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Different doses of dexmedetomidine reduce plasma cytokine production, brain oxidative injury, PARP and caspase expression levels but increase liver oxidative toxicity in cerebral ischemia-induced rats

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ABSTRACT

Cerebral ischemia-induced progression of brain, liver, and erythrocyte oxidative injuries might be modulated by dexmedetomidine (DEX) as a potent antioxidant and anti-inflammatory drug. The present study was conducted to explore whether two different doses of DEX protect against plasma cytokine and brain, liver and erythrocyte oxidative toxicity and apoptosis in cerebral ischemia-induced rats.

Forty-two rats were equally divided into 7 groups. The first and second groups were used as untreated and sham controls, respectively. The third (DEX4) and fourth (DEX40) groups received 4 mg/kg and 40 mg/kg DEX treatments. The fifth, sixth and seventh group were operated on to induce cerebral ischemia. The fifth, sixth and seventh groups are used to represent cerebral ischemia, cerebral ischemia + DEX4, and cerebral ischemia + DEX40, respectively. DEX was intraperitoneally given to the DEX groups at the 3rd, 24th and 48th hour.

Brain and erythrocyte lipid peroxidation (MDA) levels and plasma IL-1β and TNF-α levels were high in the cerebral ischemia group although they were low in the DEX4 and DEX40 groups. Decreased glutathione peroxidase (GSH-Px) and reduced glutathione (GSH) values in the brain and erythrocyte of the cerebral ischemia group were increased by the DEX treatments, although PARP, and the caspase 3 and 9 expressions in the brain were further decreased. Conversely, the cerebral ischemia-induced decrease in the liver vitamin A, vitamin E, GSH, and GSH-Px were further decreased by the DEX treatments, although MDA level, PARP, and caspase 3 and 9 expressions were further increased by the DEX treatments.

In conclusion, DEX induced protective effects against cerebral ischemia-induced brain and erythrocyte oxidative injuries through regulation of the antioxidant level and cytokine production. However, both doses of DEX induced oxidative toxicity and apoptotic effects in the rats' livers.

1. Introduction

Cerebral ischemia-induced injury is a major cause of death and disability in adults and newborns. The long-term outcome for the brain in cerebral ischemia-induced injury includes neuronal diseases such as cerebral palsy, epilepsy, and mental retardation (Lynch and Nelson, 2001). Tissue damage occurs in ischemia through energy depletion, accumulation of toxic metabolic products and activation of phospholipase (Cai et al., 2014; Kumar et al., 2014). Reperfusion leads to further neuronal damage through generating reactive oxygen species (ROS). In addition to a poor enzymatic antioxidant defense system, brain and erythrocytes have a high rate of oxygen consumption and a rich content of polyunsaturated fatty acids (PUFAs). Because of the three reasons (their high rate of oxygen consumption, high content of PUFAs and poor enzymatic antioxidant scavenger system), the brain and erythrocytes exhibit increased vulnerability to ischemia-induced oxidative stress (Nazıroğlu, 2011; Yang et al., 2014). The liver is also essential for detoxification of ROS and cytokine productions. Hence, liver is also one of the main defense systems against ischemia-induced excessive production of ROS and inflammation in the body (Gong et al., 2012; Gul et al., 2015).

Abbreviations: DEX, dexmedetomidine; GSH, reduced glutathione; GSH-Px, glutathione peroxidase; IL-1β, interleukin-1β; ISC, ischemia; MDA, malondialdehyde; NF-kB, nuclear factor kappa B; PARP, poly (ADP-ribose) polymerase; ROS, reactive oxygen species; TBAR, thiobarbituric-acid; TNF-α, tumor necrosis factor-alpha.

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Dexmedetomidine (DEX) as a selective agonist of α2-adrenoreceptor has analgesic, anxiolytic and sedative effects. Postsynaptic adrenergic activity is decreased by selective binding DEX to presynaptic α2 adrenergic receptors. In addition to its reducing postsynaptic activity, accumulating evidence from studies proved that DEX plays a key role in the ROS production through regulation of antioxidant redox and anti-inflammatory systems in the pathogenesis of oxidative brain injuries (Cai et al., 2014; Akpınar et al., 2016). Furthermore, recent experiments showed plasma cytokine and brain oxidative stress levels were diminished in spinal cord injury-induced rats after DEX treatment (Aslan et al., 2009). DEX is mainly metabolized in the liver and is induced in rat liver during the metabolize process (Gul et al., 2015). However, there is no report of DEX on plasma cytokine production, apoptosis and oxidative stress levels in the brain, erythrocyte and liver of cerebral ischemia-induced rats. The recommended safe and effective clinical dosage range of DEX is 0.5–1 μg/kg/hour and 1–25 μg/kg in humans (Li et al., 2015; Luo et al., 2016; Ren et al., 2016) and rats (Aslan et al., 2009; Cakir et al., 2015; Gul et al., 2015), respectively. It was recently reported that high doses of DEX have been used to achieve adequate sedation in patients (Mason et al., 2008; Chen et al., 2015). More recently, we observed neuroprotective action of a moderate dose of DEX on apoptosis and oxidative stress in dorsal root ganglion and hippocampus of cerebral ischemia-induced rats (Akpınar et al., 2016). In turn, there may be related to oxidative stress and cytokine production in the high doses of DEX. Further research is needed on this subject.

The generation of ROS induced by ischemia-reperfusion injury, plays a central role in the pathogenesis of oxidative brain injury (Engelhard et al., 2002) and these radicals can stimulate the cascade of cytokines through nuclear factor kappa B (NF-kB) activation (Sifringer et al., 2015). The release of proinflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-1β (IL-1β) induces chemokine synthesis in ischemic tissues (Frangogiannis, 2007). IL-1β has been reported to contribute to brain failure and apoptosis through ischemia stimulation (Engelhard et al., 2002). Furthermore, IL-1β attracts macrophages which can act as an extra source of IL-1β and ROS (Frangogiannis, 2007). Results of limited reports have indicated that the antioxidant and anti-inflammatory roles of DEX may relate to its inhibiting effect on the augmentation of pro-inflammatory cytokines and ROS in the brain of ischemic rats (Engelhard et al., 2002). To our knowledge there are scarce reports on the effects of DEX in plasma TNF-α and IL-1β levels in cerebral-ischemia-induced rats, and this subject needs further studies.

Thus, the present study aimed to investigate whether or not two different dosses of DEX have protective effects on cerebral ischemia-induced brain, liver and erythrocyte oxidative damage and apoptosis in rats. We also investigated the role of cerebral ischemia, DEX4 and DEX40 on cytokine production, PARP, and caspase 3 and 9 expressions in the brain and liver of rats. The obtained results are discussed with the intent of explaining the mechanism of actions of DEX4 and DEX40 on ischemic brain, liver and blood injury.

2. Materials and methods

2.1. Animals

Forty-two male Wistar albino rats weighing 180–200 g were obtained from the Animal Research Laboratory, Suleyman Demirel University (SDU). The animals were housed in individual cages in a temperature- controlled room with alternating 12-h light-dark cycles and acclimatized for a week before the study. Food was removed 12 h before the cerebral ischemia induction, but all the animals were allowed free access to water and rat chow diet at all other times. The study was approved by the Local Experimental Animal Ethical Committee Neuroscience Research Center (NÖROBAM) of SDU (Protocol number: 15-04). The experiments were carried out over a 2 days period after cerebral ischemia induction.

2.2. Experimental design

The rats were equally divided into seven groups (n = 6), as follows:

Control Group. Non-ischemic, non-supplemented rats receiving 0.9% w/v saline via intraperitonel injection (IP) at the 3rd, 24th and 48th hour.

Sham Group. The group was operated on to expose the cerebral artery, and the incision was then closed without inducing cerebral ischemia induction. The rats in this group received physiologic saline solution (0.9% w/v) IP injection at the 3rd, 24th and 48th hour after the operation.

DEX4 Group. Non-ischemic rats but treated with DEX (4 μg/kg and DEX hydrochloride, Meditera Inc. İstanbul, Turkey) via IP at the 3rd, 24th and 48th hour.

DEX40 Group. Non-ischemic, non-supplemented rats receiving DEX (40 μg/kg) via IP injection at the 3rd, 24th and 48th hour.

Ischemic Group. Cerebral ischemia was subjected to 30 min of cerebral artery occlusion followed by IP injection of physiologic saline solution at the 3rd, 24th and 48th hour.

Ischemic + DEX4 Group. Ischemic rats treated with DEX (4 μg/kg) via IP injection at the 3rd, 24th and 48th hour after cerebral ischemia induction.

Ischemic + DEX40 Group. Ischemic rats treated with DEX (40 μg/kg) via IP injection at the 3rd, 24th and 48th hour after cerebral ischemia induction.

Twelve hours after the last DEX and physiological saline administration, all rats were sacrificed via cervical dislocation, and blood, liver and brain samples were taken in NÖROBAM of SDU.

2.3. Induction of cerebral ischemia

All surgical procedures were performed after the rats had been sufficiently anaesthetized with an intramuscular injection of ketamine hydrochloride (50 mg/kg) and xylazine (5 mg/kg) cocktail, which was determined by assessing the eye touch response. The lower neck was shaved and sterilized with antiseptic iodine solution and then the animals were placed in a supine position. A heat lamp was used to maintain the body temperature at 37 °C during the experiment.

The right middle cerebral artery was exposed through a ventral midline incision in the neck, was carefully isolated from the vago-sympathetic trunks, and was then loosely encircled with an atraumatic microvascular clamp. A 3–0 suture was positioned so that it encircled the middle cerebral artery for further occlusion. Cerebral ischemic surgery was performed by occlusion of the right middle cerebral artery and the right middle cerebral artery for 1 h. In the current work, the sham-operated rat had the same surgical operation performed on it without the occlusion of both arteries.

2.4. Preparation of blood, brain and liver samples

Rats were sacrificed by cervical dislocation. The blood (3–6 ml) was taken into anticoagulant coated tubes protected against light. Blood samples were separated into plasma and erythrocytes by centrifugation at 1500*g* for 10 min at +4 °C. The erythrocytes samples were washed three times in cold isotonic saline (0.9%, v/w), then hemolyzed with a nine-fold volume of phosphate buffer (50 mM, pH 7.4). The hemolyzed erythrocytes and plasma samples were stored in a deep freeze (−85 °C) until processing (maximum