(11): 645-705

Sodeinde O (1992): Glucose-6- phosphate Dehydrogenase Deficiency.Brailleiress Clinical Haematology. 5(2):367-382

Swann GE and Lessor-Schlaggar (2007). Neurophysiology Review. 17(3):259-273

Titus MA, Revest P, and Shortland P (2001). The Nervous system: Basic Science and Clinical Conditions, 1st edition, Churchill Livingstone: 329- 331

U.S. Department of Health and Human Services, 2004

Wigand, J.S (2006). Additives, Cigarette Design and Tobacco Product Regulation. A Report To: World Health Organization, Tobacco Free Initiative, Tobacco Product Regulation Group, Kobe, Japan.

World Health Organization (2008): Report on the Effect of Global tobacco epidemic. The M POWER Package: 1-62. Accessed at www.whoint/tobacco/inpower.pdf.