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Effects of Pretreatment with a Xanthine Oxidase Inhibitor on Free Radical Levels during Carotid Endarterectomy

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Objective: Free radicals contribute to the tissue damage caused by ischaemia–reperfusion. The aim of the present study was to investigate whether preoperative antioxidant therapy (allopurinol) affects free radical levels in cerebral venous blood in connection with surgery for carotid artery stenosis.

Materials and methods: Twenty-five patients were randomised into the study. Thirteen were controls and 12 were pretreated with allopurinol the day before surgery. Before, during and after surgery, blood samples were drawn from the ipsilateral jugular vein. Radical levels were measured using the spin trap technique ***ex vivo*** using OXANOH as the spin trap. Multivariate statistics were used with Principal Component Analysis and Partial Least Square regression analysis.

Results: Radical levels increased with diabetes, high leukocyte count, high creatinine and a high degree of contralateral stenosis. Radical levels decreased with high age, blood pressure, collateral circulation as well as operation for left-side carotid artery stenosis. After pretreatment with allopurinol, several of the relationships noted in the control group were eliminated, i.e. leukocyte count, side of operation, Betapred pretreatment and collateral circulation.

Conclusions: Radical levels can be determined in connection with surgery for carotid artery stenosis using an ***ex vivo*** spin trap method. With preoperative antioxidant therapy the relationships between enhanced radical levels and clinical data, as seen in control subjects, disappeared. This might indicate a beneficial effect of preoperative pretreatment with antioxidants.

Keywords: Carotid artery; Stenosis; Antioxidants; Spin trap; Radical production

INTRODUCTION

Reperfusion after ischaemia causes production of free radicals in various organs, including the brain.[1–3] In animal experiments, antioxidant therapy prevented damage caused by cerebral ischaemia–reperfusion, illustrating the importance of oxidant injury in these events.[4–8] The mechanisms for the production of radicals at reperfusion of ischaemic brain tissue appear to be oxidation of accumulated hypoxanthine via the activity of xanthine dehydrogenase,[9,10] activated neutrophils[11] and metabolism of NO.[12,13]

Most earlier studies of the role of oxygen radicals in the ischaemia–reperfusion damage to the brain were performed on animals due to a lack of suitable methods for clinical studies. In animal experiments, the radical production can be measured with a spin trap technique where, e.g. OXANOH is infused in a regional artery. The local radical production is reflected in the amount of oxidized OXANO found in the regional venous blood measured with spin trap technique. OXANOH reacts with all types of radicals. This technique has been discussed in detail elsewhere.[14] OXANOH cannot be used in a similar way in clinical settings due to unknown toxicity.

In a previous study, we described a technique to analyse the production of free radicals during surgery for stenosis of the carotid artery, based on determination of radical production *in vitro* in venous blood samples.[15] The rationale behind this

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TABLE I Total number of patients, gender, age, prevalence of diabetes, hypertension, cardiac disease, previous vascular surgery, pulmonary disease, renal insufficiency and smoking in the control and treatment groups

	Total	Female/male	Age (years) median range	Diabetes	Hypertension	Cardiac disease	Previous vascular surgery	Pulmonary disease	Renal insufficiency	Smoking
Control group	13	4/9	72 (58–81)	4/13	7/13	5/13	2/13	2/13	0/13	6/13
Treatment group	12	3/9	72 (54–77)	3/12	7/12	5/12	3/12	0/12	0/12	4/12

in vitro technique is that radical production continues in regional venous blood draining a previously ischaemic area. As an example, most radicals detected in kidneys at reperfusion are not superoxide or hydroxyl radicals but rather products from the chain reaction of lipid peroxidation started by the former radicals.[16] One possible way for radical production *in vitro* is oxidation of hypoxanthine due to release of xanthine oxidase and xanthine dehydrogenase from the reperfused tissue. Such a release of xanthine oxidase and xanthine dehydrogenase has been found in venous outflow from previously ischaemic livers and intestine both in animal experiments and clinical settings.[17–19] This release of biological sources for radical production could explain damage to other organs than the previously ischaemic one at reperfusion.

Several studies demonstrate that the xanthine oxidase inhibitor allopurinol has a neuroprotective effect in conjunction with ischaemia–reperfusion of the brain. In experiments on rats, it has been demonstrated that pretreatment with allopurinol reduces neuropathological alterations and hydroxyl radical production and improves energy phosphates at reperfusion after brain ischaemia.[20–22] Preoperative allopurinol administration to infants undergoing cardiac surgery provides significant neurocardiac protection in higher risk patients with hypoplastic left heart syndrome.[23] Furthermore, allopurinol treatment of postasphyxial newborn infants leads to improved cerebral perfusion and electrical brain activity as well as fewer signs of lipid peroxidation compared to the control.[24]

Carotid endarterectomy is a prophylactic procedure aiming at reducing the rate of stroke. During this procedure the cerebral perfusion is at risk to be reduced. Five per cent of the patients suffer a cerebrovascular accident as a consequence of the procedure.[25] The aim of the present study was to analyse the importance of pretreatment with allopurinol on radical production in conjunction with carotid endarterectomy. To enhance sensitivity, multivariate statistical analysis was applied, allowing characterisation of the relationships between clinical parameters and radical production.

MATERIALS AND METHODS

Patients

From 19 May 1999 to 23 November 2000, 25 patients were included in the study. The patients were randomised to either the control group or the treatment group, where allopurinol (Zyloric®) 300 mg × 3 was given the day before the surgery. No placebo was given to the control group. Data concerning the patients are presented in Table I. There were no differences between the treatment group and the control group as for gender, age, diabetes, hypertension, cardiac disease, previous vascular surgery, pulmonary disease, renal insufficiency or smoking habits. Indications for operation, side of operation, degree of stenosis and occurrence of collaterals are presented in Table II. There were no differences in these aspects between the groups. Operation for left-side stenosis was more common than right-side stenosis.

Karlström performed 12 of the 13 operations in the control group and 10 of the 12 operations in the treatment group. This surgeon has a CVA complication rate of less than 3%.

TABLE II Indications for carotid endarterectomy, side of stenosis, degree of stenosis and degree of contralateral stenosis in the control and treatment groups

	Minor stroke	Amaurosis fugax	TIA	Left/right	Degree of stenosis (Mean ± SEM)	Contralateral stenosis (Mean ± SEM)
Control group	8/13	3/13	2/13	9/4	89.6 ± 1.4	40.0 ± 7.6
Treatment group	8/12	3/12	1/12	8/4	89.2 ± 1.5	47.7 ± 9.2

Randomisation

Patients were randomised to either the control group or the allopurinol group. The randomisation was stratified according to Pocook and Simon[26] to achieve highest possible match for age (cut-off point 68 years), gender, diabetes, severe or not severe stenosis (cut-off point 85%). The randomisation was performed the day before surgery.

Ethical

The study was approved by the Ethical Committee of the University of Gothenburg. Approval No. R297-98.

Methods

Anaesthesia

The patients were premedicated with a combination of pethidine and dixyrazine (Esucos®) according to their weight and then operated on under fentanyl-assisted general anaesthesia.

Operation

Operation was in all cases performed for symptomatic carotid stenosis >70%. During operation cerebral oxygen saturation was continuously monitored using cerebral oximetry. The carotid artery was exposed via an incision along the anterior border of the sternocleidomastoid muscle. A 1-mm catheter was inserted via the facial vein into the jugular vein and advanced up to the level above the skull base, and the facial vein was divided. The catheter was advanced 12–15 cm up until resistance and then withdrawn 1 cm until blood could be aspired without resistance. The common carotid and internal carotid artery were isolated separately without touching the carotid bifurcation, whereafter the external carotid artery was isolated.

After clamping the common and external carotid artery, stump pressure was measured using an arterial pressure monitor via a needle inserted into the internal carotid artery distal to the stenotic lesion. Stump pressure was produced by the other extracranial arteries supplying the brain, such as the contralateral carotid artery and the vertebral arteries via the circle of Willis. A shunt may sometimes be used to temporarily bypass the clamped vessels in order to avoid ischaemic damage of the brain. This was not considered necessary in any of the cases, as judged by sufficient stump pressure and cerebral oximetry. The internal and common carotid arteries were opened via a longitudinal arteriotomy and the plaque removed. In order to prevent dissection, the remaining intimal layer was secured distally using intimal sutures when considered appropriate. The arteriotomy was closed using 6-0 prolene continuous sutures. In two cases, in each group, the arteriotomy was closed using a patch to avoid narrowing of the internal carotid artery. The clamping time was 41.5 ± 4.3 min for right-side stenosis and 38.7 ± 2.1 min for left-side stenosis (mean ± SEM).

After the operation, the patients were observed in the ICU for one night. Before being discharged from hospital they were checked by a neurologist. The total length of stay averaged 4.6 days in the treatment group and 4.0 days in the control group (NS).

One patient in the control group was reoperated on a few hours after the primary procedure for hemiparesis and aphasia. At reoperation, no thrombosis was found although the patient had persistent paresis in his right arm and dysphasia. One patient in the treatment group had a postoperative transient ischaemic attack (TIA), and one in the control group had a minor stroke with only slight persistent symptoms. In one of these cases (control group) the arteriotomy was reopened before closure of the wound due to low flow and a patch was inserted. In the other case, there was a short reversible attack of dysphasia on the first postoperative day.

There was no mortality and cardiac complications.

Sampling Procedure

After declamping, blood flow was monitored using a sterile doppler probe and a flow meter (Medistim R, Vingmed, Sweden). From the catheter, inserted into the jugular vein, 4 cm^3 blood samples were drawn three times at 5-min intervals, before clamping the carotid artery, once 1 min after clamping and once 3 min before declamping. Another four 4-cm^3 samples were drawn at 1, 5, 10 and 15 min after declamping.

After heparinisation each venous blood sample was divided into two 1-ml portions (one sample and one blank) and pipetted into Eppendorf tubes. OXANOH (v.i.) (1 mM final concentration) was added to both tubes. In order to distinguish the part of the electronic spin resonance (ESR) signal attributable to superoxide and/or hydroxyl radicals, superoxide dismutase, catalase and desferrioxamine were added to the blank tube to a final concentration of 0.1 mg ml^{-1}, 16,000 units ml^{-1} and 0.4 mg ml^{-1}, respectively. The same volume of isotonic sodium chloride as used to solute the scavenger substances was added to the sample tube. Subtraction of the ESR signal seen in the samples treated with antioxidant cocktail from that of the saline samples yields the part of the signal that can be attributed to superoxide and/or hydroxyl radicals or any secondary radicals dependent on these. The tubes were shaken and centrifuged at 14,000 rpm for 1 min. The plasma was removed immediately and frozen in liquid nitrogen, thus the time from sampling to freezing was less than 2 min.

Measurement of Radical Production

OXANOH was used as a spin trap. The stable nitroxide radical 2-ethyl-2,4,4-trimethyloxazolidin-3-yloxy (OXANO$^{\cdot}$) was reduced to the hydroxylamine spin trap 2-ethyl-3-hydroxy-2,4,4-trimethyloxazolidine (OXANOH) by gassing a 10 mM solution of the radical with hydrogen for 45 min in the presence of the catalyst platinum dioxide (PtO_2). On contact with a radical, OXANOH is reoxidised to OXANO$^{\cdot}$, which can be measured by ESR. The spin trap was prepared freshly before each experiment and kept on ice until used.

The samples were transported to the ESR laboratory and thawed. The concentration of OXANO$^{\cdot}$ was analysed using a Bruker ECS 106 ESR spectrometer. The spectrometer settings were as follows: field centre, 3478 G; modulation amplitude, 1 G; microwave power, 10 mW; microwave frequency, 9.70 GHz; scan range, 5 G; scan rate, 1 G s^{-1}; time constant, 20 ms.

The concentration of OXANO in the blank sample was subtracted from that of the corresponding test sample.

Statistics

Due to the small number of patients, standard descriptive statistics did not seem optimal. Instead, principal component analysis (PCA) was performed on the total data set. All variables were scaled to zero mean and unit variance. A detailed description of the computational steps involved in a PCA is given in Ref. [27]. In essence, a variance/covariance matrix is calculated based on the scaled variables. Principal components are calculated as the eigen-vectors of this matrix, yielding the variable loadings shown in variable loading plots. The first principal component has the capacity to encompass a maximum of the variance in one single vector, which is a linear combination of all variables analysed. Each subsequent component constitutes an independent linear combination of variables, capturing a maximum of the variance remaining in the data set, and is orthogonal to all other components. In biological material, with a considerable degree of collinearity between the variables measured, the first component thus represents a large part of all information, compressed into one variable. The subsequent principal components represent independent information, in decreasing order of magnitude. In addition to reducing a large data set to a few components that can be easily overviewed, principal component-based analyses have important, inherent noise-reducing properties due to the simultaneous analysis of several variables. This is analogous to the reduction in noise gained by using large samples, where the large number of objects increases the precision of, for example, the sample mean. Results thus generated by PCA-based methods are more robust than corresponding univariate descriptors or bivariate correlation analyses. Compared with multiple regression techniques, the latter are highly sensitive to distribution and colinearities, while PCA can be applied to any kind of data, regardless of distribution, and, as outlined above, utilises the covariances to reduce noise and to compact the data. In subsequent analyses, Partial Least Square (PLS) regression analyses were applied.[27] As with PCA, the principal components are extracted with the modification that they are construed to find a regression between *X*- and *Y*-data in addition to producing a compact representation of the data. In the calculation of principal components, the components of the *X*-block that produce the strongest linear relation to the *Y*-variables are extracted. The principal difference is that in PLS, a number of (one or several) *Y*-variables are defined. For the regression coefficients yielded in PLS analyses, standard errors were estimated using the jack-knife procedure,[28] which is a non-parametric, general principle for the estimation of errors in various estimates, suitable for PLS regression coefficients. All PCA and PLS models, as well as graphics and standard error estimates, were generated using the Simca-P 8.0 Software (Umetrics, Inc.).

RESULTS

Characterisation of Data

To obtain an overview of the data and detect outliers among the patients or variables, a PCA was performed on the complete data set, i.e. controls and allopurinol-treated patients were pooled (Fig. 1). The PCA yielded two significant components, describing 35% of the information contained in the 42 variables ($R^2X_{cum} = 0.35$), $Q^2_{cum} = 0.046$. The outcome of the PCA of the complete data set is shown in Fig. 1. The OXANO radical levels (PI, I, RP) measured at different time points all show a strong positive correlation, which is apparent from their location in a cluster at the far left of the *X*-axis. They appear in random order within the cluster, reflecting the lack of an obvious time course in the OXANO levels measured during the operation. The variable "Allopurinol", denoting allopurinol treatment, is located at the origin, indicating a lack of correlation between allopurinol treatment and any of the other variables in this general overview. Collateral circulation, left-side surgery, age and betamethasone are all located in the opposite direction to the OXANO cluster, suggesting a negative correlation between these variables and the general level of radical production in these patients.

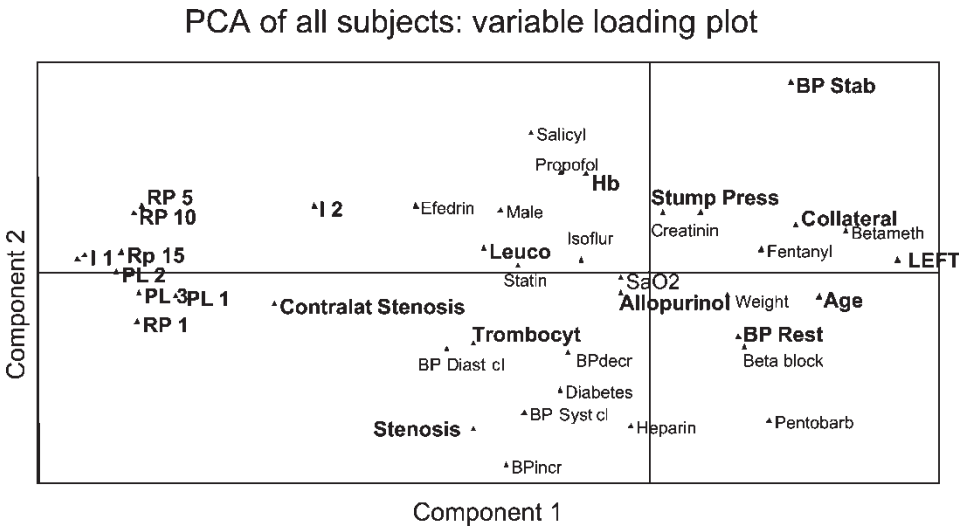

FIGURE 1 Variable loadings derived from a PCA of the full data set, including controls and allopurinol-treated patients. The position of each variable in the loading plot indicates its relationship with other variables. Strongly correlated variables are located close to each other. Abbreviations: PL1, PL2, PL3, OXANO baseline values; I1 etc., OXANO levels after 1 min during clamping etc.; RP1 etc., OXANO levels 1 min after start of reperfusion etc.; SaO_2, O_2 saturation during clamping; BP rest, resting systolic blood pressure; BP decr, per-operative BP decrease; BP inc, Per-operative BP increase; BP Stab, BP stable per-operatively; BP diast, Diastolic pressure during clamping; BP syst cl, systolic pressure during clamping; collateral, degree of collateral circulation; contralat stenosis, degree of contralateral stenosis; leuc, white blood cell count. Variables specifically discussed in the results section are highlighted (bold).

An analogous overview of the patients included in the data set is shown in Fig. 2. Patients located close to each other tend to be similar with regard to many of the variables. It is clear from the graph that the control and allopurinol-treated groups are not separated with regard to the variables measured (Fig. 2). Open and filled circles appear in a random order along both dimensions. The ellipse indicates statistical outliers at 95% probability according to the Hotelling T^2 criterion.[29] One control patient is located on the left-hand side, outside the ellipse, probably due to high values in OXANO radicals. However, since most patients are widely distributed over both dimensions, this patient still fits into the group and should not be excluded.

Effect of Allopurinol

Since the OXANO radicals show clear correlations with several clinical variables, PLS regression models describing how OXANO levels depend on the other variables were calculated separately for the controls and the allopurinol-treated group. In the control group, the PLS regression analysis yielded

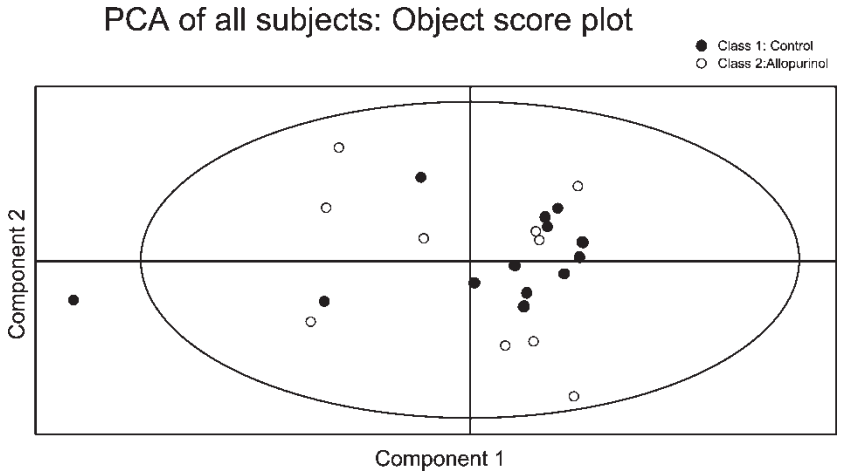

FIGURE 2 Object score plot from a PCA model of the full data set. Object scores contain information from all variables, represented in a small number of components. In this case, two significant components were obtained, based on the 46 original variables. Object scores along the 1st component (horizontal axis) vs. scores along the 2nd component (vertical axis) are shown for all patients. Each circle represents one patient, filled circles denoting controls, open circles denoting allopurinol-treated patients. Patients with close positions in the score plot tend to be similar over the full range of variables measured. The ellipse indicates statistical outliers at 95% probability according to the Hotelling T_2 criterion.

a statistically significant model, relating the various clinical measures to the radical production (two components, $R^2X_{cum} = 0.271$, $R^2Y_{cum} = 0.982$, $Q^2_{cum} = 0.57$). The regression coefficients are shown in Figs. 3a, 4a and 5a. In the allopurinol group, the PLS model came out as non-significant ($Q2 = -0.43$). For comparison, regression coefficients with error bars for the latter model are shown in Figs. 3b, 4b and 5b.

In the control group, the radical production increased with diabetes, higher leukocyte counts, higher creatinine, the occurrence of a contralateral stenosis and the use of ephedrine, and decreased with higher age, blood pressure, higher arterial

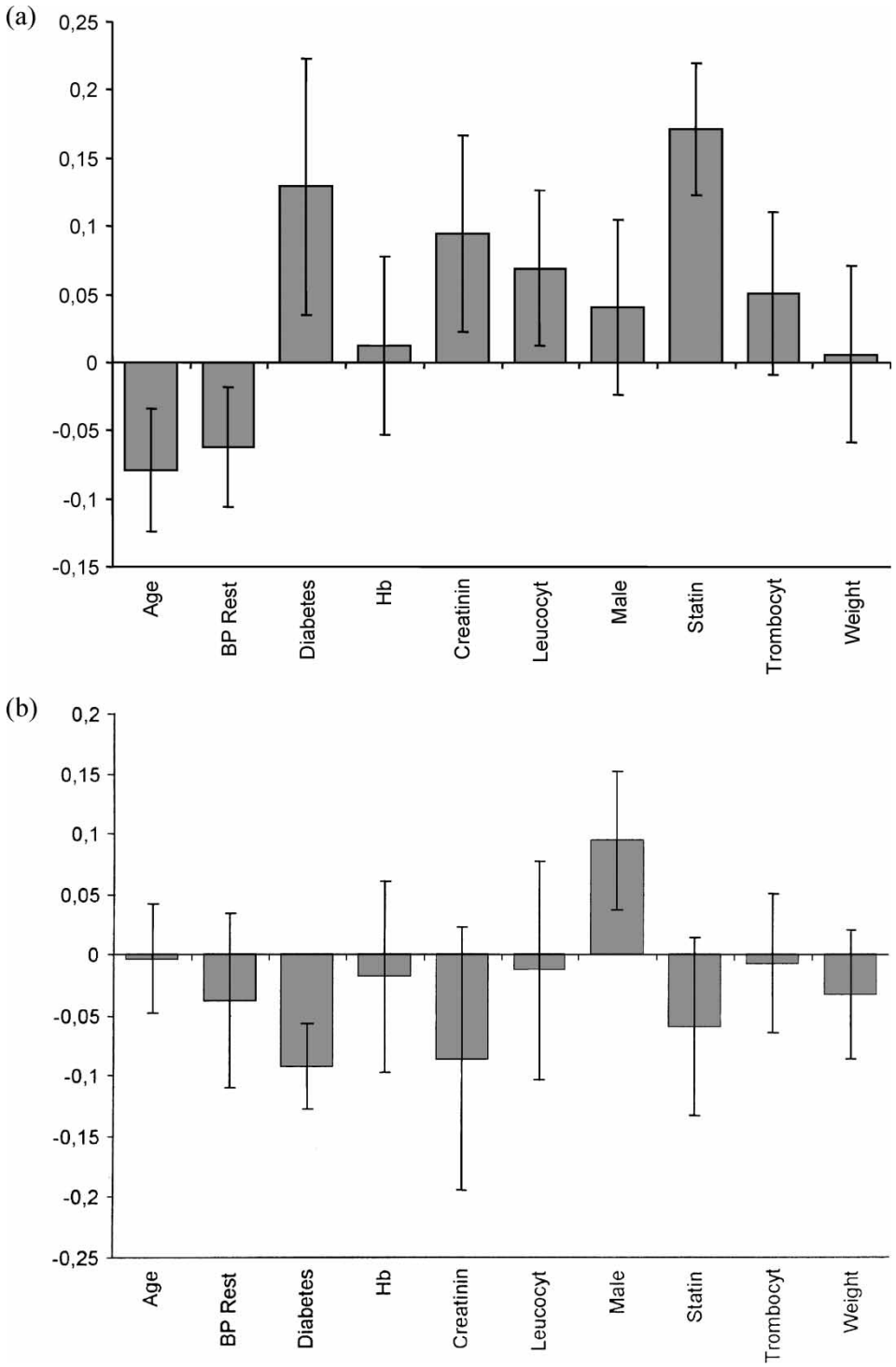

FIGURE 3 Regression coefficients in a PLS model, relating radical production to clinical variables in (a) control patients and (b) allopurinol-pretreated patients. Shown are scaled and centred regression coefficients with standard errors estimated by the jack-knife procedure. A positive coefficient indicates a positive relationship to radical production and vice versa.

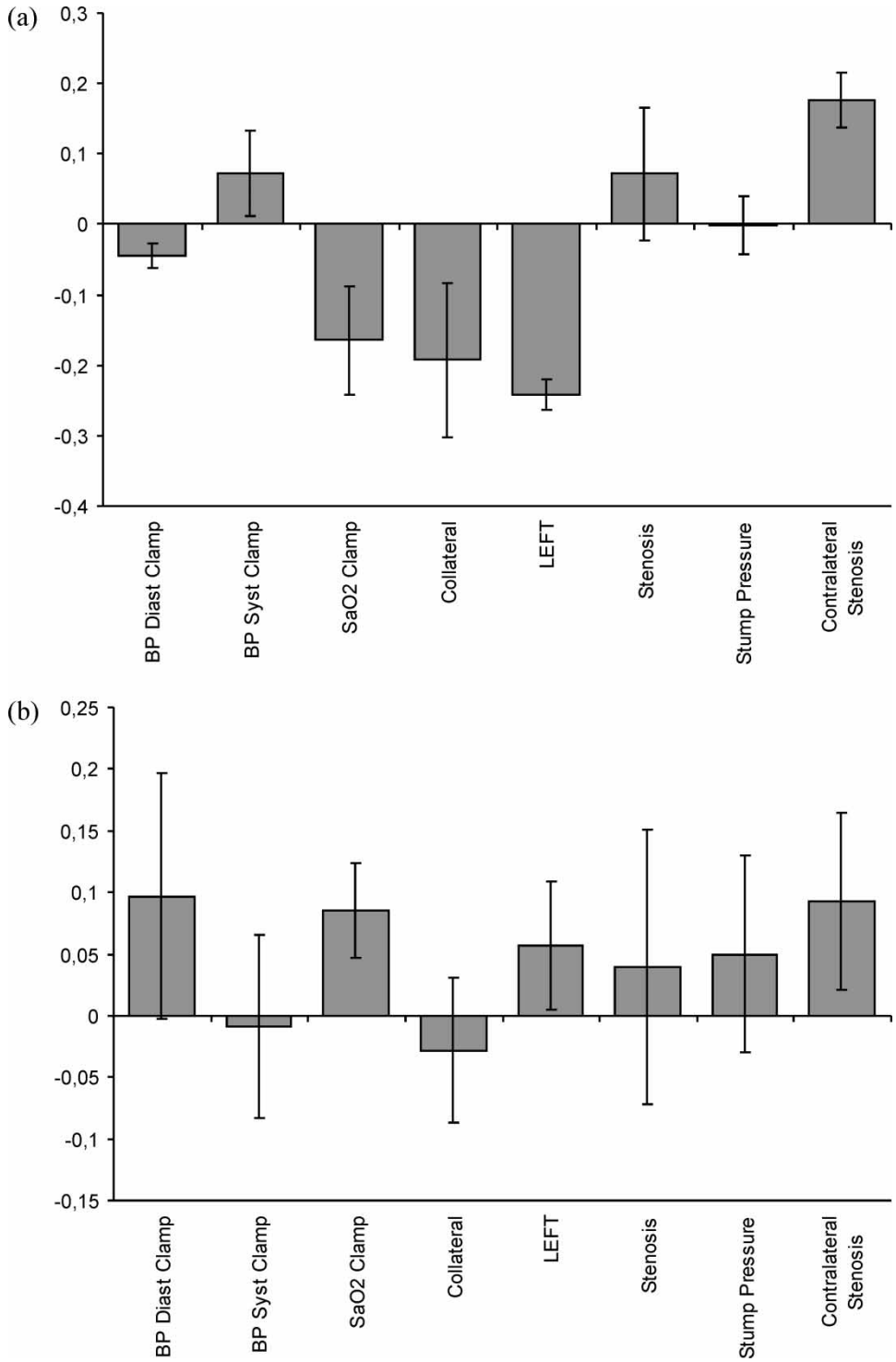

FIGURE 4 Regression coefficients in a PLS model, relating radical production to clinical variables in (a) control patients and (b) allopurinol-pretreated patients. Shown are scaled and centred regression coefficients with standard errors estimated by the jack-knife procedure. A positive coefficient indicates a positive relationship to radical production and vice versa.

oxygen saturation, the occurrence of collaterals and medication with cortisone or beta-blockers. Furthermore, operations for left-side stenosis resulted in lower radical production. After pretreatment with allopurinol, several of the relationships noted in the control group were lacking, causing this part of the model to emerge as insignificant. Notably, no correlations with leukocyte count, side of operation, betapred pretreatment and collateral circulation were seen after allopurinol pretreatment. In both groups, however, the use of ephedrin during surgery as well as the presence of contralateral stenosis was associated with reduced radical production.

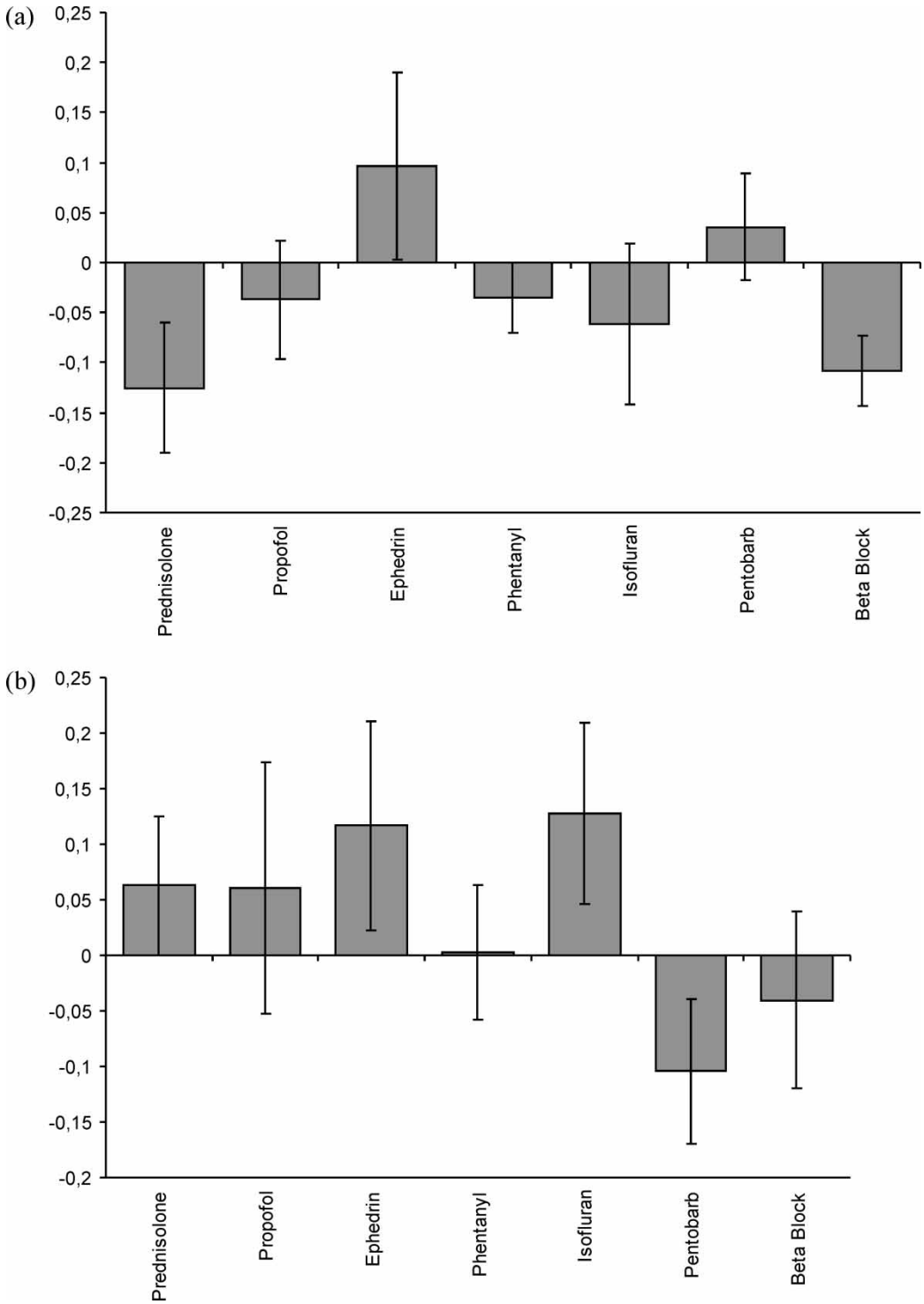

FIGURE 5 Regression coefficients in a PLS model, relating radical production to various anaesthetic variables in (a) control patients and (b) allopurinol-pretreated patients. Shown are scaled and centred regression coefficients with standard errors estimated by the jack-knife procedure. A positive coefficient indicates a positive relationship to radical production and vice versa.

DISCUSSION

The present study compares the production of free radicals during and after operation for stenosis of the carotid artery in patients not pretreated or treated with a xanthine oxidase inhibitor. Radical production has been calculated using an *ex vivo* spin trap technique with OXANOH as the spin trap.

The data collected in this study were investigated using multivariate techniques, to take maximum advantage from the large number of variables in combination with a small sample size. These

data-analytical tools are gaining increased use in the life sciences field, in particular in medical imaging, general pattern recognition, and molecular biology applications, apart from medicinal chemistry where they are standard tools to perform quantitative structure–activity relationship (QSAR) analysis. The basic idea is that in the case when several variables are collected on a sample, information can be gained by looking at variable patterns, rather than examining one variable at a time. From a statistical point-of-view, it can be shown that such an approach has inherent noise-reducing properties and gives an increased sensitivity. Principal component-based methods have the advantage that they are robust to non-normal distributions. Furthermore, correlations among variables do not impair the analysis, but are used to describe the data. PCA and PLS, which is closely related, generate principal components, which are composite variables representing the full data set in an optimal fashion. Thus, typically a complex biological data set can be adequately represented by 1–3 principal components. In some contexts, the term "principal property" is used to emphasise the data compressing nature of principal components. The results from a PCA or PLS are most conveniently examined in "object score plots", or "variable loading plots". The score plot shows the scores of each object with respect to the principal components. Objects that are similar overall, appear near each other in a score plot. Thus, the score plot is useful to detect clusters, and outliers among objects. The loading plot shows the pattern of correlations in the data set. Closely correlated variables show up near each other, while independent variables appear in orthogonal directions. Such plots give an overview on how the variables are interrelated. In addition, score plots and loading plots are super-imposable, so that for instance, the variables underlying a clustering of objects can easily be spotted.

A significant pattern of relationships between the clinical and peri-operative variables was found in a PCA (Fig. 1). Part of this pattern reflects predictable relationships in this patient population, such as the covariance between age, weight, and resting systolic blood pressure. Another reasonable feature is the observed negative correlation between contralateral stenosis and collateral circulation/stump pressure, where the latter two covary. Some of these relationships have been reported in our previous study.[14] It is conceivable that a large degree of contralateral stenosis contributes to a low degree of collateral circulation and low stump pressure, and good collateral circulation increases stump pressure. Furthermore, large contralateral stenosis is likely to be related to stenosis at other locations, e.g. ipsilaterally. Consequently, contralateral and ipsilateral stenosis are to some extent positively correlated, both variables located to the left along component 1 (Fig. 1).

The PCA variable loading plot displays the overall pattern of correlations between the variables in the data set. In general, variables located close to each other are positively correlated, whereas variables located in opposite directions are negatively correlated. Variables located at orthogonal directions to the origin are uncorrelated. In addition, variables appearing close to the origin have no strong correlation with any of the other variables. The general impression of this plot is that the variables are fairly well distributed and most variables carry independent information since they are not tightly clustered. There are no "outliers", i.e. variables with extreme loading values along an axis, which may affect the PCA model negatively since such variables tend to dominate the model and obscure relationships between other variables.

Apart from the haemodynamic interactions, it is worth noting that the degree of contralateral stenosis appears to be the variable that is most strongly related to radical production as reflected in *ex vivo* spin trap measurements. This finding is entirely reasonable. The question arises why the degree of ipsilateral stenosis is not even more important. The explanation appears to be the selection of patients. A major criterion for carotid end-arterectomy is a degree of ipsilateral stenosis between 70 and 95%, which means that the variation in this variable is rather small compared with the variation in contralateral stenosis, which ranges from 0 to 90% among these patients. Since the latter variable has greater variance, correlations with other variables is picked up more sensitively and it is allowed to have more impact on the multivariate models.

The allopurinol symbol is located close to origo, indicating a lack of correlation with any variable in this general overview. However, this does not exclude the fact that allopurinol pretreatment influences the relationship between single variables and radical production as illustrated below.

In the object score plot (Fig. 2), each patient appears as a single circle, with treatment coded by filling pattern. The location (coordinate) of each circle represents the values in all variables, reduced to two dimensions. The contribution of each variable to these two dimensions, i.e. principal components, is visualised in the variable loading plot (Fig. 1). The first principal component (*X*-axis) mainly represents OXANO levels and the degree of contralateral stenosis along with variables appearing at the far right of the horizontal axis, e.g. left-side surgery, age and collateral circulation. Thus, a location on the left in the object score plot indicates a high general level of OXANO radicals, severe contralateral stenosis and low values in age and collateral circulation. The location along the vertical

axis (second principal component) is mainly dependent on the degree of stenosis, preoperative rise in blood pressure and some variables describing medication. Allopurinol-treated and control patients are evenly distributed over the two principal components, which represent all variables in a compact fashion. There are thus no appreciable systematic differences between the groups with regard to any of the variables included in the model.

When the data set was divided into subsets consisting of either controls or allopurinol-treated patients, clear effects of allopurinol were disclosed. Although the general level of each variable was unaffected by allopurinol, the pattern of relationships between the clinical variables versus radical production is disrupted distinctly by allopurinol treatment. In the control group, the OXANO radical levels were significantly related to the other variables. This pattern of relationships appears to have been extinguished in the allopurinol group.

Comparison between radical production in the control and allopurinol groups revealed higher production in both groups when contralateral stenosis prevailed and when ephedrine was used during surgery. These factors appear to be strongly correlated to radical production and not preventable with a blockade of the xanthine oxidase system. However, the positive correlation between leukocyte number and radicals found in the control group disappeared after allopurinol pretreatment, which suggests that this pretreatment interfered with leukocyte radical formation. The effects of prednisolone and beta-blockers seen in the control group were not seen after allopurinol pretreatment. A plausible interpretation is that use of steroids and beta-blockers does not add to the effect of allopurinol once this pretreatment has been given.

In the control group, radical production is more pronounced in patients operated on for right-side carotid stenosis. This could be due to the fact that the right jugular vein drains both hemispheres while the left jugular vein more selectively drains the ipsilateral hemisphere.[30] This side difference disappeared after allopurinol pretreatment, which could be due to lowered radical production in the right-side stenosis group.

A technique, similar to the present one, to determine free radical production in connection with carotid endarterectomy was used by Bacon *et al*.[31] Venous blood was sampled from a catheter inserted into the ipsilateral jugular bulb. The plasma antioxidant potential as well as the spin trap adduct concentration using PBN as a spin trap in the blood samples collected before, during and after endarterectomy were determined. No change in PBN adduct concentration was detected during ischaemia or reperfusion. However, the plasma antioxidant potential was reduced markedly during reperfusion, indicating that free radical species were produced, which consumed antioxidants in plasma. The authors thus found supportive evidence for oxidant production during cerebral ischaemia–reperfusion in a clinical setting. Using a similar sampling technique, Weigand *et al.* found reduced plasma antioxidant status in jugular venous blood during carotid endarterectomy but only in patients with insufficient collateral circulation.[32] In the same patient group, an enhanced jugular venous–arterial malondialdehyde (MDA) difference was found during and after carotid endarterectomy, suggesting oxygen radical-mediated peroxidation of polyunsaturated fatty acids.

The present study shows that radical production can be determined in connection with surgery for carotid artery stenosis using an *ex vivo* spin trap technique with OXANOH as the spin trap. The major finding is that allopurinol pretreatment affects the relationships between the clinical variables seen in controls, which disappear after pretreatment. This might indicate a beneficial effect as the enhanced radical production in, for example, diabetes and contralateral stenosis is lost after pretreatment.

Acknowledgements

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