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Antiinflammatory activity of methionine, methionine sulfoxide and methionine sulfone

M.K. Unnikrishnan and M.N.A. Rao

Department of Pharmaceutical Chemistry, College of Pharmaceutical Sciences Manipal-576119, India

Abstract

The oxidation of methionine in peptides is often associated with the loss of biological activity. Since methionine showed good antiinflammatory activity, its oxidized products methionine sulfoxide and methionine sulfone were tested. The sulfone was more active than the sulfoxide although methionine was most active indicating that the antiinflammatory activity is not correlated with the oxidation state of sulphur. Their hydroxyl radical scavenging activity was measured. Methionine was most active and sulfone was least active. Here also no correlation with antiinflammatory activity was found.

Introduction

The oxidation of methionine in peptides is often associated with the loss of biological activity [1]. Chemotactic peptides like fMet-Leu-Phe and proteinase inhibitors like alpha-1-proteinase inhibitor are inactivated by the oxidation of methionine specifically [1]. Many of these peptides are associated with inflammation. Methionine, both DL and L isomers were reported to possess good antiinflammatory activity [2, 3]. Free radicals like superoxide, hydroxyl radical etc. are implicated in inflammation [4, 5]. Number of free radical scavengers are known to possess antiinflammatory activity [6, 7]. Methionine is also a potent hydroxyl radical scavenger [8]. Many other aminoacids which do not contain sulphur, like tryptophan [9], phenylalanine [9], creatine [10], glutamine [11] etc. are reported to possess antiinflammatory activity [12]. These aminoacids can also act as scavengers of oxygen radicals due to their "captodative" nature i.e., they have a carbon radical centre with increased stabilization due to combined effect of electron withdrawing and electron releasing group [13, 14].

We were interested to know whether the oxidation state of sulfphur influences the antiinflammatory activity of methionine and its hydroxyl radical scavenging activity. In the present study we have measured the antiinflammatory activity of methionine, methionine sulfoxide and methionine sulfone. They have also been tested for their hydroxyl radical scavenging activity.

Materials and methods

Albino rats (Charles-Foster strain) of either sex weighing 150–200 g were used for Antiinflammatory activity.

DL-methionine, DL-methionine sulfoxide, DL-methionine sulfone and p-nitroso dimethylaniline were from Sigma.

Antiinflammatory activity

Antiinflammatory activity was measured by carrageenan induced paw edema as described by Winter et al. [15]. Drugs were given orally as solution in distilled water. The solution was so prepared that

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for every 100 g body weight of the animal 0.3 to 0.8 ml of the solution was administered depending on the dose. Groups of six rats were dosed orally with drug 1 h before injection of 0.1 ml of a 1% suspension of carrageenan in normal saline into the subplantar region of the right hind paw. Control animals (six) received carrageenan only. Oedema was measured 3 h later plethysmographically. Mean increase in the paw volume and standard error of the mean (SEM) for each group were calculated and the results were expressed as percent inhibition of oedema as compared to the control group. ED_{50} values were determined by semilogarithmic plot of the % inhibition versus log dose.

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity was measured as described earlier with slight modifications [16]. Hydroxyl radical generated through Fenton reaction, can bleach p-nitrosodimethyl aniline (pNDA) specifically. Scavenging activity was measured by the extent of inhibition of bleaching of pNDA in presence of the test compound.
To a reaction mixture containing EDTA (0.1 m*M*) ascorbic acid (0.1 m*M*), H_2O_2 (2 m*M*), pNDA (0.01 m*M*), test compound in phosphate buffer pH 7.4 (20 m*M*), was added ferric chloride (0.1 m*M*) to give final volume of 3 ml. Absorbance was measured at 440 nm. Percentage scavenging was calculated from the control where not test compound was present. All solutions were prepared freshly in phosphate buffer except ferric chloride which was prepared freshly in distilled water. Experiments were done in triplicate.

Results

Antiinflammatory activity

Antiinflammatory activity of methionine and its oxidised products is given in Table 1. Methionine was found to be most active and methionine sulfoxide least active. ED_{50} of methionine and methionine sulfone are in the similar range.

Hydroxyl radical scavenging activity

Table 2 shows the hydroxyl radical scavenging of methionine and its oxidised products. All three derivatives have appreciable scavenging activity. Methionine is most effective followed by sulfoxide and sulfone. The scavening activity is proportional to the oxidation state of sulphur.

Table 1
Antiinflammatory activity of methionine and its oxidation products

Drug	Antiinflammatory activity (%) ±SEM			
	Dose (mg/kg)			ED_{50} (mg/kg)
	25	50	100	
Methionine	28.3±1.5*	60.3±4.7*	73.4±5.3	44.2
Methionine sulfoxide	0.8±2.8	24.3±1.9*	31.6±2.7	202.7
Methionine sulfone	21.8±2.1	53.3±5.8*	60.9±4.8	59.0

Antiinflammatory activity by carrageenan induced paw edema in rats at the end of 3 h. Drug was given orally as solution in distilled water (* $p < 0.05$ Mann-Whitney test). Six rats were used for each test group and the control.

Table 2
Hydroxyl radical scavenging activity of methionine and its oxidation products

Drug	Hydroxyl radical scavening (%) ±SEM			
	Concentration (mg/ml)			IC_{50} (mg/ml)
	0.2	0.4	0.8	
Methionine	42.7±0.2	57.0±0.9	87.6±1.4	0.301
Methionine sulfoxide	36.8±0.2	57.0±0.6	84.4±0.7	0.346
Methionine sulfone	32.5±0.3	52.6±0.5	74.5±1.1	0.420

Hydroxyl radical was generated through Fenton reaction. Scavenging activity was measured by the inhibition of bleaching of *p*-nitro dimethylaniline by hydroxyl radical in presence of drug.

Discussion

The present study shows that although the oxidation of methionine results in a decrease in antiinflammatory activity, this decrease is not correlated with the oxidation state of sulphur. The sulfone showed good activity despite being at a higher-oxidation state than the sulfoxide. Similarly no correlation was observed between hydroxyl radical

scavenging activity and the antiinflammatory activity. Lack of correlation between hydroxyl radical scavenging and antiinflammatory activity has been observed earlier also [7]. Mannitol, benzoate are known to be inactive as antiinflammatory agents despite being good scavengers of hydroxyl radical [17]. Thus the antiinflammatory activity of methionine may not be due to its hydroxyl radical scavenging activity. However it must be noted that the antiinflammatory activity was measured *in vivo* whereas the hydroxyl radical scavening activity was determined *in vitro*. Due to differences in polarity the absorption, distribution and metabolism may be different for methionine and its oxidised products. They may also account for the differences in activities. Methionine sulfoxide can be converted to methionine in the body by the enzyme methionine sulfoxide reductase [1].

No such enzymes are known which can reduce the sulfone to the sulfoxide or to methionine. Also sulfones are in general more stable and resist reduction compared to sulfoxides. Considering these facts it is interesting that methionine sulfone shows good antiinflammatory activity orally.

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