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DETERMINATION OF TREATMENT TIME AND EFFICACY WITH OXYGEN IN EXPERIMENTAL METHEMOGLOBINEMIA MODEL

DENEYSEL METHEMOGLOBİNEMİ MODELİNDE OKSİJEN İLE TEDAVİ SÜRESİ VE ETKİNLİĞİNİN BELİRLENMESİ

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ABSTRACT

Introduction: Methemoglobin, an abnormal form of hemoglobin, is one of the causes that should be considered primarily in severe central cyanosis. Methylene blue and ascorbic acid are used as the first-line therapies in methemoglobinemia. Experience with the use of oxygen therapy is not sufficient to conclude the duration of therapy. The aim of the present study is to find out the effects of oxygen therapy on drug or toxic agent-induced methemoglobinemia.

Material and Method: First group rats (n=6) were administered 100 mg/kg prilocaine intraperitoneally, and the rats were kept at room temperature. The second group of oxygen group rats (n=6) were administered 100% oxygen at 10 liters/min. All rats were observed for 180 minutes, and blood gas and biochemical analyses were performed at the end of the experiment period.

Results: Fifty percent of the rats in the room air group (n=3) died spontaneously at the end of the trial period, while all rats in the oxygen group (n=6) survived to the end of the trial period. When evaluated in terms of survival, a statistical difference was found between the groups ($p < 0.05$). In addition, although there was a significant difference in oxygen saturation levels between the groups ($p < 0.05$), there was no significant increase in partial arterial oxygen pressure, the primary determinant of oxygenation.

Conclusion: This study revealed that rats are not suitable for this type of experimental study. Prilocaine doses did not cause high levels of methemoglobinemia due to mortality, so the goal and target could not be met. It has been concluded that this study can serve as a guide for choosing methodologies to be employed in planned future investigations of the topic.

Keywords: Oxygen therapy, methemoglobinemia, local anesthetics, prilocaine

ÖZET

Giriş: Anormal bir hemoglobin şekli olan methemoglobin, öncelikle ağır santral siyanozda göz önünde bulundurulması gereken nedenlerden biridir. Methemoglobinemide birinci basamak tedavi olarak metilen mavisi ve askorbik asit kullanılır. Oksijen terapisi kullanımına ilişkin deneyim, tedavinin süresini sonuçlandırmak için yeterli değildir. Bu çalışmanın amacı, oksijen tedavisinin ilaç veya toksik ajan kaynaklı methemoglobinemi üzerindeki etkilerini bulmaktır.

Gereç ve Yöntem: Birinci grup sıçanlara (n = 6) periton içine 100 mg / kg prilokain verilmiş ve sıçanlar oda sıcaklığında tutulmuştur. İkinci grup oksijen grubu sıçanlara (n = 6) ise 10 litre / dak %100 oksijen uygulanmıştır. Tüm sıçanlar 180 dakika boyunca gözlenmiş ve deney süresi sonunda, kan gazı ve biyokimyasal analizler yapılmıştır.

Bulgular: Oda hava grubundaki (n = 3) sıçanların yüzde ellisi deneme süresi sonunda kendiliğinden ölmüştür, oksijen grubundaki (n = 6) tüm sıçanlar deneme süresinin sonuna kadar yaşamıştır. Sağkalım açısından değerlendirildiğinde gruplar arasında istatistiksel bir fark saptanmıştır ($p < 0.05$). Ayrıca, gruplar arasında oksijen doygunluğu seviyelerinde anlamlı bir fark olmasına rağmen ($p < 0.05$), oksijenasyonun primer belirleyicisi olan parsiyel arteriyel oksijen basıncında anlamlı bir artış görülmemiştir.

Sonuç: Yapılan bu çalışma, sıçanların bu tür deneysel çalışma için uygun olmadığını ortaya koymuştur. Prilokain dozları mortalite nedeniyle yüksek düzeyde methemoglobinemi oluşturmamış, bu nedenle amaç ve hedef karşılanamamıştır. Bu çalışmanın, konu ile ilgili ileride yapılacak araştırmalarda kullanılacak metodolojilerin seçiminde yol gösterici olabileceği sonucuna varılmıştır.

Anahtar Kelimeler: Oksijen tedavisi, methemoglobinemi, lokal anestezikler, prilokain

INTRODUCTION

One of the possible underlying causes suspected in cases of severe central cyanosis (1) is methemoglobin (MetHb), an abnormal type of hemoglobin (Hb) formed by the oxidation of the molecule's iron cation from its ferrous form (Fe^{2+}) into its ferric form (Fe^{3+}). In healthy people, the ratio of MetHb is less than 2% of total Hb and an increase in MetHb levels which lack oxygen (O_2) carrying capacity will shift the oxygen dissociation curve (ODC) to the left, causing hypoxia, lactic acidosis and death. The MetHb alters tissue oxygenation due to the lingering high-O_2 affinity of the remaining non-oxidized parts of Hb, while the O_2 carrying capacity of the overall MetHb molecule is disrupted.

Common causes of methemoglobinemia include exposure to oxidizing chemicals and drugs, and high doses of local anesthetics, or toxins, used in anesthesia rank as the most frequently observed triggers of methemoglobinemia. The second most frequent cause is the severe metabolic acidosis that develops in infants aged less than six months due to diarrhea and dehydration, which leads to the idiopathic form of the disease. The third most frequent cause is the consumption of nitrate and nitrite-containing foods, and the fourth most frequent etiological cause is

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an autosomal recessive genetic disorder. The possible presence of methemoglobinemia should certainly be considered during a differential diagnosis of infant cases in which central cyanosis cannot be associated with cardiac or pulmonary pathologies (2). The lower MetHb reductase activity in the first three months of life, combined with the easier oxidation of fetal hemoglobin, leads to a greater risk of contracting methemoglobinemia from drugs and toxic substances, and most cases reported in literature belong to this age group (3-5).

The primary treatment for toxic methemoglobinemia is methylene blue or ascorbic acid administration (6, 7), while supportive treatment involves the administration of hyperbaric O2 (8). The goal of the latter is to increase the level of dissolved O2 in the plasma so as to protect the metabolism. Hyperbaric oxygen treatment allows for a temporary improvement in oxygen delivery (DO2), while the alternative is blood transfusion, which, as in the case of hyperbaric oxygen treatment, is applied in cases where methylene blue is not immediately available or if the patient is unresponsive.

Regarding hyperbaric oxygen treatment, there is a lack regarding information and experience of the effectiveness of O2 treatment and its impact on overall treatment duration (9). The aim of the present study was to demonstrate the effect of 100 percent O2 treatment on MetHb levels, oxygenation, and hemodynamism in rats induced experimentally with methemoglobinemia through prilocaine administration and then kept under room conditions or under 100 percent O2 treatment.

MATERIALS AND METHODS

Experimental rat used, and their care and shelter conditions

Preliminary studies to determine the toxic dose of prilocaine were carried out using 16 Spraque-Dawley male rats weighing between 244 and 404 g. The study protocol was applied to 12 Spraque-Dawley rats, obtained from the Research Center of the Bezmialem Foundation University (approval number 2012/423), weighing between 318 and 419 g. Prior to the experiment, two rats were kept per cage at a room temperature of 21–22℃, and under a photoperiod of 12 hours day/12 hours night. The rats were fed 18-20% protein rat pellets and given water to drink.

Preliminary Study

To determine the toxic dose of prilocaine, 16 Sprague-Dawley male rats were used at room air conditions. Anesthesia was applied through the intraperitoneal administration of 0.3–0.4 ml of Xylazine (Rompun®) at a dose of 10 mg/kg, and Ketamine (Ketalar®) at a dose of 75 mg/kg. For monitoring purposes, a saturation probe was placed on the feet of the rats to track oxygen saturation (SpO2) and heart rate (HR) (Nihon Kohden®, Japan). Blood gas and MetHb levels were determined by collecting approximately 1000 microliters (ml) of blood intracardially with a 2.5 ml injector containing heparin, and then examining the samples with a RapidLab® (Siemens, Germany) oximeter blood gas measuring device. The baseline partial arterial oxygen pressure (PaO2), partial arterial carbon dioxide pressure (PaCO2), arterial oxygen saturation (SaO2), HR and MetHb values were recorded. On a sensitive scale, pure prilocaine powder (prilocaine hydrochloride-P9547 Sigma®) was weighed on a sensitive scale and then diluted with 1 mL of physiological saline solution to obtain doses of 600 mg/kg for one of the groups (n=6), 300 mg/kg for the second group (n=6) and 200 mg/kg for the third group (n=4). The doses were administered intraperitoneally to the rats, and 1000 ml of intracardiac blood was collected from the experimental rats at 30 min intervals. To compensate for the loss of fluid, 1 ml of physiological saline solution was given intraperitoneally every 60 minutes, although most of the rats died within 60 minutes due to prilocaine's toxic effect, the invasiveness of obtaining intracardiac blood at frequent intervals and the substantial amount of blood collected. To reduce the volume of blood collected from the 200 mg/kg prilocaine group, the decision was taken to collect approximately 300 ml of blood samples intracardially using an insulin injector containing heparin. In these blood samples, 100 ml of blood was examined using an Epoc® co-oximeter blood gas device (Epocal Inc., Canada) to determine PaO2 and PaCO2, while 200 ml of blood was kept inside the heparin-containing tubes to determine MetHb levels through a spectrophotometric analysis in the biochemistry laboratory. Because the doses had to be reduced, the study procedure was revised to include the use of Priloc®, a commonly used medication in the clinic, at a dose of 100 mg/kg, rather than the pure powder prilocaine. In addition, the decision was made to collect blood samples for MetHb measurements from the tail vein rather than intracardially.

Study protocol

In the study protocol, revised based on the results of the preliminary studies, the rats were anesthetized and monitored under the same conditions, and the obtained values were recorded accordingly. During the study, additional ketamine (Ketalar®) was administered in doses of 45 mg/kg to sustain anesthesia. Prior to prilocaine administration, blood samples of approximately 300 ml were collected from all rats kept under room air conditions using heparin-containing insulin injectors. In these blood samples, 100 ml of blood was examined using the bedside Epoc® co-blood gas device to determine PaO2 and PaCO2, while 200 ml of blood was kept inside the heparin-containing tubes to determine MetHb levels through spectrophotometric analysis in the biochemistry laboratory. Work on the room air group (n=6) was carried out in the

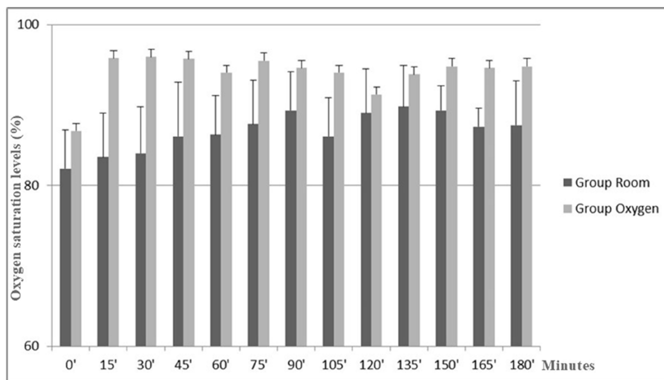

Figure 1. SpO2 results measured at 15 min intervals before prilocaine administration (0 min) and after prilocaine administration (180 min) in the room group and O2 group.

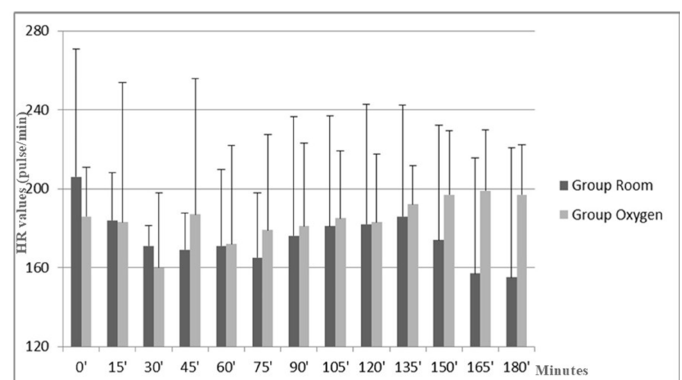

Figure 2. HR results measured at 15 min intervals before prilocaine administration (0 min) and after prilocaine administration (180 min) in the room group and O2 group.

same environment. The rats in the oxygen group (n=6) were placed inside an induction chamber (VetEquip, Inc. Pleasanton, CA, USA) 10 minutes before the start of the study, and oxygenated at a rate of 10 lt/min. Throughout the study procedures, conditions inside the chamber were maintained by adjusting and keeping the flowmeter of the anesthesia device (VetEquip, Inc. Pleasanton, CA, USA) at 10 lt/min. Blood samples of 200 ml were collected from the tail vein of all experimental rats at 30-minute intervals until minute 180 of the study, and MetHb values were measured using spectrophotometric methods. HR and SpO2 measurements were also monitored and recorded every 15 minutes, while the blood samples taken prior to prilocaine administration and at minute 180 of the study were evaluated for blood gases. The experimental rats that were still alive at the end of the 180-minute study period were sacrificed by decapitation. The livers, lungs, kidneys, and brains of all experimental rats used in the preliminary study and the main study groups were also collected, with half of the single organs and one of the double organs being preserved in formaldehyde for future studies, and the remaining organs and organ parts being kept in dry tubes at -20 ℃ for histochemical examination. The samples were kept at the pathology and biochemistry laboratory of the Cerrahpaşa Medical Faculty.

Statistical analysis

The statistical analysis was carried out using the IBM SPSS 19 statistical software, and the descriptive data was presented as mean ± standard deviation (SD) and median. Comparisons between two groups were carried out using a Mann-Whitney U-test, while variations in repeated measurements within a group were evaluated with a Friedman Test. P values of 0.05 were considered statistically significant in all tests.

RESULTS

Adult male Sprague-Dawley rats weighing between 311 and 415 g (six rats) in the room group and between 351 - 419 g (six rats) in the O2 group were used in the study. All rats received 100 mg/kg of prilocaine. The weights of the experimental rats, the applied prilocaine dose, room temperature and room humidity are given in Table 1. There was no significant difference between the weights of the experimental rats, the applied prilocaine dose, and room temperature in comparison with room group and the O2 group. There was a significant difference between the two groups in terms of room humidity ($p < 0.05$).

Table 2 shows the hematocrit (Hct) values of the rats in the room group and O2 group at the beginning of the experiment (0 min) and at the end (180 min) in the blood gas device.

Oxygen saturation (SpO2) levels

SpO2 results measured at 15 min intervals before prilocaine administration (0 min) and after prilocaine administration (180 min) in the room group and O2 group are presented in Table 3 and Figure 1. The lowest level of SpO2 in the room group before the administration of prilocaine was found in the rats (SpO2:74%) in the 5th group, and the lowest SpO2 level in the O2 group was found in the 1st group (SpO2:80%). The levels of SpO2 in the room group ranged from 74% to 89% and from 80% to 90% in the O2 group before the administration of prilocaine. There was no significant difference between the groups in terms of baseline values ($p > 0.05$). Therefore, the comparison between the groups was continued in the later periods.

After administration of prilocaine, SpO2 values in the room group ranged from 76% to 97%. The lowest value was seen in rat number 5, with an initial SpO2 value of 74%. There was a progressive increase in SpO2 values in each rat. In the O2 group, the lowest SpO2 was observed at 120 minutes (SpO2: 69%). Due to the effect of O2 support, the SpO2 value increased in the first 15 minutes and remained stable. However, these changes in the group were not statistically significant ($p > 0.05$).

Table 1. Weights of experimental rats, prilocaine amounts, room temperature and room humidity

	Room Group (21% O2)	O2 Group (100 % O2)	p value
Weight (mg)	372±33.9	375.5±24.5	0.87
Dose of Prilocaine (mg)	37.2±3.3	37.5±2.4	0.87
Room Temperature (°C)	19.6±0.4	19.8±0.0	0.10
Room Humidity (%)	0.5±0.01	0.63±00	0.002

Data are presented as mean±standard deviation. P value was calculated by Mann-Whitney U-test.

Table 2. Hct (%) values measured in room group and O_2 group before prilocaine application (0 min) and then (180 min)

Rat No	0 min Hct%	180 min Hct%	Rat No	0 min Hct%	180 min Hct%
Room 1	42	32	O2 1	42	38
Room 2	34	26	O2 2	40	39
Room 3	46	39	O2 3	39	29
Room 4	37	31	O2 4	43	35
Room 5	38	31	O2 5	43	29
Room 6	33	31	O2 6	42	37
Mean ± SD	38.3 ± 4.9	31.6 ± 4.1	Mean ± SD	41.5 ± 1.6	34.5 ± 4.4

Hct: hematocrit.

Table 3. SpO_2 levels of room group and O_2 group before prilocaine application (0 min) and after prilocaine application (180 min) (every 15 min).

SpO2 levels (%)	Room group	O2 group	p values
0 min	82.1±4.8	86.8±4.6	0.11
15 min	83.5±5.5	95.8±6.5	0.02
30 min	84.0±5.8	96.0±6.0	0.01
45 min	86.1±6.7	95.7±5.6	0.02
60 min	86.3±4.9	94.0±6.4	0.05
75 min	87.6±5.5	95.5±5.9	0.04
90 min	89.3±4.8	94.6±4.7	0.07
105 min	86.1±4.8	94.0±4.2	0.01
120 min	89.0±5.5	91.3±11.4	0.22
135 min	89.8±5.1	93.8±9.2	0.26
150 min	89.3±3.1	94.8±9.4	0.05
165 min	87.3±2.3	94.6±8.7	0.05
180 min	87.5±5.5	94.8±9.2	0.05
p values (within groups)	0.08	0.75	

Data are presented as mean ± standard deviation. P value was calculated by Mann-Whitney U-test.

Table 4. HR values of room group and O_2 group before prilocaine application (0 min) and after prilocaine application (180 min) (every 15 min).

HR values (pulse/min)	Room Group	O2 Group	p values
0 min	206.1 ± 64.9	186.8 ± 25.07	0.52
15 min	184.3 ± 24.2	183.8 ± 71.0	0.81
30 min	171.1 ± 10.4	160.6 ± 37.7	0.42
45 min	169.5 ± 18.8	187.3 ± 68.7	0.87
60 min	171.6 ± 38.6	172.8 ± 49.9	0.87
75 min	165.3 ± 33.0	179.5 ± 48.5	0.26
90 min	176.3 ± 60.5	181.0 ± 42.0	0.52
105 min	181.5 ± 55.9	185.0 ± 34.3	0.87
120 min	182.8 ± 61.0	183.6 ± 34.6	0.81
135 min	186.8 ± 56.6	192.3 ± 19.6	1.00
150 min	174.1 ± 58.0	197.8 ± 32.3	0.26
165 min	157.0 ± 58.7	199.0 ± 30.9	0.10
180 min	155.8 ± 65.9	197.3 ± 25.4	0.10
p values (within groups)	0.10	0.30	

Data are presented as mean ± standard deviation. P value was calculated by Mann-Whitney U-test. Abb. HR: heart rate.

Heart rate (HR) levels

HR results measured at 15 min intervals before prilocaine administration (0 min) and after prilocaine administration (180 min) in the room group and O2 group are presented in Table 4 and Figure 2.

In the evaluation performed before prilocaine administration, the lowest HR levels were found in rats in the group 2 (HR: 172/min) and in the O2 group in the group 3 (HR: 150/min).HR values were changed between 172/min - 303/min in room group and 150/min -224/min in the O2 group before prilocaine administration. There was no significant difference in initial HR measurements between the groups ($p > 0.05$). Since there was no difference in the measurements before prilocaine administration, a comparison was performed between the groups in other stages. However, no significant difference was observed between the groups in other stages ($p > 0.05$).

Blood gases (PaO2and PaCO2) values

Blood samples collected by intracardiac route were taken before the administration of prilocaine (0 min) and after the administration of prilocaine (180 min) in the room group and O2 group, and the values found in the blood gas device are presented in Table 5.

In the room group, rats 1, 3 and 4 died spontaneously after 180 minutes. Rats 2, 5 and 6 in the same group were sacrificed by decapitation at 180 min. In the O2 group, all rats were sacrificed after 180 min. There was a significant difference in survival between groups ($p < 0.05$).

MetHb (%) levels

MetHb results measured at 30 min intervals before prilocaine administration (0 min) and after prilocaine administration (180 min) in the room group and O2 group are presented in Table 6 and Figure 3.

The lowest level of MetHb in the room group before the administration of prilocaine was found in the rats (MetHb = 0.98%) in the 3rd group, and the lowest MetHb level in the O2 group was found in the 5th group (MetHb = 0.44%). The levels of MetHb in the room group ranged from 0.98% to 2.67% and from 0.44% to 2.01% in the O2 group before the administration of prilocaine. Since the MetHb levels were similar in the room group and the O2 group before prilocaine administration, and the rats were given prilocaine in standard doses, the comparison between the groups

Table 5. PaO_2 and $PaCO_2$ values of room group and O_2 group before prilocaine application (0 min) and after prilocaine application (180 min).

Rat No	PaO_2 (mmHg) 0 min	PaO_2 (mmHg) 180 min	$PaCO_2$ (mmHg) 0 min	$PaCO_2$ (mmHg) 180 min	Type of mortality
Room 1	73.7	63.7	69.7	55.2	Ex
Room 2	45.7	21.9	71.6	82.4	Decapitation
Room 3	35.6	67.8	64.3	58.5	Ex
Room 4	62.9	21.3	49.2	63.1	Ex
Room 5	26.1	62.8	65.3	52.8	Decapitation
Room 6	35.5	110.7	57.3	43.2	Decapitation
O_2 1	55.9	50	67.6	70	Decapitation
O_2 2	118.6	85.1	62.5	69.7	Decapitation
O_2 3	40.1	22.5	64.6	51.8	Decapitation
O_2 4	86	82.6	64	70.3	Decapitation
O_2 5	40.3	30.3	61.7	65.4	Decapitation
O_2 6	42	40.6	59.5	87.8	Decapitation

Table 6. MetHb levels (%) of room group and O_2 group before prilocaine application (0 min) and after prilocaine application (180 min) (every 30 min).

MetHb levels (%)	Room group	O_2group	p values
0 min	1.7 ± 0.6	1.2 ± 0.5	*0.26*
30 min	1.7 ± 0.6	2.6 ± 0.7	*0.33*
60 min	2.2 ± 1.1	3.7 ± 2.2	*0.26*
90 min	1.9 ± 0.9	2.8 ± 0.9	*0.07*
120 min	2.9 ± 0.76	2.8 ± 0.5	*0.52*
150 min	2.6 ± 0.9	1.6 ± 0.7	*0.15*
180 min	1.7 ± 0.5	1.6 ± 0.8	*1.00*
p values (within groups)	*0.087*	*0.006*	

Data are presented as mean ± standard deviation. P value was calculated by Mann-Whitney U-test. Abb. MetHb: methemoglobin.

was maintained at other times, and there was no significant difference between the groups ($p > 0.05$). However, the change in MetHb levels in the O2 group was significant ($p < 0.05$).

DISCUSSION

Due to the lack of information in the literature on dose levels that would result in methemoglobinemia in rat models, 600 mg/kg prilocaine was administered initially in line with the application principles of other anesthetic drugs, although all of the experimental rats died within 60 minutes of prilocaine administration due to the toxic dose. Accordingly, six other rats were given 300 mg/kg prilocaine (half the dose given to the first group) intraperitoneally after anesthesia and analgesia was ensured under room air conditions, while the other four rats received 200 mg/kg prilocaine intraperitoneally after anesthesia and analgesia. These rats also died before the end of the study period. During the main study, 50 percent (n=3) of the room air group rats died after the end of 180 minutes, while 50 percent (n=3) had to be sacrificed. In the O2 group, all of the rats (n=6) had to be sacrificed at the end of the 180-minute period.

The study protocol envisaged the collection of blood samples at 30-minute intervals for a total duration of

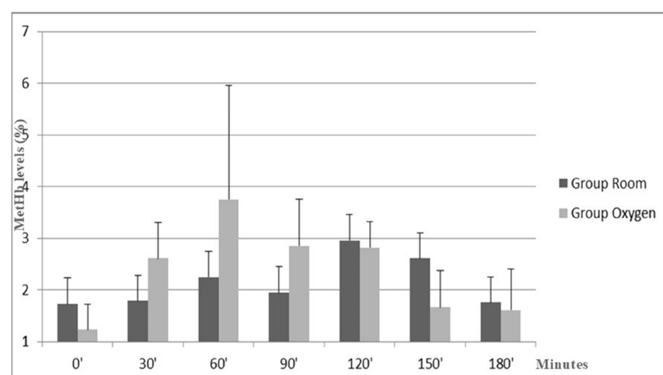

Figure 3. MetHb results measured at 30 min intervals before prilocaine administration (0 min) and after prilocaine administration (180 min) in the room group and O2 group.

180 minutes, although it is believed that collecting blood intracardially caused the death of rats due to intrapericardial/intrathoracic bleeding and pneumothorax, resulting from both the volume of blood lost and the trauma inflicted on the cardiac muscles. The highly toxic effect of prilocaine, the invasiveness of intracardiac blood collection, and the large amounts of blood lost led to rat deaths, making it more difficult to formulate a study model, thus requiring a change in the study protocol.

Accordingly, we reduced the amount of blood samples collected intracardially for blood gas measurements prior to prilocaine administration, and after anesthesia and analgesia were ensured under room air conditions. The volume of the blood samples was reduced to one-third of the amounts collected in the preliminary study. For the main study, it was envisaged that the rats would be given 100 mg/kg prilocaine intraperitoneally, and that 200 µl blood samples would be collected from the tail vein every 30 minutes throughout the 180-minute period. It was also planned that final blood samples would be collected intracardially at the end of the study (at minute 180) to examine blood gases. For the time prior to prilocaine administration and the 180 min, 200 µl blood samples were planned to be collected from the tail vein every 30 minutes for MetHB measurements through spectrophotometric methods. Since the prilocaine doses to be given in the main study were not excessive (volume-wise), we decided to use Priloc®, which is a form of prilocaine commonly used in anesthesia practice.

The pulse oximeter is designed to detect only reduced and oxygenized species of Hb, since the presence of other Hb species can cause misreading of O2 saturation. MetHb absorbs both red and infrared light in the same proportion, so the absorption ratio is 1:1, corresponding to 85 percent saturation. (The algorithms used for SpO2 calculation will show O2 saturation as 85% when the absorption ratio is 1.) When SaO2 falls below 85% under high MetHb levels, SpO2 is misread as being higher than it is (10). Despite low SaO2

levels, SpO2 rarely falls below 85% in methemoglobinemia (11). Only in the room air group did the average SpO2 level fall below 85% in our experimental study at minutes 0, 15, and 30. In a similar study, methemoglobinemia was induced in pigs with 15mg/kg of 4-dimetylhlaminophenol, and the effects of hyperoxia on O2 transport, tissue oxygenation, and survival were investigated. In the previous study, following the application of hyperoxia to seven rats under room air conditions and seven rats under ventilation, no increase in SpO2 was observed, despite the rise in dissolved O2 in the plasma (12).

Higher MetHb levels lead to lower PaO2 and SaO2 levels, which do not correlate with the SpO2 levels seen in the pulse oximeter. In methemoglobinemia cases, the SpO2 levels recorded by the pulse oximeter may be misleading, thus making it more important to determine exact oxygenation levels through blood gas measurements (13). However, our preliminary study demonstrated the difficulty in collecting arterial blood samples, while collecting blood through a percutaneous intracardial approach led to mixed (both arterial and venous) blood collections. This modification to our study is one of its limitations. The inability to demonstrate statistically the SpO2 increase observed in the room air group may have stemmed from misreading caused by MetHb.

In our study, HR levels were recorded every 15 minutes, starting from the moment prior to prilocaine administration and up until the 180th minute of the study. However, the intragroup and intergroup comparisons of the rats revealed no statistically significant differences (p>0.05). Significant differences in HR values and large standard deviations between rats may be due to differences in depth of anesthesia or invasiveness of the intracardiac blood collection method. In an experimental study in which hyperoxygenation was applied to rats with fatal methemoglobinemia, it was shown that hyperoxygenation did not result in a significant difference in HR and macrohemodynamic response (12).
At the start of the study, both groups had similar pre-prilocaine MetHb levels. In the room air group, the MetHb increase peaked at minute 120, while in the O2 group, the MetHb increase peaked at minute 60. This difference may be due to the metabolic differences of the experimental rats, or to differences in the absorption of the intraperitoneally administered drug.

Various studies have shown that hypoxia caused by carbon monoxide (CO) intoxication, which is a similar condition to methemoglobinemia, can actually be treated more effectively and over shorter periods of time with O2 support. The primary treatment of toxic methemoglobinemia involves methylene blue or ascorbic acid, and hyperoxygenation through 100 percent O2 treatment is later added during the treatment period to promote tissue oxygenation. However, no studies have been conducted to date to investigate the efficacy or effect on treatment duration of 100 percent O2 application for the treatment of hypoxia caused by methemoglobinemia.The role of hyperbaric O2 in methemoglobinemia treatment is also rat of debate (14-16). In the experimental study model, it was envisaged that 100 percent O2 hyperoxygenation would have a positive impact on the treatment period of hypoxia resulting from methemoglobinemia.

Increasing the quantity of dissolved O2 carried by plasma can lead to two different results with regards to O2 transport and the prevention of tissue hypoxia: inflammatory changes in the alveocapillary membrane due to pulmonary O2 toxicity (17); and hyperoxic arteriolar vasoconstriction (18-20). An earlier study showed that pulmonary O2 toxicity can develop as a result of long-term hyperoxygenation (>17 hours) (17). As the study duration was 180 minutes, which limited the exposure of the rats and collecting blood intracardially caused the intrapericardial/intrathoracic damage, we did not show that no inflammatory changes in the alveocapillary membrane as a result of O2 toxicity occurred in the present study. On the other hand, hyperoxic arteriolar vasoconstriction may develop within a short time following hyperoxic ventilation (21), which leads to a decrease in the amount of blood reaching the capillaries, thus reducing capillary blood perfusion, and increasing the risk of tissue hyperoxia (18-22).

While there have been numerous studies on the use of methylene blue and ascorbic acid for the treatment of methemoglobinemia, there have been very few on the use of 100% O2 application in the treatment of the condition (17). In an experimental study investigating the effects of hyperoxygenation on O2 transport, tissue oxygenation, and survival in pigs induced with fatal methemoglobinemia, hyperoxygenation was shown to have no discernible benefit on O2 transport and tissue oxygenation. The fact that half of the rats under room air conditions (n=3) died at the end of the study period, while all of the rats (n=6) in the O2 group survived until the end of the study demonstrates that, similar to another study on pigs, the application of O2 effectively lengthens survival (12). Another factor with the potential to affect survival may be anemia resulting from blood collection, although the rats that died in the room air group, namely, rats 1, 3 and 4 had hematocrit levels above 30 %. On the other hand, cardiac injury and pneumothorax due to intracardiac blood collection are the other factors affecting survival.

There were no significant differences in the MetHb

degradation rates of rats treated with 100% O2 in our study. One of the primary goals in this study was to calculate the degradation curves. However, the fact that the MetHb levels were too widely distributed prevented the formation of a graph similar to those used to illustrate the degradation of a pharmacological drug.

CONCLUSION

The aim of the present study, which was conducted on healthy experimental rats with no prior pathologies, was to demonstrate the potential benefit of O2 treatment in patients with no systematic diseases who developed toxic methemoglobinemia due to the administration of various drugs or agents. However, since the selected laboratory rats were found to be unsuitable for the study, in that the high prilocaine doses applied resulted in mortality rate before even inducing methemoglobinemia the study was unable to achieve its aims and objectives. Additionally, the method of blood collection with selected intracardiac tract affects the mortality with cardiac and thoracic trauma. The application of 100 mg/kg prilocaine resulted in the death of half the rats in the room air group. While the 100 percent O2 application obtained better survival results, there is a need to accurately examine other factors (central venous pressure, hct, SaO2, PaO2, PaCO2, pH, etc.) that may influence the interpretation of the results. The present study was beneficial in terms of showing how to develop study procedures, and in highlighting the negative and problematic aspects to avoid when designing similar studies in the future.

Ethics Committee Approval: This study was approved by the ethics committee of Bezmialem Vakif University with approval number 2012/423.

Authorship Contributions:
Idea/Concept: MK, Design: MK, Supervision: MK, Data Collection or Processing: MK, Analysis or Interpretation: MK, Literature Search: MK, Writing: MK, Critical Review: MK, References And Fundings: MK, Materials: MK.

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