Elevated activity of semicarbazide-sensitive amine oxidase in blood from patients with skeletal metastases of prostate cancer

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ABSTRACT
The semicarbazide-sensitive amine oxidases constitute a group of copper-containing enzymes whose physiological function is unclear. The enzymes are present in various tissues, including blood plasma. At present, the source of the plasma enzyme in humans is not known. Results of a recent study suggested that semicarbazide-sensitive amine oxidase is expressed in the skeleton, e.g. in the spine. Using an indirect autoradiographic method in mice, we provide evidence that semicarbazide-sensitive amine oxidase is present in high abundance in bone tissue. Specific activities of semicarbazide-sensitive amine oxidase were estimated in blood samples from subjects with femoral bone fractures. Moreover, enzyme activities were also measured in patients suffering from prostate cancer with skeletal metastases. The level of specific semicarbazide-sensitive amine oxidase activity in serum was significantly elevated in patients with skeletal metastases compared with both healthy controls and patients having prostate cancer without signs of skeletal metastases. Based on the results of the present study, we propose that semicarbazide-sensitive amine oxidase in blood plasma may originate, at least in part, from the skeleton.

INTRODUCTION
The semicarbazide-sensitive amine oxidases (SSAOs; EC 1.4.3.6) are widely distributed among both eukaryotic and prokaryotic organisms (for a review, see [1]). However, the substrate specificity and the kinetic properties of the enzymes differ markedly between different species. The enzymes in the SSAO family catalyse the oxidation of mono- and di-amines, yielding aldehydes, ammonia and hydrogen peroxide. Moreover, all SSAO enzymes are inhibited irreversibly by semicarbazide and other carbonyl agents, and they require trihydroxyphenylalanine and copper as cofactors. In humans, SSAO in blood plasma shows high specific activity towards benzylamine, which is a non-physiological substrate. The physiological role of this abundant enzyme is essentially unknown, and endogenous substrates with high affinity have so far not been identified. Considerable amounts of SSAO have been observed in the lungs, intestine and placenta. Therefore it has been postulated that SSAO may be a ‘scavenger enzyme’, protecting the organism from toxic exogenous amines. It is known that human plasma SSAO can metabolize both aromatic and aliphatic primary amines, among which are the endogenous substances methylamine and aminoacetone (for reviews, see [1,2]). Oxidation of these substrates yields formaldehyde and methylglyoxal respectively. In contrast with a protective role of SSAOs, it has been proposed by several investigators that reactive aldehydes and hydrogen peroxide produced by endogenous amine oxidases could be of importance with regard to carcinogenesis [3,4].

Key words: autoradiography, benzylamine oxidase, bone scintigraphy, mouse, prostate adenocarcinoma, skeleton.
Abbreviation: SSAO, semicarbazide-sensitive amine oxidase.
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The SSAO activity in blood plasma is changed in some pathological conditions, e.g. decreased specific activities in patients with solid tumours and increased activities associated with diabetes mellitus [5,6] and congestive heart failure [7]. The mechanism(s) underlying these alterations in enzyme activity are currently uncharacterized. With regard to previous studies on cancer, Lewinsohn and co-workers investigated plasma SSAO levels in 125 patients diagnosed with a variety of malignancies, e.g. lung, breast and colon cancers (for a review, see [8]). The mean specific activity of SSAO in blood plasma was significantly lower in this heterogeneous group compared with that in healthy age-matched controls. In that study, 71 patients were receiving either drug treatment or radiotherapy, while 54 were untreated. It was evident that some chemotherapeutic regimes had an effect on SSAO activities, whereas radiotherapy produced no apparent changes in enzyme activity. However, in that study no data were available on the stage of the cancers. In a study on experimentally induced breast cancer in rats, it was shown that an increasing degree of malignancy was associated with a progressive decrease in SSAO activity of the tumour tissue [9]. The relevance of these data for studies on SSAO in human plasma is not clear, since rats express much lower levels of SSAO activity in the blood than do humans.

The source of SSAO in human plasma is not known. It has been speculated that it may be originally synthesized and secreted from the liver or vascular smooth muscle [10]. SSAO activity in blood plasma is elevated in diabetes mellitus, and it has been proposed that late-diabetic vascular damage may result in ‘leakage’ of the enzyme protein into the bloodstream [6].

Data from a recent autoradiographic study on mice indicated that SSAO may be abundantly expressed in the skeleton [11]. Therefore, in the present study, we have used an indirect autoradiographic method to study SSAO activity in the bone of mice. The principle of this technique was to study the formation of [14C]formaldehyde adducts from [14C]methylamine. Moreover, we have investigated the activities of SSAO in blood plasma from patients with fractures or with prostate cancer with and without skeletal metastases.

**METHODS**

**Animals**

Male NMRI mice weighing 35–40 g (9–10 weeks old), purchased from B&K Universal AB (Sollentuna, Sweden), were used in the autoradiographic experiments. Studies were approved by the local ethical committee (permit no. C56/97).

**Autoradiography**

The autoradiographic study was performed using the protocol of Grönvall and collaborators [11]. Mice were pretreated either with two doses of 2.0 mg of hydralazine hydrochloride (Sigma Chemical Co., St. Louis, MO, U.S.A.) on two consecutive days or with saline. Then, 24 h after the last injection of hydralazine or vehicle, the mice were given an intraperitoneal injection of [14C]-methylamine (350 nmol, 20 μCi; Amersham Life Sciences, Little Chalfont, Bucks, U.K.). After a further 24 h, the mice were killed by CO2 asphyxiation and the bodies were frozen at −20 °C. Cryosections (20 μm thick) were collected on adhesive tape and dried at −20 °C for at least 24 h. The tapes with sections from the animals were attached to 14C-sensitive film (Structurix X-ray-film; Agfa-Gevaert), exposed at −20 °C for 3 weeks, and then developed.

**Human subjects**

All subjects included in the study gave informed written consent for their participation in the investigation, which was approved by the local ethical committee. Research was carried out in accordance with the Declaration of Helsinki (1989) of the World Medical Association.

Serum samples from 15 subjects with femoral bone fractures were analysed. Blood samples were collected by venopuncture from patients 24–72 h after the fracture-causing accident. The mean age (± S.D.) in this group was 66.1 ± 21.7 years, and nine of these 15 patients were women. In five of these cases, the accidents were associated with additional fractures of hips and arms.

In addition, 20 patients with prostatic adenocarcinoma were included in the study. The disease was verified histopathologically in all subjects. The patients were also examined by bone scintigraphy performed 2.5–4.0 h after an intravenous bolus injection of 400 MBq of m99Tc-hydroxyethylenediphosphonate (Mallinkrodt, Patten, The Netherlands). Planar images with a total of 400000 counts per image were collected. Ten of these individuals showed significant appearance of skeletal metastases, while the other 10 patients lacked any signs of metastases. The cases were selected so that the two subgroups (with/without skeletal metastases) were ideally matched with regard to tumour size, radiotherapy and pharmacotherapy. The mean ages of the two groups were 65.0 ± 17.3 and 67.4 ± 13.8 years respectively.

Two control groups were included in the study. One control group was used for comparison with subjects with bone fractures, and this group consisted of 24 healthy volunteers (six men and 18 women), i.e. they had no known history of cardiovascular disease or any other condition known to influence SSAO activity. The mean age of the subjects in this control group was 71.7 ± 5.4 years. The other control group was used for comparison with the subjects with prostate cancer, and thus only men were included in this group (n = 10). The mean age of the
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Subjects included in this ‘non-prostate cancer’ group was 70.2 ± 7.7 years.

Patients suffering from diabetes mellitus or subjects taking medication including known SSAO-inhibitory substances were not included in the study.

SSAO activity estimations

Blood samples (5 ml) from the subjects were drawn into tubes without anticoagulant. Within 24 h, the samples were centrifuged at 500 g for 10 min, and 1 ml of serum was transferred to a new tube that was kept at −70 °C for later use. At the time of enzyme analysis, SSAO activities in the serum samples were determined using a radiochemical method following the protocol described by Garpenstrand and colleagues [12], with [14C]benzylamine (Amersham) as substrate. Enzyme activity is expressed as nmol of benzylamine oxidized h⁻¹·ml⁻¹ of plasma.

All comparisons between groups were carried out using factorial ANOVA (analysis of variance).

RESULTS

Evidence of SSAO activity in mouse spine and femoral bone

In accordance with a previous study [11], long-lasting residual radioactivity was observed in various amounts in different mouse tissues after administration of [14C]-methylamine. High densities of radioactive deposits were evident in ileum, brown adipose tissue and spleen. In a control experiment, densitometric analyses were also performed in animals pretreated with hydralazine, i.e. SSAO was irreversibly inactivated. In the hydralazine-treated animals, the levels of radioactivity were below the level of detection in all tissues, suggesting that the accumulation of radioactivity was a consequence of deamination of 14C-methylamine by SSAO (results not shown). Autoradiographs of cryosections containing skeletal tissue showed substantial radioactive deposits in bone. For instance, sections of the spine (Figure 1, upper panel) and of the femoral bone (Figure 1, lower panel) revealed pronounced radioactive accumulation in these tissues.

SSAO activity in plasma from subjects with bone fractures

Samples were collected from 15 individuals with femoral bone fractures (Figure 2). The mean (± S.D.) specific activity of SSAO in serum from these patients was 14.5 ± 3.1 nmol h⁻¹·ml⁻¹. The mean activity in the control group, which consisted of samples from 24 healthy age-matched individuals, was 14.2 ± 2.2 nmol h⁻¹·ml⁻¹. Hence the mean SSAO activity of this patient group did not differ significantly from that observed in the control group. In accordance with previous studies, a strong correlation was observed between enzyme activity (standardized to volume) and protein concentration (r = 0.994).
SSAO activity in plasma from subjects with prostate cancer

SSAO activities were also measured in two groups of patients having prostatic adenocarcinomas (Figure 3). In one of these groups, the patients had skeletal metastases, as confirmed with bone scans, while the patients in the other group lacked signs of metastases. In the patient group having prostate cancer without skeletal metastases, the mean specific activity of SSAO in plasma was 14.0 ± 2.8 nmol·h⁻¹·ml⁻¹, i.e. the levels were comparable with those in the group of healthy controls. In contrast, the mean specific activity of SSAO was significantly elevated in the group of patients having prostate cancer with skeletal metastases. The mean activity in this group was 17.6 ± 2.7 nmol·h⁻¹·ml⁻¹, i.e. the enzyme activity was approx. 25% higher than in healthy controls or patients with prostate cancer without metastases (P < 0.007 and P < 0.012 respectively).

DISCUSSION

The tissue distribution of SSAO activities in mammals has been described by several investigators. For instance, it has been shown that SSAO activity is high in adipose tissue, eye, vascular smooth muscle and lung. Data from such studies have provided evidence for considerable differences in the properties of SSAO between species (for reviews, see [1,13]). Interestingly, in a recent autoradiographic study on mice, we observed high levels of formaldehyde adduct formation in bone [11]. The data from that study indicated that high specific SSAO activities were expressed in the skeleton. However, the film exposures in that autoradiographic study were not optimized for the very high adduct formation occurring in bone tissue.

The tissue origin of the SSAO enzyme(s) in human blood plasma is not known. We postulated that a fraction of the SSAO in the bloodstream may be derived from the skeleton. In order to address this question, we analysed the specific activities of plasma SSAO in samples from individuals with ‘skeletal disease’, i.e. fractures and metastases. Since no previous data were available on enzyme activities in different kinds of bone, we chose to investigate this using an autoradiographic approach in experimental animals. Ideally, estimations of enzyme activity should be performed using homogenates of human skeleton. However, in studies by Norqvist and collaborators [14,15], it was demonstrated that estimation of enzyme activities in hard tissue such as dental matter is associated with considerable intra-individual variability (sample-to-sample variation).

The results of the present study show that SSAO activity in plasma is increased in subjects with prostate cancer and associated skeletal metastases, whereas no alterations were observed in subjects with fractures. Based on the present results, one may speculate that increased SSAO activity in the bloodstream is the result of an alteration in the SSAO synthesis rate in bone, rather than ‘leakage’ from damaged tissue. The possibility cannot be excluded that increased SSAO activity may be observed in blood samples collected at later time points after fracture (>3 days) as a consequence of bone regeneration.

Methylamine is known to be mainly metabolized by SSAO, i.e. there were no radioactive deposits when the animals were pretreated with hydralazine, which is an SSAO-inhibitory agent. It is well known that formaldehyde reacts rapidly with tissue proteins. Accordingly, the substantial radioactive accumulation that was evident in bones of mice injected with [14C]methylamine suggests that SSAO is highly expressed in this tissue.

The endogenous substrates of SSAO are not yet completely characterized, and therefore the role of SSAO in the development of cancer is unknown. The data from the present study do not provide evidence for a role for SSAO in carcinogenesis. However, several groups have recently provided indirect evidence that amine oxidases may be involved in tumorigenesis via production of reactive aldehydes and hydrogen peroxide [16,17]. Moreover, in earlier studies it has been demonstrated that hydrogen peroxide produced by amine oxidases may cause spontaneous neoplastic transformation of cultured murine cells in vitro [3]. Notably, it seems that the specific activities of aldehyde dehydrogenases are reduced in benign prostate hyperplasia, as well as in prostate tumours [18]. This could imply that hyperplasic prostate cells are relatively more sensitive to malignant transformation under the influence of reactive aldehydes. One may therefore speculate that treatment with an amine-oxidase-inhibiting drug may be of therapeutic value. By analogy, it has been proposed by several authors that
treatment with a specific SSAO inhibitor may reduce or prevent the damaging effects of reactive aldehydes and hydrogen peroxide produced under the influence of these enzymes (for a review, see [19]). Drugs such as carbidopa, benserazide and hydralazine have all been shown to inhibit SSAO [20,21], and the widespread clinical experience with these drugs indicates that inhibition of SSAO is not associated with serious side-effects.

In summary, the present study provides evidence for an involvement of SSAO in skeletal metastases of prostate cancer. The mechanism for increased specific activities of SSAO in blood plasma from patients with metastases is unknown. The results of the present investigation may suggest that at least a fraction of the SSAO in the circulation may originate from the skeleton.

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