Effects of Dantrolene on Ischemia-Reperfusion Injury in Animal Models: A Review of Outcomes in Heart, Brain, Liver, and Kidney

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Background/Objectives: Ischemia-reperfusion (IR) is the restoration of blood flow to a tissue that was formerly deficient of blood flow. Tissue damage after IR is considered an IR injury (IRI). During IR, there is an increased level of cytosolic calcium ($[Ca^{2+}]_i$) due to the release of calcium from mitochondrial, sarcoendoplasmic reticulum, and nuclear organelles. Dantrolene sodium (dantrolene) is a 1-[[[5-(4-nitrophenol)-2-furanyl]methylene]amino]-2, 4-imidazolidinedione sodium salt with a nonspecific mechanism, inhibiting organelle release of Ca^{2+} into the cytosol. This work reviews the outcomes of administering dantrolene in brain, heart, liver, and kidney animal models of IRI.

Methods: An extensive PubMed, MEDLINE, and MEDLAR literature review during the last 30 years on the effect of dantrolene in IRI in animal models was analyzed to determine the clinical implications of this important study. Particular attention was given to dantrolene in heart, brain, liver, and kidney IRI.

Results: *Heart:* Nine studies of heart IRI were reviewed and include an in vivo dog model (n = 1), in vivo rabbit model (n = 1), isolated dog myocardial fibers (n = 1), and isolated rat hearts (n = 6). Four studies showed decreased infarct size and increased cardiac function after IRI. One in vivo rabbit study found no difference in infarct size or cardiac function after IRI versus controls. Dantrolene may be protective or inductive of post-IRI arrhythmias depending on preestablished myocyte cycling times. *Brain:* Nine studies of brain IRI were reviewed and include an in vivo dog model (n = 1), in vivo gerbil model (n = 2), and in vivo rat models (n = 6). Dantrolene shows protective decreases in apoptotic markers in 6 studies, but it shows no effect on the necrotic core and mixed effects on reduction of infarct volume. One study found increased mortality in the dantrolene group. *Liver:* One study of in vivo rat liver IRI found that dantrolene decreased liver function tests, tissue necrosis factor α, tissue necrosis, and increased interleukin 10. *Kidney:* One study of in vivo rat kidney IRI showed that dantrolene had no effect.

Conclusions: Dantrolene shows protective effects in animal models of heart, brain, and potentially liver IRI, reinforcing the importance of calcium homeostasis during IRI. Variations of dose, timing of administration, route of administration, and outcomes between studies make definitive conclusions difficult. The nonspecific mechanism of action of dantrolene may also account for the variation among studies. Lack of studies in the liver and kidney makes any consensus in these organs premature, and thus, emphasis for this review was put on studies of the heart and brain.

Key Words: ischemia-reperfusion injury, ryanodine receptors, dantrolene, heart, brain, liver, kidney, calcium

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Ischemia-Reperfusion Injury and Calcium Homeostasis

Ischemia-reperfusion (IR) is the restoration of blood flow to tissue that was formerly deficient of blood flow. Tissue damage after IR is considered an IR injury (IRI). Ischemia-reperfusion injury produces a series of molecular and cellular changes such as reduction-oxidative damage, high-energy depletion, molecular inflammatory dysfunction, and pH and ion imbalances that culminate in a severe pathological state directly related to the intensity of the injury.[1,2] Ischemia-reperfusion injury is one of the most frequent diseases of the western world. Therefore, attempts to understand and modify its outcomes are important.

Cellular dysregulation of calcium homeostasis plays a large role in IRI. Dysregulation of cell calcium homeostasis in IRI means increased cytosol Ca^{2+} concentration ($[Ca^{2+}]_i$), decreased organelle Ca^{2+} concentration, and sarcoplasmic reticulum Ca^{2+} overload. Increased $[Ca^{2+}]_i$ leads to the activation of many Ca^{2+}-dependent effector molecules causing cell damage, necrosis, and apoptosis. In addition, depleted organelle Ca^{2+} stores in the endoplasmic reticulum (ER) decrease the ionic concentration necessary for the proper folding of proteins. These malformed proteins activate cell stress–response proteins and an apoptotic signal cascade in what is called the unfolded protein response. Maintenance of both organelle and cytosol Ca^{2+} homeostasis is critical to mitigating loss of organ function due to cellular damage during IRI.[3–5]

Dantrolene Pharmacology

Dantrolene sodium (dantrolene) blocks organelle Ca^{2+} release.[6] It is a 1-[[[5-(4-nitrophenol)-2-furanyl]methylene]amino]-2, 4-imidazolidinedione sodium salt, originally synthesized by Snyder et al.[7] in 1967. Indicated uses for dantrolene include malignant hyperthermia and chronic spasticity due to upper motor neuron disease. Off-label uses include neuroleptic malignant syndrome, ecstasy intoxication, and heat stroke.[6]

Dantrolene is classically known as an antagonist to ryanodine receptors (RyRs). Ryanodine receptors are key components in regulating cellular calcium homeostasis. They are transmembrane receptors located in the mitochondrial, ER, sarcoplasmic,

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and nuclear membranes, regulating the release of calcium from these organelles.[8] There are 3 isoforms, namely, RyR_1, RyR_2, and RyR_3. Isoform distribution is widespread throughout the body, but it is thought that RyR_1 is predominantly located in the skeletal muscle, RyR_2 in the heart, and RyR_3 in the central nervous system (CNS).[9]

The exact mechanism of action of dantrolene' is not fully known and may be different under physiological and pathological conditions. Under physiologic conditions, evidence suggests that dantrolene works by binding a domain switch directly within the RyR_1 and RyR_3, possibly modifying the RyR's response to calmodulin (CaM) and adenine with no effect on RyR_2.[10,11] Other studies show that dantrolene is nonspecific to RyRs. Evidence suggests that dantrolene's function to decrease organelle Ca^{2+} release from RyRs overlaps with another intracellular calcium release channel, the inositol 1,4,5 triphosphate receptor (IP3R) by being able to reduce organelle Ca^{2+} release from IP3Rs in the presence of its ligand, inositol 1,4,5 triphosphate.[12] In addition, in the brain, dantrolene has been shown to bind the plasma membrane *N*-methyl-D-aspartic acid (NMDA) receptor[13] and reduce neuronal excitotoxicity when glutamate, the ligand of the NMDA receptor, was administered.[13] In contrast, studies of dantrolene's mechanism of action under pathological conditions suggest that the effects of dantrolene are dependent on the RyR conformational state and other regulatory molecules such as the Ca^{2+}/CaM–dependent protein kinase (CaMKII) where CaMKII is suggested to affect the interaction between internal RyR domains and the downstream mechanism of calcium release under pathological conditions.[14–16]

Dantrolene is highly lipophilic and, thus, poorly water-soluble. Three grams of mannitol is added to an intravenous (IV) formulation of 60 mL of water and 20 mg of dantrolene to aid in solubility. This yields a final concentration of 0.33 mg/mL and a pH of 9.5.[6] Rapid and continuous IV administration is recommended because the alkaline pH is a venous irritant. Dosing at 2.4 mg/kg (10 μmol/L) blocks up to 75% of muscle contraction in human adults.[17] Current IV dosing regimens start at 2.5 mg/kg (10 μmol/L) and increase up to a total dose of 10 mg/kg (40 μmol/L).

Dantrolene is metabolized by liver microsomes to 5-hydroxydantrolene, an active metabolite. Plasma concentrations remain therapeutic for 5 hours after administration with an estimated half-life of 10 to 12 hours.[18] Dantrolene and metabolites are excreted in bile and urine.

The most common adverse reactions are drowsiness, phlebitis, dizziness, weakness, general malaise, fatigue, and diarrhea. More serious reactions include anaphylaxis, gastrointestinal bleeding, aplastic anemia, hepatitis, respiratory failure, and hepatotoxicity.

In vivo IRI animal models and ex vivo animal IRI organ models provide a biological complexity that mimics reality more closely than most in vitro cellular studies. Although in vitro cell studies are appropriate for assessing processes within the cell, they cannot be used as appropriate assessment tools of whole organ functions/outcomes. For this reason, our review focuses on the measured outcomes of dantrolene administration in animal studies of brain, heart, liver, and kidney IRI.

HEART IRI AND DANTROLENE

The rise in $[Ca^{2+}]_i$ during IRI promotes uncontrolled hypercontracture of myofibrils and activation of cytoskeleton-degrading calpains.[19] This causes loss of sarcolemma structure/function integrity and subsequent cell death.[19] Further complications arise from myocyte gap junctions that allow the hypercontracure to spread to neighboring cells, effectively increasing the size of the IRI.[19,20]

Cardiac Infarct Size After IRI

Infarct size is a measure of myocardial IRI damage and has implications for post-IRI cardiac functioning. Infarct size is commonly assessed by histopathological measures and cardiac enzyme biomarkers such as creatine kinase (CK) and lactate dehydrogenase (LDH). Of 5 studies measuring infarct indicators, 4 showed evidence that dantrolene reduced infarct damage during IRI.[2,21–23] All 4 protective studies were performed in isolated rat hearts with the ischemic period ranging from 5 to 30 minutes. Between studies, the administration of dantrolene during the preischemic and postreperfusion periods seemed to produce similar protective effects.[2,21–23] Doses ranging from 0.2 to 45 μmol/L showed significant dose-dependent decreases versus controls (25% at 1 μmol/L to 71% at 45 μmol/L).[22,23] Enzyme biomarkers, CK and LDH, corroborated histopathology data as doses from 1 to 100 μmol/L showed significant dose-dependent decreases up to 50% versus controls for both LDH and CK.[2,21,23] Four studies supported dantrolene's protective effects during isolated rat heart IRI, but only 1 study, to our knowledge, investigated infarct size during in vivo rabbit heart IRI. This study found that 40 μmol/L dantrolene, a dose comparable to protective studies, had no protective effect during IRI. However, a variable not consistent with the protective studies was the administration of dantrolene during the postischemia period. No study to date has directly measured outcome differences between timing of dantrolene administration and infarct size after cardiac IRI.[21]

Cardiac Function After IRI

Post-IRI function is commonly assessed by cardiac contractility, relaxation, oxygen consumption, cardiac output, and left ventricular pressure development. Studies investigating post-IRI heart function showed inconsistent results.[2,11,14,21,23–25] All 4 studies supporting dantrolene's ability to protect cardiac function after IRI were carried out in isolated rat hearts with dantrolene administered either before ischemia or after reperfusion.[2,14,21,24] Dantrolene (100 μmol/L), when given after perfusion, significantly increased reperfusion oxygen consumption up to 3 times that of controls.[21] Dantrolene (12.5–45 μmol/L) was also shown to have increased the percent recovery of left ventricular developed pressure, 71% versus controls 44%,[24] and reduced ectopic beats by 75%.[14] In vivo rabbit studies suggested that dantrolene (40 μmol/L) given after ischemia might have increased cardiac output versus controls; however, this was not statistically significant ($P = 0.09$).[21] Contrary to popular thought, dantrolene did show significant negative inotropic effects during one study of isolated rat heart IRI at doses greater than 16 μmol/L, but this was not noted in similar studies.[23]

Cardiac Arrhythmias After IRI

Arrhythmias are a significant cause of morbidity and mortality after cardiac IRI. The isolated rat heart studies provided antiarrhythmogenic evidence for dantrolene when given before ischemia at doses as low as 12.5 μmol/L,[2,14] but early studies of in vivo canine IRI found that 10 μmol/L dantrolene given before ischemia significantly increased the number of fatal arrhythmias (37% vs control 11%) and also reduced the time to appearance of ventricular arrhythmias.[25] In addition to the conflicting results of the previous studies, another study showed that dantrolene may be antiarrhythmogenic or arrhythmogenic or may have no

arrhythmia effect depending on the preestablished myocyte conditions of cycle length and activation times.[11]

Cardiac IRI Discussion

These studies showed general support for the protective effects of dantrolene administration in preventing cardiac IRI in animal models. The evidence suggests that dantrolene is effective in reducing infarct size and increasing cardiac function after IRI with uncertain arrhythmogenic effects. However, the following factors make these conclusions unclear. (1) Most of the beneficial studies came from ex vivo isolated rat heart studies, whereas in vivo studies showed no protective effect during IRI. This discrepancy may be due to dantrolene's pharmacologic properties in vivo versus ex vivo or to unrecognized variables associated with different animal models. Dantrolene's recognized poor solubility when given IV during in vivo studies may have potentially limited its effectiveness; however, in in vivo studies, doses ranged from 10 to 40 μmol/L, the range of current IV dosing for human therapy for malignant hyperthermia, making these differences due to dantrolene's solubility properties less likely. (2) Dantrolene's ability to affect the RyR_2 isotype predominantly found in the heart is controversial.[10] Under physiologic conditions, evidence suggests that dantrolene has no effect on RyR_2.[10] Other studies propose that it may act indirectly on RyR_2 by working on CaM and/or may have functional overlapping effects with the IP3R, another calcium release channel found in the heart.[12,14,26] More critical to IRI is how dantrolene works under pathological conditions. New evidence has shown the effects of dantrolene to be dependent on the RyR_2 conformational state[15] and dependent on the RyR_2 accessory protein CaMKII under pathological conditions.[14] Calmodulin-dependent protein kinase is suggested to regulate the interaction between internal RyR_2 conformational domains and the downstream mechanism of calcium release.[14,16] These studies suggest that dantrolene has a nonspecific mechanism of action in the heart and that its effectiveness may be dependent on a pathological state. Currently not known is dantrolene's exact mechanism of action during cardiac IRI or at what point during IRI the RyR_2 complex may switch from its normal physiologic arrangement to a pathological one. Answers to these questions would be illuminating to heart IRI because these factors likely account for much of the variation in the results between current studies. (3) A wide range of doses from 0.2 to 100 μmol/L was used, along with variations in timing and route of administration. Ex vivo isolated heart studies theoretically received higher concentrations of dantrolene because there was little metabolism, less effective circulating volume, and no other tissues to which dantrolene was distributed. This makes conclusions on dose, timing, and route of dantrolene administration difficult to make. Future studies of cardiac IRI in animal models should seek to address these 3 variable factors to help clarify dantrolene's potential therapeutic role in cardiac IRI.

BRAIN IRI AND DANTROLENE

Brain IRI results in the disruption of the blood-brain barrier and increased inflammatory response leading to neuronal cell death, cerebral edema, and increased intracranial pressure. The area at the center of the brain IRI is usually characterized by rapid neuronal necrosis, whereas the peri-infarct area or penumbra is characterized by slower apoptosis.[4,5] Maintenance of neuronal calcium homeostasis by dantrolene administration during IRI may be beneficial to decreasing cell damage/death and preserving brain functions after IRI. Although dantrolene's precise mechanism of action is currently debated, dantrolene may have a role in mitigating neuronal calcium dysregulation and CNS IRI. For a review of dantrolene neuroprotective mechanisms in various models, see Muehlschlegel and Sims.[27]

Neuronal Apoptosis After IRI

Apoptosis is a controlled process of programmed cell death and is associated with the penumbra of IRI. Apoptosis is thought to be a preventable process if interventions are made early after an insult because it takes more time for apoptotic injuries to occur after insult. Thus, the amount of apoptosis is important in determining the extent of brain IRI and retention of brain function. Markers of apoptosis include DNA fragmentation assays (terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling [TUNEL]–positive), ER stress response proteins, free fatty acids, and lipid-creatine ratio (Lip/Cr). Increases in these markers are signs of increased apoptosis. All studies that investigated apoptotic markers during in vivo rat brain IRI showed that dantrolene (8 μmol/L to 5 mmol/L) administered intracerebroventricularly (ICV) during variable periods of the IRI significantly decreased apoptotic markers.[28–32]

Dantrolene (30 μmol/L) significantly decreased proapoptotic ER stress–response proteins (p-protein endoplasmic reticulum kinase, p-eIF2a-, activator transcription factor-4, and C/EBP-homologous protein) at the apoptotic periphery versus controls. However, not all markers were affected equally. p-protein endoplasmic reticulum kinase-positive cells were significantly reduced at 1 and 4 hours of reperfusion, peIF2a-positive cells were significantly reduced at all time points (1–24 hours), and activator transcription factor-4 and C/EBP-homologous protein were significantly reduced at 24 hours of reperfusion.[28]

Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling studies measure DNA fragmentation, an end result of apoptotic processes. Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling studies found conflicting evidence of dantrolene's effects.[29,32] One-millimolar doses of dantrolene in neonatal rats administered before ischemia found no difference in the amount of TUNEL-positive cells at 14 days after IRI,[32] whereas 5 mmol/L dantrolene administered after ischemia and continued for a longer period thereafter decreased TUNEL-positive cells (65% vs controls) and increased the number of viable neurons roughly 4 times that of the controls at 4 days after IRI.[29] In another study of gerbil CNS IRI, dantrolene administered ICV 30 minutes after ischemia found 4 times and 3 times less neuronal death than that of controls at 1.6- and 0.4-mmol/L doses, respectively. However, this effect was not seen when dantrolene was dosed 120 minutes after ischemia.[30]

Increased fatty acid levels and Lip/Cr ratios are estimates of increased apoptosis and decreased membrane stability. Dantrolene (20 μmol/L) administered ICV before ischemia significantly reduced free fatty acids elevation in adult rat brains[31] and 1 mmol/L of dantrolene given before ischemia significantly reduced the Lip/Cr on day 1 after IRI with no difference versus controls at day 14 after IRI in neonatal rat brains.[32]

Brain Infarct Size and Cell Viability After IRI

Histopathology measures of infarct size and cell viability are other common measures used to quantify the total amount of brain IRI sustained. Of the 6 studies that investigated dantrolene's effects on infarct size and cell viability, 3 found dantrolene to be protective during brain IRI.[29,32,33] Two other studies showed mixed results and 1 study found no protective effects.[28,34,35] In addition, there was no evidence of a significant effect on the ischemic core that is closely associated with necrotic cell death.[28,30,33] Those studies that showed benefit all took place in in vivo rat models receiving 30 μmol/L to 5 mmol/L of dantrolene administered ICV at variable periods of the IRI.[28,29,32,35]

TABLE 1. Overall Review of the Role of Dantrolene on Ischemia/Reperfusion Injury

	Injury	Model	Time of Dantrolene Dose	Dantrolene Dose	Significant Summary Results	Reference
Heart	Focal ischemia: 10 min	In vivo dog hearts	Before ischemia	2.5 mg/kg (10 μmol/L)	(1) Increased fatal arrhythmias vs. control. (2) Reduced time to appearance of ventricular fibrillation.	Pelleg et al.[25]
	Focal ischemia	Isolated dog Purkinje fibers	Superperfusion	35 μmol/L	(1) Complete block and suppression of reentry after 20 min superperfusion. (2) Antiarrhythmogenic at fast stimulation frequencies and arrhythmogenic at slower frequencies.	Davidenko et al.[11]
	Focal ischemia: 5 min	Isolated rat hearts	Before ischemia		(1) Protects against myocardial dysfunction and arrhythmias. (2) Decreases CK and LDH enzymes.	Balam et al.[2]
	Global ischemia: 30 min	Isolated rat hearts	First 15 min after perfusion	25, 100 μmol/L	(1) 50% Decreased peak CK (25 μmol/L). (2) Preserved viable myocardial tissue (25 μmol/L). (3) Decreased total CK 35% (100 μmol/L) and increased reperfusion oxygen consumption 3× control.	Preckel et al.[21]
	Global ischemia	In vivo rabbit hearts	25 min after ischemia	10 mg/kg (40 μmol/L)	(1) Increased cardiac output vs control ($P = 0.09$). (2) No difference in infarct size vs control	Preckel et al.[21]
	Global ischemia: 30 min	Isolated rat hearts	5 min after perfusion	0.2, 1, 4, 16, 45 μmol/L	(1) Dose-dependent increases in coronary flow. (2) Dose-dependent reduction in myocardial necrosis (significant at 1 μmol/L). (3) Dose-dependent decreases in LDH release. (4) >16 μmol/L negative inotropic effects.	Yu et al.[23]
	Global ischemia: 20 min	Isolated rat hearts	20 min before ischemia	4 μmol/L	Significantly reduced % of necrotic mass (control 53%, dantrolene 24%)	Zucchi et al.[22]
	Global ischemia: 20 min	Isolated rat hearts	13 min before ischemia	12.5 μmol/L	Increased % of left ventricular developed pressure recovered after IR vs. control.	Mitchell et al.[24]
	Acidosis	Isolated rat hearts	10 min before acidosis	45 μmol/L	(1) Prevented ectopic beats by roughly 75% and arrhythmias. (2) No effects on basal contractility and relaxation vs. controls.	Said et al.[14]
Brain	Focal ischemia: 90 min right middle cerebral artery occlusion	In vivo rat brain	Ischemia-onset, ICV	20 μg (30 μmol/L)	(1) Significantly decreased apoptotic periphery and decreased ER stress proteins. (2) 2.8× smaller infarct volume. (3) No effect on necrotic core.	Li et al.[28]
	Focal ischemia	In vivo rat neonatal hypoxic-ischemic injury	Before ischemia, ICV	1 mmol/L	(1) Significantly improved morphologic infarct area at 14 d. (2) Lip/Cr ratio 1 d after hypoxic ischemic injury was significantly lower.	Gwak et al.[32]

					(3) No difference between NAA/Cr ratios. (4) No difference 14 d TUNEL or survival.	
	Focal cerebral ischemia: 120 min	In vivo rat brain	20 min before ischemia	2 mg/kg (8 μmol/L)	No significant decrease in infarct volume	Kim et al.[36]
	Global forebrain ischemia: 5 min	In vivo rat brain	15 min after ischemia, ICV with 20-min injection at a rate of 1 μL/min, followed by a 3-d infusion rate of 1 μL/h.	5 mmol/L	(1) Increased viable pyramidal CA1 hippocampal neurons roughly 4× control. (2) Decreased TUNEL-positive nuclei roughly 65%.	Yano et al.[29]
	Global forebrain ischemia: 3 min	In vivo gerbil brain	30 or 120 min after ischemia.	1.6 mmol/L, 0.4 mmol/L	(1) 1.6 mmol/L 4.5× protection at 30 min. (2) 0.4 mmol/L 3× protection at 30 min. (3) No protection at 120 min.	Zhang et al.[30]
	Global forebrain ischemia: 20 min	In vivo rat brain	15 min before ischemia	20 μmol/L	Significantly reduced IRI-induced free fatty acid formation	Phillis et al.[31]
	Global forebrain ischemia: 5 min	In vivo gerbil brain	After ischemia, IV	10, 25, 50 mg/kg (40–200 μmol/L)	(1) 50 mg/kg group 6/12 died between days 1 and 3, 83% of sham neuronal viability. (2) 25 mg/kg 3/10 died on the third day, 78% of sham neuronal viability. (3) 10 mg/kg 0/9 died, 67% of sham neuronal viability	Wei et al.[34]
	Global forebrain ischemia: 10 min	In vivo rat brain	15 min before ischemia, ICV	5 mmol/L	(1) Dantrolene significantly reduced extracellular glutamate concentration vs. control. (2) Dantrolene significantly increased CA1 viability by roughly 40%.	Nakayama et al.[35]
	Global cerebral ischemia: 11 min	In vivo dog brain	Before ischemia, IV	4 mg/kg (16 μmol/L)	No change in neurologic score vs control. Histopathology investigations not done.	Kross et al.[33]
Liver	Right liver ischemia: 90 min	In vivo rat liver	Dan-Pre: 15 min before ischemia. Dan-RP given at reperfusion Dan-Pos: 15 min after ischemia	1 mg/kg (4 μmol/L)	(1) During ischemia: significantly decreased LFTs, TNF-α levels 48%, increased IL-10, and reduced tissue necrosis roughly 37%. (2) Before and after ischemia dosing showed some protective effects, but these were not statistically significant.	Lopez-Neblina et al.[37]
Kidney	Global ischemia: 30 min	In vivo rat kidney	15 min before ischemia	1.12 mg/kg (4.5 μmol/L) (2) No effect on renal cell apoptosis	(1) No effect on renal function. (2) No effect on renal cell apoptosis	Wu et al.[38]

Dan-Pos indicates dantrolene administered after ischemia; Dan-Pre, dantrolene administered before ischemia; Dan-RP, dantrolene administered at reperfusion; LFTs, liver function tests; NAA/Cr, *N*-acetylaspartate/creatine.

Dantrolene administration seemed most effective in reducing infarct volume and increasing cell viability in models of IRI when the ischemic period lasted 5 to 20 minutes in length and the dantrolene dose was greater than 1 mmol/L.[29,32,35] This significantly decreased the infarct area morphologically by roughly 30% at 1 mmol/L 14 days after IRI[32] and increased cell viability 4 times that of controls at 5 mmol/L 3 days after IRI.[29] Even at lower doses (30 μmol/L) and longer ischemic periods (90 minutes), dantrolene administration significantly reduced infarct volume roughly 3 times that of controls.[28] When lower dantrolene (8 μmol/L) doses were given ICV after longer ischemic periods (120 minutes), there was no effect on the infarct volume compared with controls.[36]

During in vivo gerbil studies of brain IRI, a range of dantrolene doses (40–200 μmol/L) administered IV were found to increase cell viability versus controls, but dantrolene also increased gerbil mortality rate in a dose-dependent manner 3 days after IRI.[34] At the lowest dose (40 μmol/L), there were more viable neurons (67% vs controls) without increased mortality.[34]

Brain Function After IRI

Early canine in vivo studies administered dantrolene IV at a dose of 4 μmol/L, and this was found to have no effect on improving neurological function after brain IRI versus controls.[33] No pathological investigations were made in this study, and adverse effects were not noted to be increased versus controls.

CNS IRI Discussion

There is evidence that dantrolene may provide some protective effects during IRI by reducing apoptosis and infarct volume and by increasing neuron cell viability. However, at this time, it is difficult to form a strong and clear consensus because dantrolene doses, timing, and route of administration varied significantly among the studies.

Doses of dantrolene varied from 8 μmol/L to 5 mmol/L. Doses less than 20 μmol/L had minimal effectiveness when administered either ICV or IV.[33,36] In addition, doses greater than 40 μmol/L revealed an increased mortality in 1 study of gerbils; however, 4 other studies used doses up to 5 mmol/L without a similar toxicity noted.[29,30,32,34,35] The reason for this toxicity is unclear at this time.

The optimal period to administer dantrolene is not clear because different periods were used in various studies with various results (Table 1). Investigations on dantrolene's best route of administration suggest that IV dosing decreases the effectiveness compared with ICV administration. Two IV studies showed negative effects at 16 to 200 μmol/L, whereas most ICV studies showed effectiveness at doses as low as 20 μmol/L.[31,33,34] This may be due to dantrolene's poor lipid solubility and trouble crossing the blood-brain barrier, thereby decreasing the effective concentration of dantrolene in CNS tissue when administered IV.

In summary, doses of dantrolene in the range of 20 to 30 μmol/L administered ICV are best for CNS IRI protection because lower doses seem to decrease the effectiveness of dantrolene administration regardless of animal model or route of administration. In addition, dantrolene seems to be more effective when the ischemic insult lasts less than 90 minutes. These are weak conclusions because the studies reviewed showed inconsistent results and had a high degree of variability across dose, timing, and route of administration (Table 1).

In the brain, dantrolene's mechanism of action is nonspecific. Although physiologic studies show dantrolene works by binding a domain switch directly within the RyR_3, modifying the RyR's response to CaM and adenine,[10,26] there is also evidence that dantrolene has effects on the IP3R and the NMDA receptor.[12,13] Dantrolene helped maintain calcium homeostasis in neurons by reducing organelle Ca^{2+} release from the IP3R in the presence of its ligand, inositol 1,4,5,[12] by blocking the toxic effects of a high concentration of glutamate, the NMDA receptor ligand, and by binding the NMDA receptor.[13] This effect of dantrolene on NMDA receptors may explain why in 1 study dantrolene decreased infarct size by reducing loss of calcium regulation during glutamate-induced cytotoxicity without reducing the amount of glutamate released during IRI.[35] These nonspecific mechanisms of dantrolene may account for some of the variability seen across current studies of CNS IRI.

This review reinforces the importance of maintaining calcium homeostasis during CNS IRI. Dantrolene's multiple nonspecific mechanisms of calcium homeostasis make it a potentially very useful therapy. However, at this time, only weak conclusions can be made about dantrolene's effectiveness during CNS IRI. Future studies should focus investigations to clarify dose, timing, and route of administration, as well as the risks of dantrolene and its mechanism of action and during CNS IRI.

LIVER IRI AND DANTROLENE

During 2007, more than 6000 liver transplants were performed in the United States. Ischemia-reperfusion injury of the liver remains a significant problem for successful liver transplant surgery. Heart failure, arrhythmias, dehydration, severe bleeding, and infection can also predispose the liver tissue to the development of IRI.

Interestingly, the hepatocyte RyR is a 5′ truncated RyR_1 isoform with a missing N-terminal cytosolic domain.[39] It is inferred here that dantrolene's mechanism of action is similar to that of the RyR_1 found in skeletal tissue; however, no studies have investigated dantrolene's role in the truncated RyR_1 found in liver tissue. The liver RyR_1 may have a very different interaction with dantrolene because the cytosolic domain is thought to maintain a binding region for dantrolene.[10,26] In this review, only 1 study by Lopez-Neblina et al.[37] measured liver function tests, tumor necrosis factor α (TNF-α), interleukin 10 (IL-10), and tissue necrosis levels in response to dantrolene administration during different periods of rat liver IRI. Tumor necrosis factor α, an inflammatory mediator released by Kupffer cells, activates apoptotic pathways. In contrast, IL-10 is thought to limit the inflammatory response.

Liver Inflammatory Markers and Liver Function Tests After IRI

Dantrolene administered (4 μmol/L) during reperfusion significantly decreased liver function tests, TNF-α by 48% compared with control, and increased IL-10 levels in rat livers.[37] Dantrolene dosed before and after ischemia showed no significant difference compared with controls. Dantrolene given during ischemia was also associated with better liver function tests and less necrosis (37% vs controls).[37]

Liver IRI Discussion

Because only 1 study investigated the effects of dantrolene treatment in liver IRI, any conclusions made about dantrolene's role in liver IRI would be presumptive. However, this evidence reinforces the importance of controlling calcium homeostasis during liver IRI. Investigations into other calcium blockers, such as nifedipine and diltiazem,[40–42] have also proven protective during an in vivo animal model of liver IRI. Further investigations into calcium blockers may clarify their role and dantrolene's role in liver IRI.

This study was the first to investigate the timing of dantrolene administration and found that dantrolene administered at the time of reperfusion was superior to before and after ischemia administration. This information should help guide future studies identify the best timing of dantrolene administration. Investigation of dantrolene's mechanism of action on the truncated RyR_1 found in liver tissue would also be worth exploring because heart and brain tissue studies have found novel mechanisms nonspecific to the RyR isoform in those tissues. This would help clarify how dantrolene may be affecting protective outcomes in liver IRI.

KIDNEY IRI AND DANTROLENE

Ischemia is the most common cause of renal failure. Kidney anatomy does not show as extensive collateral blood supply as other organs. Thus, even mild ischemia can lead to the priming of inflammation via neutrophils, resulting in acute renal failure.[42] Renal IRI can occur during transplantation, prolonged surgery, shock, and sepsis. It can also result from immunopathological and vascular disorders. Sites affected during renal IRI include proximal tubule brush border, distal and proximal tubule cells, and medullary regions.[43]

The RyR_2 isoform is found in kidney cells.[44] As with other RyRs, the renal RyR_2 is susceptible to oxidation on reperfusion from the production of reactive oxygen species. These superoxide radicals formed also induce cyclic ADP-ribose formation, which has been shown to increase the open probability of renal arteriole RyR_2.[44,45] Tumor necrosis factor α produced during IRI also causes increased cyclic ADP-ribose and increased release of Ca^{2+} via RyR_2 in mesangial cells of the nephron.[46]

Kidney Apoptosis and Function After IRI

Although RyRs have an integral role in kidney Ca^{2+} homeostasis, to date, only 1 study has investigated the effects of inhibition of intracellular calcium release by L-type, IP3R channels, and RyR_2 during IRI of renal tubules. In this study, Ca^{2+} release inhibition by L-type and IP3Rs significantly inhibited apoptosis and improved post-IRI renal function, whereas 4.5 μmol/L dantrolene did not.[38]

Kidney IRI Discussion

These results may be a reflection on IP3 and IP3R's predominant role in the signaling cascade of renin-angiotensin system. Angiotensin II released during decreased renal perfusion increases the levels of IP3, the ligand of IP3R. This suggests that dysregulation of organelle and cellular Ca^{2+} via IP3R plays a significant role in kidney IRI. Further investigations regarding the protection of calcium homeostasis in renal cells may prove beneficial.

Reviewing only 1 study makes drawing conclusions premature. This study used a relatively low dose (4.5 μmol/L) of dantrolene compared with other studies examined in this review and only administered dantrolene at 1 period of IRI, before ischemia, which has had mixed results in other organ IRI (Table 1). This may be a variable affecting dantrolene's effectiveness. In addition, dantrolene's controversial ability to affect the RyR_2 isotype in the heart may also apply to the kidney RyR_2. Under physiologic conditions, dantrolene seems to have no effect on RyR_2,[10] but under pathological conditions, evidence suggests that it may be dependent on the RyR_2's conformational state and altered phosphorylation or interaction with CaMKII.[14–16] Interestingly, unlike this study, another study noted dantrolene to have a functional overlap with IP3R calcium release.[12] This may be due to the inherent differences within tissues or method differences between studies, but it nonetheless highlights the lack of clarity surrounding dantrolene's mechanism of action.

CONCLUSIONS

Maintenance of calcium homeostasis during IRI is important in reducing the detrimental impacts associated with IRI in organ systems. This review explored the role that dantrolene has in mitigating animal models of heart, brain, liver, and kidney IRI. Although dantrolene is a known calcium release antagonist and has been shown here to reduce cellular calcium dysregulation, infarct area, ER unfolded protein response, inflammatory mediators, and apoptosis, it is difficult to make definitive conclusions about dantrolene's effects on animal models of IRI in the heart, brain, liver, and kidney (Table 1). Calcium, RyRs, dantrolene, and IRI act in complex systems. The effects of dantrolene noted here cannot be directly ascribed to its direct effects on RyRs as previously thought. Dantrolene's effects on RyR CaM, CaMKII, IP3R, RyR conformational state, RyR phosphorylation status, and NMDA make dantrolene nonspecific and may account for a good deal of the variation of results seen in these studies.[10,12,13,16] The mechanism of dantrolene under IRI conditions has not been investigated fully, and it is not known at what point during IRI that the RyR complex is altered if at all. A clearer understanding of dantrolene's mechanism of action during IRI would be beneficial in guiding future studies trying to understand and mitigate IRI in the heart, brain, liver, and kidney.

Dantrolene's protective effect was not consistent across studies or without adverse effects such as toxicity during CNS studies and negative inotropic effects in the heart. These discrepancies may be due to variations in methods, particularly the wide range of doses, length of IRI, time of dantrolene administration, or route of administration. In addition, having only 1 study in both liver and kidney, IRI makes any conclusions about dantrolene's IRI effects presumptive in these organs. More studies are needed to make refined conclusions about dose, timing, and route of dantrolene administration in all organ models of IRI.

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