The Effect of Isopropyl Nitrite on Hemoglobin Oxidation Studies in Diabetics Blood

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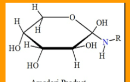
1) Introduction

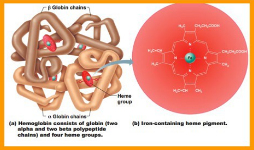
Isopropyl nitrite belongs to a class of compounds called alkyl nitrites that cause oxyhemoglobin to undergo oxidation, i.e. the iron (II) in the hemoglobin loses an electron to become iron (III) and cannot carry oxygen to the tissues and is therefore useless in oxygen transport to the tissues [1]. Nitrites are compounds that have long been known to induce this oxidation reaction [2]. Any number of nitrite esters such as ethyl nitrite, propyl nitrite, butyl nitrite, pentyl nitrite and hexyl nitrite are used as inhalants known as poppers [3]. They are easily obtained [4] and overuse can cause side effects such as tachycardia, migraine headaches, fainting and dizziness [5]. Isopropyl nitrite poppers can cause maculopathy [6-8]. Collectively, it has been discovered that alkyl nitrites may induce neurotoxicity which mainly impacts learning and memory function [9]. If swallowed poppers can cause cyanosis followed by death due to too much methemoglobin because the hemoglobin can no longer transport the amount of oxygen required by the tissues which is a condition referred to as acquired methemoglobinemia [10-12]. Because of the wide usage of these nitrite esters for recreational consumption coupled with side effects arising from their use a comparative study of diabetics blood vs. normal blood appears warranted [13-18].

As is well known people with diabetes mellitus also have hemoglobin that differs from ordinary adult blood in that it is glycosylated to a level of 6.5% or greater by the abnormally high level of glucose in the untreated diabetic's blood.

As a result hemoglobin becomes hemoglobin A1c by the Amadori rearrangement to form a 1-deoxy-1- amino-D- fructopyranose whose structure is shown below. (Note: The R group in the figure would be the beta hemoglobin polypeptide and the sugar has reacted with the N-terminal valine of this beta globin chain.)



Amadori Product



2) Aim

The goal of these studies is to help establish that people with type 2 diabetes mellitus have a greater risk of enhanced methemoglobin formation by alkyl nitrites compared with normal (non-diabetics) blood. To the end statistical methods can be employed to identify the 'at risk' group, e.g., diabetics, by using Student's t-test. This could later be followed by the requisite ad hoc statistical tests including linear regression analysis and the coefficient of determination calculation provided the diabetics blood first showed an enhanced susceptibility to oxidation by isopropyl nitrite. All of this would then provide supportive reasons to indicate that alkyl nitrite use in any form should be avoided for diabetics. In this particular study isopropyl nitrite will be investigated to see how its properties correspond to other alkyl nitrites that have been previously investigated so as to obtain a more comprehensive understanding of the enhanced susceptibility of Type 2 diabetics blood to alkyl nitrites.

3) Materials and Methods

Isopropyl nitrite was purchased from Win-Win Chemical CO., Limited. Other required chemicals were obtained from the Sigma and Aldrich Chemical Company. Blood products such as normal adult blood and diabetics blood were purchased from Physicians Plasma Alliance (PPA). All blood was tested and certified to be non-viral by PPA. All subjects who participated in the studies gave voluntary informed consent. PPA identified the source of diabetics blood as being from patients with type II diabetes mellitus. A laboratory spectrophotometer equipped with a strip chart recorder to monitor the formation of methemoglobin at 436nm was used to obtain the oxidation times for these patients blood. A small table top centrifuge to separate plasma from the red blood cells was employed in these studies to obtain the packed erythrocytes.

For these studies the data was obtained from 40 donors 20 of whom had type 2 diabetes mellitus and 20 of whom were nondiabetics. In Tables 1 and 2 the characteristics of the patients used in these studies are presented, i.e., HbA1C percentage, age, gender, weight, smoker status and oxidation times (in min). All blood was drawn into ACD tubes and stored at 2-4 C prior to use.

The Hemoglobin A1C (HbA1C) percentages were determined using a Bayer DCA-2000 test kit. Diabetes was assessed as a HbA1C percentage greater than 6.5% [19]. The sample methodology employed to obtain the oxidation times was the same as described in an earlier publication [15]. According to Colton [20] the appropriate test to use for these data is the Student's t-test for independent samples. The significance level has been considered to be P< 0.05.

4) Results and Discussion

For the isopropyl nitrite studies the findings of the HbA1C percentages revealed that the diabetics blood mean ± standard error of the mean (SEM) was 11.4 ± 0.27%, while that of the nondiabetics blood had a mean ± SEM of 5.5 ± 0.08%. Thus, the percentage differences between the two populations was statistically significant (P<0.05), and this means that these two populations are good groups on which to undertake the alkyl nitrite oxidation studies as is shown in the column comparison of the means ± SEM in Figure 1. For isopropyl nitrite the mean oxidation time of the diabetics blood ± SEM was 1.5 ± 0.05 min whereas the mean oxidation times of the non-diabetics blood ± SEM was 4.6 ± 0.13 min as shown in the column comparison of the mean ± SEM in Figure 2 . Based on an independent Student's t-test, the time taken for diabetics erythrocytes to undergo oxidation was significantly shorter (P<0.05) than the non-diabetic controls.

Interestingly, the enhanced susceptibility to both alkyl nitrite induced oxidation reactions occurred in Type 2 diabetics blood illustrates that HbA1C oxidation to methemoglobin is a direct function of the amount of HbA1C present as opposed to metabolic differences in the type 1 and type 2 diabetes [21] , i.e., a clear inverse relation appears to exist between the amount of HbA1C present and the oxidation time. Hence, any untreated diabetic simply has a greater percentage of HbA1C than a non-diabetic, e.g. 11.4% vs. 5.5%, as is shown in Figure 1. Thus, these preliminary findings with isopropyl nitrite indicate that diabetics have hemoglobin that exhibit greater oxidative stress to alkyl nitrite owing to a higher percentage of HbA1C. Moreover, this is reinforced by earlier studies wherein other alkyl nitrites such as ethyl nitrite, butyl nitrite, pentyl and hexyl nitrite all gave equivalent results with Type diabetics blood [13-18]. These similar findings could be attributed to the fact that these nitrite esters differ only in the type of alkyl group present. Whether there are two, three, four, five or six saturated carbons attached to the ester's oxygen makes no difference in terms of these compound's ability to induce enhanced methemoglobin formation in Type 2 diabetics blood.

5) Conclusion

The present study shows a statistically significant enhanced hemoglobin oxidation time for type 2 diabetics blood. This relationship between methemoglobinization time with HbA1C values for diabetics blood vs. normal blood using Student's t-test for independent samples was found to be statistically significant (P<0.05). This implies that methemoglobinization is an important indicator for oxidative stress in diabetic's blood and makes any use of alkyl nitrites to be avoided by diabetics since their blood much more easily undergoes conversion from oxyhemoglobin to methemoglobin than with non-diabetics. The strength of these findings are that they apply to human blood samples of diabetics and normal individuals, i.e., the *in vitro* findings are clear cut. Nevertheless, this does not necessarily prove these findings would be the same *in vivo*.

6) Acknowledgements

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7) Tables

Table 1. Characteristics of Diabetic Patients in the Isopropyl Nitrite Oxidation Studies

Sample ID	HbA1C (%)	Age (yrs)	Gender	Weight (lbs)	Smoker status	Oxidation Time (min)
GWB005533	13.3	50	Male	167	Smoker	1.2
GWB005544	11.4	43	Female	254	Non-Smoker	1.5
GWB005547	10.4	60	Female	223	Non-Smoker	1.7
GWB005548	10.6	48	Female	167	Non-Smoker	1.7
GWB005549	12.7	21	Female	282	Smoker	1.2
GWB005550	10.9	21	Male	158	Non-Smoker	1.5
GWB005551	11.1	46	Male	252	Non-Smoker	1.3
GWB005553	10	57	Male	207	Non-Smoker	1.8
GWB005556	13.7	41	Female	300	Smoker	1.2
GWB005564	10.1	47	Female	219	Non-Smoker	1.7
GWB005694	11.8	41	Male	255	Non-Smoker	1.5
GWB005690	10.2	45	Male	330	Non-Smoker	1.8
GWB005675	11.7	41	Male	314	Non-Smoker	1.5
GWB005683	10.7	27	Male	245	Non-Smoker	1.7
GWB005687	13.5	37	Female	225	Smoker	1.1
GWB005688	12.7	49	Female	237	Smoker	1.4
GWB005689	12.2	29	Female	137	Non-Smoker	1.4
GWB005692	9.6	41	Male	290	Non-Smoker	2
GWB005691	10.2	36	Female	165	Non-Smoker	1.8
GWB005678	11.3	46	Male	415	Non-Smoker	1.6
Mean	11.4	41.3		242.1		1.5
Standard Deviation	1.22	10.2		66.4		0.2
SEM	0.27	2.3		14.9		0.05

Table 2. Characteristics of Nondiabetic Patients in the Isopropyl Nitrite Studies

Sample ID	HbA1C (%)	Age (yrs)	Gender	Weight (lbs)	Smoker status	Oxidation Time (min)
GWB005538	5.2	25	Male	166	Non-Smoker	4.5
GWB005539	5.4	28	Male	279	Smoker	4.2
GWB005546	5.7	48	Female	167	Non-Smoker	4
GWB005552	5.8	51	Male	253	Non-Smoker	4.1
GWB005554	5.9	42	Male	250	Non-Smoker	4
GWB005555	5.6	54	Male	270	Non-Smoker	4
GWB005558	5.8	65	Male	273	Non-Smoker	5.3
GWB005565	5.3	37	Male	190	Smoker	5.7
GWB005566	5.8	61	Male	174	Non-Smoker	3.8
GWB005570	5.4	47	Male	194	Non-Smoker	4.2
GWB005698	6	51	Male	395	Smoker	5.1
GWB005697	4.8	31	Female	138	Smoker	5.8
GWB005681	5.6	43	Male	155	Smoker	4.4
GWB005676	4.9	42	Female	196	Smoker	5.2
GWB005677	5.7	54	Male	210	Non-Smoker	4.5
GWB005680	5.6	57	Male	198	Non-Smoker	4.4
GWB005699	5.3	19	Male	141	Non-Smoker	4.7
GWB005702	5.9	46	Male	265	Non-Smoker	4.9
GWB005704	5.3	64	Male	212	Non-Smoker	4.3
GRP000480	4.7	39	Female	163	Non-Smoker	5
Mean	5.5	45.2		214.5		4.6
Standard Deviation	0.37	12.4		60.8		0.57
SEM	0.08	2.8		13.6		0.13

8) Figures

Figure 1. COLUMN COMPARISON OF MEANS FOR THE PERCENT HbA1C OF THE HEMOGLOBIN OF DIABETICS AND NON-DIABETICS BLOOD

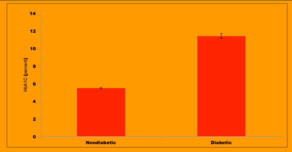
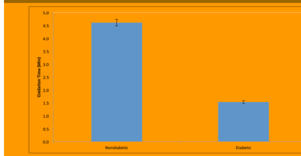


FIGURE 2.COLUMN COMPARISON OF MEANS FOR THE OXIDATION TIMES OF THE HEMOGLOBIN OF DIABETICS AND NON-DIABETICS BLOOD



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