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Plasma Carotenoid and Malondialdehyde Levels in Ischemic Stroke Patients: Relationship to Early Outcome*

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An association between ischemic stroke and increased oxidative stress has been suggested from animal studies. However, there is a lack of evidence with respect to this association in humans. Here, the time course of plasma levels of six carotenoids, which are lipophilic micronutrients with antioxidant properties, as well as of malondialdehyde (MDA), a marker of lipid peroxidation, was followed in ischemic stroke patients. Plasma levels of lutein, zeaxanthin, β-cryptoxanthin, lycopene, α- and β-carotene, as well as MDA were measured by high-performance liquid chromatography in 28 subjects (19 men and nine women aged 76.9 ± 8.7 years) with an acute ischemic stroke of recent onset (<24 h) on admission, after 6 and 24 h, and on days 3, 5, and 7. Carotenoid and MDA levels in patients on admission were compared with those of age- and sex-matched controls. Plasma levels of lutein, lycopene, α- and β-carotene were significantly lower and levels of MDA were significantly higher in patients in comparison with controls. Significantly higher levels of MDA and lower levels of lutein were found in patients with a poor early-outcome (functional decline) after ischemic stroke as compared to patients who remained functionally stable. These findings suggest that the majority of plasma carotenoids are lowered immediately after an ischemic stroke, perhaps as a result of increased oxidative stress, as indicated by a concomitant rise in MDA concentrations. Among the carotenoids, only lutein plasma changes are associated with a poor early-outcome.

Keywords: Antioxidants; Carotenoids; Outcome; Oxidative stress; Stroke

INTRODUCTION

Ischemic stroke is a major health problem in Western countries and is among the leading causes of disability. Ischemia/reperfusion injury in animal studies was found to be associated with a condition of oxidative stress, expressed both in terms of antioxidant consumption and the formation of markers of free radical-induced damage. Evidence is still limited in humans with acute cerebral ischemia. Diets rich in fruits and vegetables, containing large amounts of antioxidant vitamins and micronutrients such as carotenoids, were shown epidemiologically to protect from the occurrence of cerebrovascular disease.[1] A poor vitamin C status is related to risk of death from stroke.[2]

It has been shown recently that patients with ischemic stroke have decreased plasma vitamin C levels and erythrocyte superoxide dismutase activity immediately after the ictus, and that this decrease is significantly more pronounced in patients with the worst outcome after 1 week.[3] The aim of the present study was to assess over-time-changes of a number of carotenoids during the first hours after the occurrence of ischemic stroke. Over-time-changes of plasma levels of malondialdehyde (MDA), a marker of free radical-induced lipid peroxidation, were also assessed acutely after stroke onset.

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SUBJECTS AND METHODS

Twenty-eight patients older than 65 years (19 men, nine women, aged 76.9 ± 8.7 years) admitted to the Geriatric Ward of the University Hospital of Perugia, Italy, within 24 h from the onset of ischemic stroke (mean time 7.3 h) were consecutively enrolled. Patients were compared to 76 healthy, free-living, normolipidemic subjects (48 men, 28 women, aged 77.3 ± 10.1 years). Patients with other neurological or psychiatric disorders and major organ failure, as well as those taking iron or antioxidant vitamin supplements were excluded from the study.

Patients underwent CT scan and detailed neurological and physical examination. Information on body temperature, blood cell count, lipid profile, caloric intake, and presence of vascular risk factors and alcohol abuse were collected. The clinical type of syndrome (lacunar or non-lacunar) and the severity of the neurological deficit scored by means of the Canadian Neurological Scale[4] (CNS) were recorded.

The functional independence of patients as assessed by the Barthel Index[5] (BI) score was evaluated (by patient or close relative interview) on admission and 1 week after stroke, and compared to that of 2 weeks prior of the stroke onset. On the basis of the BI after 1 week, patients were divided *a priori* in two groups, the first including patients who experienced functional decline (Group W-worse), the second included patients who did not undergo functional decline (Group S-stable).

Patients or their close relatives gave informed consent, and the study was approved by the local Ethics Committee. One tube of heparinized blood was obtained on admission (T1), after 6 h (T2), 24 h (T3), and after 3 (T4), 5 (T5) and 7 (T6) days. Controls gave blood once in the morning after overnight fasting. Plasma was stored at −80°C until analysis.

Plasma carotenoids including lutein, zeaxanthin, β-cryptoxanthin, total lycopene, α-and β-carotene were measured by HPLC with UV/Vis. detection.[6] Plasma MDA levels were measured by HPLC with fluorescence detection.[7]

Data are presented as means ± SD. Statistics were performed with the program SIGMASTAT (version 2.03, 1997, SPSS Inc). Continuous variables were compared by the unpaired *t*-test or the Mann–Whitney test, as appropriate. Prevalences were compared by the Chi-square test. Correlation analyses were performed by means of the Pearson's test. Plasma MDA and carotenoid concentrations over time in W and S patient subgroups were compared by two-way analysis of variance (ANOVA). Tukey test was used for *post-hoc* analyses. Significance was accepted if the null hypothesis was rejected at the $p < 0.05$ level.

RESULTS

Nineteen patients were included in the Group W-worse (seven having a lacunar syndrome) and nine in Group S-stable (nine having a lacunar syndrome). Diabetes was present in three (Group-W) and in two (Group-S) patients. Hypertension was present in eight patients in Group-W and in four patients in Group-S. Seven (Group-W) and five (Group-S) patients were smokers. Indexes of renal and hepatic function, as well as lipid profile, were within the normal range in all subjects. There were no differences of body temperature between groups.

On admission, plasma levels of lutein, lycopene, α-carotene, and β-carotene were significantly lower in patients as compared to controls (Table I). MDA plasma levels on admission were significantly higher (by almost 160%) in patients as compared to controls (Table I). The plasma carotenoid concentrations showed a non-significant decrease after the ischemic event, reaching the lowest value 24 h after admission, both in the whole sample and in each of the two groups (W and S). The decrease was about 10% on average, ranging from 7% for β-carotene to 19% for β-cryptoxanthin (data not shown). During the following days, carotenoid levels increased returning close to their baseline values, both in the S-group and in the W-group, with the exception of lutein and α-carotene which remained lower in the latter group. When over-time-changes of carotenoids and MDA were compared between groups-W and S-separately, significant differences were found for lutein (Fig. 1, panel A) and MDA (Fig. 1, panel B). Plasma lutein levels significantly higher in S-group as compared to W-group patients ($p < 0.01$) (Fig. 1, panel A), and plasma MDA levels significantly lower in group-S as compared to W-group patients ($p < 0.05$) (Fig. 1, panel B). A significant inverse relationship was found between plasma MDA levels and CNS scores on day 1 ($r = 0.37$, $p < 0.05$), and a significant direct relationship was found between plasma lutein levels and CNS scores on day 7 ($r = 0.51$, $p < 0.03$).

TABLE I Plasma levels of Lutein, Zeaxanthin, β-Cryptoxanthin, Lycopene, α-Carotene, β-Carotene, and MDA in patients with ischemic stroke on admission and in healthy control subjects

	Patients ($n = 28$)	Controls ($n = 76$)
Lutein (μmol/l)	0.28 ± 0.16	0.34 ± 0.15*
Zeaxanthin (μmol/l)	0.12 ± 0.17	0.11 ± 0.04
β-Cryptoxanthin (μmol/l)	0.28 ± 0.14	0.30 ± 0.19
Lycopene (μmol/l)	0.35 ± 0.19	0.71 ± 0.27**
α-Carotene (μmol/l)	0.05 ± 0.02	0.06 ± 0.03**
β-Carotene (μmol/l)	0.35 ± 0.17	0.57 ± 0.26***
MDA (μmol/l)	0.60 ± 0.13	0.23 ± 0.12**

Values are means ± SD. $*p < 0.04$, $**p < 0.001$, $***p < 0.004$ vs. controls.

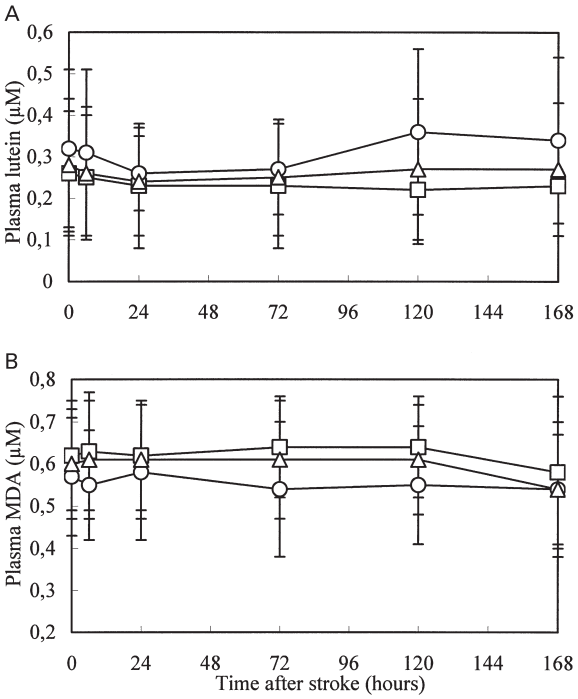

FIGURE 1 A: Plasma lutein levels (means ± SD) in all stroke patients (triangles), in patients who worsened (group-W, squares) and in patients who remained stable (group-S, circles) in the first week after stroke. Plasma lutein levels were significantly lower ($p < 0.01$; 2-way ANOVA) in group-W than in group-S patients. (B): Plasma MDA levels (means ± SD) in all stroke patients (triangles), in patients who worsened (group-W, squares) and in patients who remained stable (group S, circles) in the first week after stroke. Plasma MDA levels were significantly higher ($p < 0.05$; 2-way ANOVA) in group-W than in group-S patients.

DISCUSSION

Brain ischemia, and especially the condition of ischemia and reperfusion occurring after stroke, has been shown to be associated with free radical-mediated reactions potentially leading to neuronal death.[8] Oxidative stress in the organism can be prevented and/or reversed by a variety of anti-oxidant and repair systems, but if these systems are depleted, they might be overwhelmed and oxidative damage will occur. It has been shown that pivotal non-enzymatic hydrophilic and lipophilic antioxidants decrease acutely after stroke.[3] With this study, new information regarding significantly lowered carotenoid levels and significantly increased plasma MDA levels in stroke patients as compared to controls is added, unrelated to lipid profile and to the presence of vascular risk factors. Chang *et al.*[9] observed lowered total carotenoid levels and increased thiobarbituric acid-reactive substances (TBARs) in plasma from Taiwan stroke patients within 24 h after the stroke.

Our main finding is that only lutein among the carotenoids measured is significantly related to the outcome of stroke patients and to stroke severity. A significant inverse relation between lutein, but not other carotenoids, and risk for ischemic stroke was recently shown.[10] Our results are in agreement with this observation, and suggest that low lutein plasma levels might influence not only the risk of stroke, but also its outcome. Other studies reported that oxo-carotenoids, notably lutein and β-cryptoxanthin, might prevent coronary disease.[11]

In this study, patients followed a regular hospital diet and none of them stopped to eat after the stroke; none required intravenous feeding. Although we frequently monitored the daily nutritional status of patients both clinically and by mini-nutritional assessment, the change in plasma carotenoids shortly after admission might be partially explained by a difference between hospital diet and diet at home. This difference, however, is not likely to entirely explain the early decline (i.e. within 24 h from stroke onset) of plasma carotenoid levels, also in consideration of the long half-life (days) of carotenoids in plasma. In addition, it should be noted that plasma levels of the majority of carotenoids returned to baseline values after 1 week: this happened also in the group with the worst outcome, suggesting that the fluctuation cannot be fully explained by differences in dietary intake.

Along with the new observation of a relationship between plasma MDA levels and stroke outcome, these data are consistent with the occurrence of a condition of oxidative stress in patients with ischemic stroke, able to produce a specific oxidant/antioxidant profile.[12] Since carotenoid levels before stroke occurrence were not known, and given the fact that carotenoid levels are extremely variable among individuals, further studies in the field of stroke and nutrition[13] are needed.

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References

[1] Gillman, M.W., Cupples, L.A., Gagnon, D., Posner, B.M., Ellison, R.C., Castelli, W.P. and Wolf, P.A. (1995) "Protective effect of fruits and vegetables on development of stroke in men", *J. Am. Med. Assoc.* **273**, 1113–1117.

[2] Gale, C.R., Martyn, C.N., Winter, P.D. and Cooper, C. (1995) "Vitamin C and risk of death from stroke and coronary artery disease in cohort of elderly people", *Br. Med. J.* **310**, 1563–1566.
[3] Cherubini, A., Polidori, M.C., Bregnocchi, M., Pezzuto, S., Cecchetti, R., Ingegni, T., Di Iorio, A., Senin, U. and Mecocci, P. (2000) "Antioxidant profile and early outcome in stroke patients", *Stroke* **31**, 2295–2300.
[4] Cote, R., Battista, R.N., Wolfson, C., Boucher, J., Adam, J. and Hachinski, V. (1989) "The Canadian Neurological Scale: validation and reliability assessment", *Neurology* **39**, 638–643.
[5] Mahoney, F.I. and Barthel, D. (1965) "Functional evaluation: the Barthel Index", *MD State Med. J.* **14**, 56–61.
[6] Stahl, W., Sundquist, A.R., Hanusch, M., Schwarz, W. and Sies, H. (1993) "Separation of β-carotene and lycopene geometrical isomers in biological samples", *Clin. Chem.* **39**, 810–814.
[7] Suttnar, J., Cermak, J. and Dyr, J.E. (1997) "Solid-phase extraction in malondialdehyde analysis", *Anal. Biochem.* **249**, 20–23.
[8] Phillis, J.W. (1994) "A *radical* view of cerebral ischemic injury", *Prog. Neurobiol.* **42**, 441–448.
[9] Chang, C.Y., Lai, Y.C., Cheng, T.J., Lau, M.T. and Hu, M.L. (1998) "Plasma levels of antioxidant vitamins, selenium, total sulfhydryl groups and oxidative products in ischemic-stroke patients as compared to marched controls in Taiwan", *Free Radic. Res.* **28**, 15–24.
[10] Ascherio, A., Rimm, E.B., Hernan, M.A., Giovannucci, E., Kawachi, I., Stampfer, M.J. and Willett, W.C. (1999) "Relation of consumption of vitamin E, vitamin C, and carotenoids to risk for stroke among men in the United States", *Ann. Intern. Med.* **130**, 963–970.
[11] Howard, A.N., Williams, N.R., Palmer, C.R., Cambou, J.P., Evans, A.E., Foote, J.W., Marques-Vidal, P., McCrum, E.E., Ruidavets, J.B., Nigdikar, S.V., Rajput-Williams, J. and Thurnham, D.I. (1996) "Do hydroxy-carotenoids prevent coronary artery disease? A comparison between Belfast and Toulouse", *Int. J. Vitam. Nutr. Res.* **66**, 113–118.
[12] Polidori, M.C., Stahl, W., Eichler, O., Niestroj, I. and Sies, H. (2001) "Profiles of antioxidants in human plasma", *Free Radic. Biol. Med.* **30**, 456–462.
[13] Diplock, A.T., Charleux, J.L., Crozier, W.G., Kok, F.J., Rice-Evans, C., Roberfroid, M., Stahl, W. and Vina-Ribes, J. (1998) "Functional food science and defence against reactive oxidative species", *Br. J. Nutr.* **80**(Suppl. 1), S77–S112.