Longer Duration of Cardiopulmonary Bypass Is Associated With Greater Numbers of Cerebral Microemboli

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Background and Purpose—Many patients who undergo cardiac surgery assisted with cardiopulmonary bypass (CPB) experience cerebral injury, and microemboli are thought to play a role. Because an increased duration of CPB is associated with an increased risk of subsequent cerebral dysfunction, we investigated whether cerebral microemboli were also more numerous with a longer duration of CPB.

Methods—Brain specimens were obtained from 36 patients who died within 3 weeks after CPB. Specimens were embedded in celloidin, sectioned 100 μm thick, and stained for endogenous alkaline phosphatase, which outlines arterioles and capillaries. In such preparations, emboli can be seen as swellings in the vessels. Cerebral microemboli were counted in equal areas and scored as small, medium, or large to estimate the embolic load (volume of emboli).

Results—With increasing survival time after CPB, the embolic load declined (P<0.0001). (Lipid emboli are known to pump slowly through the brain.) Also with increasing time after CPB, the percentage of large and medium emboli became lower (P=0.0034). This decline is consistent with the concept that the emboli break into smaller globules as they pass through the capillary network. A longer duration of CPB was associated with increased embolic load (P=0.0026). For each 1-hour increase in the duration of CPB, the embolic load increased by 90.5%.

Conclusions—Thousands of microemboli were found in the brains of patients soon after CPB, and an increasing duration of CPB was associated with an increasing embolic load. (Stroke, 2000;31:707-713.)

Key Words: bypass surgery ■ cardiopulmonary bypass ■ cerebral embolism ■ cerebral ischemia, transient ■ embolism, fat
36 subjects. Our results support the hypothesis that a longer time on CPB results in a greater embolic load.

Subjects and Methods

Patients

We studied brain specimens obtained at autopsy from 36 subjects who underwent CPB-assisted cardiac surgery at our institution between 1987 and 1997 (Table). For some analyses, the patients were divided into 2 subgroups: patients who underwent coronary artery bypass graft surgery (CABG) only (n=24) and those who underwent cardiac valve repair with or without CABG (n=12). All patients included in the study were adults who died within 3 weeks after CPB that was performed with a membrane oxygenator (not a bubble oxygenator). Other exclusions thought to have potentially confounding effects on the numbers of cerebral emboli included left heart catheterization after CPB (all had left heart catheterization before CPB), retrograde cerebral perfusion, heart transplantation, and CPB more than once within 2 months.

Tissue Preparations

In each subject, a 1.5-cm-thick coronal brain slice was taken from the left hemisphere at the mamillary bodies. As previously described, brain tissue was fixed for 24 hours in cold, buffered, weak formaldehyde (0.4%) and then dehydrated in alcohols, embedded in celloidin, and sectioned 100 μm thick. Sections were dehydrated in alcohols, and celloidin was removed in acetone. The sections were stained for alkaline phosphatase, an endogenous enzyme in the...
endothelium of capillaries and arterioles, and then cleared in xylene and mounted on oversized glass slides. The brown-black lead sulfide reaction product revealed the afferent vasculature in 3 dimensions within a clear background.

Microemboli Counting

Counting was performed in a standardized area of the basal ganglia. Because SCADs occur everywhere in the microcirculation of the brain, selection of the basal ganglia was simply to provide an easily recognizable anatomic feature that contains both white matter and gray matter with numerous vessels. Microemboli were counted on a field-by-field basis with a ×10 objective lens, with focusing up and down through the sections, in 3 adjacent 1-cm² areas. Microemboli were recorded as small, medium, or large; size was estimated against a grid in the visual field. For small microemboli, the diameter and length ranged from 10×10 to 15×20 μm; for medium, up to 20×30 μm; and for large, up to ~40×50 μm. The average volumes of small, medium, and large microemboli were estimated, respectively, as 2000 μm³ (12×12×14 μm), 6000 μm³ (17×17×21 μm), and 18 000 μm³ (25×25×29 μm); the ratio was ~1:3:9. Embolic load was calculated with this ratio and the numbers of microemboli of each size: (1×small)+(3×medium)+(9×large). To determine whether microemboli sizes decreased as time after surgery increased, the proportion of large and medium microemboli was determined for each subject. Counts were done by W.R.B. In a blind study of variability in recounts, reliability was ~90%. Statistic Analysis

Statistical analysis and graphing were performed with the computer program Prism, version 2.0 (GraphPad Software). Data were examined by means of linear regression and Student’s t test. Exact P values are reported and referred to in consideration of the statistical significance of data.

Results

Microemboli (Figure 1) were found in arterioles and capillaries in all 36 subjects, often at bifurcations in the microvasculature. With increasing survival time after CPB, the general tendency was for the percentage of large and medium emboli to decline (P=0.0034) (Figure 2). However, there was considerable variability, and in 4 of the 16 patients who died between 1 and 3 weeks after CPB, the trend toward a decreased size of emboli was not apparent. With increasing time after CPB, there was a logarithmic decline in the number of microemboli (P<0.0001) and the embolic load (P<0.0001) (Figure 3). The decline in embolic load was especially rapid in the first few days after CPB. As Figure 4 shows, the rate of decline was the same for the 2 subgroups of patients (ie, CABG only or valve repair). The mean embolic load for valve repair patients was 46.2% greater than that for CABG patients, but this difference may have been due to chance (P=0.1576 in a 1-tailed t test).

Longer duration of CPB was associated with increased embolic load (P=0.0026) (Figure 5). For each 1-hour in-
increase in CPB duration, the embolic load increased by 90.5%. However, when the data for the CABG and valve groups were analyzed separately, it was found that the increase in embolic load was much greater in the valve group (145.3% per hour) than in the CABG group (30.5% per hour) (Figure 6). The increase in embolic load appeared to be significant in the valve group ($P=0.0022$) but not in the CABG group ($P=0.4854$). It can be seen in Figure 6 that the 4 longest CPB durations occurred in valve patients, and these patients tended to have relatively high embolic loads. CPB duration for valve patients averaged 17.2% longer than that for CABG patients, but the difference did not appear to be very significant ($P=0.0550$ in a 1-tailed $t$ test).

The deviation from the predicted embolic load (Figures 5 and 6) was calculated as follows: from the linear regression lines for valve and CABG in Figure 4, the Prism statistics program was used to calculate a residual for each data point. This residual was subtracted from the measured embolic load to obtain the predicted embolic load for that time after surgery. The residual and predicted values were converted from log10 to standard numbers, and the residual value was divided by the predicted value to obtain the percent deviation from the regression line. Valve data were compared with the valve regression line, and CABG data were compared with the CABG regression line.

**Discussion**

Our finding that the percentage of large and medium microemboli declined with time after CPB is consistent with the hypothesis that fat emboli break into smaller globules as they pass through the capillary network. CPB-induced cerebral microemboli in dogs also have been reported to become smaller with time, and fat emboli in the lungs of dogs became smaller within 2 hours; some passed through to the brain within 1 hour. It has also been shown that fat injected into the carotid arteries of rabbits passes through the brain to the lungs.

After CPB, good blood flow through the brain might hasten the clearance of microemboli, and increased perfusion pressure during CPB has been proposed as a means of forcing air bubbles through the cerebral microcirculation. In contrast, diminished blood flow during CPB might be expected to reduce the number of emboli carried to the brain. Consistent with this concept, microemboli counts were very low in the 2 subjects (subjects 253 and 373) in our study who had brain edema, which tends to reduce brain blood flow. The brain edema may have begun in these patients as a result of severe myocardial infarction the day before surgery. Their embolic loads were 26.6% and 6.6%, respectively, of that which would be predicted on the basis of CPB duration and survival time after surgery.

Of potential clinical significance is our finding that the embolic load tended to be higher in patients who underwent prolonged CPB. This increase was most apparent in the valve repair patients, perhaps because CPB tended to be longer for valve patients than for CABG patients. There was no indication that the rate of clearance of microemboli from the brain was different for valve and CABG patients. On the contrary, the clearance curves (Figure 4) were nearly parallel, indicating similar rates of clearance. The essential difference between CABG and valve patients, in regard to microemboli, appeared to be that valve patients tended to have longer CPB duration and survival time after surgery. One factor that was not analyzed but may be relevant is the potential effect of reduced cardiac output on cerebral blood flow and clearance of microemboli.

That longer CPB might cause more emboli has long been suspected, but until now the number and volume of cerebral microemboli have not been systematically quantified. Blauth...
et al. \(^4\) counted microvascular occlusions in retinas, but they found no correlation between CPB time and the number of vascular occlusions. However, they did find a tendency toward a correlation between CPB time and psychometric deficits (\(P<0.075\)). Others have observed an increase in microemboli with longer CPB time, but no counts were made. These reports involved fat emboli in human brain, \(^{13}\) kidney, \(^{15}\) and lung, \(^{19}\) and silicone antifoam emboli in human brain \(^{40}\) and kidney \(^{3,9}\) and in dog kidney. \(^{5}\) Contrary to those observations, in 1962 Miller et al. \(^{16}\) noted no apparent relationship between CPB duration and the degree of fat embolization in various tissues. It is important to note that none of those studies considered that survival time after CPB could affect the counts. We have shown that the counts rapidly decline with increasing survival time.

Caguin and Carter \(^{18}\) found cerebral fat emboli in CPB patients after noticing that the pattern of cerebral dysfunction in an orthopedic patient with cerebral fat embolism was the same as that in some CPB patients. Hodge et al. \(^{41}\) reported a case of fatal cerebral fat embolism resulting from CPB and suggested that the fat embolism syndrome is present subclinically in the majority of CPB patients. Such subclinical effects may correspond to the transient or permanent neuropsychological or neurological deficits that can now be detected in many CPB patients after detailed preoperative and postoperative evaluations.

Numerous reports over the years have shown that CPB duration is associated with neurological dysfunction. \(^{23-29}\) Both brain emboli and brain injury were found to increase with prolonged CPB. \(^{5,9}\) However, because current membrane oxygenators release far fewer antifoam emboli into the blood than did bubble oxygenators, the association between CPB duration and cerebral dysfunction is likely to be weaker now.

We examined archival material from a patient who died at 0.2 day after CPB with a bubble oxygenator (data not shown), and she had far more microemboli than did any other patient: 571 microemboli with an embolic load of 1532 compared with a predicted embolic load of 324 for her length of survival after surgery and duration of CPB (199 minutes).

Cerebral fat embolism produces less brain injury than might be expected, perhaps because the emboli are small and transient. \(^{22}\) However, the evidence that microemboli can cause severe brain injury is most impressively shown in the fatal case reported by Hodge et al. \(^{41}\) Furthermore, the use of filters in the CPB apparatus has reduced the number of microemboli and the incidence of neurological injury. \(^{8,23,25,42}\) Cerebral microemboli may also cause neuronal dysfunction without killing the cells, \(^{8}\) as has been observed in cases of intermittent monocular blindness. \(^{43,44}\) Vision returned when the retinal microvascular emboli moved and circulation was restored. Mild emboli-induced ischemia may cause transient dysfunction in the vital centers of the brain stem and contribute to the immediate mortality rates associated with CPB. \(^{8}\)

Sternal bone marrow is an important source of lipid microemboli. Sternotomy reportedly results in more fat in the blood \(^{24}\) and fat emboli in the tissues \(^{16}\) than does thoracotomy. During sternal division, numerous fat emboli have been found in the right atrium. \(^{45}\) Perhaps more important is fat that washes into the pool of pericardial blood from the sternum and the incised thoracic tissues, particularly the large amount of fat that is found in the sternum of older patients. Shed blood around the heart is often scavenged with a suction line and returned to the patient via the CPB apparatus. It has been reported that the majority of microemboli come from the cardiotomy suction line. \(^{46-48}\) In dogs undergoing CPB, Brooker et al. \(^{49}\) found a 10-fold increase in microemboli when pericardial blood was scavenged and returned to the circuit. Evidently, embolic fat is a major problem with the pericardial blood. \(^{17-19,30-52}\) A strong association has been reported between the reuse of scavenged blood and a negative outcome after CPB. \(^{53}\) and superior results have been shown when cardiotomy suction blood is discarded. \(^{18,54}\) Increased blood scavenging during prolonged CPB may be one cause of the association found between CPB duration and embolic load in this report and between CPB duration and cerebral dysfunction in reports of >40 other studies.

Atheromatous debris may contribute to brain emboli during CPB. \(^{55}\) Proximal aortic atherosclerosis, the strongest predictor of stroke, stupor, or coma after CPB, increases the risk by >4-fold. \(^{55}\) However, aortic atheroma does not appear to be associated with the more subtle cognitive deficits, \(^{55}\) which we suspect may be caused by lipid microemboli. In 7 subjects who had left heart catheterization after CPB (data not shown), we found more microemboli than would be expected from CPB alone. This finding is consistent with the fact that left heart catheterization is known to cause cerebral emboli. \(^{20,56}\) apparently due to the disruption of aortic and coronary artery atheroma. The disruption of aortic or carotid atheroma may release lipid microemboli as well as larger emboli of atheromatous debris and cholesterol crystals. After carotid angiography, atheromatous debris was found in the arteries, whereas the small lipid emboli lodged in arterioles and capillaries. \(^{57}\)

Retrograde cerebral perfusion, which was first used to treat air embolism during CPB, \(^{58}\) may be useful to wash out particulate emboli. \(^{59}\) We have data from a single case that supports that concept. We studied a subject who had retrograde cerebral perfusion (data not shown), and we found far fewer microemboli than would be predicted. This patient died 4.1 days after surgery and had a very long CPB duration (279 minutes). On the basis of the data for the 36 patients in the present study, the predicted embolic load for this patient would be 200; however, her embolic load was only 21.

Positioning the patient head down during CPB might help to steer fat emboli away from the origins of the arteries that supply the brain. Evidence that fat emboli float in the blood and that a change in a patient’s position can alter the route of circulating fat emboli has been shown in 12 cases of traumatic fat embolism. \(^{60}\)

In conclusion, we have shown that microemboli were numerous in all CPB patients examined within a few days after CPB and that most microemboli had cleared from the vessels within 1 week. Microemboli tended to be more numerous in patients who underwent CPB of longer duration. From this study, it is not clear why this should be so. However, previous studies have suggested that the scavenging of pericardial blood is a potential major source of lipid emboli. If prolonged CPB is associated...
with increased scavenging of pericardial blood, then elimination of the use of scavenged blood or removal of lipid emboii from scavenged blood may be important to reduce post-CPB cerebral dysfunction.

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