Improving the ischemia-reperfusion injury in vascularized composite allotransplantation: Clinical experience and experimental implications

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Long-time ischemia worsening transplant outcomes in vascularized composite allotransplantation (VCA) is often neglected. Ischemia-reperfusion injury (IRI) is an inevitable event that follows reperfusion after a period of cold static storage. The pathophysiological mechanism activates local inflammation, which is a barrier to allograft long-term immune tolerance. The previous publications have not clearly described the relationship between the tissue damage and ischemia time, nor the rejection grade. In this review, we found that the rejection episodes and rejection grade are usually related to the ischemia time, both in clinical and experimental aspects. Moreover, we summarized the potential therapeutic measures to mitigate the ischemia-reperfusion injury. Compare to static preservation, machine perfusion is a promising method that can keep VCA tissue viability and extend preservation time, which is especially beneficial for the expansion of the donor pool and better MHC-matching.

KEYWORDS ischemia-reperfusion injury (IRI), vascularized composite allotransplantation (VCA), tissue damage, transplant rejection, innate immunity, adaptive immunity

Introduction

The world of reconstructive transplantation is mature (1). The challenges of allograft rejection have focused research on the long-term success of vascularized allograft transplantation (2, 3). Ischemia-reperfusion injury (IRI) is a potential threat to long-term allograft success, which is an inevitable event that follows reperfusion after a period of cold static storage (4, 5). This review summarizes the current clinical and laboratory aspects that discuss the relationship between transplant outcomes and IRI tissue damage.
It can give some implications to reduce the IRI to achieve long-term VCA allograft survival.

**Mechanisms of IRI**

Ischemia leads to hypoxic anaerobic glycolysis and oxygen consumption, depleting adenosine triphosphate (ATP) and dysregulating ATP-dependent membrane ion exchangers (6, 7), reducing the activity of the Na+/K+/ATPase pump and increasing intracellular sodium concentration (8). Furthermore, the reduction in the intracellular concentration of ATP prevents the regeneration of glutathione, ascorbic acid and tocopherol that take part in detoxifying the metabolites present in the cytosol and the sarcoplasmic membrane. The accumulation of osmotically active particles such as lactate, sodium, inorganic phosphate and creatine leads to cell edema.

Moreover, cellular acidosis can stimulate the antiport Na+/H+ receptors, worsening the sodium overload and affecting the function of other membrane receptors such as the Na+/Ca2+ antiport. The Na+/Ca2+ antiport enables sodium exportation from cells based on the intracellular calcium concentration (9). Cellular hypercalcemia causes the breakdown of sarcoplasmic phospholipids and cytoskeleton protein, alters contractile protein’s efficiency and calcium affinity, and changes the tertiary structure of certain enzymes such as xanthine dehydrogenase to xanthine oxidase (10). These two enzymes have similar functions: the transformation of hypoxanthine in xanthine and xanthine in uric acid. Damage to calcium-dependent receptors increases cytosolic calcium, loss of homeostasis, activation of proteolytic enzymes, cell membrane disruption, and release of free fatty acids. Collectively, this dysfunction manifests as cell apoptosis or necrosis (11–13).

Reperfusion triggers a localized microvascular and systemic reaction, resulting in further tissue damage (14–16). Mitochondria respond to many different types of stress like oxidative and metabolic stresses (17–19). They are the primary source of reactive oxygen species (ROS), a by-product of respiration generated mainly at the electron transport chain complexes I and III (20). Ca2+ overload, with high ROS and Pi, changes mitochondrial membrane permeability and induces the opening of non-selective and high-conductance permeability transition pores (PTP) in the inner mitochondrial membrane (21–23). The PTP further compromises mitochondria’s bioenergetics function and structural integrity, leading to cell death (24–26). The release of ROS, mainly from mitochondria, forms the basis for IRI (27, 28).

**How the IRI influences the transplant outcome**

**Experimental implications**

**IRI in the skin and subcutaneous tissue**

IRI in the skin has been reported in several publications, not only in VCA but also in flap surgery (29–31). Skin and subcutaneous tissue are relatively resistant to the effects of anoxia, and intracellular pH changes are reversible for up to 24 h (32). Donski et al. (33) investigated the effect of cooling on the survival of free flaps in rabbit. They found 86% of flaps that were cooled for 1–3d survived. Meanwhile, other authors concluded that the maximum ischemia time of a rat flap was 6h at normal body temperatures and 48h if cooled (34). Thus, the warm IRI has more serious tissue damage than the cold IRI.

As VCA tissue is usually preserved at 4 °C for 6h, the warm ischemia time in VCA skin and subcutaneous tissue is pretty short. The tissue damage in the skin and subcutaneous tissue can be ignored. However, the IRI should be considered if warm ischemia is >6h or cold ischemia time >24h.

**IRI in the skeleton muscle**

Compared to the skin, mammalian skeletal muscle is substantially less tolerant to ischemia (35). Irreversible damage to the microcirculation of skeletal muscle in man begins at around 6 h (36). Wagh et al. (37, 38) found that skeletal muscle is much more susceptible to damage from cold (4°C) ischemic storage than skin, with an estimated critical ischemic time for rat gastrocnemius muscle flaps of approximately 16 h compared with approximately 3.5 days for rat epigastric skin flaps. Although measures have been taken to ameliorate the IRI in muscles, lots of results are based on short time warm-ischemia time (39–43). The data for VCA clinical usage is limited.

**IRI in the vessel**

The endothelium is very sensitive to I/R injuries (44, 45). It is essential to preserve the endothelium because endothelial cells have several vital functions, including controlling vascular tone and local blood flow, modulating coagulation and inflammation, participating in immune response, regulating micro and macromolecules’ movement towards the interstitium, and assisting in angiogenesis (46). Endothelium-dependent vasodilatation is more susceptible to IRI than vasoconstriction and endothelial-independent vasodilatation (47, 48). ROS and tumor necrosis factor-alpha (TNF-α) play a significant role in this process. Reperfusion also induces a critical inflammatory response, characterized by a massive production of free radicals and activation of the complement pathway, leucocytes and neutrophils (49). A little interaction between activated endothelium and neutrophils will result in a significant concentration of activated neutrophils in the interstitium, which release oxygen radicals and proteases, leading to the destruction of cells and the extracellular matrix. The migration of neutrophils from the intravascular bed to the interstitium involves several families of proteins such as selectins (P-selectin and L-selectin), integrins (intracellular adhesion molecule-1), and immunoglobulins (platelet-endothelial cell adhesion molecule-1). Lastly, oxidative stress, cytokine production, and the secondary mitochondrial lesions that occur with reperfusion induce apoptosis in parenchymal cells and the vascular structures.
In addition, vascular endothelial cells are the initial barrier to allograft-activated host immune rejection and are critical in triggering cell-mediated acute rejection (50). It has been found that circulating mitochondria in organ donors with prolonged ischemia may directly activate allograft vascular endothelial cells and promote graft rejection (51–53). Therefore, endothelial cells mediate acute graft rejection after IRI. The targeted intervention of mitochondrial damage in vascular endothelial cells, thereby reducing graft rejection events, has also been a research hot-spot in recent years.

IRI in the nerve

Although much is known, the precise pathophysiology of IRI in the peripheral nerve remains to be elucidated. Microvascular events, which may occur during reperfusion, may be important in amplifying the nerve fiber degeneration that is initiated during ischemia (54). Haruyasu Iida et al. (55) showed that reperfusion induced oxidative damage, which lowered nerve function and increased fiber deterioration, but extending the period of reperfusion to 42 days allowed for fiber regeneration. To reduce oxidative injury, Sang-Jin Shin et al. (56) investigated how inducible nitric oxide synthase (iNOS) inhibition affects the recovery of motor function in the rat sciatic nerve after IRI. Their study indicated that early inhibition of iNOS is vital for IRI reduction or prevention. Franka et al. (57) studied the critical ischemia times of individual tissues of a rat limb isograft. Histomorphometric investigation of the tibial nerve on POD 10 showed the typical signs of Wallerian degeneration that is initiated during ischemia (54). Haruyasu Iida et al. (55) showed that reperfusion induced oxidative damage, which lowered nerve function and increased fiber deterioration, but extending the period of reperfusion to 42 days allowed for fiber regeneration. To reduce oxidative injury, Sang-Jin Shin et al. (56) investigated how inducible nitric oxide synthase (iNOS) inhibition affects the recovery of motor function in the rat sciatic nerve after IRI. Their study indicated that early inhibition of iNOS is vital for IRI reduction or prevention. Franka et al. (57) studied the critical ischemia times of individual tissues of a rat limb isograft. Histomorphometric investigation of the tibial nerve on POD 10 showed the typical signs of Wallerian degeneration in all transplanted animals and the nerve transection groups. The nerve of non-transplanted controls appeared to be normal in shape without signs of injury or cell infiltration. Overall, histopathological scores for nerve damage were significantly higher in the ischemia group than transection group. In general, nerve scores increased proportionally with the duration of ischemia time.

IRI in the bone

Compared to most other organs, the bone’s IRI is poorly understood, particularly from a mechanistic perspective. However, IRI of the bone is considered to occur in various diseases/situations (58–61), such as vascular disruption or compression, fractures, limb replantation/allotransplantation, and thromboembolic disorders. Moreover, some systemic diseases such as sickle cell anemia, Caisson disease, and Cushing’s disease may initiate IRI in the bone (62–64).

As systemic diseases influence many organs, it’s hard to investigate the mechanism of reperfusion injury in bones. Thus, bone IRI has been studied by interrupting blood supply through vascular compression (clamping) or dissection. In these studies, limb or bone graft replantation/transplantation was performed after preserving the limbs/grafts at 0 – 4 °C or room temperature (21 –25°C) (65–68). The studies concluded that significant retardation of bone growth/development occurs when critical ischemia lasts between 3 to 7 h at 37 °C. But the critical ischemia time increases with decreasing temperature. In some studies, even cold ischemia time (0 – 5 °C) of 25 h and above have been found to be tolerated (69).

The fact that therapy with antioxidants resulted in considerable protection proves that reperfusion injury of the bone, or extra injury during the reperfusion period, occurs (70). This reiterates the protective function of antioxidants against ROS. ROS can only be formed in the presence of O2, which means upon reperfusion. The available results, however, suggest some similarities to the mechanisms of IRI of other organs, such as the involvement of ROS (71, 72).

From a review of the literature, we have summarized the following critical ischemia time of VCA tissues (Table 1):

Many papers reported the IRI tissue damage in VCA. We briefly introduce the tissue damage in different types of VCA tissue. The lack of blood supply does not damage all tissues in the allografts to the same degree; some tissues are more susceptible than others. Those damaged tissues may release some molecules and activate the innate immune response, which is a barrier to long-term allograft survival (5, 73–75). In this review, we are not only focused on the relationship between the IRI and tissue damage but also discuss the relationship between IRI and transplant rejection caused by tissue damage (Table 2).

Clinical experience

Ischemia is clinically an inevitable factor following donor organ procurement, cold preservation, and implantation. Though its specific role in VCA is occasionally underappreciated, the IRI can affect graft survival, function, and rejection. However, there is a paucity of studies examining IRI in VCA clinical usage. The experience in re-transplantation has opened a window for us to know the critical ischemia time related to VCA. The recommended ischemia times compatible with reliable success in replantation are 6 h of warm and 12 h of cold ischemia for major replants, although successful replantations have been reported after longer ischemia times (86–89). The ischemia time is largely influenced by skeleton muscle and causes it even more susceptible to IRI. Besides tissue damage, the literature in SOT has clearly demonstrated that IRI is a potent activator of the immune system and subsequently leads to occur rejection episodes. These clinical findings have also been

| Tissue                  | Warm | Cold  |
|-------------------------|------|-------|
| skin and subcutaneous tissue | 4–6h | up to 12h |
| muscle                  | <2h  | 8h    |
| nerve                   | 8h   | 24h   |
| vessel                  | 6h   | 12h   |
| bone                    | <3h  | 24h   |
tested in VCA. Based on the presence of a more aggressive diffuse lymphocytic infiltration and disruption of tissue architecture, Pradka et al. (90) showed that skin and muscle exposed to 3 h of ischemia had significantly higher rejection scores than when exposed to 1 h of ischemia.

To sum up, the IRI in VCA has the following effect on the graft and body.

**Graft survival**

Insult to the endothelium leads to an upregulation in the expression of bioactive agents (such as endothelin and thromboxane) and suppresses the release of nitric oxide (NO), increasing vascular tone. The presence of free radical accumulation, endothelial edema, and platelet activation can result in the cessation of blood flow through the graft. Thus, graft survival is threatened by IRI (91, 92).

**Tissue damage**

Muscle and nerve dysfunction in the donor limb has a significant impact even if the graft survives since their physiological integrity is crucial for maintaining physical function. We clearly described the IRI tissue damage in experimental implications. The muscle tissue is sensitive to the IRI, then the skin and subcutaneous, bone is more tolerant to IRI (93–95). However, the muscle normal structure and functional recovery are very important to the VCA surgical outcomes. Besides, the neuromuscular junctions are most sensitive to ischemia (96). Since most organs are still preserved in static preservation, muscle tissue

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**TABLE 2** Relationship between IRI and tissue damage or transplant rejection.

| Year | Author | Species | Model | Ischemia time | Preservation solution | Follow up | Conclusion |
|------|--------|---------|-------|---------------|-----------------------|----------|------------|
| 2009 | Pradka, S. P (76) | Rat | Allogeneic vascularized epigastric flaps | 1h or 3 h WI | Heparinized saline solution | POD 6 | Skin and muscle demonstrated increased acute rejection of allotransplants with increased subcritical ischemic time |
| 2010 | Xiao, B (77) | Rat | Allogeneic vascularized groin flaps | 0h, 6h, 12h, 18h, or 24 h CI | University of Wisconsin | POD 2-8 | Prolonged ischemia has a deleterious effect on allograft survival |
| 2010 | Fumiaki Shimazu (78) | Rat | Allogeneic vascularized groin flaps | 1h or 6 h WI | N/A | POD 14 | Longer ischemic time induces more severe rejection against allo-transplanted tissue compared with the shorter one |
| 2012 | Villamaria, C. Y (79) | Swine | Gracilis musculocutaneous flap | 1 h CI or 3 h CI | Heparinized saline solution | POD 1 to POD 14 | Skeletal muscle tissue injury (LDH, CK, and AST) showed ischemia period-dependent response |
| 2014 | Hautz (80) | Rat | Syngeneic hindlimb transplantation | 2 h CI or 10 h CI or 30 h CI | Saline or Histidine-tryptophan-ketoglutarate, or University of Wisconsin | POD 10 | Severe inflammation and tissue damage are observed after prolonged cold ischemia in muscle and nerve |
| 2016 | Bonastre, J (81) | Rat | Allogeneic orthotopic hindlimb transplantation model | 7h CI | Heparinized saline solution | 2 months | An association between cold ischemia and chronic rejection was observed in experimental vascularized composite allotransplantation |
| 2017 | Datta, N (82) | Mouse | Allogeneic orthotopic hindlimb transplantation model | 1h or 6 h CI | University of Wisconsin | POD 1 to POD 3 | Prolonged cold ischemia triggers progressive IRI with vascular endothelial damage |
| 2017 | Messner, F (57) | Rat | Syngeneic hindlimb transplantation | 2 h CI or 6 h CI, or 10 h CI | Saline or Histidine-tryptophan-ketoglutarate, or HTK-, or TiProtec | POD 10 | Muscle and nerve injury was significantly aggravated after prolonged cold ischemia |
| 2017 | Fries, C. A (83) | Swine | Gracilis musculocutaneous flap | 3h CI | Heparinized saline solution or CI esterase inhibitor | POD 1 to POD 14 | ClInhibitor is protective of IRI and may have utility in vascularized composite allotransplantation |
| 2018 | Robbins, N (84) | Swine | Heterotopic myocutaneous flap (autotransplants and allotransplants) | 5 h CI or 14 h CI, 17h machine perfusion | University of Wisconsin | 14 days for autotransplants and 60 days for allotransplants | Machine perfusion protects ischemic damage and chronic rejection following allotransplantation in the porcine model |
| 2020 | Gok, E (85) | Rat | Syngeneic hindlimb transplantation | 6h h WI or 6 h CI | Histidine-tryptophan-ketoglutarate | 12 weeks | Limb allotransplant suffer from irreversible muscle damage without circulation by 4 h and have functional deficits on cold ischemia at 6 h |

IRI, ischemia-reperfusion injury; h, hours; CI, cold ischemia; WI, warm ischemia; POD, postoperative day; N/A, not available.
is more sensitive to IRI than other organs, and it is not easy to regenerate after IRI, reducing IRI tissue damage has a positive effect on the functional recovery of VCA.

**Graft rejection**

As it is well known, there are 3 necessary steps for graft rejection. Step 1: Leukocyte infiltration (endothelial cells dysfunction, P-selectin and L-selectin, intercellular adhesion molecule-1, platelet-endothelial cell adhesion molecule-1). Step 2: Leukocyte activation (local inflammation). Step 3: Target organ (effective T cells and cytokines producing). Injured tissues activate the immune system by releasing damage-associated molecular patterns, which are endogenous molecules that mediate cellular injury. Their release upregulates endothelial adhesion molecules, migration of leukocytes into the graft and complement activation. This localized inflammatory response hampers the development of tolerance (97, 98). This explains why post-transplant graft dysfunction and rejection are proportional to the intensity and duration of IRI (Figure 1). Thus, ameliorating the IRI in VCA may reduce the incidence of VCA rejection (99).

**Systemic reperfusion injury**

Reperfusion injury may lead to systemic metabolic changes and the release of oxidized free radicals in patients, leading to cellular oxidative stress, systemic inflammatory response, multiple organ failure, and eventually death. Based on current arm replantation experiences, there is a chance of local or systemic complications, such as sepsis, remote organ failure, hyperkalemia, or acidemia (100–104). Thus, the systemic reperfusion injury should be seriously evaluated prior to VCA surgery (105).

**Strategies to reduce IRI**

Over the past 20 years, a variety of drugs and interventions have been reported in clinical and basic research to alleviate IRI. Many treatment methods are based on limb IRI models, limb autograft models, and limb replantation. These interventions have shown good therapeutic effects, and the research results are worthy of reference by VCA. Table 3 summarizes the most commonly used treatments to reduce IRI, which include adenosine agonists, endothelin antagonists, antioxidants, complement activation inhibitors, apoptosis inhibitors, anti-inflammatory and proangiogenic, metabolic inhibitors, bioactive gases, traditional Chinese medicine, cell-based therapy, etc.

Despite the aforementioned therapeutic substances, the maneuver of postconditioning or remote postconditioning are effective therapies targeting IRI (142–145). Importantly, these strategies are simple, safe, and at least relatively harmless. Although the clinical trials of ischemic preconditioning or remote ischemic preconditioning have demonstrated favorable results in cardiac, hepatic, and pulmonary surgery, large, randomized, multi-center trials are required to verify the efficacy of these interventions in human skeletal muscle and skin. Recently, cutting-edge techniques have shown promising results, especially in muscle tissue preservation. The following paragraph describes these exciting methods.

**Cryopreservation**

Cryopreservation aims to slow the deterioration of graft tissue by reducing the rate of metabolism (146–149). This requires
| Group | Substances category | Author, Year | Treatment drug | Ischemia method | Species | Number | Ischemia time | Reperfusion time | Skeleton muscle included |
|-------|---------------------|-------------|----------------|----------------|---------|--------|---------------|-------------------|------------------------|
| I     | Adenosine agonist, endothelin antagonist, prostaglandin | Rowlands, 1999 | prostaglandins (PG) E1, E2 | Hindlimb IRI model | SD rats | 82 | Warm 6h | 4h | Yes |
|       |                     | Luyt, Charles-Edouard, 2000 | mixed ETA/B receptor antagonist, LU 132532 | Hindlimb IRI model | Lewis rats | 33 | N/A | 5h, 5d, 14d | Yes |
|       |                     | Herbert, K. J, 2001 | Bosentan | Hindlimb IRI model | SD rats | 47 | Warm 120 min | 90min, 24h | Yes |
|       |                     | Jan Fräßdorf, 2006 | Prostaglandin E1 | Hindlimb IRI model | Rabbits | 64 | Warm 45 min and 3h | 2h, 3h | Yes |
|       |                     | Zheng Jingang, 2007 | Edenosine A1, A2A, and A3 receptors | Hindlimb IRI model | C57BL6 | 32 | Warm 90 min | 24h | Yes |
| II    | Complement inhibitor | Claudia Duehrkop, 2013 | C1-inh | Hindlimb IRI model | Wistar rats | 25 | Warm 3h | 24h | Yes |
|       |                     | C. Anton Fries, 2016 | C1-inh | Free musculocutaneous flap model | Swine | 12 | Cold 3h | 1d, 2d, 7d, 14d | Yes |
|       |                     | Shengye Zhang, 2018 | C1-inh | Hindlimb IRI model | Wistar rats | 28 | Warm 2h | 24h | Yes |
|       |                     | Inmaculada Massa, 2021 | C1-inh | Superficial caudal epigastric skin flaps | Wistar rats | 50 | Warm 8h | 7d | No |
| III   | Antioxidant | Cengiz Bolcal, 2007 | N-acetylcysteine, β-glucan, and coenzyme Q10 | Hindlimb IRI model | New Zealand white rabbits | 44 | Warm 1h | 3h | Yes |
|       |                     | Bradley D Medling, 2010 | Vitamin E | Gracilis Muscle Flap Model | Wistar rats | 12 | Warm 4h | 24h | Yes |
|       |                     | Gudrun Avci, 2012 | Curcumin | Hindlimb IRI model | Wistar rats | 40 | Warm 4h | 2h | Yes |
|       |                     | Gan Muneuchi, 2013 | D-allose | Abdominal skin island flap | Wistar rats | 110 | Warm 8h | 8h | No |
|       |                     | Xu Dong, 2014 | Dexamethasone | Hindlimb IRI model | Wistar rats | 40 | Warm 4h | 2h | Yes |
|       |                     | Yin, Zhuming, 2016 | Recombinant human thioredoxin-1 | Dorsal lateral thoracic artery pedicled island skin flaps | CD-1 mice | 98 | Warm 2h, 4h, 6h, 8h, 10h, and 12 h | 24h | No |
|       |                     | Mircafer Seyid, 2021 | Ceruloplasmin | Epi gastric island flaps | SD rats | 32 | Warm 6h | 24h | No |
| IV    | Anti-apoptosis | Kexin Song, 2015 | Methane-rich saline | Abdominal skin flap | SD rats | N/A | Warm 6h | 72h | No |
|       |                     | Yedong Cheng, 2016 | Pterostilbene | Hindlimb IRI model | SD rats | N/A | Warm 4h | 4h | Yes |
|       |                     | Dawei Xin, 2020 | LXA4 | Abdominal skin flap | Wistar rats | 54 | Warm 8h | 12h, 24h, 48h | No |
| V     | Anti-inflammatory angiogenesis | Elizabeth W Zhang, 2015 | Activated protein C | Gracilis muscle flap | SD rats | 60 | Warm 4h | 1h, 4h, 18h, 24h | Yes |
|       |                     | Dong Kyun Rah, 2017 | Platelet-Rich Plasma | Lateral thoracic artery island flaps | C57BL6 | 30 | Warm 4h | 1d, 3d, 5d, 7d, 10d | No |

(Continued)
freezing of the graft to temperatures below 0°C and offers the possibility of storage for many weeks. To preserve tissue viability by cryopreservation, careful control of the rate of cooling is necessary, as well as the addition of cryoprotectants to prevent intracellular ice crystal formation (150–152). Several studies have described the applicability of preserving single-cell systems, blood vessels, cutaneous tissues, bones, and nervous tissues by cryopreservation (153–155). In 2008, Rinker et al. (156) preserved rat epigastric flaps at −140°C for 2 weeks. The authors then performed isointransplantation using the flaps, which remained viable for up to 60 days, maintaining normal pigmentation and hair growth, and showing no histological signs of inflammation or necrosis. Arav et al. (157, 158) performed the first directional freezing and vitrification to preserve a syngeneic heterotopic rat hindlimb for 7 days. They demonstrated that myocytes, blood vessels, and skin layers of the hindlimb remained histologically viable 3 days after transplantation. Studies on the effects of cryopreservation on human VCA grafts are currently lacking. Although long-term VCA graft storage is possible with cryopreservation, it is still challenging to establish a standard preservation guideline because different tissues respond differently to freezing, thawing, and cryoprotectants (159).

| Therapeutic substances category | Author, Year | Treatment drug | Ischemia method | Species | Number | Ischemia time | Reperfusion time | Skeleton muscle included |
|-------------------------------|--------------|----------------|-----------------|---------|--------|---------------|---------------------|-------------------------|
| Group VI Reduce metabolic    | Sun-Young Nam, 2018 (126) | NecroX-5 | Abdominal skin flap | SD rats | 20 | Warm 7h | 24h | No |
| Group VII Traditional chinese medicine | Henderson, Peter W, 2010 (127) | Hydrogen Sulfide | Hindlimb IRI model | C57BL6 | 42 | Warm 3h | 3h | Yes |
| Group VIII Bioactive gases   | Gang Zhao, 2018 (128) | Irisin | Dorsal island skin flap | SD rats | 48 | Warm 6h | 7d | No |
| Group IX Cell based treatment or Mitochondrial transplantation | Gang Chen, 2018 (129) | Iuteolin | Abdominal skin flap | SD rats | 18 | Warm 4h | 7d | No |
| Group VIII Bioactive gases   | Huwen Ren, 2018 (130) | Polysaccharide Peptide | Dorsal lateral thoracic artery pedicled island skin flaps | CD-1 mice | 80 | Warm 4h | 24h, 7d | No |
| Group VIII Bioactive gases   | Yijia Xiang, 2018 (131) | Salvianolic acid | Hindlimb IRI model | SD rats | 60 | Warm 6h | 24h | Yes |
| Group VIII Bioactive gases   | Yan Zhao, 2019 (132) | epigallocatechin gallate | Hindlimb IRI model | SD rats | 30 | Warm 4h | 6h | Yes |
| Group VIII Bioactive gases   | Joon Pio Hong, 2003 (133) | Hyperbaric Oxygen | Abdominal skin flap | SD rats | 100 | Warm 3h | 24h | No |
| Group VIII Bioactive gases   | Aurelia Bihari, 2017 (134) | Carbon monoxide-releasing molecules | Hindlimb IRI model | Wistar rats | 14 | Warm 2h | 1.5h | Yes |
| Group VIII Bioactive gases   | Cagdas Elsurer, 2018 (135) | Oxone | Pectoralis muscle flap | Wistar rats | 28 | Warm 3h | 7d | Yes |
| Group VIII Bioactive gases   | Hao Cui, 2020 (136) | Nitric oxide (NO) | Rectangular island flap | Wistar rats | 24 | Warm 10h | 12h | No |
| Group VIII Bioactive gases   | Jian Tong, 2021 (137) | Hydrogen Gas | Hindlimb IRI model | C57BL/6 | 24 | Warm 3h | 4h | Yes |
| Group IX Cell based treatment or Mitochondrial transplantation | David W Hammers, 2015 (138) | Anti-inflammatory macrophages | Hindlimb IRI model | C57BL/6 | 21 | Warm 2h | 3d, 5d | Yes |
| Group IX Cell based treatment or Mitochondrial transplantation | Alberto Ballestin, 2018 (139) | Adipose-Derived Stem Cells | Superficial caudal epigastric skin flaps | Wistar rats | 28 | Warm 8h | 7d | No |
| Group IX Cell based treatment or Mitochondrial transplantation | Yun Bai, 2018 (140) | Adipose mesenchymal stem cell-derived exosomes | Superficial inferior epigastric vessels | SD rats | 18 | Warm 6h | 5d | No |
| Group IX Cell based treatment or Mitochondrial transplantation | Arzoo Orfany, 2020 (141) | Mitochondrial transplantation | Hindlimb IRI model | C57BL/6 | 48 | Warm 2h | 24h | Yes |

N/A, not available.
Machine perfusion

The aim of machine perfusion is to preserve organ viability by supplying oxygen and nutrients and removing metabolic by-products (160–163). This way, grafts are preserved extracorporeally for extended periods, thereby significantly increasing their geographic accessibility (164–166). Grafts can be preserved under a variety of perfusion temperatures (167); these include hypothermic (0°C–12°C), mid-thermic (13°C–24°C), sub-normothermic (25°C–34°C), and normothermic (35°C–38°C) conditions. Studies utilizing small and large animal VCA models have shown that machine perfusion can effectively preserve transplant tissue for up to 24 hours (Table 4)(173, 187–191). Human limbs were preserved by Werner et al. (181) for 24 h using plasma-based sub-normothermic machine perfusion. After being preserved for 24 hours, the grafts were still functional and continued to respond to neuromuscular electrostimulation while exhibiting no evidence of myocyte damage.

Although recent studies have demonstrated the capability of machine perfusion in preserving graft tissue for an extended period (171, 192), some challenges still exist, such as (1) a paucity of studies utilizing allografts, (2) the absence of long-term follow-up data, and (3) lack of consensus on ideal temperature or perfusate for use in clinical settings. With the development of science and technology, machine perfusion combined with cryopreservation, CRISPR/Cas 9, stem cell therapy, siRNA, etc, to achieve in vitro editing of donor organs and modify the immunogenicity of donors, which can reduce IRI and immune rejection of the graft, and help the long-term survival of the graft (193–197).

### TABLE 4 Machine perfusion in VCA.

| Year   | Author               | Species       | Model         | N   | Perfusion solution                     | Perfusion time | Perfusion temperature | Oxygenation | Outcomes                                                                 |
|--------|----------------------|---------------|---------------|-----|----------------------------------------|----------------|------------------------|--------------|--------------------------------------------------------------------------|
| 2022   | Rezaei, M. et al     | Human         | Upper extremities | 20  | Oxygenated red blood cell-based solution | 41.6 ± 9.4hr | Normothermic (38°C)    | Yes          | MP overcome the limitations of SCS extending preservation times, enabling limb quality assessment, and allowing limb reconditioning before transplantation. |
| 2022   | Goutard M. et al     | Rat           | Hindlimb      | 60  | Modified Steen solution               | 3hr           | Mid-thermic (21°C)     | Yes          | The use of MP for vascularized composite allografts could extend the preservation time and limit cold ischemia induce injury. |
| 2022   | Figueroa, A. et al   | Swine         | Forelimb      | 24  | Polymerized HBOC-201                  | 22.5 ± 1.7hr  | Normothermic (38°C)    | Yes          | MP with HBOC-201 could support isolated limb physiology, metabolism, and function. |
| 2022   | Burlage, L.C. et al  | Rat           | Hindlimb      | 74  | Acellular oxygen carrier HBOC-201    | 6hr           | Mid-thermic (21°C)     | Yes          | Six hours MP using an acellular oxygen carrier HBOC-201 results in superior tissue preservation compared to SCS. |
| 2021   | Kruijt, A. S. et al  | Swine         | Forelimb      | 24  | UW solution                           | 16hr          | Hypothermic (8°C-10°C) | No           | In-vivo muscle contraction was well preserved after 18 h machine perfusion compared to short SCS. |
| 2021   | Amin, K. R. et al    | Swine         | Forelimb      | 35  | Matched blood                        | 6hr           | Normothermic (38°C)    | Yes          | MP resulted in superior graft preservation and less reperfusion injury compared with the SCS. |
| 2020   | Said, S. A. et al    | Swine         | Forelimb      | 3   | HBOC-201                              | 21.3 ± 2.1hr  | Normothermic (39.8°C) | Yes          | MP could preserve muscle contractility and mitochondrial structure compared to SCS. |
| 2020   | Haug, V. et al       | Human         | Upper extremities | 6   | Steen solution                       | 24hr          | Hypothermic (10°C)     | Yes          | MP with an oxygenated acellular Steen solution can extend the extracorporeal preservation time compared to SCS. |
| 2020   | Haug, V. et al       | Swine         | Forelimb      | 10  | Dextran-enriched Phoxilium, Steen, or Phoxilium | 12hr           | Hypothermic (10°C or 4°C) | Yes          | MP has been shown to be a promising alternative to (SCS for preservation of vascularized composite allotransplantation. |
| 2020   | Fahradryan, V. et al | Swine         | Forelimb      | 10  | Colloid solution containing washed RBCs | 12-44hr       | Normothermic (38°C)    | Yes          | Extended normothermic MP is a feasible option for preservation of amputated limbs. |

(Continued)
Summary

Our retrospective review found that IRI not only causes tissue damage but also increases acute and chronic rejection events, with consistent results in organ transplantation and VCA. However, VCA contains different tissue components, and muscle is a highly metabolically active tissue that is most susceptible to reperfusion injury. The traditional static preservation method has been unable to meet clinical needs. Long-term cold ischemia causes great muscle damage, which is extremely detrimental to the functional recovery of VCA.

Advances in science and technology, such as cryopreservation technology, machine perfusion technology, etc, have significantly prolonged the preservation time of VCA. These effects are significantly better than static preservation. However, these technologies still need to be further improved, and certain consensus should be reached to standardize their clinical usage.

Future

Although VCA surgery is a life-improving, non-life-saving surgery, the ethics of surgery are still subject to academic controversy. The current focus of controversy is how to achieve a balance between patient cost and benefit. In order to improve the quality of life, patients need to take immunosuppressive drugs for a long time, and the side effects of these drugs greatly limit their clinical application. Recently, with the continuous deepening of basic research, the immune tolerance program of VCA has been successfully established in mice (198–201). But there are
still many hurdles in translating it into large animals, even primates (202). IRI is one of the important factors that threaten the immune tolerance of VCA. In addition, improving the IRI could break geographic boundaries, expand the donor pool, increase organ utilization, and achieve better MHC-matching. At present, there are still few studies on IRI, and the pathophysiological mechanism of its tissue injury still needs to be further studied.

Author contributions

JH wrote the article and made the figure. UK, LQ, PW and JT proofread the manuscript. JT reviewed the article. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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