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Hemostasis Abnormalities in Patients with Vascular Dementia and Alzheimer's Disease

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Summary

Since it has not been established to what extent abnormalities of hemostasis contribute to the occurrence and development of dementia, selected measurements of coagulation and fibrinolysis were obtained in elderly patients with Alzheimer's disease (n = 22) or vascular dementia (n = 29), compared with healthy individuals in the same age range (n = 61). Hemostasis abnormalities were more frequent and marked in vascular dementia, being expressed as significant increases of plasminogen activator inhibitor type 1, von Willebrand factor, D-dimer and activated factor VII. However, some hemostasis measurements (von Willebrand factor, activated factor VII) were abnormally high also in the patients with Alzheimer's disease, a condition in which vascular damage is not considered to play a major pathogenetic role. It could not be established in this study whether or not these hemostatic abnormalities play a causal role in the pathogenesis of dementia, or whether they are secondary to inflammation and chronic vascular disease. Nevertheless, their presence may contribute to aggravating vascular disease.

Introduction

It has been recently suggested that vascular damage is important in the pathogenesis not only of vascular dementia but also of Alzheimer's disease (1). It has been shown, for instance, that the cortical microvasculature is abnormal in patients with Alzheimer's disease, but it is still not clear how greatly vascular changes contribute to the cognitive impairment (1, 2). No systematic study has been carried out to evaluate whether or not the vascular changes are accompanied by hemostatic disturbances in patients with dementia, particularly in those with Alzheimer's disease. Despite this lack of information about the behaviour of hemostasis, the use of anticoagulant therapy has been proposed for the treatment of patients with Alzheimer's dementia (3).

With this as background, we evaluated some selected parameters of coagulation and fibrinolysis in elderly patients with Alzheimer's disease or with vascular dementia and healthy controls in the same age range. We measured fibrinogen, factor VII and plasminogen activator inhibitor type 1 because several studies have shown that these proteins are markers of existing or of a high risk of developing atherothrombotic disease (4-7). To see whether there is a state of heightened activation of coagulation enzymes, the plasma levels of activated factor VII (the key enzyme in the initiation of blood coagulation) (8, 9) and of peptides resulting from the enzymatic activation of the coagulation and fibrinolytic systems (fibrinopeptide A, the prothrombin fragment 1+2 and D-dimer) were also measured. Finally, we measured von Willebrand factor, which is considered a marker of dysfunction of vascular endothelial cells and a risk factor for cardiovascular disease (10).

Materials and Methods

Subjects

A total group of 61 demented patients consecutively referred for diagnostic purposes to the Aging Brain Center of Perugia University entered the study. Thirty-two of them fulfilled NINCDS-ADRDA criteria (11) for probable Alzheimer's disease, all had cerebral CT scans negative for vascular lesions and no history of cardiovascular events and/or major vascular risk factors. Twenty-nine were defined as having probable vascular dementia according to NINDS-AIREN criteria (12); all had Hachinsky Ischemic Scores (13) ≥7. Control subjects (n = 61) were judged to be physically and mentally healthy according to the criteria set by the Senieur protocol (14). In particular, all had Mini Mental State Examination (MMSE) scores ≥ 28.

Blood Sampling

Venipunctures were performed with 19-gauge butterfly infusion sets. After the first 4 ml of blood were discarded, samples were collected directly into vacutainers. For fibrinopeptide A assay, an anticoagulant provided by the manufacturer of the assay kit (see below) and containing unspecified amounts of heparin and aprotinin was used (anticoagulant/blood ratio: 0.1 : 0.9). All the remaining coagulation and fibrinolysis assays were carried out in blood collected into sodium citrate at a final concentration of 3.8% (wt/vol). All blood specimens were centrifuged at 4° C (except for activated factor VII, at room temperature) for 20 min at 2000 x g and platelet-poor plasma was stored at –80° C until assays were carried out within 2-3 months. All assays were done at the Angelo Bianchi Bonomi Hemophilia and Thrombosis Center of the University of Milan.

Assays

Fibrinogen was measured in plasma with a commercial kit based upon the rate of fibrin polymerization (Boehring Biochemia). Factor VII antigen was measured by a previously described enzyme-linked immunosorbent assay (ELISA) (15). Plasminogen activator inhibitor type 1 activity was measured by an amidolytic assay (Biopool, Umeå, Sweden). Activated factor VII, the enzymatically active form of factor VII, was measured with an assay based upon a modification of the prothrombin time, with a truncated soluble form of recombinant tissue factor (prepared by Novo Nordisk, Gentofte and kindly supplied by Dr. Nemerson, Mount Sinai Hospital Medical School, New York, NY) that upon relipidation reacts with activated factor VII but not with the zymogen factor VII (16). The prothrombin fragment 1+2, an index of prothrombin activation by activated factor X, was measured in plasma by ELISA (Enzygnost F 1+2, Behring Germany). Fibrinopeptide A is a measure of the formation of fibrin, being released from the alpha-chain of fibrinogen when thrombin converts fibrinogen to fibrin. It was assayed by ELISA in

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Table 1 Clinical details of the subjects

	Controls	Alzheimer's disease	Vascular dementia
Age (m± SE)	70.1±0.9	67.6±1.3	74.5±1.4 [A, B]
M/F	27/34	13/19	15/14
% of patients with blood groups A, B or AB	62 %	63 %	58%
Mini Mental State Examination	≥ 28	11.1±2	17.8±1.9 [B]
Disease duration (years)	=	5.3±1.0	3.0±0.8 [B]

A: p<0.05 vs controls; **B:** p<0.05 vs Alzheimer

plasma adsorbed twice with bentonite to remove fibrinogen, using a kit from Diagnostica Stago (Asnières, France). Von Willebrand factor antigen, a marker of endothelial perturbation, was assayed by ELISA using monoclonal antibodies (17). D-dimer, a marker of lysis of cross-linked fibrin, was measured by ELISA (Dimertest, Agen Biomedical, Brisbane, Australia).

Statistical Analysis

The values for patients and controls were compared, after logarithmic transformation when necessary, by analysis of covariance. Comparisons were carried out on age-adjusted means (GLM procedure, SAS/STAT Rel. 6.10, SAS Institute, SAS/STAT User Guide 6th Ed., SAS Institute, CARY, NC, USA).

Results

Clinical details of all individuals studied are reported in Table 1. Vascular dementia patients were older than Alzheimer patients and controls; they also had milder degrees of cognition impairment (as expressed by mean MMSE score) and shorter durations of disease. The frequency of blood group other than type 0 (A,B and AB) was similar in the three groups.

In Table 2, age-adjusted means and 95% confidence intervals of the coagulation and fibrinolysis parameters are reported. Activated factor VII was higher in Alzheimer's disease patients and in those with vascular dementia than in the control group (p <0.001). Plasminogen activator inhibitor type 1 was much higher in vascular dementia patients than in controls (p <0.001), being significantly different also from that of patients with Alzheimer's disease (p <0.05). von Willebrand factor antigen was higher in vascular dementia (p <0.001) and Alzheimer's disease (p <0.05) patients than in the control group. Finally, D-dimer was much higher in vascular dementia patients than in controls (p <0.001), and also significantly higher than in patients with Alzheimer's disease (p <0.05). Fibrinogen, factor VII antigen, prothrombin fragment and fibrinopeptide A showed no between-group differences. There was no correlation between biological measurements and the clinical characteristics of the disease (duration and severity of dementia) (not shown).

Table 2 Age-adjusted means and 95% confidence intervals for the biological parameters studied

	Controls (n=61)	Alzheimer's disease (n=32)	Vascular dementia (n=29)
Factor VII antigen (U/dl)	104 (99-109)	107 (100-114)	110 (102-118)
Fibrinogen mg/dl	335 (317-353)	324 (299-349)	361 (335-387)
von Willebrand factor antigen (U/dl)	152 (138-166)	178 (157-199) [C]	185 (163-207) [A]
Plasminogen activator inhibitor type 1 (IU/ml)	4.6 (2.7-6.6)	5.9 (3.2-8.7)	12.9 (10.0-15.9) [A,D]
Activated factor VII (ng/ml)	3.1 (2.8-3.4)	4.2 (3.7-4.6) [A]	4.2 (3.7-4.8) [A]
Fibrinopeptide A (n M)	1.2 (0.2-2.3)	1.9 (0-5.4)	1.3 (0-2.4)
Prothrombin fragment 1+ 2 (n M)	1.3 (1.1-1.4)	1.5 (1.3-1.7)	1.4 (1.2-1.6)
D-dimer (ng/ml)	79 (69-91)	93 (78-112)	129 (102-162) [A,D]

A: p<0.001 vs controls; **B:** p<0.001 vs Alzheimer
C: p<0.05 vs controls; **D:** p<0.05 vs Alzheimer

Discussion

Coagulation and fibrinolysis measurements were often abnormally high in patients with dementia. As expected, abnormalities were usually more frequent and marked in vascular dementia, but some of them were also present in Alzheimer's disease patients. The abnormalities in vascular dementia patients were roughly similar to those found in other groups of patients with atherothrombotic disease, being expressed as significant increases of plasminogen activator inhibitor type 1, von Willebrand factor and D-dimer (6, 7, 10, 18-20). Activated factor VII was high in vascular dementia patients. Fibrinogen, an important marker of atherothrombotic disease, was not significantly increased in the same patients, but there was a trend towards high levels. We still do not know whether the observed changes in coagulation and fibrinolysis measurements are expressions of hypercoagulability or relatively non-specific markers of systemic inflammation accompanying atherothrombotic disease. Our finding of normal levels of measurements of coagulation enzyme activity (fibrinopeptide A and prothrombin fragment 1+2) would favour the latter view. On the other hand, it is possible that, once they have developed as a result of endothelial dysfunction or inflammation, hemostatic alterations may contribute to aggravating the underlying vascular alterations.

The newest finding of this study is that some hemostatic measurements were abnormal even in Alzheimer's disease patients, a condition in which until now vascular damage was not thought to be a major pathogenetic factor. Activated factor VII and von Willebrand factor were high, and there was a positive correlation between the values of the two measurements (r = 0.36, P = 0.006), suggesting that they are associated phenomena. Perhaps high levels of activated factor VII are the results of vascular damage present in these patients, which would facilitate the expression of tissue factor on vascular endothelial cells and accelerate the transformation of factor VII into the corresponding enzyme. Vascular involvement and the resulting endothelial cell perturbation might perhaps also explain the high levels of von Willebrand factor, which would be secreted into plasma in larger amounts from damaged vascular endothelial cells. It is unlikely that the increase in von Willebrand factor was related to a higher prevalence of blood groups other than type 0 (A, B, AB) in patients with Alzheimer disease, because the distribution between blood group type 0 (associated with lower von Willebrand factor levels) and groups non-0 (associated with higher levels) was similar in the three comparison groups.

In conclusion, alterations of hemostasis were seen in patients with dementia. The pattern was similar to that found in patients with atherothrombotic disease, but there are also alterations in Alzheimer's disease, although less marked than in vascular dementia. A causal relation between these alterations and the vascular changes accompanying dementia remains to be demonstrated. On the other hand, since currently available treatments are of little help in slowing or

stopping the progress of Alzheimer's disease, the possibility of evaluating the efficacy of antithrombotic therapy should perhaps be considered for these patients.

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