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Short Communication

Platelet antiaggregatory effects and haemodynamic activity of two terfuroxan isomer pairs

Clara Cena [a], Sonja Visentin [a], Annalisa Gasco [b], Piero Antonio Martorana [c,1], Roberta Fruttero [a], Alberto Gasco [a,*]

[a] *Dipartimento di Scienza e Tecnologia del Farmaco, Università degli Studi di Torino, via Pietro Giuria 9, 10125 Turin, Italy*
[b] *Azienda Ospedaliera San Giovanni Battista, corso Bramante 88, 10126 Turin, Italy*
[c] *Dipartimento di Fisiopatologia e Medicina Sperimentale, Università di Siena, via Aldo Moro, San Miniato, Siena, Italy*

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Abstract

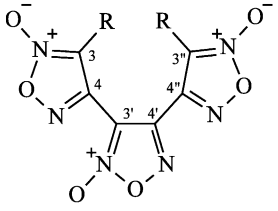
A couple of terfuroxan isomer pairs **1a**,**b** and **2a**,**b** were studied for their in vivo vasodilating activity and in vitro antiplatelet action. The haemodynamic profiles of the products resemble that of other NO-donors. Their in vitro antiaggregating activity is influenced both by terfuroxan system and by the substituents. © 2002 Published by Éditions scientifiques et médicales Elsevier SAS.

Keywords: NO; NO-donors; Furoxans; Platelet aggregation; In vivo haemodynamic effects

1. Introduction

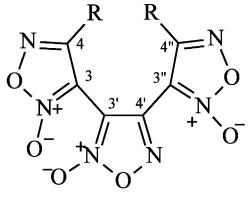
Nitric oxide (NO) is a biological messenger which triggers a wide range of physiological and pathological effects. Endothelium derived relaxing factor (EDRF) is believed to be NO and plays a crucial role in the functioning of the vascular system. It helps to maintain microvascular and macrovascular homeostasis by several mechanisms including dilation of arterial blood vessels, inhibition of platelet adhesion and aggregation, attenuation of leucocyte adhesion and activation. Activation of soluble guanylate cyclase (sGC) is involved in all of these actions [1]. Various NO-prodrugs can mimic the EDRF functions. Typical examples are the nitrate esters, which can be biotransformed to NO in vivo [2]. Another interesting class of NO-donors is the furoxan derivatives [3]. These products are able to release NO in physiological conditions, under the action of thiol cofactors. The mechanism of this release is not yet well understood. It could involve more than one NO redox species. Compared with other NO-donors, furoxans can exhibit a very desirable pharmacological profile characterised by slow onset and long duration of action with no development of nitrate tolerance.

In previous works we described synthesis and in vitro vasodilating actions of many furoxans, including a series of terfuroxan derivatives [4]. These latter products behaved as potent in vitro vasodilators. In the present note we report on the in vivo cardiovascular effects of two selected terfuroxan isomer pairs (der.s **1a**,**b** and **2a**,**b**) and on their in vitro antiplatelet action.



R = CH_3 **1a**

$CH_2N(CH_3)_2 \cdot HCl$ **2a**



R = CH_3 **1b**

$CH_2N(CH_3)_2 \cdot HCl$ **2b**

* Corresponding author.
E-mail address: gasco@pharm.unito.it (A. Gasco).

[1] The in vivo part of this work has been carried out in the Department of Pharmacology, Cassella AG, Frankfurt/Main, Germany.

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2. Experimental procedures

2.1. Materials

Terfuroxan derivatives **1a**, **2a**, **1b**, **2b** were prepared according to the procedure previously reported [4]. Purity of the compounds was checked by HPLC (Merck Purospher RP-18 column, 250 × 4.6 mm; 5 μm particles size, 40 °C, elution at 1 ml/min with MeOH/H_2O 60/40 (v/v) for derivatives **1a** and **1b**, and with MeOH/H_2O 60/40 (v/v), TFA 0.1% for derivatives **2a** and **2b**).

2.2. Inhibition of platelet aggregation in vitro

Venous blood was obtained from consenting healthy human subjects who had not taken any drug for at least 2 weeks. Volunteers were informed that blood samples were obtained for research purposes and that their privacy would be protected. Experiments were carried out in accordance with the Helsinki Declaration of 1975. PRP was prepared by centrifugation of citrated blood at 200 × *g* for 20 min. Aliquots (300 μl) of PRP were added into an aggregometer cuvette (Elvi) and aggregation was recorded as increased light transmission under continuous stirring (1000 rpm) at 37 °C for 5 min after the addition of the stimulus. ADP was used at a supramaximal concentration (8–10 μM). The inhibitory activity of the compounds was tested by adding their solution or the solvent alone (DMSO) to PRP 5 min before the addition of the stimulus at 37 °C. Haemoglobin was added to the platelets 7 min before addition of the platelet activator at 40 μM final concentration, while ODQ was added to the platelets 15 min before ADP at 100 μM. The maximal amount (0.5%) of DMSO added to PRP did not affect platelet function. The antiaggregating activity of tested compounds was evaluated as the % of inhibition of platelet aggregation with respect to the control samples. When inhibition at maximal concentration exceeded 50%, pIC_{50} values were calculated by non-linear regression analysis.

2.3. Cardiovascular effects in vivo

Male pigs of the German land race (20–25 kg) were anaesthetised with ketamine (20 mg/kg, i.m.) (Hostaket, Hoechst, Frankfurt/Main, Germany), methomidate (10 mg/kg, i.p.) (Hypnodil, Janssen, Neuss, Germany), xylacine (3 mg/kg, i.m.) (Rompun, Bayer, Leverkusen, Germany) and pentobarbital (30 mg/kg, i.v., as a bolus followed by a continuous infusion at the rate of 0.16 mg/kg/min) Nembutal, Sanofi, Hannover, Germany). After tracheal intubation, the animals were artificially ventilated with ambient air enriched with oxygen to maintain blood gas parameters within normal limits. A catheter was placed into the right femoral artery to measure blood pressure (BPs, BPd) with a Statham 23 Db pressure transducer (Gould, Cleveland, OH, USA). Left ventricular enddiastolic pressure, contractility, and heart rate (LVEDP; dP/dt_{max}, HR) were measured with a Millar PC 350 catheter-tipmanometer (Millar Instr., Houston, TX, USA) introduced into the left ventricle via the left carotid artery. When stable haemodynamic conditions were achieved for at least 30 min, the compounds were administered suspended in methyl cellulose at the dose of 3 mg/kg, through a catheter inserted in the duodenum (i.d.). This dose was chosen according to the previously obtained results in the anaesthetised pig with other NO-donor furoxan derivatives [5,6]. The haemodynamic effects were recorded for 2 h. Two animals were used for each compound and data are presented as the mean of the results obtained in the two animals. In order to investigate a dose–response relationship, compound **1a** was also administered at the dose 1 mg/kg i.d. to two pigs.

3. Results

3.1. Inhibition of platelet aggregation in vitro

Antiaggregatory effects in vitro of derivatives **1a**,**b** and **2a**,**b** were evaluated on ADP-induced platelet aggregation of human platelet rich plasma (PRP). The experiments were repeated in the presence of 40 μM oxyhaemoglobin (HbO_2), a known scavenger of NO, and of 100 μM of ODQ, a potent inhibitor of sGC. The results are shown in Table 1, where antiaggregatory activity of SNP is also reported for comparison. Data are expressed as $IC_{50} \pm SEM$ or as the % of inhibition of aggregation at maximal concentration tested (100 μM) when IC_{50} could not be calculated. All the derivatives were able to inhibit the aggregation induced by ADP in PRP in a concentration-dependent manner. Analysis of these results by comparison with SNP taken as a reference shows that derivative **1a** behaves as the most potent inhibitor of the series on ADP-induced aggregation, and it is eight times more potent than SNP. Both derivatives **1b** and **2a** are equipotent with the reference compound, while derivative **2b** is about five times less potent than SNP.

3.2. Cardiovascular effects in vivo

The cardiovascular effects in vivo of the methylderivative isomers **1a**,**b** and of the dimethylaminomethyl isomers **2a**,**b** are shown in Table 2. Tested compounds, suspended in methyl cellulose, were given by the intraduodenal (i.d.) route of administration, which simulates the clinical peroral administration, to anaesthetised pigs. Administration of the vehicle alone did not induce haemodynamic changes.

Table 1
Antiaggregatory activity of the tested compounds on ADP-induced PRP aggregation

Comp.	Antiaggregatory activity				
	$+HbO_2$ 40 μM			+ODQ 100 μM	
	EC_{50} (μM) ± SEM	EC_{50} (μM) ± SEM	% Inhibition (100 μM) [a]	EC_{50} (μM) ± SEM	% Inhibition (100 μM) [a]
1a	0.67 ± 0.24	10.8 ± 4.7	[c]	7.7 ± 2.8	[c]
1b	6.7 ± 2.5	43.0 ± 7.4	[c]	[b]	24.1 ± 12.6
2a	6.8 ± 2.9	30.8 ± 10.9	[c]	69.7 ± 8.2	[c]
2b	24.6 ± 4.8	57.6 ± 11.8	[c]	[b]	12.4 ± 5.1
SNP	5.2 ± 1.5	[b]	15.4 ± 5.8	48.6 ± 13.4	[c]

[a] Maximal concentration tested.
[b] Aggregation did not reach 50% of control effect: pIC_{50}s could not be calculated.
[c] For these compounds a complete concentration–response curve could be performed, therefore pIC_{50} values are reported in the previous column.

Table 2
Maximal haemodynamic effects and duration of action of the compounds **1a**,**b** and **2a**,**b** given intraduodenally in the anaesthetised pig

Comp.	Dose (mg/kg) i.d. [a]	BPs [a] (Δ [a] mm Hg) (min)	BPd [a] (Δ mm Hg) (min)	LVEDP [a] (Δ mm Hg) (min)	dP/dt_{max} [a] (Δ mm Hg) (min)	HR [a] (Δ b/min) (min)
1a	3	−15 (>120)	−13 (>120)	−1 (120)	−300 (>120)	−5 (>120)
1b	3	−22 (>120)	−17 (>120)	−2 (>120)	−100 (120)	15 (>120)
2a	3	−14 (>120)	−12 (>120)	−1 (30)	−200 (>120)	2 (90)
2b	3	−28 (120)	−20 (120)	−4.5 (>120)	−200 (>120)	3 (120)

[a] Abbreviations: i.d. = intraduodenal administration; N = number of animals; Δ = change from the baseline value; BPs = systolic blood pressure; BPd = diastolic blood pressure; LVDEP = left ventricular enddiastolic pressure; dP/dt_{max} = index of contractility of the heart; HR = heart rate; number in brackets correspond to the duration of action in min.

Both methylderivative isomers had a similar haemodynamic profile. In this regard **1b** was slightly more potent than **1a** as shown by the effects on the systolic and diastolic blood pressure, as well as on the left ventricular enddiastolic pressure. Isomer **1a** had practically no effect on heart rate while **1b** slightly increased this parameter. The dose–response relationship on BPs investigated for the compound **1a** is shown in Fig. 1. The dimethylaminomethyl isomers also had similar cardiovascular effects. In this regard, **2b** was clearly more potent than **2a** on the systolic blood pressure, diastolic blood and left ventricular enddiastolic pressure. Both compounds slightly decreased the contractility of the heart and did not affect heart rate.

The compound **2b** induced the most significant cardiovascular changes of all four compounds tested.

For all compounds of both series the onset of action was approximately 15 min and the duration of action (Table 2) 120 min or longer.

4. Discussion

All the compounds displayed good antiaggregating potency in vitro. Their activity is related to NO-donating properties and parallels their ability to produce nitrite in vitro in the presence of thiols, according to the data reported in a previous paper [4]. The antiaggregatory effects of these NO-donors were reversed both by oxyhaemoglobin and by ODQ.

As already shown in a previous paper [4] for NO-donating and vasodilating properties of derivatives **1a**,**b** and **2a**,**b**, their antiaggregating potency is also influenced both by terfuroxan system structure (4,3′:4′,4″ connection gives rise to more potent compounds than 3,3′:4′,3″ one) and by the substituents introduced at the ring (methyl-substituted derivatives are more potent than dimethylaminomethyl derivatives).

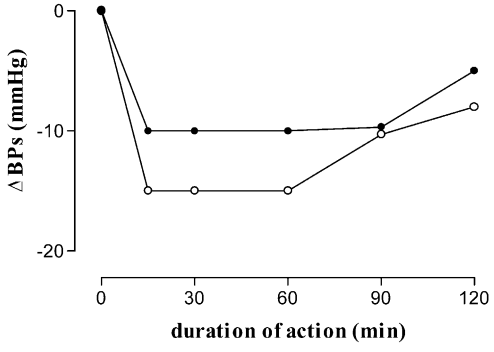

Fig. 1. Dose-effect of compound **1a** on the systolic blood pressure (BPs) of the anaesthetised pig. Compound **1a** was given at the doses 1 mg/kg (filled circles) and 3 mg/kg (open circles). Two pigs per dose were used and the data are presented as the mean of the results obtained in the two animals. Mean BPs baseline was 120 mmHg in both groups.

The results of the cardiovascular investigation have been obtained from a limited number of animals, and thus, are only indicative. However, they clearly show that all these compounds are absorbed by the intestinal tract and that they exert an activity in vivo. All compounds given i.d. lowered the preload as well as the afterload of the heart, with minimal or no effects on contractility and heart rate. The lack of a reactive tachycardia in the presence of a decrease in blood pressure is a common phenomenon of barbiturate anaesthesia, particularly when changes in pressure have a slow onset, as is the case after i.d. administration [7].

The haemodynamic profile of **2b** as well as that of the other compounds resembles that of other NO-donors, as recently reported [5–8], and thus indirectly indicates that in vivo production of NO may have been responsible for the cardiovascular changes observed here.

Acknowledgements

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