Oxidation-reduction potential parameters worsen following intraarterial therapy in patients with reduced collateral circulation and middle cerebral artery occlusions

BENJAMIN ATCHIE[1,2], STEPHANIE JARVIS[3], RICHARD BELLON[1,2], TREVOR BARTON[4], LAUREN DISALVO[4], KRISTIN SALOTTOLO[3], RAPHAEL BAR-OR[5] and DAVID BAR-OR[6]

[1]Department of Neuroradiology, Swedish Medical Center; [2]Department of Neurointerventional Surgery, Radiology Imaging Associates; [3]Department of Epidemiology, Injury Outcomes Network (ION) Research; [4]Department of Neurology, Swedish Medical Center; [5]Department of Basic Science and [6]Department of Directors, Injury Outcomes Network (ION) Research, Englewood, CO 80113, USA

Received July 22, 2022; Accepted March 24, 2023

DOI: 10.3892/etm.2023.11994

Abstract. Collateral circulation is important for cerebral perfusion in acute ischemic strokes. Monitoring the oxidation-reduction potential (ORP) may be useful to assess collateral status or treatment efficacy. The objectives of the present study were to determine if the ORP was associated with collateral circulation status in middle cerebral artery (MCA) occlusions and to identify patterns in the ORP and the collateral circulation status among patients treated with intraarterial therapy (IAT) over time. The present pilot study was nested within a prospective cohort study measuring the ORP of the peripheral venous plasma of stroke patients. The population included in the present study were patients with MCA (M1/M2) occlusions. Two ORP parameters were examined: Static ORP (sORP; mV), indicating oxidative stress, and capacity ORP (cORP; μC), indicating antioxidant reserves. Collateral status was retrospectively graded using Miteff's system as good (grade 1) or reduced (grade 2/3). Comparisons were made between collateral status groups (reduced vs. good collaterals) in all patients, within a subset including only patients who received IAT, and between thrombolysis in cerebral infraction scale score (TICI) groups (0-2a vs. 2b/3). The Fisher's exact test, Student's t-test and Wilcoxon tests were used (α<0.20). The 19 patients were categorized based on their collaterals: Good collaterals (53%) and reduced collaterals (47%). The baseline characteristics were similar with the exception that the patients with good collaterals had a lower international normalized ratio (P=0.12) and were more likely to have a stroke on the left side (P=0.18) or to have a mismatch (P=0.05). The admission sORP values were comparable (169.5 vs. 164.2 mV; P=0.65), as was admission cORP (P=0.73). When considering only the patients who received IAT (n=12), admission sORP (P=0.69) and cORP (P=0.90) were also statistically similar. On day 2, after IAT, both groups experienced a worsening in ORP measures; however, the patients with good collaterals had a significantly lower sORP (169.4 vs. 203.5 mV; P=0.02) and a higher cORP (0.2 vs. 0.1 μC; P=0.002) compared with the patients with reduced collaterals. Neither sORP nor cORP were significantly different between TICI score groups on admission or on day 2. Upon discharge, patients with a TICI of 2b-3 had a significantly better sORP (P=0.03) and cORP (P=0.12) compared with those with a TICI of 0-2a. In conclusion, upon patient admission, the ORP parameters were not significantly different between the collateral circulation status groups for MCA occlusions. The ORP parameters worsened after IAT regardless of the collateral circulation status; however, after IAT, on day 2, patients with good collaterals experienced less oxidative stress (sORP) and had higher antioxidant reserves (cORP) than patients with reduced collaterals.

Correspondence to: Professor David Bar-Or, Department of Directors, Injury Outcomes Network (ION) Research, 601 East Hampden Ave, Englewood, CO 80113, USA
E-mail: davidbme49@gmail.com

Abbreviations: ORP, oxidation-reduction potential; MCA, middle cerebral artery; sORP, static ORP; cORP, capacity ORP; IAT, intraarterial therapy; INR, international normalized ratio; CTA, computed tomography angiography; NIHSS, National Institutes of Health Stroke Severity; tPA, tissue plasminogen activator; ICH, intracerebral hemorrhage; sICH, symptomatic ICH; mRS, discharge modified Rankin Scale; TICI, thrombolysis in cerebral infraction scale; LOS, length of stay

Key words: MCA occlusion, collateral circulation, ORP

Introduction

Cerebral collateral circulation describes a network of endogenous bypass blood vessels that can provide protection in acute ischemic stroke and is important for cerebral perfusion (1). Robust collateral flow is associated with rapid recanalization of middle cerebral artery (MCA) occlusions and smaller infarcts (1). Patients with reduced collaterals at baseline may not have salvageable brain tissue even after recanalization,

whereas patients with good collateral circulation at baseline are more likely to benefit from recanalization (2,3).

The oxidation-reduction potential (ORP) in biological systems has been described as an integrated measure of the balance between total oxidants (i.e., oxidized thiols, superoxide radicals, hydroxyl radicals, hydrogen peroxide, nitric oxide, peroxynitrite and transition metal ions) and total reductants (i.e., free thiols, ascorbate, α-tocopherol, β-carotene and uric acid) (4). Oxidative stress occurs when the quantity of oxidants exceeds the capacity of the reductants (5). Previous studies have found an association between increased ORP and various injuries and illnesses, including coronary occlusions, traumatic brain injury severity, Alzheimer's disease, metabolic syndrome, type 2 diabetes, hypertension, and chronic obstructive cardiopulmonary disease (4-11). To the best of our knowledge, it is not known whether the ORP may be useful for assessing collateral circulation status or treatment efficacy among stroke patients.

In a previous study, the epidemiology of stroke patients was described, and plasma samples were collected for ORP analysis (12-15). From the initial study, three manuscripts and one abstract have been published (12-15). In the initial study, it was observed that: i) multimodal imaging did not delay thrombolytic administration beyond 60 min, the goal at the Swedish Medical Center; ii) the ORP was associated with discharge dispositions in stroke patients and a 24-h delay in redox response was associated with the most severe strokes; iii) plasma oxidized albumin and evidence of strong antioxidant buffering were associated with an improved discharge modified Rankin Scale (mRS); and iv) ORP was associated with stroke severity, length of stay (LOS) and mortality (12-15).

The present study aimed to utilize the ORP parameters previously measured to address the following objectives: i) determine if ORP measurements from peripheral venous plasma can identify, on admission, the collateral circulation status (good vs. reduced) of patients with MCA occlusions; ii) identify any differences in ORP measurements between collateral circulation status groups (good vs. reduced) over time across all patients and when considering only patients who received intraarterial therapy (IAT); and iii) identify if ORP measurements on admission can identify patients who had successful reperfusion among the patients who received IAT.

Materials and methods

Study design, setting, selection criteria and comparison groups. The present study was a pilot study nested within a previously completed prospective cohort study that measured ORP in the peripheral venous plasma of patients with stroke symptoms at a comprehensive stroke center, Swedish Medical Center (Englewood, USA) (12-15). Patients with reduced collateral circulation were compared with patients with good collateral circulation, and the definitions used for the collateral circulation status are provided below. Only patients with MCA (M1/M2) occlusions were included in the present population, as these were the only patients from the initial population who received computed tomography angiography (CTA), allowing for retrospective collateral circulation scoring. The MCA occlusions were confirmed using CTA imaging. The patients were admitted over a 2-year period between January 2010 and January 2012. No other selection criteria were applied. Among all patients, the mean age was 65 years (SD, 19 years; range, 29-86 years), and 58% of patients were female and 42% were male.

Institutional Review Board (IRB) approval. The initial prospective study that the present pilot study was nested within, #231797, was approved by the Hospital Corporation of America-HealthOne IRB (Englewood, USA) which is the IRB responsible for research conducted at Swedish Medical Center. Swedish Medical Center is owned by Hospital Corporation of America HealthOne, and Radiology Imaging Associates is a contracted interventional and diagnostic radiology company within Swedish Medical Center. Written informed consent for all study activities was provided by the patients or their legally authorized representative during the initial study #231797. All patients were recruited at Swedish Medical Center. The aforementioned objectives were novel ideas developed after the closure of the initial study but could be evaluated using the initial study data. Thus, a new study protocol for the present pilot study, #1562897, was submitted to Hospital Corporation of America-HealthOne IRB to use the previously collected data. The present pilot study, #1562987, which used a subset population drawn from the initial study, #231797, was reviewed by Hospital Corporation of America-HealthOne IRB and determined to be exempt from IRB approval, with a waiver of the requirement for an additional new informed consent for the present study.

Collateral circulation scores. Collateral grades were retrospectively assigned using admission CTAs and Miteff's system by two independent interventional radiologists (16,17). Any differences in grading were reviewed, and a consensus was reached on the grade before the analysis. Grade 1 indicated that the entire MCA distal to the occluded segment was reconstituted with contrast material, and CTA maximum image projection reconstruction clearly demonstrated MCA branches, with abrupt termination of reconstituted vessels at the distal end of the occlusion within the M1 or proximal M2 segments (16,17). Grade 2 indicated that there was some reconstitution of the MCA branches within the Sylvian fissures, whereas Grade 3 indicated that only the distal superficial MCA branches were reconstituted with contrast material (16,17). Comparisons were made between groups based on vessel collateralization: Miteff's Grade 2 and 3 were combined and were referred to as 'reduced collaterals' and Grade 1 was referred to as 'good collaterals' (16).

Sample collection and ORP analysis. Plasma samples were collected daily for ORP analyses until patient discharge. Plasma ORP was measured using an ultrahigh impedance electrometer (RedoxSYS® Analyzer; model number 100016; Aytu BioPharma, Inc.; rebranded under the name MiOXSYS® System; https://mioxsys.com/mioxsys-system/; Caerus Biotechnologies) (5,7,8,18). The process for sample collection and analysis has been previously described (5,7,8,18). Briefly, a disposable sensor strip (RedoxSYS® sensor; cat. no. 100183; Aytu BioPharma, Inc., rebranded as MiOXSYS® sensor; cat. no. 100279; https://mioxsys.com/mioxsys-system/; Caerus

Biotechnologies) was inserted into a connector model of the analyzer, and 30-40 μl of the plasma sample was applied to a filtered opening in the sensor. The sensor was a self-contained electrochemical cell with platinum working and counter electrodes and a silver/silver chloride reference cell (7). After the sample made contact with the working and counter electrodes, hydration and wetting of the electrode surface and reference cell occurred, completing the electrochemical circuit (7). The transfer of electrons from the oxidant to the reductant (referred to generally as oxidation-reduction), was measured by the instrument as a voltage potential between the working electrode and the reference cell. The voltage was measured in mV and averaged over 10 sec, while a negligible current was applied between the working and counter electrodes. In the second stage of measurement, the applied current was linearly increased until the antioxidants at the surface of the working electrode were depleted. During this time, continued measurements of the voltage were collected. The point at which the voltage rise inflected indicated that the antioxidants were consumed and was used to calculate the antioxidant reserves in μC (5,7). Two distinct ORP measurements were described in the present study: i) Static ORP (sORP), which indicated the oxidant status at the time of measurement, measured in mV; and ii) capacity ORP (cORP), which indicated reductants, or antioxidant reserves, measured in μC (5,8). A higher sORP is worse, indicating more oxidative stress, whereas a higher cORP is better, indicating a higher level of antioxidant reserves, which provides a greater ability to mitigate an oxidative insult. The averages of both ORP measurements were calculated for admission (day 1), day 2 and the last collection day, which typically occurred on the discharge day of the patient. The mean last collection day was also summarized for both groups. Among all patients the mean (SD) last collected sample day was hospital-day-6 (3). For 9 patients, the last sample was collected a mean (SD) of 4 (2) days before the discharge date, ranging between 1 and 13 days before discharge. These 9 patients with their last sample collected before their discharge day had a mean (SD) LOS of 9 (6) days, with a range of 2-19 days. Patients discharged to in-house rehabilitation experienced the largest differences between their last collection day and discharge day (range, 5-13 days) and had a LOS ranging between 7 and 19 days. The changes in ORP measurements between day 1 and 2 were calculated by subtracting the day 1 values from the day 2 values. For patients who received IAT, the average postoperative ORP values were also calculated.

Variables included. Variables collected from the electronic medical record and from an internal database used at Swedish Medical Center to track patient admissions included: age (continuous), sex [% female (n), % male (n),], ethnicity [% White (n), % Black (n), % Hispanic (n)], time from symptom to arrival (continuous; min), initial National Institutes of Health Stroke Severity (NIHSS) score (continuous) (19), IAT [% treated (n)], tissue plasminogen activator [tPA; % treated (n)], preinjury locomotion [% walking independently (n)], transfer status [% transferred (n)], symptomatic intracerebral hemorrhage (ICH) [sICH; % yes (n)], discharge mRS (continuous) (20), hospital LOS (continuous), discharge disposition [% discharged to: Home or home with health care services, hospice or death, and rehabilitation (n)], thrombolysis in cerebral infraction scale (TICI; categorized as 0-2a or 2b/3) (21), side of stroke [% left (n), % right (n)], stroke mismatch [% with a mismatch (n)], creatinine (continuous), low-density lipoprotein (continuous) and international normalized ratio (INR; continuous).

tPA treatment. Activase® Alteplase (Genentech, Inc.) was used at Swedish Medical Center for tPA treatment. The single dose was dependent on the weight of the patient per the Federal Drug Administration package label (0.09 mg/kg). Patients who arrived within 4 h of symptom onset were considered for tPA treatment with the goal of providing a single dose of tPA within the first 60 min. Administration of tPA was not recommended for patients with the following characteristics at Swedish Medical Center: ICH on CT scan, abnormal anticoagulation profile, severe head trauma within the last 3 months, posttraumatic infarction, infective endocarditis, intracranial or spinal surgery within the last 3 months, gastrointestinal (GI) malignancy, GI bleeding within the last 21 days, history of ICH, intracerebral neoplasm, CT evidence of ischemic changes, suspicion of subarachnoid hemorrhage, or aortic arch dissection.

Statistical analysis. The patient characteristics and outcomes were first compared between groups of patients based on their collateral circulation score (good collaterals vs. reduced collaterals). A subset analysis was conducted among only the patients treated with IAT, which includes catheter-based approaches to achieve recanalization using mechanical clot disruption. In the subset analysis of only patients treated with IAT, outcomes were compared between groups of patients based on the collateral circulation score (good vs. reduced collaterals). To evaluate if ORP parameters were associated with successful reperfusion, the ORP parameters were also compared between groups of patients based on their TICI score; patients with a TICI score of 0-2a (unsuccessful reperfusion) were compared with those with a TICI score of 2b, 2c or 3 (successful reperfusion). Table SI shows the effect of tPA administration (yes/no) on ORP parameters. Dichotomous and categorical data are presented as proportions (counts) and were compared using Fisher's exact test. The Shapiro-Wilk test was conducted for each continuous variable within each population analyzed to determine if the variable was parametric or not. Shapiro-Wilk is the most accurate test of normality for small sample sizes. Parametric continuous data were summarized as the mean and SD and were compared using an unpaired Student's t-test. Nonparametric data were summarized as the median and interquartile range (IQR) and were compared using the Wilcoxon rank sum test. All data were analyzed using Statistical Analysis System version 9.4 (SAS Institute, Inc.).

Statistical significance threshold. $P<0.20$ was used to indicate a statistically significant difference. The present pilot study used a convenience sample drawn from a previously completed prospective study, from which 19 patients met the selection criteria. As more patients could not be obtained and the sample size was small, the P-value was increased to reduce the likelihood of a type II error (a failure to reject the null hypothesis when it was false) and increase the power (22-25).

Table I. Clinical characteristics.

Patient characteristics	Good collaterals (n=10)	Reduced collaterals (n=9)	P-value
Age, years [mean (SD)]	62.7 (24.1)	66.7 (11.7)	0.66
Sex, % (n)			>0.99
Female	60 (6)	56 (5)	
Male	40 (4)	44 (4)	
Ethnicity, % (n)			>0.99
White	90 (9)	89 (8)	
Black	10 (1)	0 (0)	
Hispanic	0 (0)	11 (1)	
Walking independently, % (n)	100 (10)	100 (9)	N/A
Symptom to arrival, min [mean (SD)]	197.1 (142.5)	230.6 (109.9)	0.59
Initial NIHSS score [mean (SD)]	18.3 (5.2)	15.9 (6.7)	0.39
Creatinine, mg/dl [mean (SD)]	1.0 (0.2)	1.1 (0.3)	0.58
LDL, mg/dl [mean (SD)]	88.0 (36.4)	88.3 (34.7)	0.98
INR [mean (SD)]	1.0 (0.1)	1.1 (0.1)	0.12
Admission type, % (n)			0.35
Transferred	50 (5)	78 (7)	
Direct Admission	50 (5)	22 (2)	
Side of stroke, % (n)			0.18
Left	70 (7)	33 (3)	
Right	30 (3)	67 (6)	
Mismatch, % (n)			0.05
Yes	60 (6)	11 (1)	
No	40 (4)	89 (8)	
Treatment, % (n)			0.29
Both IAT and tPA	50 (5)	33 (3)	
Only IAT	20 (2)	22 (2)	
Only tPA	20 (2)	0 (0)	
Neither IAT nor tPA	10 (1)	44 (4)	

Data presented as the mean (SD) were compared using an unpaired Student's t-test, and dichotomous and categorical data were compared with Fisher's exact test. NIHSS, National Institutes of Health Stroke Severity; LDL, low-density lipoproteins; INR, international normalized ratio; IAT, intraarterial therapy; tPA, tissue plasminogen activator.

An increased sample size or P-value results in an increased power, as the three variables are positively associated with each other. Thus, since the sample size could not be increased, the P-value was increased, as described and validated previously (22-25). This method is similar to decreasing the P-value to P<0.0001 for studies using large datasets to decrease the likelihood of type I error (rejecting the null hypothesis when it is true) (26). In the present pilot study, which had a small sample size drawn from a convenience sample, the P-value was increased to maintain adequate power.

Results

Patient demographics and clinical characteristics. In the present study, 19 patients were included: 53% (n=10) had good collaterals and 47% (n=9) had reduced collaterals. The mean age was 65 years, 58% of patients were female, 79% of patients were White and all patients walked independently prior to injury. The patient demographics (age, sex and ethnicity) were statistically similar between the collateral status groups (Table I). The patients with good collaterals had a significantly lower mean arrival INR (P=0.12), were more likely to have a stroke on the left side (P=0.18), and were more likely to have a stroke mismatch (P=0.05) compared with the patients with reduced collaterals. While patients with good collaterals received both IAT and tPA, and were more likely to be treated with or received tPA alone more frequently than the patients with reduced collaterals, there were no significant differences in the treatment approaches between the groups (P=0.29).

Overall outcomes compared by collateral status. The mean admission sORP was comparable between the groups (P=0.65), and the mean admission cORP was the same for both groups, 0.2 μC (0.1 μC) (Table II). The mean day 2 sORP was significantly higher for patients with reduced collaterals compared with patients with good collaterals (P=0.07). The

Table II. ORP values over time by collateral grade.

Outcome	Good collaterals (n=10)	Reduced collaterals (n=9)	P-value
Type and day of ORP sample collection			
Day 1			
sORP, mV [mean (SD)]	169.5 (28.4)	164.2 (18.9)	0.65
cORP, μV [mean (SD)]	0.2 (0.1)	0.2 (0.1)	0.73
Day 2			
sORP, mV [mean (SD)]	168.6 (14.6)	188.7 (28.6)	0.07
cORP, μV [mean (SD)]	0.2 (0.0)	0.2 (0.1)	0.20
Change between day 1 and 2			
sORP, mV [median (IQR)]	5.8 (-20.3, 14.2)	4.1 (-2.7, 40.2)	0.48
cORP, μV [median (IQR)]	0.0 (-0.1, 0.0)	0.0 (-0.1, 0.0)	0.96
Last collected sample			
sORP, mV [mean (SD)]	160.1 (11.5)	167.7 (26.3)	0.46
cORP, μV [mean (SD)]	0.3 (0.1)	0.3 (0.2)	0.52
Last collection day, days [mean (SD)]	6.0 (4.0)	6.0 (4.0)	0.97
Discharge mRS [median (IQR)]	3.0 (2.0, 4.0)	4.0 (3.0, 4.0)	0.31
LOS, days [mean (SD)]			
Among all patients	7.2 (3.0)	8.4 (7.0)	0.63
Among survivors	7.0 (3.1)	9.4 (7.5)	0.03
Discharge disposition, % (n)			0.34
Death or hospice	10.0 (1)	33.3 (3)	
Home or home with healthcare services	40.0 (4)	11.1 (1)	
Rehabilitation	50.0 (5)	55.6 (5)	

Data presented as the median (IQR) were compared using the Wilcoxon rank sum test, data presented as the mean (SD) were compared using an unpaired Student's t-test, and dichotomous and categorical data were compared with Fisher's exact test. ORP, oxidation-reduction potential; sORP, static ORP; cORP, capacity ORP; mRS, modified Rankin Scale; LOS, length of stay; IQR, interquartile range.

mean day 2 cORP trended towards statistically different between the collateral status groups (P=0.20), while both groups had a mean of 0.2 μC, the standard deviation showed that patients with reduced collaterals had a slightly higher cORP. There were no differences in the median change from day 1 to day 2 for sORP (P=0.48) or cORP (P=0.96), or the mean last collected sample for sORP (P=0.46) or cORP (P=0.52) between collateral status groups among all patients. The last sample collection day ranged between hospital-day-2 and hospital-day-16. The last sample collection occurred on hospital-day-6 (SD, 4) both for patients with good collaterals and patients with reduced collaterals (P=0.97). The mean (SD) last sample collection day occurred significantly (P=0.07) earlier for the patients who died in-hospital or were discharged to hospice compared with the patients who did not die in-hospital or were not discharged to hospice [3 (2) vs. 7 (4)] (data not shown). The median discharge mRS was not significantly different between the groups (P=0.31). When examining LOS across all patients, there was no significant difference between the groups (P=0.63); however, among the survivors, those with good collaterals had a significantly shorter mean LOS compared with those with reduced collaterals (P=0.03). While more patients with good collaterals were discharged home compared with patients with reduced collaterals, there were no significant differences in the discharge dispositions.

Patients treated with IAT. Among only the patients treated with IAT, the admission ORP measures were similar between the groups (Table III). On day 2, after IAT, patients with good collateral circulation had a significantly lower sORP (P=0.02) and a significantly higher cORP (P=0.002) compared with patients with reduced collateral circulation. While there was an increase in sORP from day 1 to 2 in both groups, the change was significantly smaller for the patients with good collaterals, 13.0 vs. 40.2 mV, compared with patients with reduced collaterals (P=0.13). From day 1 to 2, the cORP decreased by a median of -0.1 μV for both groups but the change was also significantly different between the groups (P=0.18). In terms of the IQR for the cORP change from day 1 to 2, those with reduced collaterals experienced a greater decrease (IQR, -0.1, <-0.1 μV) than those with good collaterals (IQR, -0.1, <0.1 μV). The overall mean sORP after IAT was significantly lower (P=0.04) and the overall mean cORP after IAT was significantly higher (P=0.03) for the patients with good collaterals compared with the patients with reduced collateral circulation. On the last collection day, the sORP and cORP values remained significantly improved for the patients with good collaterals. There was no difference in the proportion of patients treated with tPA (P>0.99) or the time from stroke alert to IAT (P=0.61) between the groups. There were significantly more patients with good collaterals who had a TICI

Table III. Outcomes of patients treated with IAT.

Outcome	Good collaterals (n=7)	Reduced collaterals (n=5)	P-value
Type and day of ORP sample collection			
Day 1			
sORP, mV [mean (SD)]	162.9 (26.9)	156.3 (19.2)	0.69
cORP, μV [mean (SD)]	0.2 (0.1)	0.2 (0.1)	0.90
Day 2			
sORP, mV [mean (SD)]	169.4 (16.6)	203.5 (26.1)	0.02
cORP, μV [mean (SD)]	0.2 (<0.1)	0.1 (<0.1)	<0.01
Change between day 1 and 2			
sORP, mV [median (IQR)]	13.0 (0.2, 24.2)	40.2 (16.7, 68.2)	0.13
cORP, μV [median (IQR)]	-0.1 (-0.1, <0.1)	-0.1 (-0.1, <-0.1)	0.18
Last collected sample			
sORP, mV [mean (SD)]	156.7 (10.5)	181.4 (22.8)	0.03
cORP, μV [mean (SD)]	0.3 (0.1)	0.2 (0.1)	0.01
Average after IAT			
sORP, mV [mean (SD)]	161.4 (8.8)	184.8 (25.5)	0.04
cORP, μV [mean (SD)]	0.3 (<0.1)	0.2 (0.1)	0.03
Treatment, % (n)			>0.99
Both IAT and tPA	71.4 (5)	60.0 (3)	
tPA only	0.0 (0)	0.0 (0)	
IAT only	29.6 (2)	40.0 (2)	
Neither IAT nor tPA	0.0 (0)	0.0 (0)	
Time from stroke alert to IAT, min [mean (SD)]	95.9 (27.8)	85.0 (41.0)	0.61
TICI score, % (n)			0.18
2b, 2c or 3	100.0 (7)	60.0 (3)	
0, 1 or 2a	0.0 (0)	40.0 (2)	
sICH, % (n)			>0.99
Yes	29.0 (2)	40.0 (2)	
No	71.4 (5)	60.0 (3)	
LOS, days [median (IQR)][a]			
Among all patients	7.0 (5.0, 11.0)	3.0 (2.0, 9.0)	0.33
Among survivors	6.5 (5.0, 11.0)	3.0 (2.0, 19.0)	0.44
Discharge mRS [median (IQR)]	3.2 (1.6)	4.4 (1.7)	0.27
Discharge disposition, % (n)			0.34
Death or hospice	14.3 (1)	60 (3)	
Home or home with healthcare services	28.9 (2)	20 (1)	
Rehabilitation	57.4 (4)	20 (1)	

[a]LOS was non-parametric among the patients treated with IAT, therefore the median (IQR) is presented and compared. Data presented as the median (IQR) were compared with the Wilcoxon rank sum test, data presented as the mean (SD) were compared using an unpaired Student's t-test, and dichotomous and categorical data were compared with Fisher's exact test. sORP, static oxidation-reduction potential; cORP, capacity oxidation-reduction potential; mRS, modified Rankin Scale; sICH, symptomatic intracranial hemorrhage; LOS, length of stay; IQR, interquartile range; IAT, intraarterial therapy; tPA, tissue plasminogen activator; TICI, thrombolysis in cerebral infarction scale.

score of 2b, 2c or 3 compared with the patients with reduced collaterals (P=0.18). There was a statistically similar rate of sICH in patients with good collaterals compared with patients with reduced collaterals (29 vs. 40%, respectively; P>0.99). The LOS among all patients treated with IAT, and among survivors treated with IAT, was statistically similar between the groups. The discharge mRS was lower for patients with good collaterals (3.2 vs. 4.4) but was not significantly different. Furthermore, the discharge disposition was similar between the groups.

TICI score analysis. Upon admission, neither sORP nor cORP were significantly different between patients who had a TICI score of 0-2a and patients who had a TICI score of

Table IV. ORP measurements over time compared between TICI score groups.

Type and day of ORP sample collection	TICI 0-2a	TICI 2b, 2c and 3	P-value
Day 1			
sORP, mV [median (IQR)]	160.7 (142.0, 178.5)	164.0 (138.1, 190.6)	>0.99
cORP, μV [median (IQR)]	0.2 (0.2, 0.3)	0.2 (0.2, 0.3)	>0.99
Day 2			
sORP, mV [median (IQR)]	200.9 (189.0, 212.8)	176.9 (166.6, 197.0)	0.35
cORP, μV [median (IQR)]	0.2 (0.1, 0.2)	0.2 (0.2, 0.2)	0.34
Change between day 1 and 2			
sORP, mV [median (IQR)]	40.2 (34.3, 46.1)	13.6 (-10.7, 27.9)	0.11
cORP, μV [median (IQR)]	-0.1, (-0.1, 0.0)	-0.1 (-0.1, 0.0)	0.79
Last collected			
sORP, mV [median (IQR)]	200.9 (189.0, 212.8)	156.6 (153.8, 165.7)	0.03
cORP, μV [median (IQR)]	0.2 (0.1, 0.2)	0.3 (0.2, 0.4)	0.12

Data presented as the median (IQR) were compared with the Wilcoxon rank sum test. ORP, oxidation-reduction potential; sORP, static ORP; cORP, capacity ORP; TICI, thrombolysis in cerebral infarction scale; IQR, interquartile range.

2b-3 (Table IV). The ORP parameters remained comparable on day 2. However, the patients with a TICI score of 2b-3 experienced a significantly smaller increase in oxidative stress (sORP) from day 1 to day 2 compared with the patients with a TICI of 0-2a (P=0.11). By the time of discharge, the patients with a TICI score of 2b-3 had significantly improved sORP (P=0.03) and cORP (P=0.12) values compared with the patients with a TICI score of 0-2a.

Administration of tPA. On day 1, the patients treated with tPA had a significantly lower sORP (P=0.09) and a significantly higher cORP (P=0.06) compared with the patients who were not treated with tPA (Table SI). However, it is not known whether the tPA treatment occurred before or after the day 1 sample was collected. While the patients who were treated with tPA experienced a decrease in cORP from day 1 to day 2, the patients who were not treated with tPA experienced an increase in cORP from day 1 to day 2, and the difference was statistically significant (P=0.02). On day 2, both sORP and cORP were comparable between the groups, which remained the case on the last collection day.

Discussion

The present pilot study found that ORP could not significantly distinguish between good and reduced collateral circulation in patients with MCA occlusions on admission, which could be due to the small sample size. However, there were associations between the ORP and collateral status when examining only the patients who were treated with IAT. The biomarker for oxidative stress (sORP) increased after IAT (change day 1 to day 2), and the biomarker for antioxidant reserves (cORP) decreased after IAT (change day 1 to day 2), indicating that IAT reopened the pathway for oxidant circulation, allowing for antioxidant consumption. Patients with reduced collaterals experienced a larger increase in oxidant circulation (sORP) from day 1 to day 2 (after IAT) and a larger decrease in antioxidant availability (cORP) from day 1 to day 2 (after IAT) compared with patients good collaterals.

In the present study, neither sORP nor cORP were able to identify good or reduced collateral circulation on admission. It has been suggested that the baseline collateral circulation status may be useful in identifying the patients who will benefit from IAT (27). If repeat imaging is needed to determine the collateral circulation status, the time to IAT will increase, which is considered to contribute to poorer outcomes (28). Because of this, it was considered that a tool to detect collateral circulation status without imaging could be vital for the quick identification of patients who otherwise may not benefit from IAT. It is possible that when the admission sample was collected (immediately upon arrival) the collateral circulation had not yet been established. Yazici *et al* (29) observed that oxidative stress decreased, and antioxidant levels increased between 2 and 6 h postinjury in untreated rats with peripheral or mesenteric ischemia; the authors postulated that the improvement may be due to the development of some collateral circulation between the collection times. In the present study, although the admission ORP values were indistinguishable, the day 2 sORP was significantly higher in the patients with reduced collateral circulation. This could be due to the passage of time allowing for collateral circulation development leading to a delayed increase in oxidant circulation (sORP) compared with patients with good collateral circulation who may have experienced a more steady circulation of oxidants after their injury over time.

Alternatively, the increase in oxidative stress experienced on day 2 among the patients with reduced circulation could be the result of IAT. When considering only the patients who received IAT, there were no significant differences in the pretreatment (day 1) ORP values between the groups; however, the day 1 sORP was slightly higher among those with good collaterals. Demirbag *et al* (4) observed that patients with total coronary occlusions and good collateral circulation had higher oxidative stress levels before treatment compared with patients

with reduced collateral circulation. The present study provides evidence that not only did IAT allow for circulation of oxidants (sORP), but the degree of collateral circulation also served a role in the amount and timing of oxidant circulation (sORP). The present data indicate that there may be some pretreatment circulation of oxidants in the plasma for patients with good collaterals. The results of the present study, showing a larger increase in sORP after IAT (day 2) compared with before IAT (day 1) for patients with reduced collaterals, further indicate that for patients with reduced collaterals, the oxidants were sequestered near the infarct and were not in equilibrium with systemic circulation before IAT. This suggests that the oxidative stress level in patients with reduced collaterals was lower compared with that of the patients with good collaterals until IAT was received. While IAT caused oxidant circulation (increased sORP) for both groups, there was a greater increase in oxidants in the patients with reduced collaterals, potentially due to less pretreatment oxidant circulation. Compared with the patients with reduced collaterals, there were significantly ($P<0.01$) more antioxidant reserves (in terms of the mean cORP value) after IAT (Day 2) in the patients with good collaterals. This may indicate a lack of antioxidant consumption due to a lower volume of oxidants circulating in the peripheral blood. However, the average cORP after IAT was worse for the patients with reduced collaterals, likely due to a greater need for antioxidants after recanalization. The analysis of the last collected sample indicated that both sORP and cORP were worse for the patients with reduced collaterals compared with the patients with good collaterals, which is in line with previous research indicating that IAT is less beneficial in patients with reduced collaterals (2,3).

Among all patients, those with good collaterals appeared to have improved outcomes compared with those with reduced collaterals. The LOS among the survivors was significantly shorter for those with good collaterals compared with the patients with reduced collaterals. While not significant, the patients with good collaterals also had a lower discharge mRS and improved discharge dispositions; the patients with good collaterals were discharged home more often and died in hospital or were discharged to rehab less often than the patients with reduced collaterals. Menon *et al* (2) found that good collateral circulation status was predictive of a favorable 90-day mRS. Previous authors have discussed how patients with reduced baseline collaterals treated with IAT are at risk of reperfusion injuries (2,30-32). Among the patients who received IAT, there were still no significant differences in outcomes, but patients with good collaterals still had a lower discharge mRS and improved discharge dispositions. Wufuer *et al* (33) conducted a meta-analysis and found that patients with good collaterals were at a lower risk of mortality among the patients who received IAT.

The present findings demonstrated that a higher proportion of patients with reduced collaterals who received IAT had sICH compared with patients with good collaterals. Of the patients who suffered from sICH, 100% with reduced collaterals and 50% with good collaterals died (data not shown). Semerano *et al* (30) also reported a higher rate of sICH among patients with reduced collaterals. Tang *et al* (31,32) used deep learning technology to create a model predictive of ICH expansion, which was derived from the baseline characteristics among the patients who suffered from ICH. The model had 90% accuracy and used the age of the patient, low-density lipoprotein cholesterol, time from onset to admission, systolic blood pressure, coagulopathy, baseline Glasgow Coma Scale, baseline NIHSS and the presence of intraventricular expansion to predict ICH expansion (31). While the investigators examined the hematoma location, the authors did not evaluate occluded vessels, collateral circulation, or treatment with IAT as predictors (31,32). Further investigation of these variables as predictors of ICH expansion is warranted.

In the present study, patients with good collaterals were significantly more likely to have successful recanalization defined as a TICI score of 2b, 2c or 3 compared with the patients with reduced collaterals. Similarly, Marks *et al* (34) found that reperfusion scores were higher among patients with good collaterals compared with patients with reduced collateral circulation. Liebeskind *et al* (35) also observed that improved collaterals were closely linked to recanalization scores of 2b, 2c or 3. However, successful recanalization, as defined using the TICI, was not associated with admission sORP or cORP in the present study. Therefore, admission ORP measurements may not be useful in the identification of patients who may benefit from IAT.

In the present study, the mean admission sORP values for both groups were much higher compared with that of heathy controls in a previous study (~137 mV); similarly, the mean admission cORP was also worse for the patients in the present study compared with the mean observed in healthy controls (inverse cORP ~1.65 μV) (5). This suggests that both groups, regardless of collateral circulation status, were in a state of oxidative stress on admission. Because the ORP measurements for both groups in the present study on admission were below the normal mean result previously reported in healthy controls, antioxidant treatment may be beneficial for all patients to prevent the aggravation of complications due to oxidative stress (5,8). The stress induced after IAT was previously found to be alleviated with antioxidants and mitochondrial stabilization (36). Other studies on MCA occlusions in rodents found that vitamin C, leonurine and melatonin treatment was associated with improved outcomes, including fewer neurological deficits, increased antioxidant enzyme activity, restored mitochondrial function and redox status, reduced brain infarct, and reduced brain edema (37-39). Additional studies have reported positive outcomes (such as reduced infarct volume and neurological deficits, inhibited oxidative stress damage caused by cerebral ischemia, and reduced blood brain barrier permeability) after antioxidant treatment (such as resveratrol, ginkgolides, bilobalide and ruscogenin) in animals (mice and rats) that suffered strokes (40-42). Mixed results have been reported regarding the efficacy of using vitamin C, and there is a lack of studies examining how carotenoids or vitamin E impact stroke outcomes (43-46). One study on the parenteral administration of vitamin C demonstrated marked improvement in outcomes; the authors argued that oral vitamin C administration has limited bioavailability and a rapid clearance, not allowing for a plasma concentration high enough to be therapeutic (39). Monitoring ORP values could be useful in the identification of patients who may benefit from antioxidant treatment, such as patients whose cORP values are decreasing over time or after IAT.

The present pilot study was limited by the small sample size (n=19), especially in the analysis of only patients treated with IAT (n=12), and larger studies are required to validate the results. While samples were collected daily throughout the LOS of the patients, the LOS varied from patient to patient, thus only a small number of samples were collected after day 2. The present study was a single center study using convenience sampling, which limits the generalizability. Only patients with MCA occlusions were included as these patients underwent imaging that allowed for retrospective collateral circulation scoring. It is important to explore whether the results of the present study can be reproduced among all stroke patients. The time of plasma collection was not recorded. On admission day, there was only one collection time; a specific timepoint (e.g., 2, 4 or 6 h after admission) when the ORP can differentiate between collateral circulation status (good vs. reduced) may be found when examining ORP hourly. There is a possibility that medications can alter the ORP measurements. Because the time of plasma sample collection had not been recorded, it could not be determined if tPA treatment occurred before or after obtaining the admission sample (day 1). Future research should focus on the effect of antioxidant treatment on the outcomes of patients, especially in patients after IAT, and whether those outcomes are further improved depending on the collateral circulation status.

The ORP parameters were not able to identify the collateral circulation status for patients with MCA occlusions on admission and may not be useful in identifying the patients who may benefit from IAT. The ORP parameters worsened after IAT regardless of the collateral circulation status, but the collateral circulation status differentiated the degree of posttreatment oxidative insult (sORP). Patients with reduced collateral circulation experienced a larger increase in oxidative stress (sORP) after IAT, potentially due to the lack of good collateral circulation around the ischemia leading to a sequestering of oxidants pretreatment. After IAT, patients with good collateral circulation continued to experience less oxidative stress (sORP) and had higher antioxidant reserves (cORP) compared with the patients with reduced collateral circulation; this was also the case on the last collection day.

Acknowledgements

The authors would like to thank the IRB coordinator at Injury Outcomes Network (Englewood, USA), Ms. Tina Thompson, who assisted with IRB submissions and documentation. The authors would like to thank the clinical research coordinator at Injury Outcomes Network (Englewood, USA), Ms. Breanna Nickels, and the nurse practitioners at Swedish Medical Center (Englewood, USA), Ms. Amy Nieberlein and Ms. Jasmin Johann, who all assisted with the data collection for this project.

Funding

No funding was received.

Availability of data and materials

The datasets generated and/or analyzed during the current study are not publicly available due to data use agreements with the participating hospital but are available from the corresponding author on reasonable request.

Authors' contributions

BA, SJ, RB, TB, LD, KS, RBO and DBO contributed to the study design. DBO and RBO contributed to the study conception. SJ and DBO confirm the authenticity of all the raw data. TB, LD, BA and RB contributed to the data collection. SJ conducted the statistical analysis. KS reviewed the statistical analysis. SJ drafted the original manuscript. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The pilot study was deemed exempt from IRB approval by the Hospital Corporation of America-HealthOne IRB, Englewood, USA (#1562897). The requirement of patient consent for participation was also waived by the Hospital Corporation of America-HealthOne IRB.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Liebeskind DS: Collateral Circulation. Stroke 34: 2279-2284, 2003.
2. Menon BK, Qazi E, Nambiar V, Foster LD, Yeatts SD, Liebeskind D, Jovin TG, Goyal M, Hill MD, Tomsick TA, *et al*: Differential Effect of Baseline Computed Tomographic Angiography Collaterals on Clinical Outcome in Patients Enrolled in the Interventional Management of Stroke III trial. Stroke 46: 1239-1244, 2015.
3. Bang OY, Saver JL, Buck BH, Alger JR, Starkman S, Ovbiagele B, Kim D, Jahan R, Duckwiler GR, Yoon SR, *et al*: Impact of collateral flow on tissue fate in acute ischaemic stroke. J Neurol Neurosurg Psychiatry 79: 625-629, 2008.
4. Demirbag R, Gur M, Yilmaz R, Kunt AS, Erel O and Andac MH: Influence of oxidative stress on the development of collateral circulation in total coronary occlusions. Int J Cardiol 116: 14-19, 2007.
5. Bjugstad KB, Rael LT, Levy S, Carrick M, Mains CW, Slone DS and Bar-Or D: Oxidation-Reduction Potential as a Biomarker for Severity and Acute Outcome in Traumatic Brain Injury. Oxid Med Cell Longev 2016: 6974257, 2016.
6. Chen Z and Zhong C: Oxidative stress in Alzheimer's disease. Neurosci Bull 30: 271-281, 2014.
7. Rael LT, Bar-Or R, Kelly MT, Carrick MM and Bar-Or D: Assessment of oxidative stress in patients with an isolated traumatic brain injury using disposable electrochemical test strips. Electroanalysis 27: 2567-2573, 2015.
8. Spanidis Y, Mpesios A, Stagos D, Goutzourelas N, Bar-Or D, Karapetsa M, Zakynthinos E, Spandidos DA, Tsatsakis AM, Leon G, Kouretas D: Assessment of the redox status in patients with metabolic syndrome and type 2 diabetes reveals great variations. Exp Ther Med 11: 895-903, 2016.
9. Sinha N and Dabla PK: Oxidative Stress and Antioxidants in Hypertension-A Current Review. Curr Hypertens Rev 11: 132-142, 2015.
10. Bourg P, Salottolo K, Klein J and Bar-Or D: Can a biomarker for oxidative stress and antioxidant reserves identify frailty in geriatric trauma patients? Injury 52: 2908-2913, 2021.
11. Kirkham PA and Barnes PJ: Oxidative stress in COPD. Chest 144: 266-273, 2013.

12. Salottolo KM, Fanale CV, Leonard KA, Frei DF and Bar-Or D: Multimodal imaging does not delay intravenous thrombolytic therapy in acute stroke. AJNR Am J Neuroradiol 32: 864-868, 2011.
13. Bjugstad KB, Fanale C, Wagner J, Jensen J, Salottolo K, Rael LT and Bar-Or D: A 24 h Delay in the Redox Response Distinguishes the most Severe Stroke Patients from Less Severe Stroke Patients. J Neurol Neurophysiol 07: 395, 2016.
14. Rael LT, Leonard J, Salottolo K, Bar-Or R, Bartt RE, Wagner JC and Bar-Or D: Plasma oxidized albumin in acute ischemic stroke is associated with better outcomes. Front Neurol 10: 709, 2019.
15. Wagner JC, Salottolo K, Fanale CV, Whaley M, McCarthy KL and Bar-Or D: Abstract T P219: A Novel Method for Measuring Oxidative Stress in Patients with Stroke Symptoms. Stroke 46 Suppl 1: ATP219, 2015.
16. Miteff F, Levi CR, Bateman GA, Spratt N, McElduff P and Parsons MW: The independent predictive utility of computed tomography angiographic collateral status in acute ischaemic stroke. Brain 132(Pt 8): 2231-2238, 2009.
17. Seker F, Potreck A, Möhlenbruch M, Bendszus M and Pham M: Comparison of four different collateral scores in acute ischemic stroke by CT angiography. J Neurointerv Surg 8: 1116-1118, 2016.
18. Bar-Or D, Bar-Or R, Rael LT and Brody EN: Oxidative stress in severe acute illness. Redox Biol 4: 340-345, 2015.
19. National Institutes of Health and National Institutes of Neurological Disorders and Stroke. NIH Stroke Scale. 1-5. https://www.stroke.nih.gov/resources/scale.htm. Accessed 3/28/2023.
20. Joint Commission National Quality Measures: Modified Rankin Score (mRS) Specifications Manual. 1-3. 2023. https://manual.jointcommission.org/releases/TJC2023B/DataElem0773.html. Accessed 3/28/2023.
21. Higashida RT, Furlan AJ, Roberts H, Tomsick T, Connors B, Barr J, Dillon W, Warach S, Broderick J, Tilley B, *et al*: Trial design and reporting standards for intra-arterial cerebral thrombolysis for acute ischemic stroke. Stroke 34: e109-e137, 2003.
22. Lewis M, Bromley K, Sutton CJ, McCray G, Myers HL and Lancaster GA: Determining sample size for progression criteria for pragmatic pilot RCTs: The hypothesis test strikes back! Pilot Feasibility Stud 7: 40, 2021.
23. Schoenfeld D: Statistical considerations for pilot studies. Int J Radiat Oncol Biol Phys 6: 371-374, 1980.
24. Kianifard F and Islam MZ: A guide to the design and analysis of small clinical studies. Pharm Stat 10: 363-368, 2011.
25. Stallard N: Optimal sample sizes for phase II clinical trials and pilot studies. Stat Med 31: 1031-1042, 2012.
26. Gómez-de-Mariscal E, Guerrero V, Sneider A, Jayatilaka H, Phillip JM, Wirtz D and Muñoz-Barrutia A: Use of the p-values as a size-dependent function to address practical differences when analyzing large datasets. Sci Rep 11: 20942, 2021.
27. Boers AM, Jansen IG, Berkhemer OA, Yoo AJ, Lingsma HF, Slump CH, Roos YB, van Oostenbrugge RJ, Dippel DW, van der Lugt A, *et al*: Collateral status and tissue outcome after intra-arterial therapy for patients with acute ischemic stroke. J Cereb Blood Flow Metab 37: 3589-3598, 2017.
28. Sarraj A, Goyal N, Chen M, Grotta JC, Blackburn S, Requena M, Kamal H, Abraham MG, Elijovich L, Dannenbaum M, *et al*: Direct to angiography vs repeated imaging approachs in transferred patients undergoing endovascular thrombectomy. JAMA Neurol 78: 916-926, 2021.
29. Yazici S, Demirtas S, Guclu O, Karahan O, Yavuz C, Caliskan A and Mavitas B: Using oxidant and antioxidant levels to predict the duration of both acute peripheral and mesenteric ischemia. Perfusion 29: 450-455, 2014.
30. Semerano A, Laredo C, Zhao Y, Rudilosso S, Renú A, Llull L, Amaro S, Obach V, Planas AM, Urra X and Chamorro Á: Leukocytes, collateral circulation, and reperfusion in ischemic stroke patients treated with mechanical thrombectomy. Stroke 50: 3456-3464, 2019.
31. Tang Z, Zhu Y, Lu X, Wu D, Fan X, Shen J and Xiao L: Deep learning-based prediction of hematoma expansion using a single brain computed tomographic slice in patients with spontaneous intracerebral hemorrhages. World Neurosurg 165: e128-e136, 2022.
32. Tang ZR, Chen Y, Hu R and Wang H: Predicting hematoma expansion in intracerebral hemorrhage from brain CT scans via K-nearest neighbors matting and deep residual network. Biomed Signal Process Control 76: 103656, 2022.
33. Wufuer A, Wubuli A, Mijiti P, Zhou J, Tuerxun S, Cai J, Ma J and Zhang X: Impact of collateral circulation status on favorable outcomes in thrombolysis treatment: A systematic review and meta-analysis. Exp Ther Med 15: 707-718, 2018.
34. Marks MP, Lansberg MG, Mlynash M, Olivot JM, Straka M, Kemp S, McTaggart R, Inoue M, Zaharchuk G, Bammer R, *et al*: Effect of collateral blood flow on patients undergoing endovascular therapy for acute ischemic stroke. Stroke 45: 1035-1039, 2014.
35. Liebeskind DS, Jahan R, Rogueira RG, Zaidat OO and Saver JL; SWIFT Investigators: Impact of collaterals on successful revascularization in solitaire FR with the intention for thrombectomy. Stroke 45: 2036-2040, 2014.
36. Tan BL, Norhaizan ME and Liew WP: Nutrients and oxidative stress: Friend or foe? Oxid Med Cell Longev 2018: 9719584, 2018.
37. Loh KP, Qi J, Tan BK, Liu XH, Wei BG and Zhu YZ: Leonurine protects middle cerebral artery occluded rats through antioxidant effect and regulation of mitochondrial function. Stroke 41: 2661-2668, 2010.
38. Liu ZJ, Ran YY, Qie SY, Gong WJ, Gao FH, Ding ZT and Xi JN: Melatonin protects against ischemic stroke by modulating microglia/macrophage polarization toward anti-inflammatory phenotype through STAT3 pathway. CNS Neurosci Ther 25: 1353-1362, 2019.
39. Chang CY, Chen JY, Wu MH and Hu ML: Therapeutic treatment with vitamin C reduces focal cerebral ischemia-induced brain infarction in rats by attenuating disruptions of blood brain barrier and cerebral neuronal apoptosis. Free Radic Biol Med 155: 29-36, 2020.
40. Mota M, Porrini V, Parrella E, Benarese M, Bellucci A, Rhein S, Schwaninger M and Pizzi M: Neuroprotective epi-drugs quench the inflammatory response and microglial/macrophage activation in a mouse model of permanent brain ischemia. J Neuroinflammation 17: 361, 2020.
41. Liu Q, Jin Z, Xu Z, Yang H, Li L, Li G, Li F, Gu S, Zong S, Zhou J, *et al*: Antioxidant effects of ginkgolides and bilobalide against cerebral ischemia injury by activating the Akt/Nrf2 pathway in vitro and in vivo. Cell Stress Chaperones 24: 441-452, 2019.
42. Cao G, Jiang N, Hu Y, Zhang Y, Wang G, Yin M, Ma X, Zhou K, Qi J, Yu B and Kou J: Ruscogenin attenuates cerebral ischemia-induced blood-brain barrier dysfunction by suppressing TXNIP/NLRP3 inflammasome activation and the MAPK pathway. Int J Mol Sci 17: 1418, 2016.
43. Bahonar A, Saadatnia M, Khorvash F, Maracy M and Khosravi A: Carotenoids as potential antioxidant agents in stroke prevention: A systematic review. Int J Prev Med 8: 70, 2017.
44. Törnwall ME, Virtamo J, Korhonen PA, Virtanen MJ, Albanes D and Huttunen JK: Postintervention effect of alpha tocopherol and beta carotene on different strokes: A 6-year follow-up of the alpha tocopherol, beta carotene cancer prevention study. Stroke 35: 1908-1913, 2004.
45. Rabadi MH, Kristal BS: Effect of vitamin C supplementation on stroke recovery: A case-control study. Clin Interv Aging 2: 147-151, 2007.
46. Suter PM: Effect of Vitamin E, Vitamin C, and beta-carotene on stroke risk. Nutr Rev 58: 184-187, 2000.