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N-Nitrosylation potential of mono-*N*-desethylamiodarone at physiological pH

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Abstract

Amiodarone (AMI) is frequently used for the treatment of supraventricular arrhythmias. The parent drug is rapidly dealkylated to mono-*N*-desethylamiodarone (MDEA) and the plasma concentrations of AMI and MDEA are comparable. MDEA is a secondary amine and may thus undergo formation to the corresponding *N*-nitrosamine in combination with coadministered nitrovasodilators. Previous studies have shown that nitrovasodilators release the vasoactive NO• which may nitrosylate thiol or secondary amine groups in aqueous solutions. Therefore, the nitrosylation potential of MDEA at physiological pH was investigated. *N*-Nitroso-monodesethylamiodarone (NO-MDEA) was synthesized, characterized and used as a reference product for the detection of the corresponding *N*-nitrosamine. HPLC and NMR results have shown that the NO-MDEA product is an equilibrium of two configurational isomers (*syn* and *anti*). NO-release was generated by sodium nitroprusside (SNP) which was exposed to light. The formation to NO-MDEA was assayed by HPLC-UV. It has been found that MDEA is nitrosylated in the higher nanomolar range and that varying oxygenation of the reaction mixture did not significantly affect the reaction yields. The addition of thiols such as serum albumin (0.6 mM), L-cysteine (2.5 mM) or *N*-acetylcysteine (2.5 mM) inhibited the NO-MDEA formation indicating that they may prevent *N*-nitrosamine formation in vivo. However, as *S*-nitrosothiols may also release NO•, in long term exposure to elevated levels of nitric oxide the nitrosylation of secondary amines may be taken into account.

Keywords: Amiodarone (AMI); Mono-*N*-desethylamiodarone (MDEA); *N*-Nitroso-monodesethylamiodarone (NO-MDEA); Nitric oxide (NO•); *N*-Nitrosylation of secondary amines

1. Introduction

Amiodarone (AMI) is a potent drug for the treatment of supraventricular arrhythmias [1–3]. In humans AMI is rapidly dealkylated to its main metabolite mono-*N*-desethylamiodarone (MDEA) [4,5]. Plasma concentrations of MDEA may be higher than those of AMI [6]. Furthermore, AMI and MDEA have a high plasma protein binding ratio [7] and are accumulated in tissues [8].

Supraventricular arrhythmias may be caused by a myocardial infarct and can include the coadministra-

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tion of AMI with nitrovasodilators such as glyceryl trinitrate, isosorbiddinitrate or sodium nitroprusside (SNP). The mechanism of its vasodilative action is based on the release of nitric oxide ($NO^{\bullet}$) which functions as an endothelium derived relaxing factor (EDRF) [9]. In aqueous solutions nitric oxide may undergo hydrolysis to nitrous acid or form nitrosylating agents such as nitrous anhydride (N_2O_3) [10,11].

N-Nitrosamines were usually synthesized under acidic conditions [12] but the nitrosylation of secondary amines at higher pH has been investigated as well. First, the potential of *N*-nitrosamine formation was investigated by the reaction of gaseous nitrogen oxides ($NO^{\bullet}$, N_2O_3 and N_2O_4) and amines [10]. Later the kinetics of the nitrosylation of morpholine was investigated at physiological pH and the influence of phosphate and chloride ions was determined [11]. Recently, it was observed that the formation of *N*-nitrosamines and *N*-nitramines occurs by the reaction between secondary amines and peroxynitrite or released $NO^{\bullet}$ and superoxide [13].

In human plasma $NO^{\bullet}$ may react rapidly with oxyhemoglobin to nitrate and methemoglobin [14,15]. Another pathway of nitric oxide was found to be the binding to deoxy-hemoglobin to form nitrosyl-hemoglobin (HbFe(II)NO). It has been shown that nitrosyl-hemoglobin further reacts to *S*-nitrosohemoglobin where $NO^{\bullet}$ is bound to the β-93 cysteine of hemoglobin [16–18] which in contrast to the oxyhemoglobin reaction preserves $NO^{\bullet}$. The binding location of hemoglobin within erythrocytes prevents the rapid decay of the plasma $NO^{\bullet}$ concentration [19]. The formation of *S*-nitrosothiols depends on the rate of NO-release [20] suggesting that this is also the case for the nitrosylation of secondary amines. Furthermore, the formation of *N*-nitrosamines was investigated by incubating morpholine with macrophages. This experiment revealed that *N*-nitrosomorpholine was formed by released nitrogen species (derived from L-arginine) and not from the nitrite dissolved in the cell culture medium [21,22].

The present study was designed to examine the potential of MDEA to react to the corresponding *N*-nitrosamine at physiological pH. *N*-Nitrosomonodesethylamiodarone was synthesized and characterized by HPLC–ESI–MS and NMR. The synthesized product was then used as a reference to measure the nitrosylation of MDEA.

2. Materials and methods

2.1. Chemicals

Mono-*N*-desethylamiodarone [2-*n*-butyl-3-(3,5-diiodo-4-β-ethylaminoethoxybenzoyl)benzofuran hydrochloride] was a kind gift of the Sanofi-Synthélabo Research Laboratories, Montpellier, France. Sodium nitroprusside and di-sodium hydrogen phosphate were obtained from Merck, Darmstadt, Germany. Dichloromethane, ethyl acetate, methanol and acetonitrile were purchased from Scharlau, Barcelona, Spain. Cysteine, *N*-acetylcysteine, amonium acetate, sodium acetate, sodium nitrite, sodium sulfate, acetic acid, sulfanilic acid, 1-naphthylamine, hydrochloric acid, *n*-hexane and potassium carbonate were ordered from Fluka Chemicals, Buchs, Switzerland. Serum albumin (bovine) was purchased from Sigma Chemicals, Buchs, Switzerland.

2.2. HPLC-UV

HPLC-UV measurements were performed on a Merck–Hitachi (Darmstadt, Germany) La Chrom HPLC system equipped with a L-7100 pump, a UV-detector L-7400, an autosampler L-7200 and an interface D-7000 with a 250 mm × 4 mm Merck LiChroCART column packed with LiChrospher 60 RP-select B (particle size 5 μm) as a stationary phase. The mobile phase consisted of a phosphate buffer (pH 6; 20 mM)–methanol–acetonitrile (19:66:15, v/v/v) mixture with a flow rate of 1 ml/min. The detection wavelength was 242 nm and the pressure 170 bar (room temperature).

2.3. HPLC–ESI–MS

HPLC–ESI–MS experiments were performed on an HP 1100 HPLC system (Hewlett-Packard, Palo Alto, CA, USA). The assay was operated under the following conditions: HPLC column (RP-C8 Waters Symmetry 150 mm × 2 mm; Waters, Milford, MA, USA) maintained at 40 °C; variable-wavelength detector setting at 242 nm. The mobile phase consisted

of NH_4OAc (50 mM) as solvent A and acetonitrile as solvent B. The gradient started from 60% solvent A and 40% solvent B and increased to 100% solvent B in 9 min. The final composition of 100% solvent B and 0% solvent A was kept until 30 min of total run time elapsed. The ESI–MS detector was interfaced directly to the output of the UV. ESI–MS conditions were similar to those reported previously [23]. The ion-polarity was operated with negative-mode (cap exit offset: −65 V; skimmer 1: −15 V). Acquisitions were performed in the range between *m*/*z* 50 and 1000 and six spectra were averaged.

2.4. NMR-measurements

^1H, ^{13}C and inverse gated ^{13}C NMR spectra were recorded on a Bruker AMX-300 spectrometer (operating at 300.13 MHz for ^1H and at 75.47 MHz for ^{13}C) at 298 K. DQF-COSY-, HMBC- and HSQC-spectra were recorded on a Bruker DRX-500 spectrometer (operating at 500.13 MHz for ^1H and at 125.77 MHz for ^{13}C) at 295 K. The samples were measured in $CDCl_3$ and the residual resonance of the solvent was used as internal reference.

2.5. Synthesis of N-nitroso-monodesethylamiodarone (NO-MDEA)

NO-MDEA (2-*n*-butyl-3-(3,5-diiodo-4-β-*N*-nitroso-ethylaminoethoxybenzoyl)benzofuran) was synthesized according to methods described previously for *N*-nitrosodiphenylamine [24]: 16.2 mg (0.025 mmol) of MDEA hydrochloride (2-*n*-butyl-3-(3,5-diiodo-4-β-ethylaminoethoxybenzoyl)benzofuran hydrochloride) were dissolved in 0.1 ml of dichloromethane before adding 0.3 ml of glacial acetic acid. Then 40 μl of a 5 M solution of $NaNO_2$ were pipetted into the solution and the progress of the reaction was monitored by TLC (*n*-hexane/ethylacetate 85:15, v/v). The R_f-value of the formed product was 0.3. After 2 h of reaction 10 ml of dichloromethane were added to the mixture which was then washed with 70 ml of saturated potassium carbonate. The organic phase was dried (Na_2SO_4) and evaporated under reduced pressure to give a viscous and colorless oil. Yield: 14.9 mg (0.023 mmol; 93%). NMR (for numbering see Fig. 3): δ_H [300 MHz, $CDCl_3$] *syn*-NO-MDEA: 0.92 (3H, t, *J* 7.3 Hz, C(1)), 1.37 (2H, m, *J* 7.6 Hz, C(2)), 1.54 (3H, t, *J* 7.3 Hz, C(21)), 1.78 (3H, m, *J* 7.6 Hz, C(3)), 2.86 (2H, t, *J* 7.7 Hz, C(4)), 4.09 (2H, t, *J* 4.5 Hz, C(19)), 4.15 (2H, t, *J* 5.0 Hz, C(18)), 4.46 (2H, q, *J* 7.3 Hz, C(20)), 7.25 (1H, ddd, *J* 7.7, 7.3, 0.9 Hz), 7.32 (1H, ddd, *J* 8.3, 7.1, 1.1 Hz), 7.38 (1H, d, *J* 7.5 Hz), 7.49 (1H, d, *J* 8.3 Hz), 8.23 (2H, s, C(15)); *anti*-NO-MDEA: 0.92 (3H, t, *J* 7.3 Hz, C(1)), 1.21 (3H, t, *J* 7.2 Hz, C(21)), 1.37 (2H, hex, *J* 7.6 Hz, C(2)), 1.78 (2H, quin, *J* 7.6 Hz, C(3)), 2.86 (2H, t, *J* 7.7 Hz, C(4)), 3.90 (2H, q, *J* 7.2 Hz, C(20)), 4.41 (2H, t, *J* 5.1 Hz, C(18)), 4.65 (2H, t, *J* 5.2 Hz, C(19)), 7.25 (1H, ddd, *J* 7.7, 7.3, 0.9 Hz), 7.32 (1H, ddd, *J* 8.3, 7.1, 1.1 Hz), 7.38 (1H, d, *J* 7.5 Hz), 7.49 (1H, d, *J* 8.3 Hz), 8.23 (C(15)H-, s); δ_C [75.47 MHz, $CDCl_3$] *syn*-NO-MDEA: 13.7 (q, $C(1)H_3$), 14.1 (q, $C(21)H_3$), 22.5 (t, $C(2)H_2$), 28.2 (t, $C(4)H_2$), 30.0 (t, $C(3)H_2$), 43.6 (t, $C(19)H_2$), 49.2 (t, $C(20)H_2$), 69.1 (t, $C(18)H_2$), 90.6 (d, C(16)I), 111.1 (d, C(7)H), 115.7 (s, C(12)), 121.0 (d, C(10)H), 123.9 (d, C(9)H), 124.7 (d, C(8)H), 126.3 (s, C(11)), 138.8 (s, C(14)), 140.7 (d, C(15)H), 153.6 (s, C(6)), 160.0 (s, C(17)), 166.3 (s, C(5)), 187.6 (s, C(13)); *anti*-NO-MDEA: 11.2 (q, $C(21)H_3$), 13.7 (q, $C(1)H_3$), 22.5 (t, $C(2)H_2$), 28.2 (t, $C(4)H_2$), 30.0 (t, $C(3)H_2$), 40.7 (t, $C(20)H_2$), 51.2 (t, $C(19)H_2$), 71.6 (t, $C(18)H_2$), 90.6 (d, C(16)I), 111.1 (d, C(7)H), 115.7 (s, C(12)), 121.0 (d, C(10)H), 123.9 (d, C(9)H), 124.7 (d, C(8)H), 126.3 (s, C(11)), 138.8 (s, C(14)), 140.7 (d, C(15)H), 153.6 (s, C(6)), 160.0 (s, C(17)), 166.3 (s, C(5)), 187.6 (s, C(13)); LC–ESI–MS $[M - H]^-$ *m*/*z* 645 (100%; ret. time 15.6–15.8 min), *m*/*z* 645 (100%; ret. time 16.0–16.2 min).

2.6. $NO^\bullet$ release from SNP

A 10 mM solution of SNP in phosphate buffer (pH 7.4; 10 mM) was exposed to laboratory light conditions. After 0, 1, 2, 5, 10, 15, 20, 25, 30 and 50 min 1 ml of the solution were transferred to the prepared Griess-reagent mixture consisting of 125 μl sulfanilic acid (30 mM; dissolved in 0.2*N* hydrochloric acid), 125 μl 1-naphthylamine (40 mM; dissolved in 0.12*N* hydrochloric acid), 125 μl sodium acetate solution (25%), 1 ml acetic acid (96%) and 125 μl of deionized water. The glass tubes were protected against light exposure to prevent further release of nitric oxide. A buffer solution without SNP was used

as blank solution. The absorbance was measured at $\lambda = 525$ nM in a polystyrene cuvette. The system was previously calibrated with dissolved $NaNO_2$ in increasing concentrations. The data points were fitted by linear regression analysis using Origin Software (Redacom, Nidau, Switzerland).

2.7. N-Nitrosylation of MDEA

Ten millilitres of phosphate buffer (pH 7.4; 10 mM) were introduced into a 50 ml flask and purged with nitrogen for 5 min. Then 10 ml of air purged phosphate buffer (pH 7.4; 10 mM) were pipetted into the flask (dilution of oxygenated solution to levels measured in arterial plasma [25]). MDEA was dissolved in ethanol and added to the stirred buffer solution to result in a concentration of 2 μM which corresponds to levels measured in human plasma [6]. The reaction was run in laboratory light conditions and was started by the addition of 59.6 mg of SNP (10 mM). After 10 min the reaction was stopped by extracting the solution with two times with 20 ml of dichloromethane. The organic phase was collected in a 100 ml flask and evaporated under reduced pressure at 40 °C. Then the residue was dissolved in two times 3 ml of dichloromethane. The dichoromethane solution was collected in a reaction tube and evaporated under a flow of nitrogen. The residue was dissolved in 200 μl of mobile phase (HPLC-UV), vortexed and centrifuged before injecting 70 μl into the HPLC.

2.8. Oxygen dependence

The oxygen dependence of the reaction was investigated by mixing varying volumes of air- or nitrogen-purged phosphate buffer (pH 7.4; 10 mM) assuming that the oxygen concentration in the air purged buffer solution corresponds to tabulated values of oxygen solubility in aqueous solutions at room temperature (250 μM). Removal of oxygen was achieved by purging the reaction solution with nitrogen for 10 min. The reactions were run and analyzed as described above.

2.9. Influence of high and low molecular weight thiols

After pipetting the oxygenated phosphate buffer (pH 7.4; 10 mM) to the nitrogen purged buffer solution the thiols such as serum albumin (792 mg; 600 μM), L-cysteine (6.1 mg; 2.5 mM) or *N*-acetylcysteine (8.2 mg; 2.5 mM) were added and dissolved by stirring before the addition of MDEA and SNP. The reactions were run and analyzed as described above. Reactions with serum albumin were extracted with diethylether instead using dichloromethane.

2.10. Peak identification by HPLC-ESI–MS

The identity of the measured peaks was additionally investigated by HPLC–ESI–MS. Therein, the AMI-derivatives of the solution (0.5 ml) were extracted with diethylether (three times 1 ml) and the combined organic phase was evaporated to dryness. The samples were stored at −20 °C and analyzed within 24 h.

3. Results

3.1. Synthesis of NO-MDEA

The product of the *N*-nitrosylation of MDEA was a mixture of two compounds as can be seen in the ^{1}H NMR spectrum (Fig. 1) and the HPLC-UV chromatogram (Fig. 2). Previous work has shown that *N*-nitrosamines contain partial double-bond character as predicted from one of their resonance structures (⇄ N^+=N–O^-) leading to a high rotation barrier of the N–N-bond of ca 23–25 kcal/mol [26–29]. Thus, it was suspected that the two peaks detected by HPLC represent the *syn*- and *anti*-forms of *N*-nitroso-monodesethylamiodarone (NO-MDEA) (Fig. 3). ^{1}H NMR analysis of the synthesis product mixture resulted in the detection of eight signals for the protons 18–21 (arbitrarily numbered according to [23]). Based on chemical shifts and spin system information, proton frequencies could be easily assigned to the butyl, the ethyl and the ethylene moieties. Therein, resonances were then assigned by standard correlation techniques. Thereafter, the carbon chemical shifts were determined from the HSQC and HMBC spectra. The complete assignment of ^{1}H and ^{13}C frequencies of the ethyl and ethylene moieties is presented in Table 1. As shown in Fig. 1 the signals at 4.09 and 4.14 ppm are more intense than those at 4.41 and 4.65 ppm. We suspected that these pairs belong to either the *syn*- or the *anti*-configurational isomer of the molecule. Data from DQF-COSY spectrum

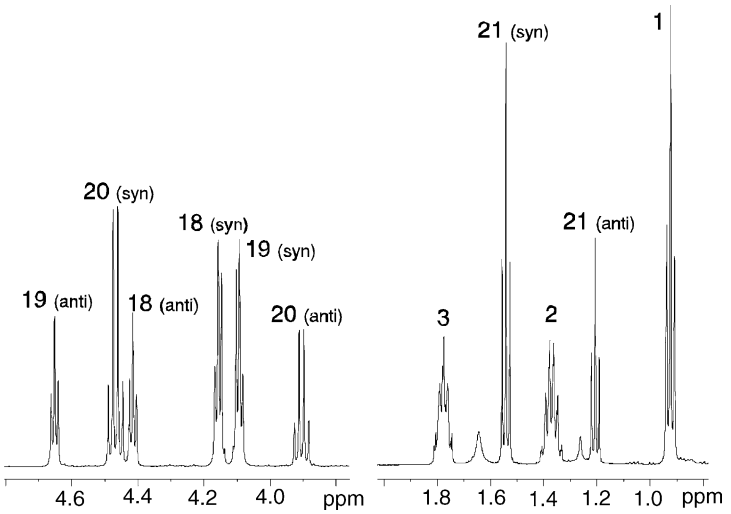

Fig. 1. ^{1}H NMR spectrum of the protons attached to carbons 18–21 (and 1–3) of the configurational mixture of NO-MDEA (*syn* and *anti*) synthesis. Carbons were numbered according to [23].

did not show any cross-correlation peaks between the two pairs of signals indicating that the protons are located in different molecules. The assignment of the signals to either the *syn*- or *anti*-configuration could only be achieved by comparing the ^{1}H- and ^{13}C-chemical shifts with published data. It is known that α-protons and α-carbons situated *anti* to the N–NO group are shifted further downfield than the atoms in *syn*-position [30]. ^{13}C NMR chemical shifts of carbon atoms in α- or β-position to the nitrogen atom of *anti*- or *syn*-positioned ethylgroups are published [31]. The α-carbon chemical shifts for heterocyclic compounds (*syn*: 39.2 ppm; *anti*: 50.2 ppm) were close to those published before [30] indicating that primarily the conformation of the nitroso-group influences the chemical shifts. HSQC, HMBC and DQF-COSY measurements support the assumption that the protons attached to C-18 resonate at 4.14 ppm in case of the *syn*- and at 4.41 ppm in case of the *anti*-isomer. These data suggest that the product mixture obtained from chemical synthesis can be

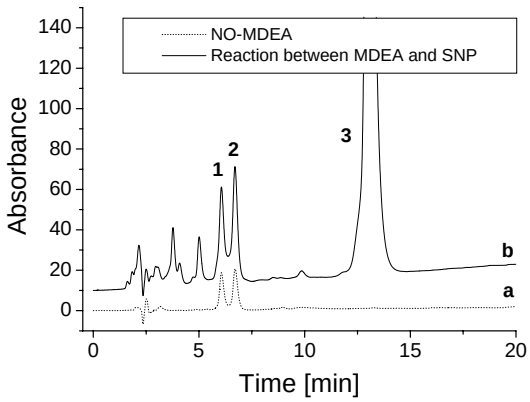

Fig. 2. HPLC-UV chromatogram of MDEA where peaks 1, 2 and 3 eluted at 6.1, 6.7 and 13.0 min and were identified as *anti*-NO-MDEA, *syn*-NO-MDEA and MDEA, respectively. Chromatogram (a) represents the injection of synthesized NO-MDEA dissolved in mobile phase while (b) is the chromatogram of the extract of the reaction of 2 μM MDEA with 10 mM SNP after 10 min (dissolved in mobile phase).

Table 1
^{1}H NMR chemical shifts of the protons bound to carbons C(18) to C(21) of nitrosylated MDEA conformeres expressed in ppm with the corresponding coupling constant in Hz (in parenthesis) in combination with chemical shifts obtained from ^{13}C NMR (in ppm relative to TMS)

Carbon no.	^{1}H NMR		^{13}C NMR	
	Syn	*Anti*	*Syn*	*Anti*
18	4.15 ($J = 5.0$)	4.41 ($J = 5.1$)	69.1	71.6
19	4.09 ($J = 4.5$)	4.65 ($J = 5.2$)	43.6	51.2
20	4.46 ($J = 7.3$)	3.90 ($J = 7.2$)	49.2	40.7
21	1.54 ($J = 7.3$)	1.21 ($J = 7.2$)	14.1	11.2

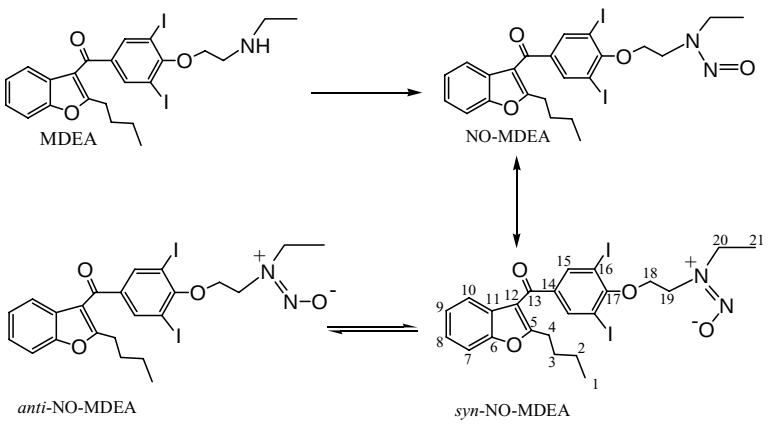

Fig. 3. Nitrosylation of MDEA is either obtained from chemical synthesis or by incubation of the substrate in phosphate buffer (pH 7.4; 67 mM) together with SNP. The mesomeric structure of the formed *N*-nitrosamine favors the configurational isomers denoted as *syn*- and *anti*-NO-MDEA.

explained by the equilibrium of two configurational isomers of the corresponding *N*-nitrosamine.

Integration of ^{1}H NMR peaks (Fig. 1) revealed that the mixture consists of about 58% *syn*- and 42% *anti*-NO-MDEA. Assuming that the two NO-MDEA conformeres have the same response in HPLC-UV analysis, the *syn*-NO-MDEA has the higher retention time compared to *anti*-NO-MDEA. In HPLC–ESI–MS analysis, quasi-molecular ions of ($[M - H]^-$) at *m*/*z* 645 were found for both peaks. This may correspond to the expected values for NO-MDEA.

3.2. Formation of NO-MDEA

It is known that nitric oxide is released from SNP by exposure to light [32,33]. As NO$^\bullet$ reacts to nitrite in oxygenated aqueous solutions the release was calibrated indirectly using the Griess reaction. The release was estimated to be about 0.5 μM/min. A blank solution (protection from light) did not show any formation of nitrite suggesting that no NO$^\bullet$ is released from SNP in darkness. The release per time was linear during the time of the measurement (30 min).

As shown in Fig. 2, the HPLC-UV analysis of the extracts of the reaction mixtures containing MDEA and SNP revealed the formation of two peaks at 6.1 and 6.7 min. Those coeluted with synthesized reference NO-MDEA. In HPLC–ESI–MS measurements, the quasi-molecular ions ($[M - H]^-$) of both peaks were detected at *m*/*z* 645 in agreement with the results for synthesized NO-MDEA. These results suggest that the peaks detected at 6.1 and 6.7 min of retention time correspond to *anti*- and *syn*-NO-MDEA, respectively.

The time dependence (Fig. 4A) revealed a linear formation rate of combined *syn*- and *anti*-NO-MDEA for the initial 30 min. under the present reaction conditions MDEA was nitrosylated by about 10 nM/min. No NO-MDEA peaks were detected at 0 min of reaction time indicating that the extraction procedure did not allow the reaction to proceed.

Surprisingly the variation of the oxygenation of the reaction solution (Fig. 4B) did not cause major changes in the reaction yield. Moreover, the highest amounts of nitrosylated MDEA after 10 min of reaction time were achieved in nitrogen purged reaction buffer. However, it has been proposed that the nitrosylation of secondary amines does not run under strictly anaerobic conditions [11]. In the present experiment, traces of oxygen may have been sufficient since the release of NO$^\bullet$ was measured to be low.

The variation of the MDEA concentration (Fig. 4C) has shown that the increase of the MDEA concentration may affect an increased production rate of the NO-MDEA conformeres. This observation may as well be related to the limitation of the concentration of nitric oxide.

The influence of high and low molecular weight thiols was examined by adding either 0.6 mM serum albumin (bovine), 2.5 mM cysteine or 2.5 mM

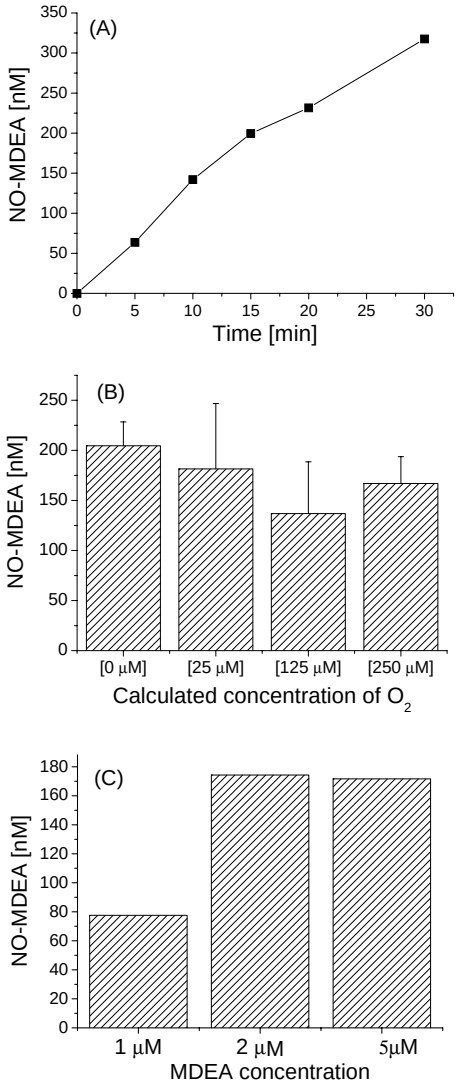

Fig. 4. MDEA was incubated in phosphate buffer (pH 7.4; 10 mM) with SNP under laboratory light conditions where (A) shows the time dependence of the nitrosylation of MDEA to *syn*- and *anti*-NO-MDEA. The reaction was further investigated concerning (B) the addition of varying concentrations of oxygen ($n = 3$; for 125 μM oxygen: $n = 5$) and (C) varying initial MDEA concentration.

N-acetylcysteine to the reaction mixture. In this reactions the nytrosylated MDEA products were below the detection limits indicating that the nitric oxide merely formed *S*-nitrosothiols with the introduced thiol groups (results not shown).

4. Discussion

It was our aim to investigate the *N*-nitrosylation potential of MDEA in physiological solutions. MDEA is a secondary amine and the major metabolite of a cardiovascular drug. The reaction parameters were adapted to physiological conditions concerning the concentrations of MDEA, phosphate and oxygen. However, changes of parameters such as the concentrations of phosphate and chloride may influence the nitrosylation of secondary amines at physiological conditions. In the present assay serum albumin as well as cysteine and *N*-acetylcysteine were successful to inhibit the nitrosylation of MDEA. It is known that thiols may be rapidly nitrosylated but may also release $NO^{\bullet}$ [34].

As can be seen in the experiments of this work nitric oxide is a highly reactive molecule and the amount of MDEA nitrosylation appears to depend strongly on the presence of $NO^{\bullet}$ in the reaction solution. The nitrosylating agent is believed to be N_2O_3 [11] while it was described that anions such as phosphate or chloride may acts as inhibitors of this reaction to form nitrosyl anions (XNOs).

Elevated levels of $NO^{\bullet}$ may arise by the administration of nitrovasodilators which are metabolized rapidly [35–38]. One part the released nitric oxide will be degraded by the reaction with oxyhemoglobin while another part will react with thiol groups. It has been found that thiol groups may function as carriers of $NO^{\bullet}$ in the human body. The release of $NO^{\bullet}$ from the *S*-NOs may result in a steady state level of nitric oxide in blood plasma. Consequently the nitrosylation of other thiol groups or secondary amines may occur.

Between 120 and 150 μM of oxygen have been found in human arterial blood. The present experimental conditions were adapted to this concentration. However, at low levels of $NO^{\bullet}$ the nytrosylation of secondary amines may not be prevented by low oxygen levels.

5. Conclusions

The nitrosylation amount of MDEA will depend on its local plasma concentration together with peak $NO^{\bullet}$ concentrations. Thiol groups may bind NO before

its releasing and thus may lower the reaction rate of NO-MDEA formation. *N*-Nitrosamines are more stable than *S*-nitrosothiols and therefore, reactions with longer duration time may lead to an increased formation of those compared to the present experimental conditions.

MDEA plasma levels were measured to be 2 μM while a larger fraction of MDEA and its parent drug AMI is bound to serum albumin [7] or accumulated in tissues e.g. adipose, heart or lung tissue [8]. AMI's pulmonary toxicity is the most feared adverse effect. In addition, the lung may as well be a way for $NO^{\bullet}$ to enter the human body.

Finally, one can state that the nitrosylation of a secondary amine such as MDEA may be taken into consideration. AMI and MDEA are highly lipophilic which leads to a long terminal elimination half life of these substrates. The conformeres of NO-MDEA are more hydrophilic as can be seen from the significantly shorter retention times in RP-HPLC analysis. Therefore, they may be eliminated more rapidly.

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