Vinpocetine protects liver against ischemia–reperfusion injury
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Abstract: Hepatic ischemia–reperfusion (IR) injury is a clinical problem that leads to cellular damage and organ dysfunction mediated mainly via production of reactive oxygen species and inflammatory cytokines. Vinpocetine has long been used in cerebrovascular disorders. This study aimed to explore the protective effect of vinpocetine in IR injury to the liver. Ischemia was induced in rats by clamping the common hepatic artery and portal vein for 30 min followed by 30 min of reperfusion. Serum transaminases and liver lactate dehydrogenase (LDH) activities, liver inflammatory cytokines, oxidative stress biomarkers, and liver histopathology were assessed. IR resulted in marked histopathological changes in liver tissues coupled with elevations in serum transaminases and liver LDH activities. IR also increased the production of liver lipid peroxides, nitric oxide, and inflammatory cytokines interleukin-1β and interleukin-6, in parallel with a reduction in reduced glutathione and interleukin-10 in the liver. Pretreatment with vinpocetine protected against liver IR-induced injury, in a dose-dependent manner, as evidenced by the attenuation of oxidative stress as well as inflammatory and liver injury biomarkers. The effects of vinpocetine were comparable with that of curcumin, a natural antioxidant, and could be attributed to its antioxidant and anti-inflammatory properties.

Key words: liver ischemia–reperfusion, vinpocetine, curcumin, cytokines, oxidative stress.

Introduction
Hepatic ischemia–reperfusion (IR) injury is an important clinical problem that complicates liver surgery and transplantation. The pathophysiology underlying hepatic IR injury is complicated, involving oxidative stress as well as inflammatory and apoptotic mechanisms (Vardanian et al. 2008). Interruption of blood flow to the liver and the subsequent reperfusion lead to an acute oxidative stress response that may cause significant cellular damage and organ dysfunction (Lanteri et al. 2006).

Hepatocellular injury during both the initial and later phases of reperfusion is partially caused by reactive oxygen species (ROS) (Jaeschke and Farhood 1991; Jaeschke 2003). Accordingly, antioxidant therapy can be used to limit IR injury (Glantzounis et al. 2005).

Curcumin (CR; 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5 dione), a yellow-orange dye extracted from the Indian spice turmeric, has been reported to possess multiple effects including antioxidant, anticarcinogenic, antimicrobial, and anti-inflammatory activities (Aggarwal et al. 2007). CR administration has been shown to ameliorate IR injury in rat intestine, liver, and kidney (Shen et al. 2007; Awad and El-Sharif 2011; Lin et al. 2012; Onder et al. 2012).

Vinpocetine, a derivative of the alkaloid vincamine, has long been used for cerebrovascular disorders and cognitive impairment. The neuroprotective effects of vinpocetine are mediated via the blockade of sodium channels, reducing calcium influx into neuronal cells, as well as acting as an antioxidant (Zelles et al. 2001; Mendoza et al. 2007; Sitges et al. 2007). Vinpocetine has been found to interfere with various stages of the ischemic cascade: ATP depletion, activation of voltage-sensitive Na+ and Ca2+ channels, release of glutamate and free radicals (Hadjiev 2003; Sitges et al. 2007).

The neuroprotective effect of vinpocetine has been reported with respect to IR injury in hippocampal neuronal cells, both in vivo and in vitro. (Rischke and Kriegstein 1991; Solanki et al. 2011). Its role in protecting against IR injury in the liver, however, remains unexplored. This study aimed to investigate the protective effect of vinpocetine in IR injury in the liver. CR was chosen as.
a reference standard. The parameters chosen to assess IR damage included estimation of serum transaminases in addition to liver lactate dehydrogenase (LDH) activity as markers of liver injury and ischemia, respectively. Moreover, the levels of inflammatory cytokines and oxidative stress biomarkers were assessed in the liver, as well as histopathologic examination of liver tissues.

Material and methods

Drugs and chemicals

CR and vinpocetine were obtained from Sigma–Aldrich, USA. They were suspended in 1% Tween 80 shortly before administration to the animals. The concentrations of the drugs were adjusted so that each animal received 1 mL of either the CR or vinpocetine suspension (containing the appropriate dose) per 100 g of body mass. All of the other chemicals were of the highest grade commercially available.

Animals

Male, albino, Wistar rats, 150–200 g body mass, were purchased from the National Cancer Institute, Cairo, Egypt, and left to acclimatise in the animal facility of the Faculty of Pharmacy, Cairo University, for 1 week before being subjected to experimentation. All animals were allowed free access to feed and tap water. The study was conducted in accordance with the ethical procedures and policies approved by the Ethics Committee of Faculty of Pharmacy, Cairo University.

Induction of hepatic IR injury

Animals were anesthetized with thiopental sodium (70 mg/kg body mass; intraperitoneal injection (i.p.)). Ischemia was induced in rats by clamping the common hepatic artery and portal vein, according to the method described by Colletti et al. (1990), for 30 min followed by 30 min of reperfusion. Throughout the operation the animal’s abdomen was covered with plastic wrap to prevent dehydration.

Experimental design

Animals were randomly allocated to 5 groups, each consisting of 8 rats. Groups I and II received 1% Tween 80 daily (per oral (p.o.)) and served as the sham-operated and the control IR groups, respectively. The remaining 3 groups were treated daily, p.o., with 100 mg CR/kg body mass, or 10 mg vinpocetine/kg, or 20 mg vinpocetine/kg, respectively for 2 weeks. The doses of the test agents used were chosen from the published literature (Rischke and Kriegstein 1991; Banji et al. 2001).

Twenty-four hours after the last treatment, rats in groups 2–5 were subjected to IR injury. Thereafter, animals were sacrificed by decapitation; blood samples were collected from the neck vein and used for the serum separation that was used for the estimation of serum activities of alanine transaminase (ALT) and aspartate transaminase (AST). The livers were then excised, washed and used for histopathologic examination of liver tissues.

Biochemical and histological assays

Serum ALT and AST activities were estimated using commercial kits and expressed in units per litre (U/L). Liver LDH activity was assessed using commercial kits from Stanbio (USA) and expressed in U/L. Liver content of GSH was determined using Ellman’s reagent according to the method described by Ahmed et al. (1991) and expressed in milligrams per gram of wet tissue. NO metabolites were measured as total nitrate/nitrite (NOx) using Griess reagent according to the method described by Miranda et al. (2001) and expressed in milligrams per gram of wet tissue. Liver lipid peroxides were determined as thiobarbituric acid reactive substances (TBARS) using malondialdehyde (MDA) as a standard according to the method described by Mihara and Uchiyama (1978) and expressed in nanomoles per gram of wet tissue. Estimation of liver contents of TNF-α, IL-1β, IL-6, and IL10 was carried out using ELISA reagent kits (Quantikine, R&D Systems, USA) and expressed in picograms per gram of wet tissue. Tissue samples preserved for histopathology were fixed in 10% formalin. The samples were washed with tap water, then serial dilutions of alcohol were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 °C in hot air oven for 24 h. Paraffin blocks were prepared for sectioning by Slidge microtome (slices 4 μm thick). The obtained tissue sections were collected on glass slides, deparaffinized, and stained with hematoxylin and eosin for histopathological examination using an electron microscope (Banchroft et al. 1996). Images were captured and processed using Adobe Photoshop (version 9).

Statistical analysis

Values are the mean ± SE from 8 rats. Results were analyzed using one-way analysis of variance (ANOVA) followed with a Tukey–Kramer multiple comparisons test. Values for p < 0.05 were considered statistically significant. Graph Pad Software InStat (version 2) was used to carry out these statistical tests.

Results

Rats subjected to liver IR showed a significant increase in both serum ALT (155.58 ± 3.01 U/L) and AST (111.31 ± 4.93 U/L) activities, reaching 129% and 149% of that in the sham-operated group, respectively. Pretreatment with CR (100 mg/kg) reduced the activity of AST to 83% of that in the IR treatment group. Treatment with vinpocetine (20 mg/kg) controlled the elevation in both ALT and AST to 88.3% and 80.6% of that in IR group, respectively (Fig. 1A and 2B).

Untreated rats subjected to IR suffered from a 2-fold increase in TBARS (51.70 ± 1.79 mmol/g wet tissue) as compared with the sham-operated group (21.20 ± 1.48 mmol/g wet tissue); meanwhile the GSH content in the IR treatment group (158.75 ± 11.43 mg/g) was reduced to almost half of the value in the sham-operated group (259.43 ± 8.53 mg/g). This effect was prevented by the administration of CR, and vinpocetine. The level of TBARS was reduced to 68% of the rats in the IR treatment group, 71% of the rats treated with CR, and 64.5% of that in the rats treated with vinpocetine (10 or 20 mg/kg). In addition, GSH levels were elevated to 163% of that in the IR treatment group, 122% of that for the rats treated with CR, and 145.5% of that in the rats treated with vinpocetine (10 or 20 mg/kg) (Fig. 2A and 2B).

Hepatic LDH activity was increased following induction of IR to 1043.73 ± 119.72 U/g wet tissue, reaching 435% of that in the sham-operated group (204.14 ± 22.7 U/g wet tissue). Pretreatment with CR or vinpocetine (10 or 20 mg/kg) daily for 2 weeks protected against increased LDH activity: enzyme activity was almost normalized in all 3 groups to be 22%, 25%, and 21% of that for rats in the IR group treatment group, respectively (Fig. 2C).

Hepatic NOx content was increased following induction of IR to 555.63 ± 17.23 mg/g wet tissue, and reached 134.5% of that in the sham-operated group (413.06 ± 21.78 mg/g). Pretreatment with CR, and 145.5% of that in the rats treated with vinpocetine (10 or 20 mg/kg). In addition, NOx levels were elevated to 163% of that in the IR treatment group, 122% of that for the rats treated with CR, and 145.5% of that in the rats treated with vinpocetine (10 or 20 mg/kg) (Fig. 2A and 2B).

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Fig. 1. The effect of pretreatment with curcumin (100 mg/kg body mass) or vinpocetine (10 or 20 mg/kg) on (A) serum aspartate transaminase (AST), and (B) serum alanine transaminase (ALT) activities in male albino Wistar rats subjected to ischemia–reperfusion (IR) injury. Curcumin and vinpocetine were administered daily, per oral, for 2 weeks. Twenty-four hours after the last dose, rats were subjected to 30 min of ischemia followed by 30 min of reperfusion. Statistical analysis was carried out with one-way ANOVA followed by the Tukey–Kramer multiple comparisons test. Value are the mean ± SE (n = 8 rats); *, p < 0.05 compared with the normal control group; @, p < 0.05 compared with the IR group. SO, sham operated; CR, curcumin; V10, vinpocetine (10 mg); V20, vinpocetine (20 mg).

Fig. 2. The effect of pretreatment with curcumin (100 mg/kg body mass) or vinpocetine (10 or 20 mg/kg) on (A) liver content of reduced glutathione (GSH), (B) liver content of thiobarbituric acid reactive substances (TBARS), (C) lactate dehydrogenase (LDH) activity in the liver, and (D) total nitrate/nitrite (NOx) content in the liver of male, albino, Wistar rats subjected to ischemia–reperfusion (IR) injury. Curcumin and vinpocetine were administered daily, per oral, for 2 weeks. Twenty-four hours after the last dose, rats were subjected to 30 min of ischemia followed by 30 min of reperfusion. Statistical analysis was carried out using one-way ANOVA followed by the Tukey–Kramer multiple comparisons test. Values are the mean ± SE (n = 8 rats); *, p < 0.05 compared with the normal control group; @, p < 0.05 compared with the IR group. SO, sham operated; CR, curcumin; V10, vinpocetine (10 mg); V20, vinpocetine (20 mg).

treatment group, and increased IL-10 content to 116% of that in IR group. Similarly, 10 mg vinpocetine/kg reduced both IL-1β and IL-6 levels to 55.5% and 71%, respectively, of that in IR treatment group, and increased IL-10 content to 109% of that in IR group; meanwhile, 20 mg vinpocetine/kg reduced both IL-1β and IL-6 contents to 37% and 66%, respectively, of that in IR group and increased IL-10 content to 141% of that in IR group (Figs. 3A–3C). On the other hand, TNF-α wasn’t significantly altered in the IR treatment group or in any of the treated groups, compared with the sham-operated group (Fig. 3D).
Histological examination of tissues of sham-operated group revealed that the histologic structure of the liver was normal (Fig. 4A), whereas in the IR treatment group the hepatocytes surrounding the central vein showed coagulative necrosis associated with diffuse Kupffer cell proliferation and inflammatory cell infiltration in between the hepatocytes, while the central vein and sinusoids were dilated (Fig. 4B).

In the CR-pretreated group, some of the hepatocytes surrounding the congested central vein showed fatty changes associated with slight Kupffer cell proliferation and inflammatory cell infiltration in between the hepatocytes, while the central vein and sinusoids were dilated (Fig. 4B).

In the CR-pretreated group, some of the hepatocytes surrounding the congested central vein showed fatty changes associated with slight Kupffer cell proliferation and inflammatory cell infiltration in between the hepatocytes, while the central vein and sinusoids were dilated (Fig. 4B). Pretreatment with 10 mg vinpocetine/kg produced dilation and congestion in the portal vein and dilation in both the central vein and sinusoids that was associated with infiltration by a few inflammatory cells (Fig. 4D). Meanwhile, pretreatment with 20 mg vinpocetine/kg revealed dilation in both central vein and sinusoids associated with infiltration by a few inflammatory cells (Fig. 4E). The severity of histopathological alterations in the liver tissues is also presented in Table 1, with the highest severity recorded following IR (++++) and CR (++). CR offered moderate protection against histological alterations (++), whereas treatment with both dose levels of vinpocetine showed better protection against these alterations than CR (+).

Discussion

In this study, clamping the common hepatic artery and portal vein of rats affected 70% of the liver mass. It has been reported that the resultant hepatic insult is similar to the clinical situation in which the liver is rendered ischemic during total vascular exclusion for liver resection (Arab et al. 2009). Indeed, in the present study, 30 min of hepatic ischemia followed by 30 min of reperfusion caused severe liver injury, as demonstrated by the structural damage to the liver and the increased serum activities of AST and ALT in addition to liver LDH activity. Similar results were reported by Taha et al. (2012).

Gao and Li (2012) concluded that oxidant stress and inflammation are the most critical mechanisms contributing to the organ pathophysiology after warm hepatic IR. This was evidenced in this study by the depletion in hepatic GSH and the parallel increase in MDA.

IR injury in the current investigation resulted in a significant increase in levels of pro-inflammatory cytokines as IL-1β and IL-6 in the liver, with a parallel decrease in IL-10, an anti-inflammatory cytokine. Increased expression of pro-inflammatory cytokines as
TNF-α and IL-6 during hepatic IR was previously reported (Taha et al. 2012). The activation of inflammatory cytokines is mediated by ROS and is associated with the induction of protective genes that are essential for the maintenance of liver functions (Llacuna et al. 2009). Kupffer cells and liver-infiltrating monocyte-derived macrophages are the primary sources of cytokines (Tacke et al. 2009). Moreover, Beraza et al. (2007) have suggested that the hepatic inflammatory response to IR is driven largely by the activation of NF-κB in hepatocytes.

Induction of hepatic IR injury was coupled with an increase in NO production. The role of NO in IR injury remains controversial. NO produced by endothelial nitric oxide synthase (eNOS) serves many physiological purposes, such as the promotion of vasodilation and protection against IR injury (Jeyabalan et al. 2008). However, excessive levels of NO via inducible nitric oxide synthase (iNOS) in macrophages may cause cellular damage via a phenomenon known as nitrosative stress (Kimura et al. 2003; Vardanian et al. 2008). This process leads to the production of peroxynitrite, which is a potent oxidant that induces cell death (Schwentker and Billiar 2002).

Indeed, reperfusion of the ischemic liver was shown to result in the generation of oxidative and nitrosative stresses as well as in the production of peroxynitrite, which induces rapid cytotoxicity and liver injury (Lin et al. 2012).

In this study, pretreatment with CR prevented IR-induced liver injury, as evidenced from the observed decrease in liver lipid peroxides and NOx levels, coupled with an increase in GSH and IL-10. Similarly, pretreatment with CR prevented IR-induced increases in IL-1β and IL-6 in the liver. Improvements in the structure of the liver correlated with a reduction in serum AST and liver LDH activities.

In a study performed by Lin et al. (2012), liver ischemia for 30 min followed by 80 min of reperfusion resulted in significant elevations in serum transaminases and LDH activity, as well as increases in blood levels of NO and TNF-α. All such changes were attenuated by pretreatment with CR (25 mg/kg). Moreover, CR in the same dose was shown to prevent methotrexate-induced hepatotoxicity (Banji et al. 2001).

In a study performed by Onder et al. (2012), CR (200 mg/kg) as a single dose protected against histopathologic damage and oxidative stress in the intestine and distant organs including liver, kidney, and lungs, and also protected against mesenteric IR injury.
Pretreatment with CR protected the liver and kidney against IR injury through multiple pathways involving immune-mediated and anti-apoptotic mechanisms, mediated via inhibition of tumor growth factor-beta (TGF-β) and caspase-3 (Shen et al. 2007; Awad and El-Sharif 2011). CR was shown to increase antioxidant enzyme expression and activity in tissue, inhibit neutrophil infiltration, and protect cell function under different stress conditions (Araujo and Leon 2001). Moreover, CR has been reported to be an effective scavenger for ROS and RNS in vitro. These effects are mediated through the regulation of various transcription factors, growth factors, and inflammatory cytokines, as well as protein kinases and other enzymes (Aggarwal et al. 2007).

Pretreatment with vinpocetine protected against IR injury to the liver in a dose-dependent manner. Vinpocetine in the large dose (20 mg/kg) prevented all IR-induced biochemical and histological changes. Vinpocetine is readily absorbed from the small intestine following oral administration. It undergoes extensive metabolism yielding its main metabolite, apovincamine, which is absorbed from the stomach (Pudleiner and Verczeyek 1993).

Zhao et al. (2011) demonstrated that by reducing the expression of inflammation-related molecules as nuclear factor-κB (NF-κB) and activator protein-1 (AP-1), vinpocetine can inhibit the production of NO and pro-inflammatory cytokines in microglia exposed to oxygen, and glucose deprivation. Hence, the observed anti-inflammatory effects of vinpocetine could be mediated via inhibition of pro-inflammatory cytokine induced NF-κB activation, as well as inhibition of monocyte adhesion and chemotaxis, which are clinical processes during inflammation (Jeon et al. 2010). Phosphodiesterase inhibitors such as vinpocetine are regarded as useful anti-inflammatory agents that down-regulate inflammatory cytokines and up-regulate inhibitory cytokines such as IL-10 in the central nervous system (Yoshikawa et al. 1999).

The effect of vinpocetine on acute hepatic injury caused by the administration of CCl4 in rats has been investigated (Abdel Salam et al. 2007). Vinpocetine reduced CCl4-induced elevation in serum transaminases and reduced associated hepatic necrosis. The ability of vinpocetine to block calcium channels (Sitges et al. 2007) may explain its hepatoprotective effect. In this context, limiting the influx of Ca2+ into the hepatocytes by calcium channel antagonists ameliorated hepatic injury in a number of experimental models (Sippel et al. 1993; Romero et al. 1994).

Another proposed mechanism for the protective effect of vinpocetine is its marked antioxidant activity from the scavenging of hydroxyl radicals and other ROS (Santos et al. 2000; Herrera-Mundo and Sitges 2012). Solanki et al. (2011) showed that vinpocetine prevented ROS generation and reduced antioxidant levels associated with IR injury in primary hippocampal cell cultures. Moreover, vinpocetine was reported to possess cytoprotective activity and to prevent apoptosis in hyphoxia (Gabryel et al. 2002).

Conclusions

In conclusion, the experimental IR model used herein was reliable with respect to the promotion of parenchymal and functional liver damage as evidenced by the observed biochemical and histopathological changes in liver tissues. Pretreatment of rats with CR or vinpocetine protected against IR-induced liver injury by virtue of their antioxidant and anti-inflammatory properties.

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References


