Published in final edited form as:
Acta Neurochir (Wien). 2008 August ; 150(8): 779–784. doi:10.1007/s00701-008-1618-6.

Elevation of monocyte chemoattractant protein-1 in patients experiencing neurocognitive decline following carotid endarterectomy

W. J. Mack[1], **A. F. Ducruet**[1], **Z. L. Hickman**[1], **J. Zurica**[2], **R. M. Starke**[1], **M. C. Garrett**[1], **R. J. Komotar**[1], **D. O. Quest**[1], **R. A. Solomon**[1], **E. J. Heyer**[2,3], and **E. Sander Connolly**[1,3]

1*Department of Neurological Surgery, Columbia University Medical Center, New York, NY, USA*

2*Department of Anesthesiology, Columbia University Medical Center, New York, NY, USA*

3*Department of Neurology, Columbia University Medical Center, New York, NY, USA*

Summary

Background—Previous studies have demonstrated that elevated pre-operative monocyte count is an independent predictor of acute neurocognitive decline following carotid endarterectomy (CEA). Monocyte chemoattractant protein-1 (MCP-1), secreted by human endothelial and monocyte-like cells, is a potent mediator of inflammation and mononuclear cell trafficking. This study examines the relationship between peri-operative serum MCP-1 elevation and post-operative neurocognitive injury following CEA.

Methods—Fifty-two patients undergoing CEA and 67 lumbar laminectomy (LL) controls were administered a battery of five neuropsychological tests pre-operatively and on post-operative day 1 (POD 1). Change in individual test scores from baseline to POD 1 were converted into Z-score and used to develop a point system quantifying the degree of neurocognitive dysfunction relative to change within the LL group. Neurocognitive injury following CEA was defined as a score greater than 2 standard deviations above mean total deficit scores of LL controls. Serum MCP-1 levels were measured pre-operatively and on POD 1 by enzyme-linked immunosorbent assay.

Findings—Mean percent MCP-1 elevation was higher for the 13 injured CEA patients (147.7 ± 32.4%) in our cohort compared to 39 age- and sex-matched uninjured CEA patients (76.0 ± 16.5%). In unconditional multivariate logistic regression analysis, percent elevation in serum MCP-1 level was associated with neurocognitive injury one day after CEA (OR = 2.19, 95% CI = 1.13-4.26, P = 0.021, for a 100% elevation from pre-operative levels).

Correspondence: Ricardo J. Komotar MD, Department of Neurosurgery, Columbia University, 710 West 168th Street, Room 431, New York, NY 10032, USA. e-mail: E-mail: rjk2103@columbia.edu.

Conflict of interest disclosure

The authors have no competing interests to declare in association with this study.

Comment

In this interesting paper the Authors convincingly report that peri-operative changes in serum monocyte chemoattractant protein-1 correlate with acute decline of cognitive performances following CEA. This study expands and corroborates previous investigations demonstrating that elevated pre-operative monocyte count is an independent predictor of neurocognitive impairment following CEA. These data are important both from the clinical point of view and the understanding of the pathophysiological mechanisms of inflammation involved in post-CEA hypoxic and hypoperfusion microvascular insults.

Clearly, further clinical and laboratory research on this topic is needed before its definitive translation from bench to bedside.

Dr d'Avella

Conclusions—Peri-operative elevations in serum MCP-1 levels correlate with acute neurocognitive dysfunction following CEA. These data implicate an inflammatory mechanism in the pathogenesis of Ischaemic neurocognitive decline.

Keywords

Arteriolosclerosis; brain ischemia; carotid endarterectomy; monocyte chemoattractant protein-1; neuropsychological tests

Introduction

Acute neurocognitive decline following CEA is believed to be ischaemic in nature, with cortical injury resulting from hypoperfusion or plaque embolization. Recent studies suggest that pre-existing microvascular disease may render CEA patients susceptible to cerebral hypoperfusion, initiating neuronal injury during a brief episode of ischaemia. We have previously shown that advanced age, pre-operative monocyte count and history of diabetes mellitus all independently predict neuropsychological decline following CEA [16,17]. We have also demonstrated that neuropsychological changes are not associated with positive lesions on diffusion weighted imaging (DWI), further implicating a role for regional hypoxia and hypoperfusion [9].

The cellular inflammatory response is a critical mediator of both acute microvascular failure and chronic arteriolosclerosis [7,21,23]. Chemokine-driven migration of inflammatory cells has been implicated in the pathogenesis of cerebral ischaemia/reperfusion injury and may play a central role in cortical dysfunction following CEA [5]. Monocyte chemoattractant protein-1 (MCP-1) is a potent chemokine secreted by endothelial and monocyte-like cells [5]. While it is believed to be inactive in the normal brain [26], increased MCP-1 concentrations facilitate the inflammatory response, initiating mononuclear cell recruitment in a state of vascular injury [21]. Animal models studies suggest that MCP-1 contributes to exaggerate responses to ischaemia/reperfusion injury [24]. Following CEA, cerebral hypoperfusion may elevate serum chemokine levels in patients primed for cortical injury by pre-existing small vessel disease. The resultant pro-inflammatory milieu would render the neural tissue susceptible to further microvascular changes and ischemic injury. This study examines the relationship between serum MCP-1 levels and neurocognitive decline following CEA.

Materials and methods

Participants

Fifty-two prospectively enrolled patients undergoing elective CEA for both symptomatic and asymptomatic carotid artery stenosis at Columbia University Medical Center (CUMC) between 1998 and 2004 were included in this CUMC Institutional Review Board-approved study. All patients had 60% or greater stenosis of the operative carotid artery and none had undergone previous ipsilateral carotid endarterectomy. After obtaining informed consent, patients were evaluated with a battery of 5 neuropsychological tests before surgery and on POD 1. As described previously, a control group of 67 contempo-raneous patients undergoing lumbar laminectomy (LL) with a similar anaesthetic regimen were included to account for effects of general anesthesia on neuropsychological test performance [10]. All tests were performed more than 3 h after administration of any analgesic or sedative medication. Patients (both CEA and LL) who reported a pain score of greater than 5 (on a 10-point scale) during testing were excluded from this study, as we have previously shown that pain confounds neuropsychological test performance [11]. All CEA and LL patients received general anesthesia with routine hemodynamic and temperature monitoring as previously described [10]. Surgical times averaged 155 ± 6 and 144 ± 6 min for the CEA and LL cohorts, respectively (mean ± SEM).

Neuropsychological evaluation

All patients were assessed pre-operatively and on POD 1 by a trained full-time research assistant using a battery of 5 neuropsychological tests chosen to represent a range of cognitive domains as we have described previously [9,10,12,16,17]. These tests are representative of the outcome measures recommended by a consensus group [19] along with tests used in previous studies of neuropsychological testing after carotid endarterectomy to better elucidate neurocognitive changes [1,13]. The Boston Naming Test evaluated patients' ability to verbally identify objects pictured on a series of cards. Halstead-Reitan Trails Parts A and B evaluated visual conceptual and visuomotor tracking by timing how long it took a subject to connect consecutively numbered circles with a single line (Part A) and then connect the same number of consecutively numbered and lettered circles by alternating between the two sequences (Part B). The Controlled Oral Word Association test assessed verbal fluency, providing information on dominant hemisphere function. Patients were asked to generate as many words as possible that began with a certain letter within 60 sec. Three separate trials were performed at each testing session, one each with the letters C, F, and L. The Copy Portion of Rey Complex Figure test evaluated visuospatial organization, providing insight into the function of the non-dominant hemisphere. Patients were instructed to copy the figure and a standardized scoring system was used to evaluate the presence of design-specific features and the accuracy of their locations [10].

Analysis of MCP-1 Levels

Serial serum samples were collected from CEA patients via indwelling arterial lines or venipuncture prior to (pre-operative level) and one day following (POD 1) their procedure. Each 5 ml specimen was centrifuged at 5000 RPM for 15 min, and the resulting supernatant was stored at -80 °C until it was assayed. MCP-1 levels were quantitatively measured in picograms per milliliter using commercially available enzyme-linked immunosorbent assays (BD Biosciences, San Jose, California, USA). Percent change in MCP-1 value was then calculated for each CEA patient.

Statistical analysis

Thirteen injured patients and thirty-nine age- and sex-matched uninjured patients (1:3 ratio) were analyzed in this study. Matching by sex was confirmed using McNemar's test and by age using paired *t*-test (p = ns).

Each neuropsychological test was scored individually for CEA and LL patients as previously described [10]. The change in individual test scores from baseline to POD 1 was converted into Z-score relative to change within the LL group. Z scores were converted into a point system quantifying the degree of cognitive dysfunction associated with each neuropsychological test at POD 1. For each CEA patient, these deficit points were summed to generate a total deficit score (TDS) that measured the global level of cognitive decline. Neurocognitive injury following CEA was defined as a score greater than 2 standard deviations above mean TDS of LL controls. Using this method, neurocognitive outcome is expressed as a dichotomous variable: "injured" or "uninjured".

Non-parametric Mann-Whitney and Kruskal-Wallis tests were used to perform univariate analyses to investigate potential associations between percent change in MCP-1 levels and the following variables: symptomatic presentation, diabetes mellitus, hypertension, hypercholesterolemia, history of smoking, current use of statin medication, previous myocardial infarction, and previous contralateral CEA. Unconditional univariate logistic regression was employed to examine the relationship between the aforementioned variables and neurocognitive injury on POD 1. Variables with $P \leq 0.25$ in the univariate analysis were included in the initial multivariate model. A backward elimination variable selection method

was then employed to arrive at the final set of independent variables. The likelihood ratio test was used to assess significant differences in multivariate models after controlling for dominant hemisphere and side of surgery. $P \leq 0.05$ were considered significant in this final model.

Results

No significant associations existed in univariate analysis between demographic variables (symptomatic presentation, diabetes mellitus, hypertension, hypercholesterolemia, history of smoking, current use of statin medication, previous myocardial infarction, and previous contralateral CEA) and percent change in MCP-1 levels (p = ns). Demographic variables and percent change in MCP-1 for injured and uninjured CEA patients are presented in Table 1. Included are probability values for unconditional univariate and multivariate logistic regression analyses and multivariate odds ratios with respect to post-operative injury. In our final model, diabetes mellitus ($P = 0.041$, OR = 5.70, 95% CI = 1.07-30.10) and peri-operative increase in MCP-1 level ($P = 0.021$, OR = 2.19, 95% CI = 1.13-4.26 for a POD 1 level 100% greater than pre-operative level) were both independently associated with neurocognitive injury, while smoking history demonstrated an inverse association ($P = 0.044$, OR = 0.20, 95% CI = 0.04-0.95). There was no significant difference between models or odd's ratios when controlling for side of surgery and/or dominant hemisphere.

Discussion

Studies have demonstrated that conventional neurological assessment is insufficient for determining the neurocognitive sequelae of CEA. Neuropsychological tests are sensitive measures of cerebral functioning and predictors of neurological injury. Cognitive decline, not revealed on routine examination, can be demonstrated through a relevant battery of neuropsychological tests. Our clinical CEA model thus affords a controlled paradigm in which to critically examine the role of inflammation in the setting of cerebral ischemia.

In a recent study of post-operative diffusion weighted imaging in a cohort of patients who underwent CEA, we found that patients with post-operative cognitive dysfunction had significantly longer carotid cross-clamp times and that neurocognitive injury did not seem to be associated with new DWI-positive lesions [9]. Injury in this cohort may be more consistent with regional hypoperfusion of the distal watershed, rather than an embolic shower. Additionally, the neuropsychological tests employed are measures of global cerebral functioning and may be more sensitive to the affects of flow-related hypoxia than that of small embolic insults.

Strong associations exist between inflammatory processes and atherosclerosis [23]. Chronic inflammation and small vessel disease likely render patients susceptible to neuronal injury following global cerebral hypoperfusion. Our group has demonstrated that advanced age and history of diabetes mellitus are independent predictors of neurocognitive outcome following CEA [17]. We have also shown that elevated preoperative monocyte count independently predicts neuropsychological decline [16]. Each of these factors is associated with microvascular disease and may "prime" patients for an exaggerated inflammatory response and resultant cortical injury (not evident on magnetic resonance imaging) following CEA. To this end, our study examined whether patients sustaining neurocognitive decline after CEA exhibited increases in serum MCP-1 levels, a robust indicator of active inflammation.

MCP-1 is a member of the cysteine-cysteine chemokine gene family. Produced by endothelial and macrophage-like cells, it is a potent monocyte chemoattractant factor that causes vascular inflammation, angiogenesis, smooth muscle cell proliferation, and oxidative stress via CC

chemokine receptor 2-mediated interactions [6,18,22]. MCP-1 has been implicated in the pathogenesis of both chronic and acute cerebrovascular injury [2,5,24].

High MCP-1 levels have been detected in atherosclerotic carotid arteries [3]. Nelken *et al.* identified MCP-1 mRNA in 16% of cells counted in human carotid endarterectomy specimens, with highest expression seen in organizing thrombi and macrophage rich areas bordering the necrotic lipid core. By contrast, few cells expressing MCP-1 mRNA were found in normal arteries [20]. The authors further documented increased MCP-1 in carotid plaques using immunohistochemical staining and *in situ* hybridization [8,20].

In vitro, MCP-1 can induce chemotaxis of monocytes at subnanomolar concentrations [5]. Wang *et al*. detected increased levels of MCP-1 mRNA in rodent models of permanent and temporary middle cerebral artery occlusion [25]. Endothelial cells, macrophage-like cells, and neurons all express MCP-1 in the ischemic brain [4,14,25]. Animal models studies suggest that MCP-1 contributes to exaggerate responses to ischaemia/reperfusion injury, and immunoneutralization of MCP-1 results in decreased infarct size [24]. Furthermore, elevated MCP-1 protein levels have been demonstrated in human cerebrospinal fluid 24 h after stroke [15]. These authors note that further studies must be done to demonstrate that this change was not due to underlying patient characteristics. In this study using logistic regression to account for underlying patient characteristics, we provide further evidence that MCP-1 rise acutely in response to ischemia.

That post-operative serum MCP-1 levels are significantly elevated in patients suffering neurocognitive change following CEA implies an inflammatory mechanism of injury. These findings are in concordance with our previous studies, suggesting a pivotal role for monocyte activation [16]. A pre-existing inflammatory state, coupled with blood brain barrier breakdown and vasogenic edema induced by regional hypoperfusion, could result in neuronal injury significant enough to cause post-operative neurocognitive decline.

As stated, our previous studies demonstrate that age and history of diabetes mellitus also predict neurocognitive decline following CEA [17]. In order to control for the former, we selected an age- and sex-matched cohort of injured and uninjured patients for comparison in our analysis. The association between diabetes mellitus and post-operative injury was rein-forced by our data. Additionally, we found smoking history to be linked with favorable outcome, although this relationship was not observed in prior, larger studies [17]. Percent change in peri-operative MCP-1 levels, however, demonstrated the strongest correlation with outcome in our multivariate analysis. Further investigation is necessary to better elucidate the mechanism of interaction between regional cerebral hypoperfusion and an activated inflammatory infiltrate in the setting of neurocognitive injury following CEA. Inhibition of MCP-1 in CEA patients may result in decreased neurocognitive decline and better outcomes.

Acknowledgments

Funding

This research was supported in part by NIH RO1AG17604 and The Irving Clinical Research Center NIH RR00645. Funding sources had no involvement in study design, collection, analysis, or interpretation of data.

Abbreviations

MCP, monocyte chemoattractant protein-1; CEA, carotid endarterectomy; DWI, diffusion weighted imaging; LL, lumbar laminectomy; POD, postoperative day.

References

1. Berman L, Pietrzak RH, Mayes L. Neurocognitive changes after carotid revascularization: a review of the current literature. J Psychosom Res 2007;63:599–612. [PubMed: 18061750]
2. Boyle JJ. Macrophage activation in atherosclerosis: pathogenesis and pharmacology of plaque rupture. Curr Vasc Pharmacol 2005;3:63–68. [PubMed: 15638783]
3. Campanella M, Sciorati C, Tarozzo G, Beltramo M. Flow cytometric analysis of inflammatory cells in ischemic rat brain. Stroke 2002;33:586–592. [PubMed: 11823674]
4. Che X, Ye W, Panga L, Wu DC, Yang GY. Monocyte chemoattractant protein-1 expressed in neurons and astrocytes during focal ischemia in mice. Brain Res 2001;902:171–177. [PubMed: 11384610]
5. Chen Y, Hallenbeck JM, Ruetzler C, Bol D, Thomas K, Berman NE, Vogel SN. Overexpression of monocyte chemoattractant protein 1 in the brain exacerbates ischemic brain injury and is associated with recruitment of inflammatory cells. J Cereb Blood Flow Metab 2003;23:748–755. [PubMed: 12796723]
6. Egashira K. Molecular mechanisms mediating inflammation in vascular disease: special reference to monocyte chemoattractant protein-1. Hypertension 2003;41:834–841. [PubMed: 12624005]
7. Elkind MS. Inflammation, atherosclerosis, and stroke. Neurologist 2006;12:140–148. [PubMed: 16688015]
8. Furukawa Y, Matsumori A, Ohashi N, Shioi T, Ono K, Harada A, Matsushima K, Sasayama S. Anti-monocyte chemoattractant protein-1/monocyte chemotactic and activating factor antibody inhibits neointimal hyperplasia in injured rat carotid arteries. Circ Res 1999;84:306–314. [PubMed: 10024304]
9. Heyer EJ, DeLaPaz R, Halazun HJ, Rampersad A, Sciacca R, Zurica J, Benvenisty AI, Quest DO, Todd GJ, Lavine S, Solomon RA, Connolly ES Jr. Neuropsychological dysfunction in the absence of structural evidence for cerebral ischemia after uncomplicated carotid endarterectomy. Neurosurgery 2006;58:474–480. [PubMed: 16528187]discussion 474-480
10. Heyer EJ, Sharma R, Rampersad A, Winfree CJ, Mack WJ, Solomon RA, Todd GJ, McCormick PC, McMurtry JG, Quest DO, Stern Y, Lazar RM, Connolly ES. A controlled prospective study of neuropsychological dysfunction following carotid endarterectomy. Arch Neurol 2002;59:217–222. [PubMed: 11843692]
11. Heyer EJ, Sharma R, Winfree CJ, Mocco J, McMahon DJ, McCormick PA, Quest DO, McMurtry JG 3rd, Riedel CJ, Lazar RM, Stern Y, Connolly ES Jr. Severe pain confounds neuropsychological test performance. J Clin Exp Neuropsyc: Official J Int Neuropsych Soc 2000;22:633–639.
12. Heyer EJ, Sharma R, Winfree CJ, Mocco J, McMahon DJ, McCormick PA, Quest DO, McMurtry JG 3rd, Riedel CJ, Lazar RM, Stern Y, Connolly ES Jr. Severe pain confounds neuropsychological test performance. J Clin Exp Neuropsychol 2000;22:633–639. [PubMed: 11094398]
13. Irvine CD, Gardner FV, Davies AH, Lamont PM. Cognitive testing in patients undergoing carotid endarterectomy. Eur J Vasc Endovasc Surg 1998;15:195–204. [PubMed: 9587331]
14. Kim JS, Gautam SC, Chopp M, Zaloga C, Jones ML, Ward PA, Welch KM. Expression of monocyte chemoattractant protein-1 and macrophage inflammatory protein-1 after focal cerebral ischemia in the rat. J Neuroimmunol 1995;56:127–134. [PubMed: 7860708]
15. Losy J, Zaremba J. Monocyte chemoattractant protein-1 is increased in the cerebrospinal fluid of patients with ischemic stroke. Stroke 2001;32:2695–2696. [PubMed: 11692036]
16. Mocco J, Wilson DA, Ducruet AF, Komotar RJ, Mack WJ, Zurica J, Sciacca RR, Heyer EJ, Connolly ES. Elevations in preoperative monocyte count predispose to acute neurocognitive decline after carotid endarterectomy for asymptomatic carotid artery stenosis. Stroke 2006;37:240–242. [PubMed: 16322501]
17. Mocco J, Wilson DA, Komotar RJ, Zurica J, Mack WJ, Halazun HJ, Hatami R, Sciacca RR, Connolly ES Jr, Heyer EJ. Predictors of neurocognitive decline after carotid endarterectomy. Neurosurgery 2006;58:844–850. [PubMed: 16639318]discussion 844-850
18. Mukaida N, Harada A, Matsushima K. Interleukin-8 (IL-8) and monocyte chemotactic and activating factor (MCAF/MCP-1), chemokines essentially involved in inflammatory and immune reactions. Cytokine Growth Factor Rev 1998;9:9–23. [PubMed: 9720753]

19. Murkin JM, Newman SP, Stump DA, Blumenthal JA. Statement of consensus on assessment of neurobehavioral outcomes after cardiac surgery. Ann Thorac Surg 1995;59:1289–1295. [PubMed: 7733754]

20. Nelken NA, Coughlin SR, Gordon D, Wilcox JN. Monocyte chemoattractant protein-1 in human atheromatous plaques. J Clin Invest 1991;88:1121–1127. [PubMed: 1843454]

21. Nilsson J. Cytokines and smooth muscle cells in atherosclerosis. Cardiovasc Res 1993;27:1184–1190. [PubMed: 8252576]

22. Rollins BJ. Chemokines. Blood 1997;90:909–928. [PubMed: 9242519]

23. Ross R. Atherosclerosis - an inflammatory disease. N Engl J Med 1999;340:115–126. [PubMed: 9887164]

24. Terao S, Yilmaz G, Stokes KY, Ishikawa M, Kawase T, Granger DN. Inflammatory and injury responses to ischemic stroke in obese mice. Stroke 2008;39:943–950. [PubMed: 18239178]

25. Wang X, Yue TL, Barone FC, Feuerstein GZ. Monocyte chemoattractant protein-1 messenger RNA expression in rat ischemic cortex. Stroke 1995;26:661–665. [PubMed: 7709415]discussion: 665-666

26. Yamagami S, Tamura M, Hayashi M, Endo N, Tanabe H, Katsuura Y, Komoriya K. Differential production of MCP-1 and cytokine-induced neutrophil chemoattractant in the ischemic brain after transient focal ischemia in rats. J Leukoc Biol 1999;65:744–749. [PubMed: 10380894]

Table 1

Injured and uninjured patient variables and prediction of post-operative neurocognitive decline following CEA

	Injured	Uninjured	Univariate *P*-value	Multivariate *P*-value	Multivariate OR *P*-value (95% CI)
Patients (*n*)	13	39	-	-	-
Age* (mean years ± SEM)	75.7 ± 1.7	73.9 ± 0.9	-	-	-
Male gender* (*n*, %)	7 (54)	21 (54)	-	-	-
Symptomatic presentation (*n*, %)	5 (38)	17 (44)	0.75	-	-
Diabetes mellitus (*n*, %)	5 (38)	7 (20)	0.13	0.041	5.70 (1.07, 30.10)
Hypertension (*n*, %)	10 (77)	24 (62)	0.32	-	-
Hypercholesterolemia (*n*, %)	10 (77)	28 (72)	0.72	-	-
Smoker (*n*, %)	7 (54)	29 (74)	0.17	0.044	0.20 (0.04, 0.95)
Statin medication (*n*, %)	6 (46)	13 (33)	0.41	-	-
Previous myocardial infarction (*n*, %)	3 (23)	12 (31)	0.60	-	-
Previous contralateral CEA (*n*, %)	2 (15)	4 (10)	0.62	-	-
% Change MCP-1 (mean SEM)	+147.7 ± 32.4	+76.0 ± 16.5	0.048	0.021	2.19 (1.13, 4.26)†

CI Confidence interval; *OR* odds ratio; *SEM* standard error of the mean.

*Injured and uninjured patients were matched for age and gender, therefore these variables were not included in univariate or multivariate unconditional logistic regression analyses.

†OR and 95% CI for 100% increase in MCP-1 from pre-operative to POD 1 levels.

Acta Neurochir (Wien). Author manuscript; available in PMC 2009 June 18.