® Postepy Hig Med Dosw (online), 2013; 67: 331-338
e-ISSN 1732-2693

www.phmd.pl
Original Article

Received: 2012.09.25
Accepted: 2013.03.15
Published: 2013.04.24

In Vivo Anti-inflammatory Activity of Lipoic Acid Derivatives in Mice*

Aktywność przeciwzapalna pochodnych kwasu liponowego w badaniach *in vivo* u myszy

Brunon Kwiecień[1,A,B,D,E,F], Magdalena Dudek[2,A,B,C,D,E,F], Anna Bilska-Wilkosz[3,A,D,E,F], Joanna Knutelska[4,B], Marek Bednarski[4,B], Inga Kwiecień[5,A,B,D,E], Małgorzata Zygmunt[4,A,B], Małgorzata Iciek[3,D,E,F], Maria Sokołowska-Jeżewicz[3,D], Jacek Sapa[4,B], Lidia Włodek[3,D,E]

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

[1] Department of Chemistry and Physics, Hugon Kołłątaj University of Agriculture, Kraków, Poland
[2] Department of Pharmacodynamics, Jagiellonian University, Medical College, Kraków, Poland
[3] Chair of Medical Biochemistry, Jagiellonian University, Medical College, Kraków, Poland
[4] Laboratory of Pharmacological Screening, Department of Pharmacodynamics, Jagiellonian University, Medical College, Kraków, Poland
[5] Chair and Department of Pharmaceutical Botany, Medical College, Jagiellonian University, Kraków, Poland

Summary

Background: In mammals lipoic acid (LA) and its reduced form dihydrolipoic acid (DHLA) function as cofactors for multienzymatic complexes catalyzing the decarboxylation of α-ketoacids. Moreover, LA is used as a drug in a variety of diseases including inflammatory diseases. The aim of the study was to examine anti-inflammatory properties of LA metabolites.

Material/methods: The present paper reports the chemical synthesis of 2,4-bismethylthio-butanoic acid (BMTBA) and tetranor-dihydrolipoic acid (tetranor-DHLA). BMTBA is one of the biotransformation products of LA, while tetranor-DHLA is an analogue of DHLA. Structural identity of these compounds was confirmed by ^{1}H NMR. These compounds were assessed for their anti-inflammatory activity in mice. For this purpose, the zymosan-induced peritonitis and the carrageenan-induced hind paw edema animal models were applied.

Results/conclusions: The obtained results indicated that the early vascular permeability measured at 30 min of zymosan-induced peritonitis was significantly inhibited in groups receiving BMTBA (10, 30, 50 mg/kg). The early infiltration of neutrophils measured at 4 hours of zymosan-induced peritonitis was inhibited in the group receiving BMTBA (50 mg/kg) and tetranor-DHLA (50 mg/kg). The results indicated that the increase in paw edema was significantly inhibited in the groups receiving BMTBA (50, 100 mg/kg) and tetranor-DHLA (30, 50 mg/kg).
In summary, the present studies clearly demonstrated that both BMTBA and tetranor-DHLA were able to act as anti-inflammatory agents. This is the first study examining in vivo the anti-inflammatory properties of LA metabolites.

Keywords: **lipoic acid • dihydrolipoic acid • tetranor-dihydrolipoic acid • 2,4-(bismethylthio)-butanoic acid • carrageenan • zymosan • anti-inflammatory properties • hydrogen sulfide**

*This work was supported by the Ministry of Science and Higher Education, Warsaw, Poland, grant no. 2335/B/P01/2007/33, and by statutory funds of the Faculty of Pharmacy, Medical College, Kraków, Poland.

Full-text PDF: http://www.phmd.pl/fulltxt.php?ICID=1046290

Word count: 2980
Tables: 4
Figures: 4
References: 35

Author address: Lidia Włodek, Chair of Medical Biochemistry, Jagiellonian University, Medical College, Kopernika 7, PL 31-034 Kraków, Poland. e-mail: mbwlodek@cyf-kr.edu.pl

Introduction

Lipoic acid (LA, 1,2-dithiolane-3-pentanoic acid; $C_8H_{14}O_2S_2$) and its reduced form dihydrolipoic acid (DHLA, 6,8-dimercapto-octanoic acid; $C_8H_{16}O_2S_2$) are present in all prokaryotic and eukaryotic cells. DHLA is formed by reduction of LA. In humans LA is synthesized by the liver and other tissues, and functions as a cofactor for pyruvate dehydrogenase, α-ketoglutarate dehydrogenase and branched-chain α-ketoacid dehydrogenase. Moreover, LA and DHLA have been proposed to act as antioxidants [1,15,18]. LA is used as a therapeutic in a variety of diseases including diabetic polyneuropathy, heavy metal intoxication and liver diseases [2,7,11]. Furthermore, LA was suggested to play a role in cardiovascular protection and can also act as an anti-inflammatory agent [3,9,17,22,23,29,34,35].

However, in spite of numerous studies confirming the beneficial action of LA for therapy of many diseases, the mechanism of its action has not been explained in detail, yet. Therefore, it seems interesting to examine pharmacological properties of LA metabolites. It is generally accepted that biotransformation (phase I and II) of xenobiotics, including drugs, may yield metabolites that are either pharmacologically inactive or have stronger, weaker or different activity than the parent compound.

Literature data have indicated that (1) LA biotransformation involves mainly β-oxidation of the carboxylic acid side chain and S-methylation of the reduced 1,2-dithiolane moiety; (2) 4,6-bismethylthio-hexanoic acid is the main circulating LA metabolite in humans; (3) while 6,8-bismethylthio-octanoic acid and 2,4-bismethylthio-butanoic acid were shown to be minor metabolites of LA [21,25].

The present paper reports the chemical synthesis of 2,4-bismethylthio-butanoic acid (BMTBA), which is one of the LA biotransformation products, and tetranor-dihydrolipoic acid (tetranor-DHLA), a DHLA analogue. Structural identity of these compounds was confirmed by ^{1}H NMR.

The safety of BMTBA and tetranor-DHLA was evaluated by determining their potential toxicity after acute administration in mice. Furthermore, these compounds were assessed for their anti-inflammatory activity in mice. For this purpose, the zymosan-induced peritonitis and the carrageenan-induced hind paw edema animal models were applied.

The obtained results clearly demonstrated that both BMTBA and tetranor-DHLA were able to act as anti-inflammatory agents. This is the first study examining in vivo the anti-inflammatory properties of LA metabolites.

Materials and Methods

Chemical syntheses

The majority of chemicals were purchased from Sigma-Aldrich and Fluka. IR spectra were recorded on a Unicam Mattson 3020 spectrophotometer as potassium bromide discs. The ^{1}H NMR spectra were measured on a Varian Mercury VX 300 MHz spectrometer using TMS as the internal standard. Coupling constant (*J*) values are estimated in Hertz (Hz) and spin multiples are given as s (singlet), d (double), t (triplet), m (multiplet) and br (broad). Mass spectra were measured on a Finnigan MAT 900. Reaction courses were monitored by TLC on silica gel precoated F254 Merck plates. Developed plates were examined with UV lamps (254 nm).

General procedure for preparation of 2,4-dibromobutanoic acid (2)

To 100 g of γ-butyrolactone, 2 ml of phosphorus tribromide were added and the reaction mixture was heated to 100°C. The mixture was stirred while 164 g of bromine were added dropwise for 3 hours beneath the surface of the liquid (temp. 110-115°C). When the rate of bromine uptake decreased, 0.5 ml of phosphorus tribromide was added and heat was applied to maintain the reaction temperature. The addition of bromine was continued until hydrogen bromide evolution was evident. At that stage the product was stirred and cooled to room temperature.

General procedure for preparation of methyl 2,4-dibromobutanoate (3)

To 68 g of 2,4-dibromobutanoic acid, 120 ml of methanol was added and the resulting solution was saturated

with concentrated sulfuric acid. The reaction mixture was allowed to stand at room temperature overnight and methanol was evaporated under vacuum. The residue was extracted with ether. The ether extract was washed with 3% sodium bicarbonate solution to remove unchanged acid and then dried over anhydrous sodium sulfate. The solvent was removed and the residue was distilled under reduced pressure.

General procedure for preparation of 2,4-bis-(methylthio)-butanoic acid (4)

100 g of methyl mercaptan were added to a cold solution of 78 g of sodium methoxide in 750 ml of methanol. In the course of 15 minutes, 105 g of 2,4-dibromobutanoic acid were added to the cold stirred mercaptide solution. After that the mixture was refluxed for one hour and concentrated under vacuum in a water bath. The residue was diluted with 500 ml of water and acidified to pH 3 with 6 M HCl. The acidic fraction was separated by bicarbonate extraction followed by acidification and chloroform extraction. The chloroform solution of the acidic product was dried over anhydrous magnesium sulfate, filtered and concentrated in a vacuum evaporator.

General procedure for preparation of 2,4-dimercaptobutanoic acid (5)

Thioacetic acid (14.7 g) was cooled in an ice-bath and neutralized with a 10% solution (w/v) of potassium hydroxide in ethanol (approx. 135 ml). To this solution methyl 2,4-dibromobutanoate (29 g) was added and the mixture was stirred and heated under reflux in an atmosphere of nitrogen for 5 hours. After cooling of the mixture, 35 g of potassium hydroxide were added. Stirring was continued until the potassium hydroxide had dissolved and the mixture was then allowed to stand at room temperature in an atmosphere of nitrogen overnight. The reaction mixture was acidified (pH<1) with 6 M HCl and concentrated under vacuum until an oily layer appeared. Water was added to dissolve the inorganic solids. The mixture was extracted twice with chloroform, dried over anhydrous magnesium sulfate, filtered and concentrated under a vacuum evaporator.

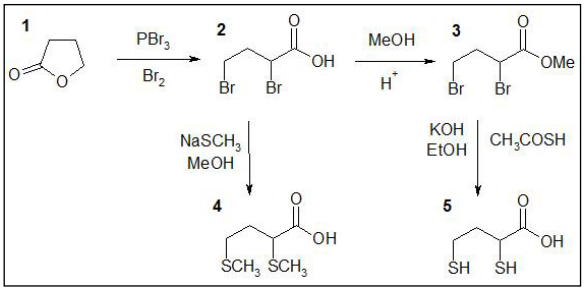

Fig.1. Synthesis of lipoic acid derivatives; (1) γ-butyrolactone; (2) 2,4-dibromobutanoic acid [BMTBA]; (3) methyl 2,2-dibromobutanoate; (4) 2,4-bismethylthio-butanoic acid [BMTBA]; (5) 2,4-dimercaptobutanoic acid (tetranor-dihydrolipoic acid, tetranor-DHLA)

2,4-dibromobutanoic acid (2)
Yield 63%; EI-MS m/z 246 (M^+).

Methyl 2,4-dibromobutanoate (3)
Yield 86%; EI-MS m/z 274 (M^+); ^{1}H NMR ($CDCl_3$, 300 MHz, ppm): 2.4-2.6 (m, 2H), 3.54 (t, 2H, *J* = 6.0 Hz), 3.8 (s, 3H), 4.52 (t, 1H, *J* = 7.0 Hz).

2,4-bis-(methylthio)-butanoic acid (4)
Yield 43%; IR (KBr) cm^{-1}: 1702 (C=O), 678 (C-S); EI-MS m/z 180 (M^+); ^{1}H NMR ($CDCl_3$, 300 MHz, ppm):1.95 (dt, 2H, *J* = 7.2 Hz; *J* = 6.9 Hz), 2.11 (s, 3H), 2.20 (s, 3H), 2.62 (t, 2H, *J* = 7.2 Hz), 3.49 (t, 1H, *J* = 6.9 Hz).

2,4-dimercaptobutanoic acid (5)
Yield 93%; IR (KBr) cm^{-1}: 2560 (S-H), 1703(C=O), 670 (C-S); EI-MS m/z 152 (M^+); ^{1}H NMR ($CDCl_3$, 300 MHz, ppm): 2.175 (dt, 2H, *J* = 6.9 Hz; *J* = 7.2 Hz), 3.35 (t, 2H, *J* = 6.9 Hz), 4.4 (t, 1H, *J* = 7.2 Hz), 6 (br, 2H).

PHARMACOLOGICAL PART

Animals

The experiments were carried out on male Albino-Swiss mice (body weight 20-26 g). The animals were housed in constant temperature facilities exposed to a 12:12 h light-dark cycle, and were maintained on a standard pellet diet with tap water available *ad libitum*. Control and experimental groups consisted of eight animals each. All experiments were conducted according to guidelines of the Animal Use and Care Committee of the Jagiellonian University (no. 50/2011 and no. 96/2011, Kraków, Poland).

Acute toxicity

The compounds were suspended in 1% Tween 80 and pitched in an ultrasonic cleaner. Acute toxicity was assessed by the methods of Litchfield and Wilcoxon [13] and presented as LD_{50} calculated from mortality of mice after 24 h. The animals were divided randomly into six groups of six animals each. The groups studied were as follows:

group I – tetranor-DHLA (200 mg/kg bw; ip)
group II – tetranor-DHLA (100 mg/kg bw; ip)
group III – tetranor-DHLA (75 mg/kg bw; ip)
group IV – BMTBA (200 mg/kg bw; ip)
group V – BMTBA (400 mg/kg bw; ip)
group VI – BMTBA (1000 mg/kg bw; ip).

Zymosan-induced peritonitis

Peritoneal inflammation was induced as described previously [10]. Zymosan A was freshly prepared (2 mg/ml) in sterile 0.9% NaCl. Thirty min after subcutaneous (*sc*) injection of the investigated compounds into the loose skin over the flank, zymosan A was injected *ip*. Four hours later the animals were killed. The peritoneal cavity was lavaged with 1.5 ml of saline and after 30 s of gentle manual massage the exudates were retrieved. Cells were counted

using an automatic cell counter (Countess, Invitrogen) following staining with Turk's solution. The investigated compounds were suspended in 1% Tween 80 and pitched in an ultrasonic cleaner. The control group was given *sc* 1% Tween 80 30 minutes prior to zymosan.

Vascular permeability

Evans blue was suspended in saline (10 mg/ml) and injected intravenously (*iv*) into the caudal vein, which was immediately followed by *ip* injection of zymosan A. Thirty minutes later the animals were killed and their peritoneal cavities were lavaged with 1.5 ml of saline as described above. The lavage fluid was centrifuged and the absorbance of the supernatant was measured at 620 nm as described previously [10]. The investigated compounds suspended in 1% Tween 80 were injected *sc* 30 min before Evans blue and zymosan. The control group was given *sc* 1% Tween 80 30 min prior to zymosan. Indomethacin in a dose of 10 mg/kg bw. was used as a reference compound.

Carrageenan-induced paw edema in mice

The test was conducted in 6-week-old mice according to the method of Winter with the modification of Yazawa [28,31]. The volume of the right hind paw of animals was measured. Thirty minutes after the injection *ip* of the investigated compounds suspended in 1% Tween 80, mice were treated with 0.05 ml of 1% carrageenan by *sc* injection into the right hind paw to induce acute inflammation. The control group received the vehicle (1% Tween 80). Indomethacin in a dose of 20 mg/kg bw was used as a reference compound. At 1, 2, 3 h after carrageenan treatment, the degree of paw edema was evaluated by measuring the volume of the paw using an aqueous plethysmometer (Plethysmometer 7140, Ugo Basile).

The mean values were calculated and the percentage change from baseline was determined according to the formula:

$$K = \frac{V_t - V_0}{V_0} x\,100$$

K – the percentage change,
V_0 – initial paw volume,
V_t – volume at time t,
t – time after the measurements.

Statistical analysis

All statistical calculations were carried out with the GraphPad Prism 5 program. The statistical significance was calculated using Student's t-test. Differences were considered statistically significant at $p \leq 0.05$. The LD_{50} values and their confidence limits were calculated according to the method of Litchfield and Wilcoxon [13].

Results

Synthesis of natural LA metabolites

The 2,4-bismethylthio-butanoic acid (BMTBA), which is one of the LA biotransformation products, and tetranor-dihydrolipoic acid (tetranor-DHLA), which is a DHLA analogue, were synthesized with good yields by using the methods reported for LA preparation with our modifications (Fig. 1). γ-Butyrolactone (1) was converted to 2,4-dibromobutanoic acid (2) [19]. 2,4-Di-(methylthio)-butanoic acid (4) was prepared by adding 2,4-dibromobutanoic acid (2) to an excess of sodium methyl mercaptide in methanolic solution and purified by distillation [14]. The carboxylic group of 2,4-dimercaptobutanoic acid (2) was protected by esterification to methyl ester (3). Treatment of the dibromo ester with potassium thiolacetate in ethanol, after alkaline hydrolysis, gave 2,4-dimercaptobutanoic acid (5) [20].

Acute toxicity

The LD_{50} values of the investigated compounds determined in mice after intraperitoneal (*ip*) administration are shown in Table 1. Tetranor-DHLA was proven to be the most toxic compound (LD_{50} = 75.4 mg/kg). The toxicity of BMTBA was over 400 mg/kg. Toxic doses of all the tested compounds caused sedation but later increased activity and elicited tonic seizures in mice.

Table 1. Acute toxicity in mice

Tetranor-DHLA LD_{50} ip in mice	BMTBA LD_{50} ip in mice
75.4 mg/kg bw (67.35 – 84.42)	588.57 mg/kg bw (469.22 – 738.27)

LD_{50} – lethal dose, 50 percent kill

Zymosan-induced peritonitis in mice

The effect of tetranor-DHLA and BMTBA on vascular permeability was tested at three doses of the former (10, 30, 50 mg/kg bw) and four doses of the latter (10, 30, 50 and 100 mg/kg bw). Indomethacin at a dose of 10 mg/kg bw was used as a reference compound (IND10 group). The early vascular permeability measured at 30 min of zymosan-induced peritonitis was significantly inhibited in groups receiving BMTBA (BMTBA10, BMTBA30, BMTBA50 groups) compared to the control group, which was given zymosan alone (Fig. 2, Table 2). On the other hand, BMTBA at a dose of 100 mg/kg bw and tetranor-DHLA in any of the administered doses did not reduce vascular permeability (Fig. 2, Table 2).

The effect of the compounds under study on infiltration of neutrophils was tested at one dose of BMTBA (50 mg/kg bw) or tetranor-DHLA (50 mg/kg bw). The early infiltration of neutrophils measured at 4 hours of zymosan-induced peritonitis was significantly inhibited in

the group receiving BMTBA (BMTBA50 group) and in the group treated with tetranor-DHLA (tetranor-DHLA50 group) compared to the control group, which was given zymosan alone (ZYM group) (Fig. 3, Table 3).

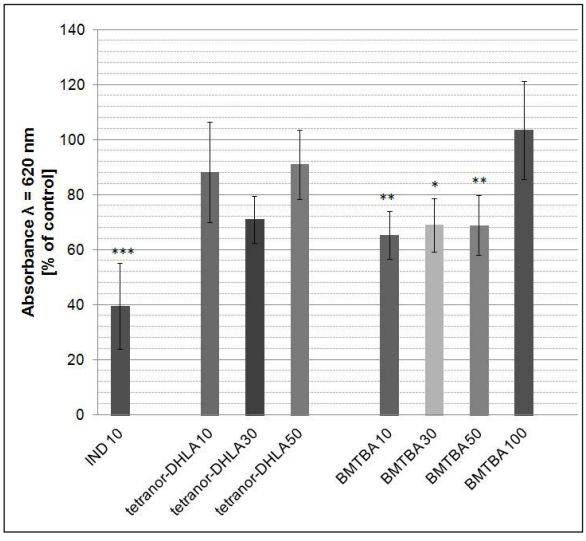

Fig. 2. Vascular permeability changes during zymosan-induced peritonitis in mice. Data are presented as the mean ± SEM, percentage of control absorbance in zymosan-induced peritonitis group, n = 8. Student's t-test, differences significant vs. control group – zymosan-induced peritonitis: * p <0.05; ** p < 0.02; *** p < 0.01. Groups: IND10 – indomethacin 10 mg/kg bw; tetranor-DHLA10 – tetranor-DHLA 10 mg/kg bw; tetranor-DHLA30 – tetranor-DHLA 30 mg/kg bw; tetranor-DHLA50 – tetranor-DHLA 50 mg/kg bw; BMTBA 10 – BMTBA 10 mg/kg bw; BMTBA 30 – BMTBA 30 mg/kg bw; BMTBA 50 – BMTBA 50 mg/kg bw; BMTBA 100 – BMTBA 100 mg/kg bw

Table 2. Percent inhibition of vascular permeability in zymosan-induced peritonitis in mice

Compounds	% inhibition
IND10	60.53
tetranor-DHLA10	11.75
tetranor-DHLA30	28.95
tetranor-DHLA50	8.99
BMTBA10	34.64
BMTBA30	31
BMTBA50	31.01
BMTBA100	0

IND10 – indomethacin 10 mg/kg bw; tetranor-DHLA10 – tetranor-DHLA 10 mg/kg bw; tetranor-DHLA30 – tetranor-DHLA 30 mg/kg bw; tetranor-DHLA50 – tetranor-DHLA 50 mg/kg bw; BMTBA 10 – BMTBA 10 mg/kg bw; BMTBA 30 – BMTBA 30 mg/kg bw; BMTBA 50 – BMTBA 50 mg/kg bw; BMTBA 100 – BMTBA 100 mg/kg bw

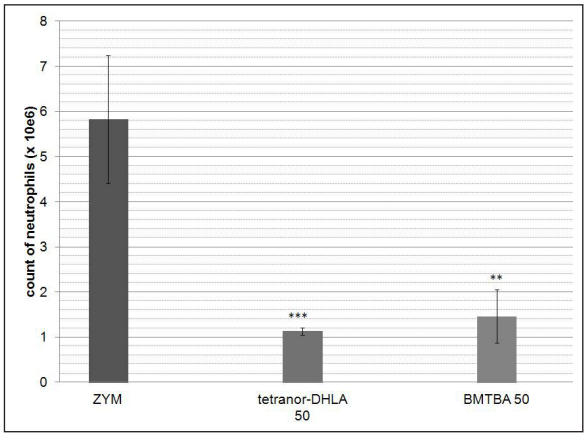

Fig. 3. Changes in neutrophil infiltration during zymosan-induced peritonitis in mice. Data are presented as the mean ± SEM of neutrophil count, n = 8. Student's t-test, differences significant vs. control group – zymosan-induced peritonitis: ** p < 0.02; *** p < 0.01. Groups: tetranor-DHLA50 – tetranor-DHLA 50 mg/kg bw; BMTBA 50 – BMTBA 50 mg/kg bw; ZYM – zymosan

Table 3. Percent inhibition of neutrophil infiltration in zymosan-induced peritonitis in mice

Compounds	% inhibition
Tetranor-DHLA50	80.65
BMTBA50	75.04

tetranor-DHLA50 – tetranor-DHLA 50 mg/kg bw; *ip*.
BMTBA50 – BMTBA 50 mg/kg bw; *ip*.

Carrageenan-induced paw edema in mice

The influence of the studied compounds in the paw edema model was examined at three doses of BMTBA (30, 50 and 100 mg/kg bw) and at two doses of tetranor-DHLA (30, 50 mg/kg bw). Indomethacin at a dose of 20 mg/kg bw was used as a reference compound (IND20 group). The mouse paw became edematous after the injection of carrageenan, and edema reached a peak at 3 h in the control group (increase by 86.71% of the initial volume). The obtained results indicated that the increase in paw edema was significantly inhibited in the groups receiving BMTBA (BMTBA50 and BTMBA100 groups) or tetranor-DHLA (tetranor-DHLA30 and tetranor-DHLA50 groups) compared to the control group, which was given carrageenan alone (Fig. 4; Table 4).

Discussion

This work showed that administration of both tetranor-DHLA and BMTBA significant ameliorated the inflammatory response in the zymosan-induced peritonitis and in the carrageenan-induced hind paw edema models in mice. It is noteworthy that the anti-inflammatory action of the compounds under study was dose-dependent.

Our results indicated that tetranor-DHLA at a dose of 50 mg/kg bw significantly reduced hind paw edema, when

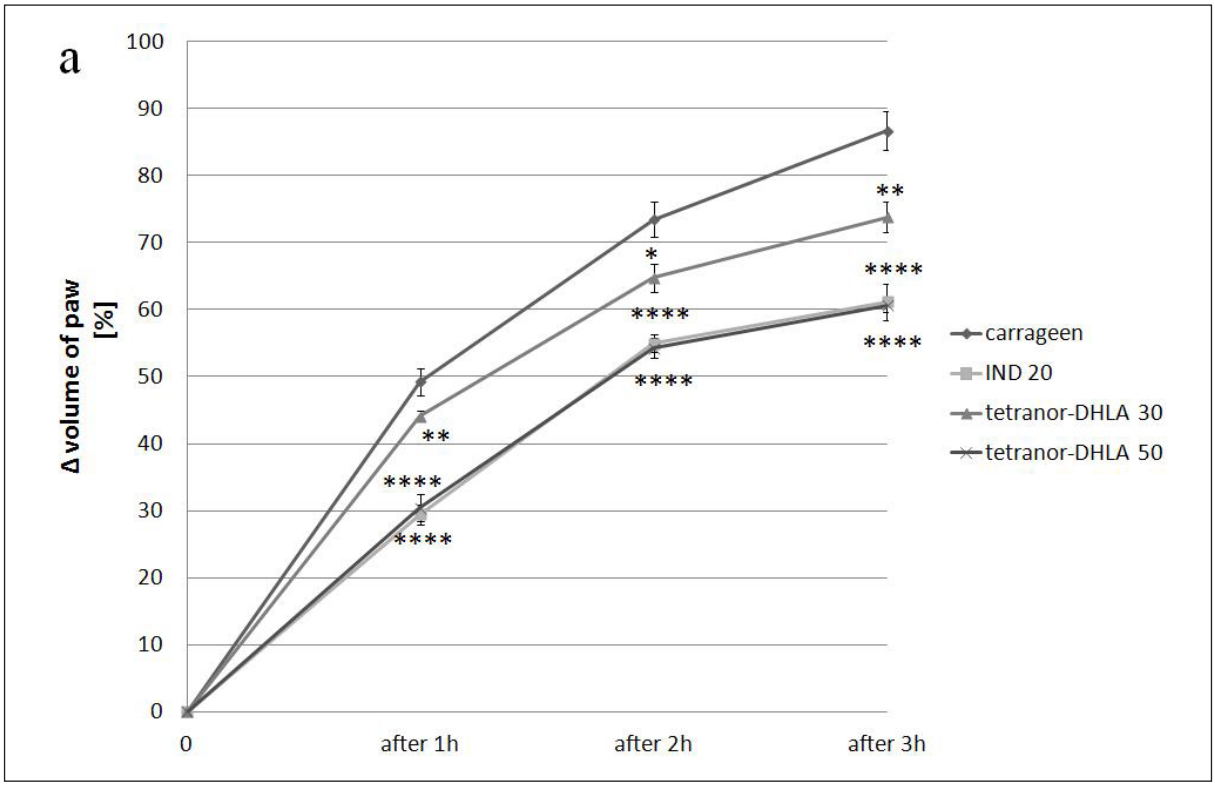

Fig. 4. Effects of pre-treatment with intraperitoneal (a) tetranor-DHLA or (b) BMTBA on paw edema induced by 1% carrageenan (0.05 ml/paw) in mice. The figures show percent change in paw volume in relation to the initial volume (before carrageenan injection). Data are expressed as the mean ± SEM of 8 animals per group. Student's t-test, differences significant vs. control group – carrageenan group – * $p < 0.05$, ** $p < 0.02$, **** $p < 0.001$. IND 20 – indomethacin 20 mg/kg bw; tetranor-DHLA30 – tetranor-DHLA 30 mg/kg bw; tetranor-DHLA50 – tetranor-DHLA50 mg/kg bw; BMTBA30 – BMTBA 30 mg/kg bw; BMTBA50 – BMTBA 50 mg/kg bw; BMTBA100 – BMTBA 100 mg/kg bw

Table 4. Percent inhibition of carrageenan-induced paw edema

Compounds	% inhibition after 1 h	% inhibition after 2 h	% inhibition after 3 h
IND20	40.27	25.16	29.5
tetranor-DHLA30	10.51	11.87	14.9
tetranor-DHLA50	38.2	26.1	30.04
BMTBA30	-2.78	-10.05	-12.96
BMTBA50	38.71	27.35	26.49
BMTBA100	35.42	38.13	42.34

IND 20 – indomethacin 20 mg/kg bw., *ip*.; tetranor-DHLA30 – tetranor-DHLA 30 mg/kg bw., *ip*; tetranor-DHLA50 – tetranor-DHLA50 mg/kg bw., *ip*.; BMTBA30 – BMTBA 30 mg/kg bw., *ip*.; BMTBA50 – BMTBA 50 mg/kg bw., *ip*.; BMTBA100 – BMTBA 100 mg/kg bw., *ip*.

its anti-inflammatory effect after 1 h, 2 h and 3 h was compared to the reference non-steroidal anti-inflammatory drug (NSAID) indomethacin. The obtained data also demonstrated that administration of tetranor-DHLA at the same dose (50 mg/kg bw) significantly inhibited infiltration of neutrophils. It is surprising that tetranor-DHLA at the same dose (50 mg/kg bw) did not reduce vascular permeability in the zymosan-induced peritonitis in mice but its lower dose of 30 mg/kg bw was efficient in that test.

The LD_{50} value in mice for tetranor-DHLA was 75.4 mg/kg bw; thus the doses used in our experiments were below the LD_{50} for this compound.

BMTBA was the second LA metabolite examined in this study. The anti-inflammatory effect of BMTBA at the dose of 100 mg/kg bw was evidenced by a significant reduction of hind paw edema in the BMTBA-treated group. The paw edema 1 h after BMTBA treatment was comparable to that after the reference NSAID indomethacin, whereas after 2 and 3 h BMTBA was even more efficient than indomethacin in paw edema reduction.

Surprisingly, BMTBA at the same dose (100 mg/kg bw) did not reduce vascular permeability in the zymosan-induced peritonitis model in mice. BMTBA effects at the dose of 30 mg/kg bw were also ambiguous. Namely, while the early vascular permeability measured at 30 min of

zymosan-induced peritonitis was significantly reduced in the BMTBA30 group, BMTBA at the same dose acted as an edemagenic agent, like carrageenan.

On the other hand, BMTBA at the dose of 50 mg/kg bw significantly reduced both the early vascular permeability and paw edema formation. For this reason, to test the inhibition of neutrophil infiltration in zymosan peritonitis in mice, we chose the BMTBA50 group.

The obtained data indicated that administration of BMTBA at the dose of 50 mg/kg bw resulted in significant inhibition of infiltration of neutrophils. The BMTBA LD_{50} value in mice reached 400 mg/kg bw; therefore, the dose of 50 mg/kg bw was approximately one tenth of its LD_{50}. Thus, based on the obtained results, it appears that BMTBA is a potential candidate for a safe and efficacious medication in inflammatory diseases.

This study is the first to show the anti-inflammatory effects of both tetranor-DHLA and BMTBA. However, the mechanism of their action is unknown. Both compounds under study appear to be efficient antioxidants, like DHLA and LA. It is well known that oxidation and inflammation are closely interrelated in biological systems [4,12]. Although so far there are no experimental literature data on biological activity of LA biotransformation products, antioxidant properties of bisnorlipoic acid and tetranorlipoic acid and their reduced forms bisnor-DHLA and tetranor-DHLA were confirmed by theoretical studies using quantum-chemical computations [24].

In our opinion, the pharmacological effects of tetranor-DHLA are also associated with the formation of hydrogen sulfide (H_2S), a novel endogenous, gaseous mediator. Zanardo et al. reported that H_2S reduced leukocyte infiltration and edema formation, using the air pouch paradigm and the carrageenan-induced hind paw edema model in rats [33]. Xu et al. indicated that H_2S protected MC3T3-E1 osteoblastic cells against hydrogen peroxide (H_2O_2)-induced oxidative injury [30]. Several authors revealed that H_2S was able to act as an inhibitor of phosphodiesterases (PDE), thereby elevating cyclic AMP and/or cyclic GMP levels, which could contribute to its anti-inflammatory effects [27]. H_2S was also shown to reduce expression of many pro-inflammatory cytokines and chemokines [5,6].

Already in the 1960s Villarejo and Westley indicated that rhodanese (thiosulfate/cyanide sulfurtransferase, TST, EC 2.8.1.1) catalyzed the reduction of thiosulfate to sulfate and H_2S when DHLA (or dihydrolipoamide) was used as a reducing agent [26]. In 2011 Mikami et al. demonstrated that H_2S was also produced by 3-mercaptopyruvate sulfurtransferase (3-mercaptopyruvate/cyanide sulfurtransferase, MST, EC 2.8.1.2) from 3-mercaptopyruvate, when, as in the case of TST, DHLA was used as a reducing agent [16].

Considering the structural similarity of DHLA and tetranor-DHLA, it can be expected that tetranor-DHLA, as a reducing agent, participates in H_2S formation catalyzed by TST and MST.

As for BMTBA, this compound can act as an antioxidant since it is known that S-alk(en)-yl derivatives of thiol compounds (compounds S-substituted by alk(en)yl groups) exhibit antioxidant and anti-inflammatory properties.

Yin et al. reported that S-methyl cysteine (SMC) and S-ethyl cysteine (SEC) intake significantly decreased malonyldialdehyde (MDA) level and increased glutathione (GSH) content in the kidney of diabetic mice [32]. Hsu et al. in a study in mice found that SEC and SMC, as well as S-propyl cysteine (SPC), S-allyl cysteine (SAC) and N-acetyl cysteine (NAC), provided a marked antioxidant protection of enzymes [8]. In the light of structural similarities of BMTBA and SMC, the hypothesis assuming antioxidant properties of BMTBA seems justified.

References

[1] Biewenga G.P., Haenen G.R., Bast A.: The pharmacology of the antioxidant lipoic acid. Gen. Pharmacol., 1997; 29: 315-331

[2] Bilska A., Włodek L.: Lipoic acid - the drug of the future? Pharmacol. Rep., 2005; 57: 570-577

[3] Choi Y.H., Chai O.H., Han E.H, Choi S.Y., Kim H.T., Song C.H.: Lipoic acid suppresses compound 48/80-induced anaphylaxis-like reaction. Anat. Cell Biol., 2010; 43: 317-324

[4] De la Fuente M., Miquel J.: An update of the oxidation-inflammation theory of aging: the involvement of the immune system in oxi-inflamm-aging. Curr. Pharm. Des., 2009; 15: 3003-3026

[5] Ekundi-Valentim E., Santos K.T., Camargo E.A., Denadai-Souza A., Teixeira S.A., Zanoni C.I., Grant A.D., Wallace J., Muscará M.N., Costa S.K.: Differing effects of exogenous and endogenous hydrogen sulphide in carrageenan-induced knee joint synovitis in the rat. Br. J. Pharmacol., 2010; 159: 1463-1474

[6] Fiorucci S., Mencarelli A., Caliendo G., Santagada V., Distrutti E., Santucci L., Cirino G., Wallace J.L.: Enhanced activity of a hydrogen-sulfide releasing mesalamine derivative (ATB-429) in a mouse model of colitis. Br. J. Pharmacol., 2007; 150: 996-1002

[7] Gorąca A., Huk-Kolega H., Piechota A., Kleniewska P., Ciejka E., Skibska B.: Lipoic acid - biological activity and therapeutic potential. Pharmacol. Rep., 2011; 63: 849-858

[8] Hsu C.C., Yen H.F., Yin M.C., Tsai C.M., Hsieh C.H.: Five cysteine-containing compounds delay diabetic deterioration in Balb/cA mice. J. Nutr., 2004; 134: 3245-3249

[9] Jesudason E.P., Masilamoni J.G., Jebaraj C.E., Paul S.F., Jayakumar R.: Efficacy of DL-α lipoic acid against systemic inflammation-induced mice: antioxidant defense system. Mol. Cell Biochem., 2008; 313: 113-123

[10] Kołaczkowska E., Barteczko M., Plytycz B., Arnold B.: Role of lymphocytes in the course of murine zymosan-induced peritonitis. Inflamm. Res., 2008; 57: 272-278

[11] Konrad D.: Utilization of the insulin-signaling network in the metabolic actions of alpha-lipoic acid-reduction or oxidation? Antioxid Redox Signal., 2005; 7: 1032-1039

[12] Ley K., Deem T.L.: Oxidative modification of leukocyte adhesion. Immunity, 2005; 22: 5-7

[13] Litchfield T., Wilcoxon F.: A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Ther., 1949; 96: 99-113

[14] Livack J.E., Britton E.C., Vander Weele J.C., Murray M.F.: Synthesis of dl-Methionine. J. Am. Chem. Soc., 1945; 67: 2218-2220

[15] Malińska D., Winiarska K.: Lipoic acid: Characteristics and therapeutic application. Postępy Hig. Med. Dośw., 2005; 59: 535-543

[16] Mikami Y., Shibuya N., Kimura Y., Nagahara N., Ogasawara Y., Kimura H.: Thioredoxin and dihydrolipoic acid are required for 3-mercaptopyruvate sulfurtransferase to produce hydrogen sulfide. Biochem. J., 2011; 439: 479-485

[17] Odabasoglu F., Halici Z., Aygun H., Halici M., Atala F., Cakir A., Cadirci E., Bayir Y., Suleyman H.: α-Lipoic acid has anti-inflammatory and anti-oxidative properties: an experimental study in rats with carrageenan-induced acute and cotton pellet-induced chronic inflammations. Br. J. Nutr., 2011; 105: 31-43

[18] Packer L., Witt E.H., Tritschler H.J.: α-Lipoic acid as a biological antioxidant. Free Radic. Biol. Med., 1995; 19: 227-250

[19] Plieninger H.: Die Aufspaltung des γ-Butyrolactons und α-Amino-γ-butyrolactons mit Natriummethylmercaptid bzw.-selenid. Eine Synthese des Methionins. Chem. Ber., 1950; 83: 265-268

[20] Reed L.J., Niu C.I.: Syntheses of DL-α-Lipoic acid. J. Am. Chem. Soc., 1955; 77: 416-419

[21] Schupke H., Hempel R., Peter G., Hermann R., Wessel K., Engel J., Kronbach T.: New metabolic pathways of alpha-lipoic acid. Drug. Metab. Dispos., 2001; 29: 855-862

[22] Skibska B., Józefowicz-Okonkwo G., Gorąca A.: Protective effects of early administration of alpha-lipoic acid against lipopolysaccharide induced plasma lipid peroxidation. Pharmacol. Rep., 2006; 58: 399-404

[23] Smith A.R., Shenvi S.V., Widlansky M., Suh J.H., Hagen, T.M.: Lipoic acid as a potential therapy for chronic diseases associated with oxidative stress. Curr. Med. Chem., 2004; 11: 1135-1146

[24] Szeląg M., Mikulski D., Molski M.: Quantum-chemical investigation of the structure and the antioxidant properties of α-lipoic acid and its metabolites. J. Mol. Model., 2012; 18: 2907-2916

[25] Teichert J., Hermann R., Ruus P., Preiss R.: Plasma kinetics, metabolism, and urinary excretion of alpha-lipoic acid following oral administration in healthy volunteers. J. Clin. Pharmacol., 2003; 43: 1257-1267

[26] Villarejo M., Westley J.: Mechanism of rhodanese catalysis of thiosulfate-lipoate oxidation-reduction. J. Biol. Chem., 1963; 238: 4016-4020

[27] Wallace J.L., Ferraz J.G,. Muscara M.N.: Hydrogen sulfide: an endogenous mediator of resolution of inflammation and injury. Antioxid. Redox. Signal., 2012; 17: 58-67

[28] Winter C.A., Risley E.A., Nuss G.W.: Carrageenin-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. Proc. Soc. Exp. Biol. Med., 1962; 111: 544-547

[29] Wollin S.D., Jones P.J.: Alpha-lipoic acid and cardiovascular disease. J. Nutr., 2003; 133: 3327-3330

[30] Xu Z.S., Wang X.Y., Xiao D.M., Hu L.F., Lu M., Wu Z.Y,. Bian J.S.: Hydrogen sulfide protects MC3T3-E1 osteoblastic cells against H2O2--induced oxidative damage-implications for the treatment of osteoporosis. Free Radic. Biol. Med., 2011; 50: 1314-1323

[31] Yazawa K., Suga K., Homma A., Shirosaki M., Koyama T.: Anti-inflammatory effects of seeds of the tropical fruit Camu-Camu (Myrciaria dubia). J. Nutr. Sci. Vitaminol., 2011; 57: 104-107

[32] Yin M.C., Hsu C.C., Chiang P.F., Wu W.J.: Antiinflammatory and antifibrogenic effects of s-ethyl cysteine and s-methyl cysteine in the kidney of diabetic mice. Mol. Nutr. Food Res., 2007; 51: 572-579

[33] Zanardo R.C., Brancaleone V., Distrutti E., Fiorucci S., Cirino G., Wallace J.L.: Hydrogen sulfide is an endogenous modulator of leukocyte-mediated inflammation. FASEB J., 2006; 20: 2118-2120

[34] Zhang W.J., Wei H., Hagen T., Frei B.: Alpha-lipoic acid attenuates LPS-induced inflammatory responses by activating the phosphoinositide 3-kinase/Akt signaling pathway. Proc. Natl. Acad. Sci. USA, 2007; 104: 4077-4082

[35] Ziegler D.: Thioctic acid for patients with symptomatic diabetic polyneuropathy: a critical review. Treat. Endocrinol., 2004; 3: 173-189

The authors have no potential conflicts of interest to declare.