



ORIGINAL

DNA and RNA oxidative damage are associated to mortality in patients with cerebral infarction



L. Lorente^{a,*}, M.M. Martín^b, A.F. González-Rivero^c, A. Pérez-Cejas^d,
P. Abreu-González^e, L. Ramos^f, M. Argueso^g, J.J. Cáceres^h, J. Solé-Violánⁱ,
A. Álvarez-Castillo^j, A. Jiménez^k, V. García-Marín^l

^a Intensive Care Unit, Hospital Universitario de Canarias, Ofra, s/n, La Laguna, 38320 Santa Cruz de Tenerife, Spain

^b Intensive Care Unit, Hospital Universitario Nuestra Señora de Candelaria, Crta del Rosario s/n, Santa Cruz de Tenerife 38010, Spain

^c Laboratory Department, Hospital Universitario de Canarias, Ofra, s/n, La Laguna, 38320 Santa Cruz de Tenerife, Spain

^d Laboratory Department, Hospital Universitario de Canarias, Ofra, s/n, La Laguna, 38320 Tenerife, Spain

^e Department of Physiology, Faculty of Medicine, University of the La Laguna, Ofra, s/n, La Laguna, 38320 Santa Cruz de Tenerife, Spain

^f Intensive Care Unit, Hospital General La Palma, Buenavista de Arriba s/n, Breña Alta, La Palma 38713, Spain

^g Intensive Care Unit, Hospital Clínico Universitario de Valencia, Avda. Blasco Ibáñez nº 17-19, Valencia 46004, Spain

^h Intensive Care Unit, Hospital Insular, Plaza Dr. Pasteur s/n, Las Palmas de Gran Canaria 35016, Spain

ⁱ Intensive Care Unit, Hospital Universitario Dr. Negrín, Barranco de la Ballena s/n, Las Palmas de Gran Canaria 35010, Spain

^j Intensive Care Unit, Hospital Universitario de Canarias, Ofra, s/n, La Laguna, 38320 Santa Cruz de Tenerife, Spain

^k Research Unit, Hospital Universitario de Canarias, Ofra, s/n, La Laguna, 38320 Santa Cruz de Tenerife, Spain

^l Department of Neurosurgery, Hospital Universitario de Canarias, Ofra, s/n, La Laguna, 38320 Santa Cruz de Tenerife, Spain

Received 30 April 2019; accepted 14 July 2019

KEYWORDS

Deoxyribonucleic acid and ribonucleic acid oxidative damage;
Cerebral infarction;
Mortality;
Prognosis

Abstract

Objective: Secondary injury due to oxidation may occur during ischemic stroke, possibly leading to oxidative damage to deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Higher blood concentrations of 8-hydroxy-2'-deoxyguanosine (8-OHdG) (through the oxidation of guanosine from DNA) have been found in ischemic stroke patients than in healthy subjects, and in patients with versus without post-ischemic stroke depression. The present study was carried out to explore the possible association between serum DNA and RNA oxidative damage and mortality in patients with cerebral infarction.

Methods: A prospective, multicenter observational study was carried out in the Intensive Care Units of 6 Spanish hospitals. We included patients with severe malignant middle cerebral artery infarction (MMCAI) defined as ischemic changes evidenced by computed tomography in more than 50% of the middle cerebral artery territory and a Glasgow Coma Score (GCS) < 9. Serum

Abbreviations: APACHE II, Acute Physiology and Chronic Health Evaluation; aPTT, activated partial thromboplastin time; CT, computer tomography; FIO₂, pressure of arterial oxygen/fraction inspired oxygen; GCS, Glasgow Coma Scale; INR, international normalized ratio; IQR, interquartile range; OGS, oxidized guanine species; PaO₂, pressure of arterial oxygen.

* Corresponding author.

E-mail address: lorentemartin@msn.com (L. Lorente).

concentrations of the three oxidized guanine species (OGS) (8-hydroxyguanine from DNA or RNA, 8-hydroxyguanosine from RNA, and 8-OHdG from DNA) on the day of MMCAI diagnosis were determined. The study endpoint was 30-day mortality.

Results: We found higher serum OGS levels ($p < 0.001$) in non-surviving ($n = 34$) than in surviving patients ($n = 34$). Logistic regression analyses showed serum OGS levels to be associated to 30-day mortality controlling for lactic acid, GCS and platelet count (OR = 1.568; 95%CI = 1.131–2.174; $p = 0.01$).

Conclusions: The novel observation in this study is the association between global serum OGS concentration and mortality in ischemic stroke patients.

© 2019 Elsevier España, S.L.U. y SEMICYUC. All rights reserved.

PALABRAS CLAVE

Daño oxidativo del ácido desoxirribonucleico y del ácido ribonucleico; Infarto cerebral; Mortalidad; Pronóstico

El daño oxidativo del ADN y ARN se asocian con la mortalidad en los pacientes con infarto cerebral

Resumen

Objetivo: En el infarto cerebral puede aparecer una lesión cerebral secundaria debido a la oxidación del ácido desoxirribonucleico (ADN) y del ácido ribonucleico (ARN). Se han encontrado concentraciones sanguíneas de 8-hidroxi-2'-desoxiguanosina (8-OHdG) (por la oxidación de la guanosina del ADN) más altas en pacientes con infarto cerebral que en individuos sanos, y en pacientes con depresión tras un infarto cerebral. El objetivo de nuestro estudio fue determinar si existe una asociación entre el daño oxidativo del ADN y del ARN, y la mortalidad de los pacientes con infarto cerebral.

Métodos: Estudio prospectivo, observacional y multicéntrico realizado en unidades de cuidados intensivos de 6 hospitales españoles. Se incluyeron pacientes con un infarto maligno grave de la arteria cerebral media (MMCAI), definido como la presencia de cambios isquémicos en la tomografía en más del 50% del territorio de la arteria cerebral media y menos de 9 puntos en la escala Glasgow Coma Scale (GCS). Se determinaron los niveles séricos de las 3 especies oxidadas de la nucleobase guanina (OGS) (8-hidroxiguanina del ADN o ARN, 8-hidroxiguanosina del ARN y 8-OHdG del ADN) en el día del diagnóstico del MMCAI. La variable principal fue la mortalidad a 30 días.

Resultados: Encontramos concentraciones séricas de OGS ($p < 0,001$) más altas en los pacientes fallecidos ($n = 34$) que en los supervivientes ($n = 34$). La regresión logística mostró que los niveles séricos de OGS se asociaban con la mortalidad a los 30 días controlando por ácido láctico, GCS y recuento plaquetario (*odds ratio* = 1,568; IC 95% = 1,131-2,174; $p = 0,01$).

Conclusiones: El nuevo hallazgo de nuestro estudio fue la asociación entre los niveles séricos de OGS globales y la mortalidad de los pacientes con infarto cerebral.

© 2019 Elsevier España, S.L.U. y SEMICYUC. Todos los derechos reservados.

Introduction

Ischemic stroke causes high consumption resources, disabilities and deaths.¹ Apart from the primary injury due to brain vasculature obstruction that causes a reduction of oxygenated blood and substrates to neurons, a secondary injury due to oxidation could appear during ischemic stroke.^{2–5} The hyperoxidative state of the ischemic stroke could produce oxidative damage on the ribonucleic acid (RNA),^{6,7} deoxyribonucleic acid (DNA),^{8–11} lipids, and proteins.

Four types of nucleobases compound DNA and RNA. The nucleobases guanine, cytosine and adenine are present in RNA and DNA, uracil in RNA, and thymine in DNA. Guanine, with the lowest redox potential, is the most prone nucleobase to oxidation.^{8–11} There is three oxidized guanine species (OGS) that are 8-oxo-guanine (8-oxo-Gua) or

8-hydroxyguanine (8-OHGua) from DNA or RNA oxidation, 8-oxo-guanosine (8-oxo-G) or 8-hydroxyguanosine (8-OHG) from RNA oxidation, and 8-oxo-deoxyguanosine (8-oxo-dG) or 8-hydroxy-2'-deoxyguanosine (8-OHdG) from DNA oxidation.

Higher levels of 8-OHG^{12–15} and 8-OHGua^{16,17} have been found in patients with different diseases than in healthy subjects. However, 8-OHdG is the most studied OGS, and higher 8-OHdG levels in patients with cardiovascular disease, heart failure and periodontal disease than in healthy subjects have been found in some meta-analyses.^{18–20}

There is scarce data about nucleic acid oxidative damage in ischemic stroke patients.^{21–23} In a study higher 8-OHdG plasma concentrations were found in ischemic stroke patients than in healthy subjects.²¹ High concentrations of 8-OHdG in serum²² and urine²³ were associated with

post-ischemic stroke depression. The objective of our study was to determine whether an association between serum DNA and RNA oxidative damage and mortality in patients with cerebral infarction exists.

Methods

Design and subjects

This was an observational and prospective study. This multicentre study was carried out after the approval of the Institutional Review Board of all participating hospitals and with the written informed consent from the next to kin of each patient. This study was performed in the Intensive Care Units of 6 Spanish hospitals: H. Universitario Dr. Negrín (Las Palmas de Gran Canaria), H. Clínico Universitario de Valencia, H. General de La Palma, H. Insular de Las Palmas de Gran Canaria, H. Universitario Nuestra Señora de Candelaria (Santa Cruz de Tenerife), and H. Universitario de Canarias (San Cristóbal de La Laguna).

We included patients with severe malignant middle cerebral artery infarction (MMCAI), defined as ischemic changes in computer tomography in more than 50% of the middle cerebral artery territory and a Glasgow Coma Scale (GCS)²⁴ lower than 9. Patients with inflammatory or malignant disease, pregnancy, age less than 18 years, intracerebral hemorrhage or subarachnoid hemorrhage were excluded of the study. The patients were recruited between 2009 and 2012.

Variables recorded

We recorded the following variables at the moment of MMCAI diagnosis: age, sex, chronic obstructive pulmonary disease (COPD), chronic renal failure, diabetes mellitus, arterial hypertension, heart failure, temperature, GCS, sodium, lactic acid, glycemia, bilirubin, creatinine, pressure of arterial oxygen (PaO₂), fraction inspired oxygen (FIO₂), platelets, leukocytes, leukocytes, leukocytes, leukocytes, hemoglobin, international normalized ratio (INR), fibrinogen, activated partial thromboplastin time (aPTT), Acute Physiology and Chronic Health Evaluation II (APACHE II) score,²⁵ infarction volume, hemorrhagic transformation, midline shift, and decompressive craniectomy. The endpoint study was 30-day mortality.

Determination of serum concentrations of OGS

Serum blood samples on the day of MMCAI diagnosis (within the first 4h after diagnosis) were collected and frozen at –80 °C until serum concentration determinations. Serum concentration of the three OGS were determined with the DNA/RNA Oxidative Damage ELISA Kit[®] (Cayman Chemical Corporation, Ann Arbor, USA), which has an assay detection limit of 10 pg/mL, intra-assay coefficient of variation (CV) of 4.7–11.6%, and inter-assay CV of 4.5–10.7%. To check the adequate dilution of the samples we follow the recommendations of the kit. A first test was performed with 9 samples diluted at 1/25, 1/50 and 1/100 with ELISA Buffer preparation that is included in the kit. After verifying that the best

dilution was 1/50, the rest of the samples were diluted to 1/50. Those determinations were carried out in the Laboratory Department of Hospital Universitario de Canarias (Tenerife, Spain).

Statistical methods

We used medians and interquartile ranges to report continuous variables, and frequencies and percentages to report categorical variables. We used Wilcoxon–Mann–Whitney test for the comparison of continuous variables between patient groups, and chi-square test for the comparison of categorical variables. We carried out a multiple logistic regression analysis to determine the association between serum OGS levels and other variables with 30 day-mortality. We performed a receiver operating characteristic (ROC) curve to explore the prediction capacity of 30-day mortality by serum OGS levels. We constructed Kaplan–Meier 30-day mortality curves with patients showing higher and lower serum OGS levels than 4.82 ng/mL (cut-off value selected by Youden J index). Jouden Index was used to select the cut-off of serum OGS levels since it shows the maximum prognostic capability with the best ratio between sensibility and specificity.²⁶ We considered as statistically significant all *p*-values < 0.05. We used NCSS 2000 (Kaysville, Utah), LogXact 4.1 (Cytel Co., Cambridge, MA), and SPSS 17.0 (SPSS Inc., Chicago, IL, USA) to perform the statistical analyses.

Results

Table 1 shows that non-surviving (*n*=34) compared to surviving (*n*=34) patients showed higher serum levels of OGS (*p*<0.001) (Fig. 1) and lactic acid (*p*=0.049), and lower GCS (*p*=0.01) and platelet count (*p*=0.02). We did not find statistically significant differences between non-surviving and surviving patients in age, sex, chronic renal failure, COPD, diabetes mellitus, arterial hypertension, body temperature, sodium, creatinine, glycemia, bilirubin, PaO₂, PaO₂/FIO₂ ratio, leukocytes, aPTT, INR, fibrinogen, hemoglobin, APACHE-II score, infarction volume, midline shift, hemorrhagic transformation, and decompressive craniectomy.

Logistic regression analysis showed that serum OGS levels were associated with 30-day mortality after control for lactic acid, GCS, and platelet count (Odds Ratio = 1.568; 95% CI = 1.131–2.174; *p*=0.01) (Table 2).

We found an area under the curve to predict 30-day mortality for serum OGS levels of 78% (95% CI = 66–87%; *p*<0.001) (Fig. 2). We found in Kaplan–Meier analysis that patients with serum OGS levels >4.82 ng/mL showed a higher 30-day mortality (Hazard ratio = 3.3; 95% CI = 1.66–6.72; *p*<0.001) (Fig. 3).

Discussion

Previously, higher 8-OHdG plasma concentrations in ischemic stroke patients than in healthy subjects,²¹ and higher concentrations of 8-OHdG in serum²² and urine²³ in patients with post-ischemic stroke depression were found. We determined in our study serum levels of global OGS, which included

Table 1 Clinical and biochemical characteristics of surviving and non-surviving patients.

	Surviving (n = 34)	Non-surviving (n = 34)	p-Value
Age (years) – median (IQR)	59 (47–68)	63 (53–70)	0.36
Gender female – n (%)	14 (41.2)	13 (38.2)	0.99
Diabetes mellitus – n (%)	4 (11.8)	9 (26.5)	0.22
Arterial hypertension – n (%)	19 (55.9)	16 (47.1)	0.63
COPD – n (%)	1 (2.9)	1 (2.9)	0.99
Chronic renal failure – n (%)	2 (5.9)	2 (5.9)	0.99
Heart failure – n (%)	1 (2.9)	1 (2.9)	0.99
APACHE-II score – median (IQR)	20 (16–25)	22 (19–27)	0.06
GCS score – median (IQR)	7 (6–8)	6 (3–7)	0.01
Temperature (°C) – median (IQR)	36.4 (36.0–37.0)	36.9 (36.0–37.3)	0.15
Sodium (mEq/L) – median (IQR)	139 (136–145)	140 (139–145)	0.38
Glycemia (g/dL) – median (IQR)	127 (100–170)	136 (118–162)	0.40
Creatinine (mg/dl) – median (IQR)	0.80 (0.60–1.13)	1.00 (0.70–1.25)	0.19
Bilirubin (mg/dl) – median (IQR)	0.60 (0.40–0.83)	0.60 (0.33–1.10)	0.95
Lactic acid (mmol/L) – median (IQR)	1.20 (0.90–1.70)	1.55 (1.00–2.70)	0.049
PaO ₂ (mmHg) – median (IQR)	156 (105–293)	115 (94–267)	0.26
PaO ₂ /FIO ₂ ratio – median (IQR)	300 (198–369)	254 (192–325)	0.24
Leukocytes – median × 10 ³ /mm ³ (IQR)	12.4 (9.6–16.9)	13.9 (9.7–20.1)	0.32
Hemoglobin (g/dL) – median (IQR)	12.1 (11.4–14.0)	12.5 (11.0–14.8)	0.81
Platelets – median × 10 ³ /mm ³ (IQR)	202 (171–265)	175 (136–216)	0.02
INR – median (IQR)	1.06 (1.00–1.20)	1.20 (1.01–1.31)	0.07
aPTT (seconds) – median (IQR)	28 (25–30)	27 (26–32)	0.91
Fibrinogen (mg/dl) – median (IQR)	443 (416–489)	419 (337–631)	0.90
Infarction volume (ml) – median (IQR)	173 (100–231)	180 (60–277)	0.64
Midline shift (mm) – median (IQR)	6.0 (2.5–11.5)	9.0 (3.5–15.0)	0.43
Thrombolysis – n (%)	11 (32.4)	10 (29.4)	0.99
Hemorrhagic transformation – n (%)	7 (20.6)	6 (17.6)	0.99
Decompressive craniectomy – n (%)	9 (26.5)	7 (20.6)	0.78
OGS (ng/mL) – median (IQR)	3.86 (2.53–5.30)	6.00 (4.86–9.46)	<0.001

IQR = interquartile range; COPD = Chronic Obstructive Pulmonary Disease; APACHE II = Acute Physiology and Chronic Health Evaluation; GCS = Glasgow Coma Scale; PaO₂ = pressure of arterial oxygen/fraction inspired oxygen; FIO₂ = pressure of arterial oxygen/fraction inspired oxygen; INR = international normalized ratio; aPTT = activated partial thromboplastin time; OGS = oxidized guanine species.

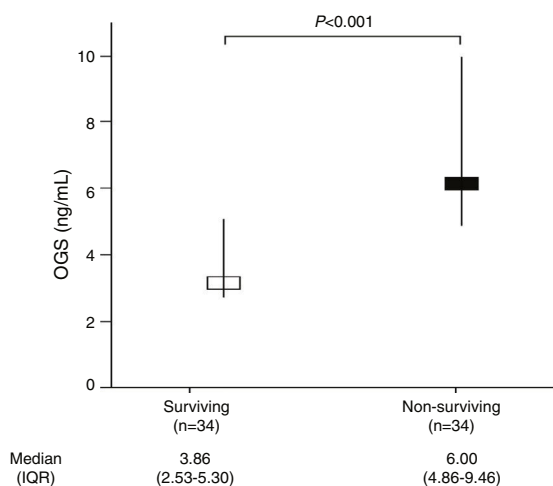


Figure 1 Serum oxidized guanine species (OGS) levels in surviving and non-surviving patients at 30 days.

8-OHdG and also of the other two OGS (8-oxo-guanosine or 8-hydroxyguanosine from RNA, and 8-oxo-guanine or 8-hydroxyguanine from DNA or RNA). In our study, we found for

the first time, higher serum OGS levels in non-surviving than in surviving MMCAI patients, and the association between serum OGS concentrations and mortality of MMCAI patients. Those findings of our study are in accordance with the findings of other studies that found higher concentrations in patients with worst evolution of ischemic stroke defined as the presence of post-ischemic stroke depression^{22,23}; although the end-point of our study was more severe (30-day mortality).

In addition, we found differences between non-surviving and surviving patients in other variables that previously were associated with mortality in those patients, such as lower GCS, lower platelet count, and higher lactic acid^{29–31}; however, we only found that GCS was associated with 30-day mortality in the logistic regression analysis.

The mortality rate in our series (50%) was similar to the rate reported in other studies (38–61%),^{27,28} the mortality rate in our patients with decompressive craniectomy (44%) and without it (52%) was also similar to the rate reported in other studies (36–43% with decompressive craniectomy and 40–76% without it),^{27,28} and the rate of decompressive craniectomy in our series (24%) was similar to the rate reported in other studies (18–21%).^{27,28}

Table 2 Multiple binomial logistic regression analysis to predict 30-day mortality.

Variable	Odds ratio	95% confidence interval	P
Serum OGS (ng/mL)	1.568	1.131–2.174	0.01
Lactic acid (mmol/L)	1.269	0.702–2.292	0.43
GCS (points)	0.705	0.507–0.980	0.04
Platelet count (each 1000/mm ³)	0.996	0.987–1.005	0.34

OGS = oxidized guanine species; GCS = Glasgow Coma Scale.

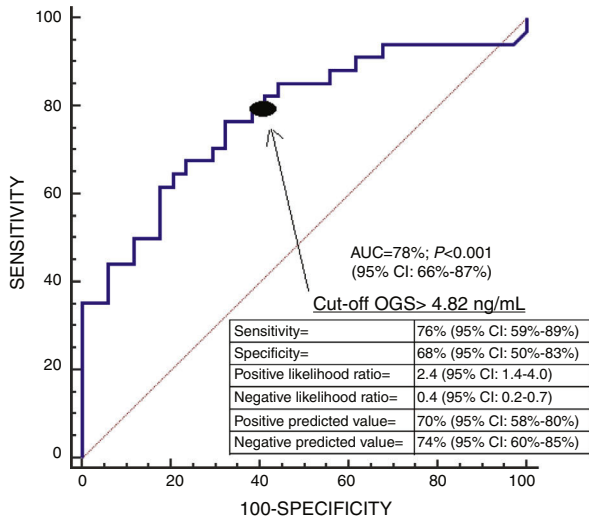
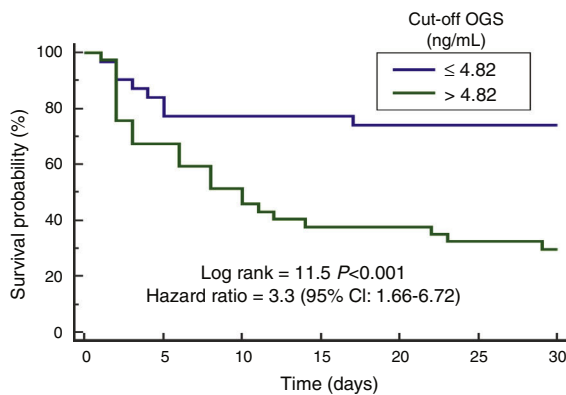


Figure 2 Receiver operating characteristic analysis using serum oxidized guanine species (OGS) levels as a predictor of mortality at 30 days.



Number of patients at risk

Group:	≤ 4.82	> 4.82				
Group: ≤ 4.82	31	24	24	24	23	23
Group: > 4.82	37	25	17	14	14	11

Figure 3 Survival curves at 30 days using serum oxidized guanine species (OGS) levels lower or equal vs higher than 4.82 ng/mL.

In our study, the mean age was 58 ± 14 years and 40% of patients were women. The incidence of ischemic stroke is higher in men, but in middle-age the rate begins to increase in women concomitant with the onset of menopause (and female sex hormone loss), and the rate is even higher in elderly females (>85 years) than in elderly males.^{32–35}

We must recognize some limitations of our study. First, we only determined serum OGS levels at the time of MMCAI diagnosis. Second, we have not determined serum OGS levels in healthy subjects. Third, higher circulating 8-OHdG levels have been found in patients with cardiovascular diseases and we have not excluded those patients in our study; however, we have not found significant differences between surviving and non-surviving patient groups. Fourth, we did not report data about number and causes of exclusion. However, we think that the strength of our study is that we reported serum levels of the three OGS and not only of 8-OHdG levels.

We believe that the new findings of our study with cerebral infarction patients and the findings in animal models of cerebral infarction about the benefits of administration of antioxidant agents reducing DNA oxidative damage in brain samples, infarction volume and neurological deficit^{36–48} could motivate the interest to research about the use of serum DNA and RNA oxidative damage levels as biomarkers of mortality prediction and to research about the use of antioxidant agents in cerebral infarction.

Conclusions

The association between serum concentrations of global OGS and mortality of ischemic stroke patients was the novel finding of our study.

Author contributions

LLo conceived, designed and coordinated the study, participated in acquisition and interpretation of data, and drafted the manuscript.

MMM, PAG, LR, MA, JJC, JSV, AAC and VGM participated in acquisition of data.

AGC and APC carried out the analyses of DNA and RNA oxidative damage.

AJ participated in the interpretation of data.

All authors revised the manuscript critically for important intellectual content and made the final approval of the version to be published.

Fundings

This study was supported by a grant (OA18/011) from Fundación DISA a la Investigación Médica 2017 (Santa Cruz de Tenerife, Spain). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflicts of interests

The authors declare that they have no competing interests.

References

1. Powers WJ, Rabinstein AA, Ackerson T, Adeoye OM, Bambakidis NC, Becker K, et al. American Heart Association Stroke Council. 2018 Guidelines for the early management of patients with acute ischemic stroke: a guideline for Healthcare Professionals From the American Heart Association/American Stroke Association. *Stroke*. 2018;49:e46–110.
2. Sun MS, Jin H, Sun X, Huang S, Zhang FL, Guo ZN, et al. Free radical damage in ischemia-reperfusion injury: an obstacle in acute ischemic stroke after revascularization therapy. *Oxid Med Cell Longev*. 2018;2018:3804979.
3. Li P, Stetler RA, Leak RK, Shi Y, Li Y, Yu W, et al. Oxidative stress and DNA damage after cerebral ischemia: potential therapeutic targets to repair the genome and improve stroke recovery. *Neuropharmacology*. 2018;134:208–17.
4. Davis SM, Pennypacker KR. Targeting antioxidant enzyme expression as a therapeutic strategy for ischemic stroke. *Neurochem Int*. 2017;107:23–32.
5. Smith JA, Park S, Krause JS, Banik NL. Oxidative stress, DNA damage, and the telomeric complex as therapeutic targets in acute neurodegeneration. *Neurochem Int*. 2013;62:764–75.
6. Li Z, Wu J, Deleo CJ. RNA damage and surveillance under oxidative stress. *IUBMB Life*. 2006;58:581–8.
7. Nunomura A, Moreira PI, Takeda A, Smith MA, Perry G. Oxidative RNA damage and neurodegeneration. *Curr Med Chem*. 2007;14:2968–75.
8. Ba X, Boldogh I. 8-Oxoguanine DNA glycosylase 1: Beyond repair of the oxidatively modified base lesions. *Redox Biol*. 2018;14:669–78.
9. Markkanen E. Not breathing is not an option: how to deal with oxidative DNA damage. *DNA Repair (Amst)*. 2017;59:82–105.
10. Kino K, Hirao-Suzuki M, Morikawa M, Sakaga A, Miyazawa H. Generation, repair and replication of guanine oxidation products. *Genes Environ*. 2017;39:21.
11. AbdulSalam SF, Thowfeik FS, Merino EJ. Excessive reactive oxygen species and exotic DNA lesions as an exploitable liability. *Biochemistry*. 2016;55:5341–52.
12. Che Y, Wang JF, Shao L, Young T. Oxidative damage to RNA but not DNA in the hippocampus of patients with major mental illness. *J Psychiatry Neurosci*. 2010;35:296–302.
13. Kumagai S, Jikimoto T, Saegusa J. Pathological roles of oxidative stress in autoimmune diseases. *Rinsho Byori*. 2003;51:126–32.
14. Kikuchi A, Takeda A, Onodera H, Kimpara T, Hisanaga K, Sato N, et al. Systemic increase of oxidative nucleic acid damage in Parkinson's disease and multiple system atrophy. *Neurobiol Dis*. 2002;9:244–8.
15. Aschbacher K, O'Donovan A, Wolkowitz OM, Dhabhar FS, Su Y, Epel E. Good stress, bad stress and oxidative stress: insights from anticipatory cortisol reactivity. *Psychoneuroendocrinology*. 2013;38:1698–708.
16. Malins DC, Haimanot R. Major alterations in the nucleotide structure of DNA in cancer of the female breast. *Cancer Res*. 1991;51:5430–2.
17. Shin CS, Moon BS, Park KS, Kim SY, Park SJ, Chung MH, et al. Serum 8-hydroxy-guanine levels are increased in diabetic patients. *Diabetes Care*. 2001;24:733–7.
18. Di Minno A, Turnu L, Porro B, Squellerio I, Cavalca V, Tremoli E, et al. 8-Hydroxy-2-deoxyguanosine levels and cardiovascular disease: a systematic review and meta-analysis of the literature. *Antioxid Redox Signal*. 2016;24:548–55.
19. Di Minno A, Turnu L, Porro B, Squellerio I, Cavalca V, Tremoli E, et al. 8-Hydroxy-2-deoxyguanosine levels and heart failure: a systematic review and meta-analysis of the literature. *Nutr Metab Cardiovasc Dis*. 2017;27:201–8.
20. Paredes-Sánchez E, Montiel-Company JM, Iranzo-Cortés JE, Almerich-Torres T, Bellot-Arcís C, Almerich-Silla JM. Meta-analysis of the use of 8-OHdG in saliva as a marker of periodontal disease. *Dis Markers*. 2018;2018:7916578.
21. Lien LM, Chiou HY, Yeh HL, Chiu SY, Jeng JS, Lin HJ, et al. Significant association between low mitochondrial DNA content in peripheral blood leukocytes and ischemic stroke. *J Am Heart Assoc*. 2017;6, pii:e006157.
22. Liu Z, Cai Y, He J. High serum levels of 8-OHdG are an independent predictor of post-stroke depression in Chinese stroke survivors. *Neuropsychiatr Dis Treat*. 2018;14:587–96.
23. Chen CY, Chen CL, Yang YH, Ho CH, Tseng WC. Poststroke depressive symptoms are associated with increased oxidative deoxyribonucleic acid damage. *J Neuropsychiatry Clin Neurosci*. 2018;30:139–44.
24. Teasdale G, Jennett B. Assessment of coma and impaired consciousness. A practical scale. *Lancet*. 1974;2:81–4.
25. Knaus WA, Draper EA, Wagner DP, Zimmerman JE. APACHE II: a severity of disease classification system. *Crit Care Med*. 1985;13:818–29.
26. Youden WJ. Index for rating diagnostic tests. *Cancer*. 1950;3:32–5.
27. Frank JI, Schumm LP, Wroblewski K, Chyatte D, Rosengart AJ, Kordeck C, et al. Hemicraniectomy and durotomy upon deterioration from infarction-related swelling trial: randomized pilot clinical trial. *Stroke*. 2014;45:781–7.
28. Jüttler E, Unterberg A, Woitzik J, Bösel J, Amiri H, Sakowitz OW, et al. Hemicraniectomy in older patients with extensive middle-cerebral-artery stroke. *N Engl J Med*. 2014;370:1091–100.
29. Katzan IL, Spertus J, Bettger JP, Bravata DM, Reeves MJ, Smith EE, et al. Risk adjustment of ischemic stroke outcomes for comparing hospital performance: a statement for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke*. 2014;45:918–44.
30. Jo S, Jeong T, Lee JB, Jin YH, Yoon J, Jun YK, et al. Initial hyperlactatemia in the ED is associated with poor outcome in patients with ischemic stroke. *Am J Emerg Med*. 2012;30:449–55.
31. Yang M, Pan Y, Li Z, Yan H, Zhao X, Liu L, et al. Platelet count predicts adverse clinical outcomes after ischemic stroke or TIA: subgroup analysis of CNSR II. *Front Neurol*. 2019;10:370.
32. Roy-O'Reilly M, McCullough LD. Age and sex are critical factors in ischemic stroke pathology. *Endocrinology*. 2018;159:3120–31.
33. Navis A, Garcia-Santibanez R, Skliut M. Epidemiology and outcomes of ischemic stroke and transient ischemic attack in the adult and geriatric population. *J Stroke Cerebrovasc Dis*. 2019;28:84–9.
34. Appelros P, Nydevik I, Viitanen M. Poor outcome after first-ever stroke: predictors for death, dependency, and recurrent stroke within the first year. *Stroke*. 2003;34:122–6.
35. Gibson CL. Cerebral ischemic stroke: is gender important? *J Cereb Blood Flow Metab*. 2013;33:1355–61.
36. Yunoki T, Deguchi K, Omote Y, Liu N, Liu W, Hishikawa N, et al. Anti-oxidative nutrient-rich diet protects against acute ischemic brain damage in rats. *Brain Res*. 2014;1587:33–9.
37. Kusaki M, Ohta Y, Inufusa H, Yamashita T, Morihara R, Nakano Y, et al. Neuroprotective effects of a novel antioxidant mixture Twendee X in mouse stroke model. *J Stroke Cerebrovasc Dis*. 2017;26:1191–6.
38. Shichinohe H, Tan C, Abumiya T, Nakayama N, Kazumata K, Hokari M, et al. Neuroprotective effects of cilostazol are mediated by multiple mechanisms in a mouse model of permanent focal ischemia. *Brain Res*. 2015;1602:53–61.
39. Ueda M, Inaba T, Nito C, Kamiya N, Katayama Y. Therapeutic impact of eicosapentaenoic acid on ischemic brain damage

- following transient focal cerebral ischemia in rats. *Brain Res.* 2013;1519:95–104.
40. Nito C, Ueda M, Inaba T, Katsura K, Katayama Y. FK506 ameliorates oxidative damage and protects rat brain following transient focal cerebral ischemia. *Neurol Res.* 2011;33: 881–9.
 41. Colín-González AL, Ortiz-Plata A, Villeda-Hernández J, Barrera D, Molina-Jijón E, Pedraza-Chaverrí J, et al. Aged garlic extract attenuates cerebral damage and cyclooxygenase-2 induction after ischemia and reperfusion in rats. *Plant Foods Hum Nutr.* 2011;66:348–54.
 42. Cheng CY, Su SY, Tang NY, Ho TY, Chiang SY, Hsieh CL. Ferulic acid provides neuroprotection against oxidative stress-related apoptosis after cerebral ischemia/reperfusion injury by inhibiting ICAM-1 mRNA expression in rats. *Brain Res.* 2008;1209: 136–50.
 43. He M, Xing S, Yang B, Zhao L, Hua H, Liang Z, et al. Ebselen attenuates oxidative DNA damage and enhances its repair activity in the thalamus after focal cortical infarction in hypertensive rats. *Brain Res.* 2007;1181:83–92.
 44. Lee EJ, Chen HY, Lee MY, Chen TY, Hsu YS, Hu YL, et al. Cinnamophilin reduces oxidative damage and protects against transient focal cerebral ischemia in mice. *Free Radic Biol Med.* 2005;39:495–510.
 45. Hayashi T, Hamakawa K, Nagotani S, Jin G, Li F, Deguchi K, et al. HMG CoA reductase inhibitors reduce ischemic brain injury of Wistar rats through decreasing oxidative stress on neurons. *Brain Res.* 2005;1037:52–8.
 46. Lee EJ, Lee MY, Chen HY, Hsu YS, Wu TS, Chen ST, et al. Melatonin attenuates gray and white matter damage in a mouse model of transient focal cerebral ischemia. *J Pineal Res.* 2005;38:42–52.
 47. Zhang WR, Hayashi T, Sasaki C, Sato K, Nagano I, Manabe Y, et al. Attenuation of oxidative DNA damage with a novel antioxidant EPC-K1 in rat brain neuronal cells after transient middle cerebral artery occlusion. *Neurol Res.* 2001;23:676–80.
 48. Mackensen GB, Patel M, Sheng H, Calvi CL, Batinic-Haberle I, Day BJ, et al. Neuroprotection from delayed postischemic administration of a metalloporphyrin catalytic antioxidant. *J Neurosci.* 2001;21:4582–92.