Cent. Eur. J. Med. • 7(2) • 2012 • 269-273
DOI: 10.2478/s11536-011-0132-z

VERSITA

Central European Journal of **Medicine**

Comparative study of the antioxidant activity of some thiol-containing substances

Research Article

Lubomir Petrov, Mila Atanassova , Albena Alexandrova*

Institute of Neurobiology, Bulgarian Academy of Sciences,
23, Acad. G. Bonchev str, 1113 Sofia, Bulgaria

Received 13 June 2011; Accepted 11 November 2011

Abstract: Background: Therapeutic thiol administration has been shown to have great potential in a variety of pathological conditions associated with oxidative stress. In the present study, the free radical scavenging effects against superoxide anion (O_{2^-}) and hydroxyl (·OH) radicals of captopril were compared with those of cysteamine and mercaptoethanol. Methods: The O_{2^-} and ·OH were generated *in vitro*. Deoxyribose (DR) was used as a detector of ·OH radicals. The degradation of DR was measured in terms of the formation of thiobarbituric acid reactive substances, which were quantified spectrophotometrically. Superoxide anion radicals were generated photochemically and O_{2^-}-produced nitro-blue tetrazolium (NBT) reduction was measured. Results: Using two distinct ·OH generating systems, the DR test showed that in the absence of the chelator diethylene triamine pentaacetic acid (DTPA) cysteamine was much more potent inhibitor of the formation of thiobarbituric acid reactive substances (TBARs) than captopril and mercaptoethanol, and that in the presence of DTPA captopril and mercaptoethanol decreased the TBARs formation in presence of H_2O_2 better than cysteamine. Captopril in concentration of 9.34 mM and cysteamine in concentration of 1.21 mM inhibited the O_{2^-} provoked NBT reduction by 50%. Mercaptoethanol up to 10 mM did not manifest an inhibitory effect. Conclusions: Captopril and mercaptoethanol are potent free radical scavengers, reacting rapidly with ·OH, whereas cysteamine acts preferentially as a chelator of iron and in this way prevents the formation of ·OH.

Keywords: *Captopril • Cysteamine • Hydroxyl radical • Mercaptoethanol • Superoxide anion radical*

Abbreviations

DR - deoxyribose;
·OH: hydroxyl radical;
O_{2^-}: superoxide anion radical;
ROS: reactive oxygen species

1. Introduction

Captopril is an angiotensin-converting enzyme (ACE) inhibitor widely used in the treatment of hypertension and congestive heart failure. It has been observed that the application of captopril in various diseases related to free radical damage ameliorates the oxidative stress provoked injury. In the "stunned" canine myocardium, a phenomenon associated with the production of oxygen radicals, the administration of this ACE inhibitor improved recovery of contractile function [1]. In transplanted lungs studied after an extended cold preservation period, the use of captopril in the preservation solution reduced ischemia-reperfusion injury (free radicals are involved in the pathology of ischemia/reperfusion injury) [2].

The mechanisms by which captopril is protective remain elusive. It might be assumed that the beneficial response of captopril against heart and peripheral vascular disease could be complemented by its action as a free radical scavenger [3]. Captopril contains a sulphydryl (-SH) group that accounts for its electron-donating character, and therefore, a potential antioxidant effect against free radicals could be hypothesized. In the organism, not all captopril is bound to ACE. Much of it is carried as adsorbed captopril or as disulphydes with endogenous sulphydril-containing compounds such as glutathione and plasma proteins [4]. In this way, it can serve as the depot of the drug and could function as recyclable antioxidant agent [5].

* *E-mail: a_alexandrova_bas@yahoo.com*

Data describing the pro/antioxidant capacity of mercaptoethanol and cysteamine, two other sulphydril-containing compounds, are scanty. According to the literature, they could also exhibit antioxidant activity. Gupta *et al*. [6] demonstrated that the addition of beta-mercaptoethanol to vitrification and culture medium partially annihilated the ROS activity of vitrified-warmed oocytes. Cysteamine confers protection against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine(MPTP)-induced oxidative toxicity in the dopaminergic neurons in a mouse model for Parkinson's disease [7].

Thiol compounds could act as antioxidants through a variety of mechanisms, including their roles as components of the general thiol/disulfide redox buffer, as metal chelators, as radical quenchers, as substrates for specific redox reactions (glutathione in particular), and as specific reductants of individual protein disulfide bonds (thioredoxin). However, although the presence of a sulfhydryl group suggests antioxidant properties, some thiol-containing compounds can exhibit pro-oxidant activity. Under certain circumstances, thiols cause apoptosis, and the hypothesis that thiols cause apoptosis via production of hydrogen peroxide (H_2O_2) during thiol oxidation raised. HL-60 cells treated with 2 mM dithiothreitol begin to show generation of ROS within 5 min of its addition; the ROS generation appears to be maximal at 15 min [8]. Therefore, thiols are able to act as antioxidants by reacting with ROS and can be prooxidants producing ROS—at least *in vitro*—and in particular, H_2O_2 via copper-catalyzed thiol oxidation and ·OH via the Fenton reaction [9,10]. ROS have a dual role in cellular function: on one hand, they are hazardous for living organisms and damage all cellular constituents, but on other hand, they play an important role as regulatory mediators in signalling processes, the determination of biological substances, and in particular for drugs, their pro/antioxidant capacity is essential.

For the present study we chose captopril, a clinically applied drug, to compare it with cysteamine and mercaptoethanol; all are sulfhydryl-containing substances with indications for providing an antioxidant effect. Based on the mechanism of antioxidant action, the free radical scavenging and/or metal chelating effects of those substances were examined against superoxide anion (O_{2^-}) and hydroxyl radical (OH.) generated *in vitro*.

2. Materials and methods

2.1 Materials

Captopril ((2S)-1-[(2S)-2-methyl-3-sulfanylpropanoyl]pyrrolidine-2-carboxylic acid), cysteamine (2-aminoethanethiol) and mercaptoethanol (2-Hydroxy-1-ethanethiol), as well as deoxyribose (DR) and catalase were purchased from Sigma Chemical Co. Nitro-blue tetrazolium (NBT) was purchased from Serva; diethylene triamine pentaacetic acid (DTPA), ethylene diamine tetraacetic acid (EDTA), H_2O_2, methionine and riboflavin – from Merck. All other reagents were analytical grade; all solutions were prepared with de-ionized water.

2.2 OH-generating system

The degradation of DR (a detector of ·OH radicals) was measured in terms of formation of thiobarbituric acid reactive substances (TBARs), according to the method of Gutteridge [11]. The TBARs were quantified spectrophotometrically. Hydroxyl radicals (·OH) were generated in a system containing either (1) 10 mM potassium phosphate buffer, pH 7.4; 0.1 mM FeSO4 and 2 mM deoxyribose (DR) or (2) 10 mM potassium phosphate buffer, pH 7.4; 0.1 mM DTPA-Fe_{2+} (the DTPA-Fe2+ complex was prepared according to Cohen [12]), 0.5 mM H_2O_2 and 2 mM deoxyribose (DR). After 30-min incubation at 37°C in the presence of increasing concentrations of the tested drugs, the reactions were stopped by the addition of catalase (20 µg/ml). After addition of 0.2 ml 2.8% trichloroacetic acid, 0.1 ml 5 N HCl and 0,2 ml thiobarbituric acid (2% w/v in 50 mM NaOH), the samples were heated at 100°C for 15 min to develop color. After cooling, the absorbance was read at 532 nm against a blank sample (without drug); A_{600} was considered to be a non-specific base-line drift and was subtracted from A_{532}.

2.3 O_{2^-}-generating system

Superoxide anion radicals (O_{2^-}) were generated photochemically in a medium containing 50 mM potassium phosphate buffer, pH 7.8; 1.17x10-6 M riboflavin; 0.2 mM methionine; 2x10-5 M KCN and 5.6x10-5 M nitroblue tetrazolium (NBT); and 1x10-4 M EDTA. The O_{2^-}-produced NBT reduction was measured in the presence of increasing concentrations of the tested drugs after a 7-min lighting of the samples, according to the method of Beauchamp & Fridovich [13].

2.4 Statistical analysis

Data were reported as mean ± SEM. The results were statistically analysed by two-way ANOVA (Bonferroni post-tests), $P < 0.001$ being accepted as the minimum level of statistical significance for the differences in population mean values.

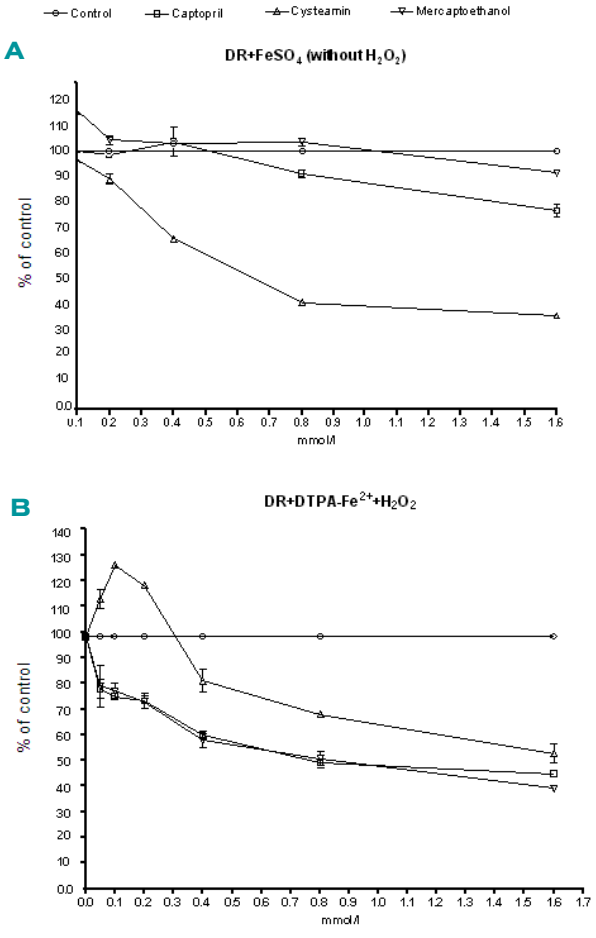

Figure 1. Effects of capropril, cysteamine and mercaptoethanol on TBARs formation.

*Reaction mixtures: A) 10 mM potassium phosphate buffer, pH 7.4; 0.1 mM $FeSO_4$ and 2 mM deoxyribose. B) 10 mM potassium phosphate buffer, pH 7.4; 0.1 mM DTPA-Fe^{2+}, 0.5 mM H_2O_2 and 2 mM deoxyribose. Incubation was carried out for 30 min at 37°C in the presence of increasing concentrations of the tested compounds. Values are the mean ± SEM of 10 experiments and are expressed in % inhibition NBT reduction. Significant differences versus corresponding controls at *p <0.001.*

3. Results

Figure 1 shows the percentage inhibition of TBARs formation by increasing doses of captopril, cysteamine and mercaptoethanol. The inhibition increased with the concentration of the drugs in both ·OH generating systems. In absence of the chelator, cysteamine was a much more potent inhibitor of the formation of TBARs than were captopril and mercaptoethanol (Figure 1A). When Fe_{2+} ions of the reaction medium were chelated to DTPA, the inhibitory potential of cysteamine decreased and both captopril and mercaptoethanol demonstrated a stronger effect than that of cysteamine (Figure 1B). Captopril and mercaptoethanol decreased the formation of TBARs in presence of H_2O_2 to an almost equal extent. The tested drugs dose-dependently inhibited O_{2^-}-provoked NBT-reduction. Captopril in the highest concentration tested (10 mM) inhibited the O_{2^-}-provoked NBT reduction by about 60%, whereas cysteamine, even at the lowest concentration tested (1 mM), achieved more than 40% inhibition (Table 1). Mercaptoethanol up to 10 mM did not exhibit any inhibitory effect.

4. Discussion

In the present study, we used two distinct ·OH generating systems with DR as a detector molecule. The first deoxyribose test evaluates the possibility of DR to bind iron ions. Bound on this detector molecule, the latter could undergo autoxidation with a production of superoxide anion radicals (O_{2^-}), which dismutate to H_2O_2 and catalyze the site-specific generation of ·OH radicals:

$DR + Fe_{2+} \rightarrow DR\text{-}Fe_{2+}$

$DR\text{-}Fe_{2+} + O_2 \rightarrow DR\text{-}Fe_{3+} + O_{2^-}$

$2\ O_{2^-} + 2\ H+ \rightarrow H_2O_2 + O_2$

$DR\text{-}Fe_{2+} + H_2O_2 \rightarrow DR\text{-}Fe_{3+} + OH^- + \cdot OH$

If a scavenger molecule has a higher binding affinity for iron than the detector, then the scavenger can protect the

Table 1. Effects of capropril, cysteamine and mercaptoethanol on the O_2 – provoked NBT reduction

Drugs / Concentration	Inhibition of O_2 – provoked NBT reduction (%)		
	Captopril	Cysteamine	Mercaptoethanol
1 mM	0	42±1.25	0
6 mM	9.96±0.56	77.98±1.15	0
8 mM	47.2±0.55	100±1.02	0
10 mM	61.9±0.7	100±0.98	0

*The values represent the mean ± SEM of 10 experiments and are expressed in % inhibition NBT reduction. Statistically significant differences versus corresponding controls at *p <0.001.*

detector molecule by transferring the damage to itself. The protection depends on the concentration of scavenger with respect to the detector molecule and on the scavenger's second-order rate constant of reaction with ·OH. Using this method for generation of ·OH, we found that (1) the inhibition was dose dependent and (2) cysteamine was a potent inhibitor of TBARs formation (Figure 1A). It seems that cysteamine acts preferentially as a chelator of iron rather than as a radical scavenger, because when Fe_{2+} is chelated to DTPA (see below), the inhibitory potential of this drug decreases and shows the weakest result compared with captopril and mercaptoethanol (Figure 1B).

Hydroxyl radicals may also be generated *in vitro* by exposing the detector molecule (DR in our experiments) to DTPA-Fe_{2+} in the presence of H_2O_2. Chelation of the metal ion will prevent its association with DR, and the generated ·OH will have equal access to all components of the reaction mixture:

$$DTPA\text{-}Fe_{2+} + H_2O_2 \rightarrow DTPA\text{-}Fe_{3+} + OH^- + \cdot OH$$

Under these conditions, we have found that captopril and mercaptoethanol decreased the TBARs formation in presence of H_2O_2 better than cysteamine, which suggests that they possess predominantly free radical scavenging activity (Figure 1B).

We also investigated the scavenging effects of captopril, mercaptoethanol and cysteamine against O_{2^-}. Our results indicated that both captopril and cysteamine can dose-dependently inhibit the O_2-provoked NBT reduction and achieve a 50% reduction at concentrations of 9.34 mM and 1.21 mM, respectively (Table 1). Indeed, these concentrations are very high, in particular for captopril, given that the peak blood concentrations of captopril are around 0.5 µM in patients with heart failure after peroral drug administration of 25 mg [14]. The superoxide radical-scavenging activity of captopril has been studied by several investigators, and the results have been contradictory. Some reported a high superoxide scavenging activity, whereas others found that captopril removed superoxide inefficiently. Chopra *et al*. [5], using the technique described by Misra and Fridovich [15], demonstrated that captopril specifically scavenges O_{2^-}. On the other hand, Winterbourn & Metodiewa [16] as well as Kukreja *et al*. [17], using a number of superoxide-generating systems such as xanthine-xanthine oxidase, phorbol myristate acetate-activated neutrophils, auto-oxidizing dihydroxyfumarate, and auto-oxidation of epinephrine to adrenochrome, asserted that captopril does not scavenge superoxide anion. However, in the organism, the compounds could have diverse lines of activity, and the effect could be different from that observed in chemical systems.

Captopril has been reported to inhibit neutrophil NADPH oxidase [18], which decreases leukocyte pro-oxidant generation. Upon activation of proinflammatory mediators, the membrane-associated NADPH oxidase reduces molecular oxygen by one electron to yield the superoxide anion radicals, which push the ROS generating machine, yielding H_2O_2 (by spontaneous dismutase) or hydroxyl radicals (by interaction with H_2O_2 in the presence of trace amounts of metal). In reperfusion-induced myocardial dysfunction, it appears that captopril acted as a nonspecific antioxidant, probably by reducing an intermediate in the complex oxidation of epinephrine to adrenochrome [17]. In its turn, cysteamine is constitutively produced by all tissues in human and animal body as part of the coenzyme A (an acyl carrier group required for many metabolic processes) catabolic pathway [19]. Recent work has shown that many tissues are capable of converting cysteamine to hypotaurine and subsequently to taurine *in vivo* [20]. Cysteamine seems to be the main source of taurine, the second most abundant amino acid in the brain, with membrane-stabilizing and antioxidant properties [21]. Oral administration of cysteamine to mice significantly increased levels of the antioxidant enzyme superoxide dismutase (SOD) in plasma, liver and brain, and obviously up-regulated the activity of glutathione peroxidase (GSH-Px) in plasma [22]. Also, β-mercaptoethanol, in addition to its direct antioxidant activity, can lead to up-regulation of glutathione synthesis [23]. In accordance with our results, there are abundant examples of the free radical scavenger activity of mercaptoethanol in the literature. In this study, we compared the captopril antioxidant activity with those of cysteamine and mercaptoethanol not only among them in the same medium, but also in regard to two different ROS (hydroxyl radicals and superoxide anion radicals). We hope that our study will contribute to the knowledge of these thiol-containing substances. Although the present results cannot be directly extrapolated to the clinical setting, they contribute to an understanding of the mechanism by which these compounds act as antioxidants, and further, suggest that by their use, lowering of free radical concentration may be possible in both laboratory and clinical practice.

5. Conclusion

According to our results, captopril and mercaptoethanol are potent free radical scavengers, reacting rapidly with ·OH, whereas cysteamine acts preferentially as a chelator of iron and in this way prevents the formation of ·OH. In regard to superoxide anion radicals, both captopril and cysteamine *in vitro* demonstrate unsatisfactory scavenging activity, and mercapoethanol does not scavenge these radicals in concentrations up to 10 mM. Although the beneficial role of the compounds we tested result from their immediate antioxidant properties, the chelator and scavenger activity of these thiols should not be neglected and should be taken into consideration as an overall therapeutic and experimental treatment.

References

[1] Przyklenk K., Kloner R., Angiotensin converting enzyme inhibitors improve contractile function of stunned myocardium by different mechanisms of action. Am. Heart J., 1991, 121(5), 1319-30

[2] Fischer S., Maclean A.A., Liu M., Kalirai B., Keshavjee S., Inhibition of angiotensin-converting enzyme by captopril: a novel approach to reduce ischemia-reperfusion injury after lung transplantation, J. Thorac. Cardiovasc. Surg., 2000, 120(3), 573-80

[3] Hanif K., Snehlata P., Pavar M.C., Arif E., Biswas P., Fahim M., Pasha M.A., Pasha S., Effect of 3-thienylalanine-ornithine-proline, new sulfur-containing angiotensin-converting enzyme inhibitor on blood pressure and oxidative stress in spontaneously hypertensive rats. J. Cardiovasc. Pharmacol., 2009, 53(2), 145-50

[4] Heel R.C., Brogden R.N., Speight T.M., Avery G.S., Captopril: a preliminary review of its pharmacological properties and therapeutic efficacy. Drugs, 1980, 20(6), 409-52

[5] Chopra M., Scott N., McMurray J., McLay J., Bridges A., Smith W.E., Belch J.J.F., Captopril: a free radical scavenger. Br. J. Clin. Pharmac., 1989, 27, 396-9

[6] Gupta M.K., Uhm S.J., Lee H.T., Effect of vitrification and beta-mercaptoethanol on reactive oxygen species activity and in vitro development of oocytes vitrified before or after in vitro fertilization. Fertil Steril., 2010, 93(8), 2602-7

[7] Sun L., Xu S., Zhou M., Wang Ch., Wu Y., Chan P., Effects of cysteamine on MPTP-induced dopaminergic neurodegeneration in mice. Brain Research, 2010, 1335, 74-82

[8] Tartier L., McCarey Y.L., Biaglow J.E, Kochevar I.E., Held K.D., Apoptosis induced by dithiothreitol in HL-60 cells shows early activation of caspase 3 and is independent of mitochondria. Cell Death and differentiation, 2000, 7(10), 1002-10

[9] Kachur A.V., Held K.D., Koch C.J., Biaglow J.E., Mechanism of production of hydroxyl radicals in the copper-catalyzed oxidation of dithiothreitol. Radiat. Res., 1997 147, 409-15

[10] Biaglow J.E., Manevich Y., Uckun F., Held K.D., Quantitation of hydroxyl radicals produced by radiation and copper-linked oxidation of ascorbate by 2-deoxy-D-ribose method. Free Radic. Biol. Med., 1997, 22, 1129-38

[11] Gutteridge J.M., Ferrous-salt-promoted damage to deoxyribose and benzoate. The increased effectiveness of hydroxyl-radical scavengers in the presence of EDTA. Biochem. J., 1987, 243(3), 709-14

[12] Cohen G., In: Greewald R.A. (Ed.), Handbook of Methods for Oxygen Radical Research, CRC Press, Boca Raton, Florida, 1985, pp. 55-64.

[13] Beauchamp C., Fridovich I., Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal. Biochem., 1971, 44(1), 276-87

[14] Cody R.J., Schaer G.L., Covit A.B., Pondolfino K., Williams G., Captopril kinetics in chronic congestive heart failure. Clin. Pharmacol. Ther., 1982, 32(6), 721-6

[15] Misra H.P., Fridovich I., Superoxide dismutase: a photochemical augmentation assay. Arch. Biochem. Biophys., 1977, 181(1), 308-12

[16] Winterbourn C.C., Metodiewa D., Reactivity of biologically important thiol compounds with superoxide and hydrogen peroxide. Free Radic. Biol. Med., 1999, 27 (3-4), 322-8

[17] Kukreja R.C., Kontos H.A., Hess M.L., Captopril and enalaprilat do not scavenge the superoxide anion. Am. J. Cardiol., 1990, 65(19), 24I-7I

[18] Egan T.M., Minta J.O., Scrimgeour K.G., Cooper J.D., Captopril--a potential free radical scavenger: inhibition of PMN NADPH oxidase. Clin. Invest. Med., 1988, 11(5), 351-6

[19] Pisoni R.L., Park G.Y., Velilla V.Q., Thoene J.G., Detection and characterization of a transport system mediating cysteamine entry into human fibroblast lysosomes. Specificity for aminoethylthiol and aminoethylsulfide derivatives. J. Biol. Chem., 1995, 270(3), 1179–84

[20] Coloso R. M., Hirschberger L. L., Dominy J. E., Lee J. I., Stipanuk, M. H., Cysteamine dioxygenase: evidence for the physiological conversion of cysteamine to hypotaurine in rat and mouse tissues. Adv. Exp. Med. Biol., 2006, 583, 25–36

[21] Dominy J.E., Simmons C.R., Hirschberger L.L., Hwang J., Coloso R.M., Stipanuk M.H., Discovery and characterization of a second mammalian thiol dioxygenase: Cysteamine dioxygenase. J. Biol. Chem., 2007, 282(35), 25189-98

[22] Liu H.-Z., Zhong J.-P., Effects of Cysteamine on Antioxidant Ability of Mice. Journal of Henan University of Science & Technology (Natural Science), 2009, en.cnki.com.cn/Article_en/CJFDTOTAL-LYGX200901018.htm

[23] Abeydeera L. R., Wang W. H., Cantley T. C., Prather R. S.,Day B. N., Presence of beta-mercaptoethanol can increase the glutathione content of pig oocytes matured in vitro and the rate of blastocyst development after in vitro fertilization. Theriogenology, 1998, 50, 747–56