ONLINE SUPPLEMENT

Synergistic interaction between zinc and reactive oxygen species amplifies ischemic brain injury in rats

Yongmei Zhao, MD, PhD; Feng Yan, MS; Jie Yin, MD; Rong Pan, PhD; Wenjuan Shi, MS; Zhifeng Qi, MD, PhD; Yalan Fang, MD; Yuyou Huang, MD; Sen Li, MD; Yumin Luo, MD, PhD; Xunming Ji, MD, PhD*; Ke Jian Liu, PhD*

Supplementary Table Ⅰ Physiological parameters were not changed by treatment with TPEN, EUK-134, R(+) PPX or DPI

Groups	MABP (mmHg)	Rectal temperature (°C)	Heart rate (times/min)
Sham	97.55±9.49	37.11±0.21	333.45±9.32
MCAO	96.91±9.48	36.97±0.42	330.63±10.29
MCAO+TPEN	97.34±9.29	37.08±0.31	332.01±9.98
MCAO+EUK-134	96.56±9.56	36.99±0.39	328.11±11.02
MCAO+R(+) PPX	97.25±9.54	37.11±0.32	333.68±9.47
MCAO+DPI	97.67±9.34	37.05±0.34	332.67±10.98

N,N,N′,N′-tetrakis(2-pyridylmethyl) ethylenediamine (TPEN) at 15 mg/kg (i.p.) was administered to the rats 30 minutes before middle cerebral artery occlusion (MCAO). EUK-134 at 10 mg/kg (s.c.) for a total of three doses were administered to the rats at 30 minutes before MCAO, 3 and 8 hours after reperfusion, respectively. R(+) pramipexole [R(+) PPX] at 1 mg/kg (i.p.) for a total of five doses were administered to the rats at 8 hours and 30 minutes before MCAO, immediately after reperfusion, 8 and 16 hours after reperfusion, respectively. Diphenyliodonium (DPI) at 0.2 mg/kg (i.p.) was administered to the rats 30 minutes before MCAO. The physiological parameters were recorded during MCAO. Data are shown as mean ± SD, n = 10 per group. There were no statistically significant differences in any of the parameters between the groups.

Stroke Online Supplement

Table I. Checklist of Methodological and Reporting Aspects for Articles Submitted to *Stroke* Involving Preclinical Experimentation

Methodological and Reporting Aspects	Description of Procedures
Experimental groups and study timeline	☑ The experimental group(s) have been clearly defined in the article, including number of animals in each experimental arm of the study. ☑ An account of the control group is provided, and number of animals in the control group has been reported. If no controls were used, the rationale has been stated. ☑ An overall study timeline is provided.
Inclusion and exclusion criteria	☑ A priori inclusion and exclusion criteria for tested animals were defined and have been reported in the article.
Randomization	☑ Animals were randomly assigned to the experimental groups. If the work being submitted does not contain multiple experimental groups, or if random assignment was not used, adequate explanations have been provided. ☑ Type and methods of randomization have been described. ☑ Methods used for allocation concealment have been reported.
Blinding	☑ Blinding procedures have been described with regard to masking of group/treatment assignment from the experimenter. The rationale for nonblinding of the experimenter has been provided, if such was not feasible. ☑ Blinding procedures have been described with regard to masking of group assignment during outcome assessment.
Sample size and power calculations	☑ Formal sample size and power calculations were conducted based on a priori determined outcome(s) and treatment effect, and the data have been reported. A formal size assessment was not conducted and a rationale has been provided.
Data reporting and statistical methods	☑ Number of animals in each group: randomized, tested, lost to follow-up, or died have been reported. If the experimentation involves repeated measurements, the number of animals assessed at each time point is provided, for all experimental groups. ☑ Baseline data on assessed outcome(s) for all experimental groups have been reported. ☑ Details on important adverse events and death of animals during the course of experimentation have been provided, for all experimental arms. ☑ Statistical methods used have been reported. ☑ Numeric data on outcomes have been provided in text, or in a tabular format with the main article or as supplementary tables, in addition to the figures.
Experimental details, ethics, and funding statements	☑ Details on experimentation including stroke model, formulation and dosage of therapeutic agent, site and route of administration, use of anesthesia and analgesia, temperature control during experimentation, and postprocedural monitoring have been described. ☑ Different sex animals have been used. If not, the reason/justification is provided. ☑ Statements on approval by ethics boards and ethical conduct of studies have been provided. ☑ Statements on funding and conflicts of interests have been provided.