Cisplatin treatment is immediately followed by a decrease in circulating levels of endothelial and platelet-derived MPs. These findings support the hypothesis that cisplatin-induced stroke may be mediated by release of tumor necrosis factor (TNF) and other pro-inflammatory cytokines that induce endothelial dysfunction. The results also suggest that circulating endothelial-derived and platelet-derived MPs may serve as biomarkers for the prediction of cisplatin-induced stroke.

Key Words: brain infarction • cancer & stroke • cisplatin • microparticles

Multiple cerebrovascular infarctions after cisplatin-based chemotherapy may affect young patients. The mechanisms of cisplatin-induced stroke remain unknown and may be mediated by endothelial dysfunction, toxicity, or apoptosis. Microparticles (MPs) are plasma membrane submicron vesicles shed by activated or apoptotic cells. Endothelial or platelet-derived MPs represent a circulating reservoir of effectors involved in myocardial infarction, stroke, and endothelial dysfunction. MPs exert prothrombotic activity by exposing negatively charged phospholipids and tissue factor. In order to investigate the mechanisms of the cisplatin-induced stroke, we performed a clinical trial to evaluate whether infusion of cisplatin is followed by shedding of endothelial-derived MPs in plasma.

Methods
We included 18 patients treated for cancer with cisplatin. Fasting citrated blood was drawn before and 3 days after cisplatin infusion. Plasma was centrifuged 12 000g to obtain platelet-free plasma and stored at −80°C until analysis. The prespecified delay of 3 days was chosen by analogy with the delay described between cisplatin and stroke, and was confirmed by additional measures at day 1, 2, or 5 for the first patients. Plasma samples were incubated 30 minutes at room temperature with different fluorochrome-labeled antibodies, or their respective isotypic immunoglobulins. Platelet and endothelial epitopes on MP were labeled with anti-CD31-PE or anti-CD41-PC5 (Immunotech). MP expressing phosphatidylserine on their surface were labeled with fluorescein-conjugated Annexin V solution (Roche Diagnostics), in presence of CaCl₂ (5 mmol/L). Unspecific Annexin V-labeling was evaluated in absence of calcium. Flow cytometry analysis was performed with a Coulter EPICS XL in presence of Flowcount calibrator beads (Beckman Coulter). Events were labeled with fluorescein-conjugated Annexin V solution (Roche Diagnostics), in presence of CaCl₂ (5 mmol/L). Unspecific Annexin V-labeling was evaluated in absence of calcium. Flow cytometry analysis was performed with a Coulter EPICS XL in presence of Flowcount calibrator beads (Beckman Coulter). Events were labeled with fluorescein-conjugated Annexin V solution (Roche Diagnostics), in presence of CaCl₂ (5 mmol/L). Unspecific Annexin V-labeling was evaluated in absence of calcium.

Concentrations of MPs are expressed as mean±SD. MPs were log-transformed to obtain a normal distribution before statistical comparison. Change in MPs for each patient and difference between patients and controls were analyzed by paired t test. Statistical significance was defined as P<0.05. Analysis was performed with Stata 9.0 software (Stata Corp).
Results

Eighteen patients (8 women) aged 57±11 years with solid cancer were included. Cisplatin (40 to 150 mg) was administered with etoposide (6 patients), 5-FU (4 patients) and vinorelbine (3 patients). Height patients were having their first cycle of chemotherapy. There was no difference in gender and age between patients and the 18 controls (8 women), age 54±24 years (P=0.67).

Baseline MP were not different between patients and controls (endothelial-derived MPs 435±560/μL versus 460±228/μL, P=0.86; platelet-derived MPs 840±1613/μL versus 1023±810/μL, P=0.67; prothrombotic MPs 1646±2756/μL versus 1612±3664/μL, P=0.97). After cisplatin treatment, patients’ circulating endothelial-derived MPs decreased to 138±133/μL (P=0.003). Platelet-derived MPs decreased to 231±313/μL (P=0.007). Prothrombotic MPs decreased to 316±499/μL (P=0.02; Figure 1). This decrease was not paralleled by a decrease in blood leukocytes and platelets at day 3. The mean baseline MP levels at different chemotherapy cycles are shown in Figure 2. MP of all origins were higher in the 5 patients at the second and third cycle, compared with the 8 patients at the first cycle (all P<0.07).

Thrombin-antithrombin complex and F1+2 levels showed a slight but nonsignificant increase at day 3 (10.0±8.9 μg/L to 11.6±14.0 μg/L, P=0.57 and 1.31±0.97 nmol/L to 1.41±1.09 nmol/L, P=0.58; Figure 1).

Discussion

This study shows that cisplatin infusion was accompanied by an important and significant decrease of circulating MPs originating from either endothelial cells or platelets. This rapid decrease does not support the hypothesis of a direct cisplatin-induced vascular toxicity mediated by increased circulating levels of prothrombotic MPs, together with activation of the coagulation.

The patients included in this trial are representative of the target population, because cisplatin-induced stroke has been observed in patients aged 25 to 72 years, 2 to 6 days after infusion of cisplatin. The small sample size of our study population may appear as a limitation; however, the decrease in MP was consistent for all MP types.

The decrease in circulating MPs may be explained by direct inhibiting effects of cisplatin on hematopoiesis or dilution by perfusion during chemotherapy. However, both hypotheses are unlikely because numbers of platelets and leukocytes were unchanged at day 3. Alternatively, cisplatin infusion might activate MP clearance. It has been demonstrated that MPs are rapidly removed from the blood by liver Kupffer cells.10 Cisplatin infusion might have increased circulating MP phagocytosis by activating macrophage Kupffer cells, as observed in animal models.11

In conclusion, the present data do not support our first hypothesis that cisplatin-induced stroke is associated with a rapid release of circulating procoagulant microparticles. However, a relation between cisplatin and MP release is not

Figure 1. Circulating levels of microparticles and markers of coagulation activity, before and 3 days after administration of cisplatin in 18 patients treated by a cisplatin-based chemotherapy for solid cancer and in 18 healthy subjects.

Figure 2. Baseline levels of MPs displayed by the number of the current cisplatin chemotherapy.
definitely ruled out, because we observed high levels of circulating MPs for the patients who were receiving their second or third cisplatin infusion. This observation should be taken with caution but may be relevant because most cisplatin-induced stroke have been observed after the second or third cisplatin infusion. Further studies on the vascular effects of cisplatin are warranted.

Disclosures
None.

References
Are Circulating Endothelial-Derived and Platelet-Derived Microparticles a Pathogenic Factor in the Cisplatin-Induced Stroke?
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