rons innervating the melanophores. Fujii[29] states that very little is known about the melanosome dispersive action of Na^+ ions and the phenomenon has been disregarded since dispersed melanosomes have been equated with the resting state of melanophores. Fujii[29] also suggests that Na^+ ions may act by stimulating melanosome dispersing neurons, but Fernando and Grove[30] have disputed this interpretation. Neuromelanophore transmission and neural signal to melanosome motility transduction appear to be independent of Na^+ channels, in contrast with the neuronal component[28]. However, a possible relationship between membrane polarization and melanosome migration involving a Na^+ pump has been suggested[31]. K^+-rich AF and Na^+-rich DF represent means of clamping membrane polarity in the components of this physiological model and changes in K^+:Na^+ ratio in the incubation media will influence polarization. K^+ potency probably reflects a positive feedback in which a small degree of neuronal depolarization establishes an excitation threshold opening voltage sensitive Na^+ channels, and thereby triggering noradrenaline release. This would explain the almost complete melanosome aggregation in response to a relatively small increase in K^+. Thus, increasing K^+:Na^+ ratio effectively simulates the effect of electrical stimulation of scale slips of *Labrus ossifagus*[14] in inducing melanosome aggregation. In vivo dispersed melanosomes probably represent a 'resting' state of melanophores in relation to body fluids rich in Na^+ ions, irrespective of any additional melanosome dispersive neural and hormonal factors that may regulate these effectors.

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Determination of cerebrospinal fluid and serum lead levels in patients with amyotrophic lateral sclerosis and other neurological diseases

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Summary. In a total of 62 samples of cerebrospinal fluid (CSF) and an equal number of serum samples obtained from 16 patients suffering from amyotrophic lateral sclerosis, 22 patients suffering from miscellaneous neurological diseases, and 24 controls, lead was measured by atomic absorption spectrophotometry. No statistical difference in lead concentration was found between the above three groups.
Key words. Lead; cerebrospinal fluid (CSF); amyotrophic lateral sclerosis (ALS).

The lead (Pb) level in the cerebrospinal fluid (CSF) in neurological disorders, mainly in amyotrophic lateral sclerosis (ALS), has already been studied[2–7]. However, the reported results are uncertain and often contradictory; this is probably due to methodological errors. The purpose of this paper is to present our results for CSF and serum Pb content in patients suffering from ALS compared with other neurological diseases and controls.

Material and methods

In a total of 62 CSF samples, obtained from the same number of patients after lumbar puncture performed for diagnostic purposes, Pb was measured. 16 out of 62 samples were from patients suffering from amyotrophic lateral sclerosis (13 males and 3 females with a mean age of 52 years), 22 samples were from patients suffering from miscellaneous neurological diseases (21 males, 1 female with a mean age of 48 years), such as multiple sclerosis (N = 5), polyneuropathy (N = 8), cerebellar atrophy (N = 4), myelopathy (N = 5), and 24 samples were from healthy controls (17 males and 7 females with a mean age of 42 years). In all the above subjects serum was also obtained for Pb determination. CSF and serum samples were collected in acid-washed polypropylene tubes and were stored in small aliquots (Eppendorf tubes) at − 20 °C until analysis. In order to avoid contamination all the necessary precautions were taken during all the steps of the whole procedure. The laboratory utensils, the pipettes and Eppendorf tubes were soaked in 1% nitric acid solution for at least 16 h and then rinsed 5–6 times with water (Analar BDH). Furthermore we made sure that the needles were not contaminated with lead.

The lead CSF and serum concentrations were measured by an atomic absorption spectrophotometer (Perkin Elmer model 2380) with a graphite furnace (HGA) 300. Pyrolytically coated graphite tubes were used and a lead hollow-cathode lamp (operating at 25 mA). The atomic absorption was monitored at 283.3 nm with background correction on. The spectral slit width was 0.7 nm and the purge gas was argon at a flow rate of 300 ml/min. Operating conditions of the graphite furnace were: drying 100 °C/10 R, 30 H s, ashing 490 °C/10 R, 60 H s, atomization 2600 °C/10 s (gas stop flow). The sample volume was 20 µl and as diluent Triton-X-100 2% was used.

The interassay coefficient of variation was 3.5% and the statistical analysis was performed using the Mann-Whitney U-test. The concentration of lead was expressed in µg/l.

Results

As can be seen in the table, the CSF Pb mean value in the group of patients with ALS was 0.70 ± 0.47 SD µg/l which does not statistically differ ($p > 0.1$) from the mean value for the control group (0.62 ± 0.41 SD µg/l), or from the mean value for the group of patients with miscellaneous neurological diseases (0.65 ± 0.25 SD µg/l) ($p > 0.1$). The mean value for this group of different neurological diseases does not differ statistically from the mean value for the control group ($p > 0.1$).

Also, no difference was found in the concentration of lead in the serum of patients with ALS as compared to the controls and to the group of patients with various neurological disorders ($p > 0.1$ and $p > 0.1$, respectively). The obtained mean values of serum Pb were 0.76 ± 0.34 SD µg/l (ALS), 0.61 ± 0.31 µg/l (controls) and 0.65 ± 0.35 SD µg/l (miscellaneous).

Discussion

The CSF and serum lead values obtained in this investigation for both normal subjects and patients were low as compared to the values reported in previous communications. The modern method of analysis used was accurate and highly sensitive. The low values may well have been due to the fact that strictly controlled conditions to avoid contamination were followed during the whole procedure.

As opposed to previous studies in which a relationship between ALS and lead levels in CSF and serum[2,3] was reported, our results show that the levels of lead both in CSF and serum of patients with ALS do not differ statistically from those of the control group ($p > 0.1$). Also, no difference in CSF and serum lead levels was noted between the group of ALS patients and the group with miscellaneous neurological diseases ($p > 0.1$). Lead has been claimed to be an etiological or initiative factor of amyotrophic lateral sclerosis. This opinion is mainly based on the correlation between the disease and the exposure of the patients to lead[4]. However, the results reported in previous studies concerning the role of Pb in the pathogenesis of ALS are contradictory. Thus the relationship between lead and ALS has been supported by Conradi et al.[2] and Ronnevi et al.[3] in a series of publications. In 12 patients suffering from ALS they found the lead content of CSF significantly elevated as compared to 28 controls but, according to the above authors, this finding does not prove a pathogenic effect of the metal in ALS. They consider that lead reaches the motor neurons from the skeletal muscles. Cambell et al.[5], in a series of 74 cases of ALS, found that 15% of these patients had a history of extensive exposure to lead compared with 5.4% of the controls. They consider that the lead released from bone may be a factor in the production of the disease. This opinion is based on the symptomatic improvement which was noted after treatment with chelating agents. In contrast to the above-mentioned investigators, Stober et al.[6] do not support the

Group of patients	No. of cases	CSF Pb mean ± SD(µg/l)	Serum mean ± SD (µg/l)
Controls	24	0.62 ± 0.41	0.61 ± 0.31
Amyotrophic lateral sclerosis	16	0.70 ± 0.47*	0.76 ± 0.34*
Miscellaneous neurological diseases	22	0.65 ± 0.25[a]	0.65 ± 0.35[a]

*$p > 0.1$ N.S. Comparison of ALS to controls. [a]$p > 0.1$ N.S. Comparison of ALS to miscellaneous neurological diseases.

assumption of lead poisoning as a pathogenic factor in ALS. They found that the CSF, blood, plasma and erythrocyte values of the metal did not differ appreciably from the controls (CSF Pb 0.89 ± 0.44 µg/l for the patients and 0.85 ± 0.91 µg/l for the controls). These results are in accordance with those of other investigators[7,8], who found no difference in CSF concentrations between patients and controls. Our results also could not confirm the findings of Conradi et al.[2] or the consideration of Cambell et al.[5]. The role of lead in producing the symptoms of ALS is as yet obscure, and the CSF levels of the metal found in this study do not indicate any correlation between lead and the disease, as concerns the CSF and serum Pb levels. Further investigations are required to elucidate this matter.

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Comparative platelet anti-aggregant activity of D-cysteinolic acid analogues

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Summary. D-Cysteinolic acid (**1**) analogues with an S-C-C-N skeleton showed increased platelet anti-aggregant activity in the following order: 2-aminoethanesulfonic acids, thiazolidines, 2-aminoethanethiols and 2-aminoethyl disulfides. Methyl substitutions at the 2-position potentiated the activity. Of these analogues, bis [(*R*)-2-aminopropyl] disulfide was the most potent inhibitor of platelet aggregation, with about 600-fold the activity of (**1**).
Key words. D-Cysteinolic acid; platelet aggregation; inhibitor; marine product; 2-aminoethyl disulfide; sardine.

During our search for pharmacological activities of marine products we focused on D-cysteinolic acid (**1**), isolated from sardine which possesses platelet anti-aggregant activity, as it is a potentially good source from which to derive useful bioactive substances[1,2]. In the work described in this paper, in order to derive a more potent platelet inhibitor from **1**, comparative activities of analogues of **1** which have an S-C-C-N skeleton have been investigated.

Materials and methods

D-Cysteinolic acid prepared in our laboratory[2] was used in this experiment. Analogues **2**–**5**, listed in the figure, were synthesized according to standard procedure[3–8]. The details of the syntheses will be presented shortly. The structural proof of the analogue was based on proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy (fig.). Thiazolidine derivatives were synthesized as previously described[9]. The other analogues consisting of an S-C-C-N skeleton were purchased from Nacalai Tesque, Inc., Kyoto, Japan. Collagen (Type I), arachidonic acid (AA), platelet-activating factor (PAF), adenosine 5'-diphosphate 2Na (ADP), calcium ionophore (A-23 187), thrombin and epinephrine were obtained from Sigma Chemical Co., Mo, USA. Collagen (Horm) was provided by Niko Bioscience Ltd., Osaka, Japan, while all other reagents and solvents came from Nacalai Tesque. Platelet aggregation studies in vitro were performed according to the turbidometric method of Born and Cross[10] in an NKK Hema Tracer1 aggregometer. 25 test samples were added to 225 µl of platelet-rich plasma (PRP) 3 min prior to the addition of an aggregating agent. PRP was obtained from Wistar male rat, albino male rabbit or human blood[11]. Assay for AA-induced mortality in ddy male mice was carried out according to the method of Griffett et al.[12]. AA was administered 30 min after the injection of the sample, and mortality within 3 min after the AA injection was observed.

Results and discussion

Thiol (**2**) and disulfide (**3**) analogues of **1** were more potent inhibitors than **1** against collagen (Type III)-induced aggregation of rat PRP (fig.). Analogues **4** and **5**, with a methyl group were more potent than **2** and **3**, respectively, with a hydroxymethyl group. Of these analogues, **5** was the most potent inhibitor. The EC_{50} (estimated concentration to produce 50% inhibition) value was 4 µM for **5**. This analogue had about 600-fold the