Amyl Nitrite Induced Hemoglobin Oxidation Studies in Diabetics and Non-diabetics Blood

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Abstract
The effect of amyl nitrite on human diabetics blood oxidation times was undertaken using non-diabetics blood as the control group. Based on a statistical analysis using Student’s t-test, human diabetics hemoglobin was found to be more susceptible to oxidation to methemoglobin compared to the control group (p<0.05).

Keywords: Amyl nitrite; Methemoglobin; Diabetics; Oxidation of hemoglobin

Background
Amyl nitrite is a chemical compound that causes the oxyhemoglobin to undergo oxidation, i.e. the iron (II) in the hemoglobin loses an electron to become iron (III). When this occurs the ruby red oxyhemoglobin changes in color to a chocolate brown which signifies it has become methemoglobin or iron (III) hemoglobin. While iron (II) hemoglobin can carry oxygen to the tissues as oxyhemoglobin, iron (III) hemoglobin or methemoglobin cannot carry oxygen to the tissues and is therefore useless in oxygen transport to the tissues [1]. With wide usage of amyl nitrite in the treatment of coronary heart disease and its effect to cause methemoglobinization and possible side effects there from, a study of diabetics blood’s susceptibility to enhanced methemoglobin formation appears warranted. Furthermore methemoglobinization is an important indicator for oxidative stress in diabetes [2].

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 [3].

Recently Moussa reported that methemoglobinization, that is the oxidation of iron (II) of oxyhemoglobin into iron (III) to form methemoglobin, is an important indicator of oxidative stress in certain diabetic patients. Specifically, those afflicted with Type 1 diabetes mellitus have a higher hemoglobin auto-oxidation rate than those with Type 2 diabetes mellitus or non-diabetics [4].

Increased susceptibility to the oxidation of diabetes blood by amyl nitrite would be worthwhile information to establish as it would be a possible contraindication for the use of this drug to treat certain diabetics with heart disease (angina pectoris). In addition these studies may establish that certain diabetics blood (e.g., those with type 2 diabetes mellitus) is less stable than those of a normal adult which could put them at even greater risk of all heart disease (both angina pectoris and myocardial infarction) due to hemoglobin with a higher auto-oxidation rate.

Materials and Equipment
Materials
Isoamyl nitrite was purchased from Acros Organics. Other required chemicals were obtained from the Sigma and Aldrich Chemical Company. Blood products such as normal adult blood and diabetic blood were purchased from Physicians Plasma Alliance. All blood was tested and certified to be non-viral by PPA. All the data was obtained from 40 donors, 20 of whom had type 2 diabetic mellitus and rest 20 of were non-diabetics. The samples were provided as matched sets of diabetics and non-diabetics blood wherein the two groups were evenly matched with respect to age, gender, number of obese and number of cigarette smokers. Also these donors took similar vitamins and medications according to their medical histories.

Equipment
Laboratory spectrophotometer equipped with a strip chart recorder to monitor the formation of methemoglobin at 436 nm. A small table top centrifuge to separate plasma from the red blood cells was used.

Methodology
The HbA1C percentages were determined using a Bayer DCA-2000 test kit.

To determine the oxidation time blood samples were centrifuged for 2000 g for 20 min to remove any remaining plasma. The remaining packed RBCs were aerated and washed in 20 mM phosphate buffer saline (PBS) at physiological pH (pH 7.2) followed by re-centrifugation to remove the saline. This procedure of centrifugation, aeration and washing was repeated. The RBCs were then resuspended in 20 mM PBS (pH 7.2) for a maximum of 60 min prior to testing. Isoamyl nitrite was diluted by the addition of ethanol so as to obtain a final concentration of 140 micromoles per liter after its addition to the hemoglobin solution. A 0.01 mL portion of resuspended RBCs was hemolyzed by the addition of 1 mL of distilled water and adjusted to a final volume of 2.6 mL by the addition of 20 mM PBS (pH 7.2). The hemoglobin solution was then adjusted to a standard absorbance (e.g., A=1.0 ± 0.2) at a wavelength of 436 nm with more 20 mM PBS (pH 7.2). The 2.6 mL aliquot of this hemoglobin solution was then added to a 0.05 mL aliquot of isomyl nitrite in ethanol. The above gave a final hemoglobin concentration...
between 6 and 9 micromoles per liter [5]. The above solution was then placed in a cuvette and the reaction measured in a spectrophotometer equipped with a chart recorder set at a wavelength of 436 nm. This is a suitable wavelength for measuring and distinguishing oxyhemoglobin and methemoglobin. The spectrophotometer chart recorder then generated graphic representations of the conversion of oxyhemoglobin into methemoglobin as a function of time. The terminal period or asymptotic phase corresponds to essentially 100% methemoglobin formation. The final absorbance was found to be approximately A=0.5 ± 0.1.

Results

The findings of the HbA1C percentages revealed that the diabetics blood averaged 11.4 ± 1.2%, while that of the nondiabetics blood averaged 5.5 ± 0.2%. 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 amyl nitrite oxidation studies as shown in figure 1 [6]. The mean oxidation time of the diabetics blood was 1.5 ± 0.2 min whereas the mean oxidation times of the nondiabetics blood was 3.1 ± 0.5 min. Thus, the comparative study of human adult diabetics blood revealed that the diabetics oxyhemoglobin was oxidized by amyl nitrite at about twice the rate of nondiabetics blood as shown in the histogram comparison 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 nondiabetic controls.

Discussion

Interestingly the enhanced susceptibility to oxidation occurred in Type 2 diabetics blood which implies 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 [4]. This finding appears to be well supported by the fact that glycation of hemoglobin is an irreversible chemical reaction that is non-enzymatic in nature irrespective of the type of diabetes. Essentially, any untreated diabetic simply has a greater percentage of HbA1C than a nondiabetic, e.g. 11.4% vs. 5.5% in this study as shown in figure 1. Thus, these preliminary findings indicate that diabetics have hemoglobin that exhibit greater oxidative stress to amyl nitrite owing to a higher percentage of HbA1C. In fact HbA1C has been reported to be more thermolabile than nonglycated hemoglobin (HbA0). This supports the view that structural modification of hemoglobin due to glycation causes the HbA1C to become less stable and more prone to oxidation by glycation-induced stuctural modification of hemoglobin which leads to a functional modification resulting in oxidative stress in diabetic patients [7].

References
