Tropical Journal of Pharmaceutical Research January 2021; 20 (1): 11-16
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v20i1.2

Original Research Article

Ginkgolide K potentiates the protective effect of ketamine against intestinal ischemia/reperfusion injury by modulating NF-κB/ERK/JNK signaling pathway

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Sent for review: 12 April 2020 *Revised accepted: 18 December 2020*

Abstract

Purpose: *To investigate the effect of ginkgolide K and ketamine treatments, alone and in combination, on intestinal ischemia/reperfusion injury (I/R)-induced injury in rats, as well as the mechanism involved.*
Methods: *Rats were treated with ginkgolide K (GK, 15 mg/kg i.v) and ketamine (KTM, 100 mg/kg i.p.), either alone or in combination 30 min before the induction of intestinal I/R. The effects of GK and KTM were determined by assessing the levels of cytokines in serum, and parameters of oxidative stress and ROS production in the intestinal tissues of I/R rats. Moreover, intestinal mRNA expressions of JNK, ERK, p38 and NF-kB were determined by quantitative reverse transcription polymerase chain reaction (qRT-PCR).*
Results: *GK and KTM treatments, alone and in combination, reduced cytokine levels in serum and oxidative stress parameters in intestinal tissues, when compared to I/R group of rats. Treatments with GK and KTM, alone and in combination, mitigated the altered mRNA expressions of JNK, ERK, p38 and NF-kB in intestinal tissues of I/R-injured rats.*
Conclusion: *These results reveal that GK potentiates the protective effect of KTM100 on I/R-induced intestinal injury in rats by regulating the NF-kB/ERK/JNK signaling pathway. Therefore, GK and KTM may find use in the management of I/R*

Keywords: *Ginkgolide K, Ketamine, Intestinal injury, Ischemia/Reperfusion, Inflammation*

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INTRODUCTION

Mesenteric obstruction leads to intestinal ischemia, and it is associated with complications of surgical emergencies such as hypovolemic shock, sepsis and trauma [1]. The mortality of patients suffering from intestinal ischemia has been found to be in the range of 60 to 100 % [2]. The intestine is very sensitive to alterations in blood supply. Intestinal tissue reperfusion enhances the production of cytokines and free radicals which alter gastrointestinal motility and damage the mucosal layer, thereby altering the integrity of

intestinal tissues [3]. Intestinal I/R also enhances the protein expression levels of adhesion molecules such as ICAM-1 and selectin [4]. Moreover, it has been reported that intestinal contractility is altered by intestinal I/R due to damage to the neurons of myenteric plexus [5]. Intestinal ischemia results in necrosis of intestinal tissues which is managed through surgical excision [6]. However, very few treatment therapies are available for managing intestinal ischemia.

The anesthetic agent ketamine shows promising effect in the treatment of intestinal ischemia [7]. Ketamine, a surface anesthetic agent with anti-inflammatory and analgesic properties, pharmacologically antagonizes NMDA receptor [8]. Studies have shown that ketamine exerts dose-dependent protective effect against intestinal I/R, although the molecular mechanisms involved are not yet understood [9]. Ginkgolide K is a diterpene lactone isolated from Ginkgo biloba [10]. It has been reported that GK mitigates I/R-induced cardiovascular and cerebrovascular disorder [11]. It protects against neuronal injury by reducing oxidative stress and inflammatory markers in the NF-kB pathway [12]. The present study was carried out to determine the synergistic effect of combination of GK and ketamine on the management of intestinal ischemia/reperfusion injury.

EXPERIMENTAL

Animals

Male Sprague–Dawley rats weighing 250–300 g were kept under a 12-h light/12-h dark cycle at 60 ± 5% humidity average room temperature of 24 ± 3 °C. The study was carried out in line with the guidelines of the Association for the Assessment and Accreditation of Laboratory Animal Care International (AAALAC) [13]. The protocols used in the animal study were approved by institutional animal ethical committee of The Second Affiliated Hospital of Xi'an Jiaotong University, China (approval no. IAEC/SAH-XJU/2018/12).

Study design and treatments

Anesthesia was induced in the rats via i.p. injection of pentobarbital sodium at a dose of 35 mg/kg. Laparotomy was performed under controlled body temperature. The small intestine was taken out after opening the abdomen. Ischemia was induced through obstruction of the portal vein and superior mesenteric artery for 30 min using microvascular clamps. Then, the clamp was removed to produce reperfusion for 1 h. Ischemia was confirmed by the presence of dark red color and loss of pulse in the intestines. The rats were divided into five different groups: control, I/R and KTM100 groups. The KTM100 group received ketamine at a dose of 100 mg/kg, i.p. 30 min before the induction of I/R. The GK group received GK at a dose of 15 mg/kg i.v. 20 min before induction I/R. Rats in the KTM100 + GK group received the GK and KTM treatments together 30 min before the induction of I/R.

Serum was separated by centrifugation of blood withdrawn from each rat at 3000 rpm. The serum samples were used to determine biochemical parameters. Moreover, the rats were sacrificed via cervical dislocation, and intestinal tissues were isolated and fixed in 10 % formaldehyde solution. The intestinal tissues were homogenized in phosphate buffer, pH 7.2, and the homogenate was centrifuged for 30 min at 3000 rpm. The supernatant was removed and stored at −20 °C.

Determination of cytokines

The serum levels of the inflammatory mediators i.e. interleukin (IL)-1β, IL-6, nuclear factor kappa B (NF-κB) and tumor necrosis factor (TNF)-α were determined using ELISA commercial kits (R&D Systems) in accordance with the manufacturer's protocols.

Evaluation of ROS

MitoSOX red mitochondrial superoxide indicator was used to estimate the intestinal tissue levels of ROS. The tissue homogenates were stained at 37 °C in the dark for 30 min with 5 μM MitoSOX red. A fluorescent plate reader was used to estimate the intracellular ROS levels at excitation and emission wavelengths of 510 and 580 nm, respectively.

Assessment of oxidative stress

Malondialdehyde (MDA), nitric oxide (NO), and reduced glutathione (GSH) levels and superoxide dismutase (SOD) activities were estimated in intestinal tissues using ELISA kits according to the manufacturer's instructions.

qRT-PCR

Trizol Reagent was used to isolate total RNA from intestinal tissues as per the directions given by the manufacturer. Then, reverse transcription kit was used to synthesize cDNA from the RNA as per the instruction given by the

kit manufacturer. Thereafter, PCR was carried using SYBR green/fluorescein qPCR Master Mix kit with ABI Prism 7500 system. The reaction conditions were as follows: 50 °C for 2 min; 95 °C for 10 min; and 40 cycles of 95 °C for 30 sec and 60 °C for 30 sec. The resultant data were analyzed using the comparative $2^{-\Delta\Delta Ct}$ method. The sequences of primers used are shown below:

Primer	Forward	Reverse
ERK:	5' TAATACGACTCACTATAGGG 3'	5' TAGAAGGCACAGTCGAGG 3'
JNK:	5'-AACTCTTTGACGCTGCTTGC-3'	5'-TGAAGCACTGTGCCTTTACC-3'
P38:	5' GCCAGGGTTTTCCCAGTCACGAC 3'	5'GAGCGGATAACAATTTCACACAGG 3'
NF-κB:	5' ATGGCAGACGATGATCCCTAC 3'	5' CGGATCGAAATCCCCTCTGTT 3'
β-Actin:	5' GGCTGTATTCCCCTCCATCG 3'	5' CCAGTTGGTAACAATGCCATGT 3'

Statistical analysis

All data are expressed as mean ± standard error mean (SEM; n = 10). Statistical analysis was done using one-way analysis of variance (ANOVA). Post-hoc comparisons of means were carried out with Dunnett's post hoc test using GraphPad Prism software (ver. 6.1; San Diego, CA, USA). Values of $p < 0.05$ were considered statistically significant.

RESULTS

Effect of ginkgolide K and ketamine on cytokines

The levels of cytokines in ginkgolide K and ketamine-treated I/R intestinal injury rats are presented in Figure 1. There were higher levels of cytokines in the serum of I/R group than in control group of rats. However, the level of cytokines were reduced in the serum of KTM100, GK and KTM100 + GK rat groups, relative to I/R group of rats. Moreover, treatment with KTM100 + GK significantly reduced the levels of cytokines, when compared to KTM100-alone group of rats.

Effect of ginkgolide K and ketamine on ROS levels

The levels of ROS in intestinal tissues of ginkgolide K and ketamine- treated rats with I/R-induced intestinal injury were estimated. There was enhanced production of mitochondrial ROS in the I/R group, when compared to control group of rats. However, KTM100 and GK reduced ROS production, relative to the I/R group of rats. Thus, GK potentiated the effect of KTM100 on ROS production in the intestinal tissue of rats with I/R-induced injury. These results are shown in Figure 2.

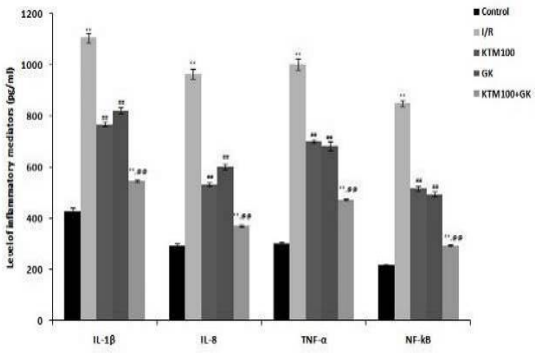

Figure 1: Effect of ginkgolide K and ketamine treatments on levels of cytokines in the serum of I/R-induced intestinal injury in rats: Data are mean ± SEM (n = 10); $^{**}p < 0.01$, compared to control group; $^{\#\#}p < 0.01$, compared to I/R group; $^{@@}p < 0.01$, compared to KTM100 and GK group

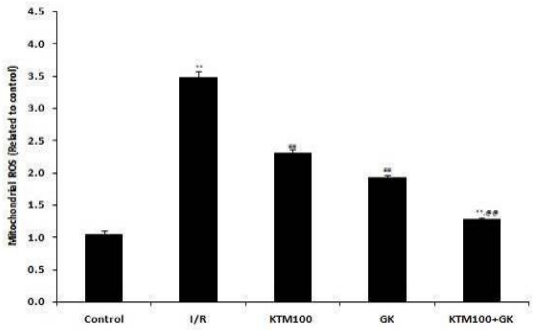

Figure 2: Effect of ginkgolide K and ketamine treatments on the production of ROS in the intestinal tissue of rats with I/R-induced intestinal injury. Data are mean ± SEM (n = 10); $^{**}p < 0.01$, compared to control group; $^{\#\#}p < 0.01$, compared to I/R group; $^{@@}p < 0.01$, compared to KTM100 + GK group

Effect of ginkgolide K and ketamine on oxidative stress

Oxidative stress parameters such as NO, MDA and GSH, as well as SOD activity were determined in ginkgolide K and ketamine-treated rats with I/R-induced intestinal injury. The results are shown in Figure 3. There were increases in the levels of NO and MDA, while the level of GSH was decreased in the intestinal tissue of I/R group, when compared with the control group. Moreover, SOD activity was reduced in I/R group, relative to control group. However, treatments with ginkgolide K and ketamine alone, and their combination ameliorated the altered levels of MDA, NO and GSH, as well as SOD activity in the intestinal tissues of rats with I/R-induced intestinal injury. These data indicate that GK treatment potentiated the effect of KTM100 on oxidative

stress parameters in rats with I/R-induced intestinal injury.

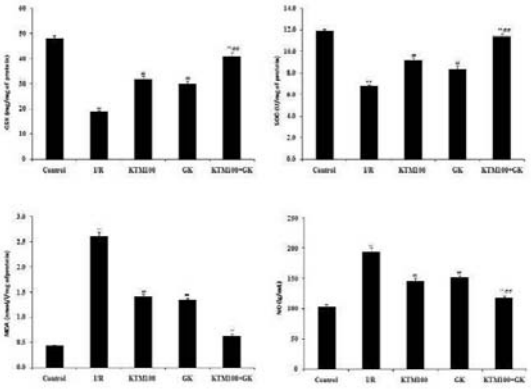

Figure 3: Effect of ginkgolide K and ketamine treatments on intestinal oxidative stress parameters of rats with I/R- induced intestinal injury: Data are mean ± SEM (n = 10); $^{**}p < 0.01$, compared to control group; $^{\#\#}p < 0.01$, compared to I/R group; $^{@@}p < 0.01$, compared to KTM100 + GK group

Effect of ginkgolide K and ketamine on mRNA expressions of JNK, ERK, p38 and NF-kB

Figure 4 shows the intestinal mRNA expression levels of JNK, ERK, p38 and NF-kB in I/R rats treated with ginkgolide K and ketamine. There were increases in the mRNA expression levels of JNK, p38 and NF-kB, while the mRNA expression level of ERK was decreased in the intestinal tissue of I/R group, when compared to control group. However, treatments with GK and KTM100, alone and in combination, ameliorated the altered intestinal mRNA expressions of JNK, ERK, p38 and NF-kB. Moreover, GK potentiated the effect of KTM100 on the intestinal expressions of JNK, ERK, p38 and NF-kB in rats with I/R-induced intestinal injury.

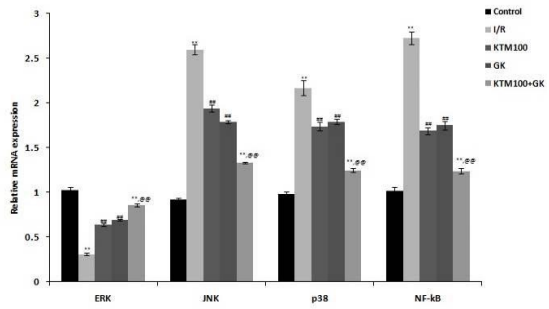

Figure 4: Effect of ginkgolide K and ketamine treatments on the intestinal mRNA expressions of JNK, ERK, p38 and NF-kB in rats with I/R-induced intestinal injury. Data are mean ± SEM (n = 10); $^{**}p < 0.01$, compared to control group; $^{\#\#}p < 0.01$, compared to I/R group; $^{@@}p < 0.01$, compared to KTM100 + GK group

DISCUSSION

Obstruction of blood supply to the intestinal tissue causes intestinal damage which leads to impairment of the motility of GIT as well as deleterious changes in the mucosal layer [14]. The drugs currently used for the management of obstructed intestinal blood supply have several limitations. Thus, the present study was carried out to determine the effects of ginkgolide K and ketamine, alone and in combination, on I/R-induced intestinal injury in rats. The effects of GK and KTM100 were determined by estimating the levels of cytokines in the serum, and parameters of oxidative stress and ROS in the intestinal tissues. Moreover, the intestinal mRNA expressions of JNK, ERK, p38 and NF-kB were determined using qRT-PCR, while histopathological changes were examined using H&E staining.

Intestinal I/R alters the functions of the intestine as a result of injury due to several factors [15]. For example, I/R induces changes in intestinal motility through some mediators, including NO [16]. Moreover, parameters of oxidative stress contribute to the pathogenesis of intestinal injury [17]. This is consistent with the results of this study. Treatments with GK and KTM100 (alone and in combination) attenuated the altered parameters of oxidative stress in I/R-induced intestinal injury rats. Ketamine has been reported to protect the intestine against injury [9]. In this study, treatment with GK potentiated the protective effect of ketamine against intestinal injury. It has been reported that the levels of ROS are enhanced in damaged intestinal tissue [18]. In this study, there were reductions in the levels of ROS in rats treated with KTM100 and GK, relative to I/R group of rats.

Cytokines contribute to the development of intestinal injury [19]. Moreover, it has been reported that ketamine reduces the intestinal levels of cytokines, thereby protecting against I/R-induced intestinal injury [9]. The results of the present study suggest that KTM100 and GK treatments (alone and in combination) significantly reduced the levels of cytokines in the intestinal tissues of rats with I/R-induced intestinal injury. Several factors such as p38, ERK and JNK are involved in intestinal injury and cellular apoptosis [20]. The results of this study showed that treatments with KTM100 and GK (alone and in combination) mitigated the changes in mRNA expressions of JNK, ERK, p38 and NF-kB in the intestinal tissue of rats with I/R-induced intestinal injury. Moreover, GK potentiated the effect of KTM100 on the expressions of JNK, ERK, p38 and NF-kB in intestinal tissue.

CONCLUSION

The results obtained in this study suggest that GK potentiates the protective effect of KTM100 against I/R-induced intestinal injury in rats by regulating NF-kB/ERK/JNK signaling pathway. Thus, GK could be used clinically for the management of intestinal injury.

DECLARATIONS

Acknowledgement

The authors are thankful to the Second Affiliated Hospital of Xi'an Jiaotong University, China for providing the facilities to conduct the presented work.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Weina Zhu, Zhili Zhao, Xiongtao Liu, performed all the experiments. Xiumei Ni, Xiaoming Lei collected materials and offered help in statistical analysis. Xiaoying Li, Rui Deng gave suggestion in designing this experiments and revision of this manuscript. The whole study was supervised by Liyan Zhao.

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