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Review

An Overview of Therapy Guidelines for Cardiac Arrest and the Potential Benefits of Hemoglobin-Based Oxygen Carriers

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Abstract: Currently, there is an unmet therapeutic need for the medical management of cardiac arrest, as is evident from the high mortality rate associated with this condition. These dire outcomes can be attributed to the severe nature and poor prognosis of this disorder. However, the current treatment modalities, while helping to augment survival, are limited and do not offer adequate improvements to outcomes. Treatment modalities are particularly lacking when considering the underlying pathophysiology of the metabolic phase of cardiac arrest. In this study, we explore the three phases of cardiac arrest and assess the factors related to positive clinical outcomes and survival for these events. Furthermore, we evaluate the present guidelines for resuscitation and recovery, the issues related to ischemia and tissue reperfusion, and the benefit of oxygen-delivery therapeutic methods including blood transfusion therapy and synthetic hemoglobins (HBOCs). The current therapy protocols are limited specifically by the lack of an efficient method of oxygen delivery to address the metabolic phase of cardiac arrest. In this article, we investigate the next generation of HBOCs and review their properties that make them attractive for their potential application in the treatment of cardiac arrest. These products may be a viable solution to address complications associated with ischemia, reperfusion injury, and organ damage.

Keywords: cardiac arrest; tissue reperfusion; cardiac resuscitation; oxygen delivery; hemoglobin-based oxygen carriers (HBOCs)

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1. Introduction

During recent decades, modern medicine has benefited from many advancements addressing common and familiar ailments, allowing for improved mortality and morbidity for a large variety of pathologies. One area which has seen relatively little improvement is the treatment of cardiac arrest. Many studies have been conducted and published on this topic to increase our understanding of the underlying pathology, mechanisms, and sequence of events involved in this condition, and these have guided the search for new therapeutic approaches. However, the mortality in patients suffering from cardiac arrest still remains very high. Ischemic heart disease is a major cause of death worldwide and is categorized as either out-of-hospital cardiac arrest (OOHCA) or in-hospital cardiac arrest (IHCA). In the USA, approximately 360,000 people suffer from OOHCAs and this pathology has a staggeringly low survival rate, estimated to be 10% to time of discharge from the hospital [1]. Meanwhile, recent studies from the literature estimate that between 370,000 to 750,000 IHCAs occur annually in the US, with recent meta-analyses showing a pooled survival rate of 15% with minimal change over time. This event also confers significant morbidity afterwards, with an estimated 1-year survival rate of 13.4% [2,3].

This review article aims to explore cardiac arrest as a currently significant and prominent pathology that also has significant mortality. First, we investigate predictors of survival and also define the three phases of cardiac arrest: the electrical (first) phase, the circulatory (second) phase, and the metabolic (third) phase. We then delve into the third phase of cardiac arrest, focusing on ischemia, complications of this pathology, and reperfusion injury. We then explore the current guidelines addressing oxygen delivery, which theoretically should address the underlying pathology of the third phase of cardiac arrest. Finally, we first investigate hemoglobin-based oxygen carriers (HBOCs) broadly and then perform an in-depth analysis of the novel generation. It is possible that this class of products may offer a benefit to the mortality associated with cardiac arrest. This review does explain the breadth of etiologies and pathophysiology associated with cardiac arrest but ultimately focuses on cardiac arrest in the setting of arrhythmias that are considered shockable rather than non-shockable rhythms. This review was based on a comprehensive PubMed search of the topic of cardiac arrest, blood transfusions, and hemoglobin-based oxygen carriers. Additional details regarding this search are described in Supplementary File 1.

2. Cardiac Arrest and Predictors of Survival

The term cardiac arrest is a broad term with a variety of potential etiologies ranging from underlying heart disease and arrhythmia to sepsis and trauma [4–6]. Arrhythmic causes of cardiac arrest are the most common, the most classically described, and the target of modern-day therapeutics for cardiac arrest, and as such they are the focus of this review article. These arrhythmic causes are subdivided based on their therapeutic approach, with "shockable" rhythms including ventricular fibrillation and pulseless ventricular tachycardia, while "non-shockable" rhythms include pulseless electrical activity and asystole [4,7]. The therapeutic approach applied for arrhythmic cardiac arrest is described in the form of an algorithm in the advanced cardiovascular life support (ACLS) guidelines. Generally, shockable rhythms are managed through a combination of cardiopulmonary resuscitation (CPR) and defibrillation with medications, while non-shockable rhythms are managed primarily with CPR in addition to various medications [8].

The predictors of survival differ between IHCA and OOHCA. Considering OOHCA, the elements of the chain of survival first described in 1991 are paramount. The chain of survival requires the timely functioning of bystanders, dispatchers, first responders, paramedics, and, finally, hospital care [9]. Ultimately, survival has been linked to appropriate timely response by the earliest members of the chain of survival, which includes prompt recognition, initiation of CPR, and, ideally, minimizing time to defibrillation, if applicable [4]. It has been shown that survival to hospital discharge is improved for OOHCA that is witnessed by a bystander/EMS (Emergency Medical Service), in patients who received bystander CPR, in patients with shockable rhythms, and in patients who were able to achieve in-field return of spontaneous circulation (ROSC) [10]. Furthermore, it has been shown that patients who suffer from OOHCA have improved outcomes when transported to medical centers that are specialized and capable of managing cardiac resuscitation with available services such as cardiac catheterization [11]. When considering IHCA, the chain of survival is not as prominent in ensuring improved survival, as patients theoretically are already at the highest level of care required to manage this condition. Instead, survival is influenced by patient factors, with reduced survival in older-aged patients, in black patients, and in those with various clinical diagnoses including sepsis, renal failure, metastatic cancer, stroke, and house-bound lifestyle [3]. More generally, improved outcomes have been linked with shorter durations of CPR and more rapid attainment of ROSC [12]. The benefit that has been shown with the application of thrombolytics in cardiac arrest suggests that myocardial blood-flow occlusion is a major component of negative outcomes [13]. Studies have also shown dysfunction of the hypothalamic–pituitary–adrenal axis after cardiac arrest, with decreased levels of cortisol, which suggests that a decrease in the body's response to stress likely plays a role in negative outcomes as well [14]. Furthermore, there are a select few accessory therapies that should be applied in specific scenarios

to help improve mortality. One such therapy is therapeutic hypothermia, which is also known as targeted temperature management. When applied following cardiac arrest, this intervention has been shown to confer a 30% improvement in mortality [15].

3. The Three Phases of Cardiac Arrest

The classically described therapeutic approach to cardiac arrest resuscitation utilizes a rhythm-based approach but does not account for time elapsed after the onset of cardiac arrest. Conversely, cardiac arrest can be viewed using the three-phase time-dependent model first described in 2002 [16]. This three-phase model remains the basis for the current treatment paradigm and each stage features different degrees of ischemia, risks of reperfusion injury, and therapeutic approaches, which target the unique underlying pathophysiology.

3.1. First (Electrical) Phase

The electrical phase begins with the onset of cardiac arrest and lasts for about 5 min. The therapeutic gold standard for this phase is early and rapid defibrillation. Patients who are able to receive appropriate early treatment have survival rates exceeding 60% [16].

3.2. Second (Circulatory) Phase

The circulatory phase of cardiac arrest is classically defined as existing between 5 and 10 min after the initiation of arrhythmic cardiac arrest. Improvements to mortality within this phase are most seen with maximization of blood flow to the myocardium. As such, the greatest benefit arises from chest compressions with intermittent defibrillation, if applicable. However, the emphasis on chest compressions that is required to improve mortality is not commonly employed, as it becomes very difficult to pinpoint and distinguish the phases of cardiac arrest in real-life clinical situations [16].

3.3. Third (Metabolic) Phase

The metabolic phase of cardiac arrest typically begins 10 min after the initiation of cardiac arrest and is responsible for the largest proportion of deaths among the phases. The failure of treatment modalities during the metabolic phase suggests that both defibrillation and CPR are not adequate as resuscitation measures [16]. The underlying pathophysiology of the metabolic phase of cardiac arrest involves a lack of adenosine triphosphate (ATP) generation due to ischemia, which disrupts the intracellular redox balance and causes the generation of reactive oxygen species. The decline in ATP levels cause failure of cellular machinery responsible for maintaining ion gradients, which causes secondary swelling of the mitochondria [17]. Meanwhile, the body suffers from a state of global ischemia, which causes a variety of pathologic processes including the release of endotoxins and cytokines. These factors suppress myocardial contractility and can be lethal to cardiomyocytes [18].

While the exact cytokine profile that categorizes the metabolic phase has not been established, it is likely very similar to the cytokine profile that is seen in the systemic ischemia/reperfusion response that arises with ROSC following a cardiac arrest. The systemic ischemia/reperfusion cytokine profile is similar to that of sepsis and is characterized by polymorphonuclear leukocyte activation, activation of inducible nitric oxide synthase, and the production of cytokines such as tumor necrosis factor alpha and interleukin-6, both vitally important for generalized immune activation. This response produces a pathological vasodilatory response while causing cardiodepression, which impedes resuscitative efforts [19]. From this comes the benefit of the application of epinephrine during cardiac arrest, which acts on α1-adrenergic receptors to cause vasocontriction in non-vital peripheral organs and increase coronary and cerebral blood flow [20]. Furthermore, in the metabolic phase, the health-care provider is placed in a complicated scenario, where successful CPR can re-introduce the metabolically deranged body to near-normal levels of oxygenation, which induces reperfusion injury as discussed below.

4. Ischemia and Supplemental Oxygen

Ischemia is a state of oxygen deprivation which occurs as a consequence of cardiac arrest when the heart is unable to appropriately circulate oxygenated blood. Ischemia in cardiac arrest is not only found in systemic tissues but is a major effector, as it occurs in the myocardium. The global ischemia characteristic to cardiac arrest is evidenced by elevated lactate levels, a hallmark of this pathology. Furthermore, the persistence of an elevated lactate level may be due to complications following cardiac arrest, including systemic inflammatory response, ongoing hypoxia, and cardiogenic shock due to myocardial dysfunction [21]. This widespread ischemia is known to produce a large quantity of reactive oxygen species (ROS), which are potentially toxic to most biological tissues, causing enzyme inactivation, protein oxidation, and mitochondrial respiratory chain inhibition [22].

The generation of this various detrimental cellular free radical generations are believed to be the culprit for the consequences of hyperoxia—the alveolar oxygen concentration exceeds that of normal breathing conditions. Supplemental oxygen is currently ubiquitous in the management of cardiac arrest, but more recent studies have shown that targeting oxyhemoglobin saturations of 94–98% can prove more beneficial to overall survival [22]. The underlying pathophysiology behind the detrimental effects of oxygen supplementation in cardiac arrest extends beyond simply the generation of ROS and is not completely understood. It has been suggested that elevated levels of inspired oxygen in ischemic heart disease reduce coronary blood flow while increasing coronary vessel resistance [23].

5. Reactive Oxygen Species and Reperfusion Injury

Under physiologic conditions, reactive oxygen species (ROS) are tightly regulated within cardiac myocytes and act as molecules that regulate gene transcription and alter enzymatic activity [24]. Reactive oxygen species are produced within cardiac myocytes by NADPH oxidases (nicotinamide adenine dinucleotide phosphate oxidase), xanthine oxidase, and nitric oxide synthase within the mitochondria as byproducts of cellular respiration. The balance within myocytes is maintained similarly to cells throughout the body by a careful equilibrium of ROS-producing pathways and neutralizing enzymes, including superoxide dismutase, catalase, and glutathione peroxidase [25]. Notably, during periods of ischemia, elevated ROS production from mitochondrial dysfunction opens various mitochondrial channels to cause ROS-induced ROS release [24]. The increase in ROS goes on to cause significant oxidative stress to myocytes and impair ATP generation [24,26]. This explains the myocardial component of reperfusion injury that typically occurs following restoration of blood flow after cardiac arrest. Reperfusion injury can be viewed within the myocardium specifically, but it can also be viewed systemically as a similar mechanism occurs in all tissues deprived of oxygen, causing increased production of ROS and resultant cellular dysfunction. Reperfusion injury systemically results in reperfusion syndrome, which can involve platelet aggregation, complement activation, capillary leak, tissue edema, and impaired microcirculation to vital organs [27]. The ROS generated due to ischemia are the basis of the ischemia/reperfusion injury, involving the release of cytokines like interleukin-6 and tumor necrosis factor alpha [19].

6. Therapeutic Hypothermia and Preconditioning

Therapeutic hypothermia is a treatment option for select patients following cardiac arrest that targets the generation of ROS to help provide benefits [28]. Specifically, hypothermia is therapeutically indicated for unconscious survivors of cardiac arrest, following ventricular fibrillation, and its application outside of these parameters and for pathologies other than cardiac arrest is not well-described [29]. This therapy involves cooling comatose survivors to target temperatures of 33–36 °C (91.5–96.8° F), with cooler target temperatures being indicated in patients with evidence of severe brain injury [28]. Therapeutic hypothermia is employed primarily for its neuroprotective effect. In neurons, the depletion of ATP in the setting of ischemia causes a buildup of cellular calcium, causing depolarization and release of neurotransmitters such as dopamine and glutamate, which

go on to enact downstream effectors to induce cellular damage [30]. Since the release of these neurotransmitters from neurons is temperature-dependent, therapeutic hypothermia helps to reduce this process and preserves neurons [30]. This therapy also helps to improve neurologic outcomes by reducing neuron metabolism and ROS generation [31]. However, it is important to note that therapeutic hypothermia is known to be arrhythmogenic and malignant arrhythmia is an unfortunate complication of this therapy [32]. Hypothermia prolongs the action potential duration and increases the heterogeneity of ventricular repolarization to predispose the myocardium to arrhythmia [33]. This has been explained by potassium shifts that occur during hypothermia resulting in systemic hypokalemia, which is linked to the development of polymorphic ventricular tachycardia [32]. It is interesting to contrast these studies with those investigating hypothermia as an adjunct to cardioplegia during cardiac surgery. In this setting, a decrementing increase in sodium influx helps to impair the severity of calcium influx following restoration of calcium levels and reduce hypercontractility and cellular injury [34]. During surgery, hypothermia functions to significantly reduce metabolism and oxygen consumption and attenuate the injury incurred by ischemia [35].

Other therapies that target the generation of ROS have been studied but are not applied clinically today. It has been shown that the administration of polyethylene glycol-superoxide dismutase 24 h prior to global ischemia events helped to reduce reperfusion injury [36]. Many of the studies and potential therapies associated with ROS level modification are currently impractical in the clinical setting and their continued research is invasive and costly.

Preconditioning is a phenomenon associated with myocardial ROS that has been described within the myocardium in which brief episodes of ischemia activate cardioprotective mechanisms, which help to minimize the effect of ischemia and ROS on the myocardium [37]. This phenomenon is believed to occur due to the production of ROS, which then modify gene expression to improve cellular tolerance to impending ischemia [24]. This phenomenon can be observed naturally in patients with intermittent arrhythmias and coronary artery disease but is also studied for application in cardiac surgery [37,38].

7. Clinical Complications of Cardiac Ischemia

The clinical complications that follow cardiac arrest are varied and contribute to the high mortality rate associated with this condition. There are a variety of complications that stem from the ischemia and metabolic dysfunction that arise within the myocardium, and they share a similar pathophysiology to the third phase of cardiac arrest. These complications include ventricular free-wall rupture, papillary muscle rupture, and aneurysm formation, which arise along different timeframes following myocardial infarction and result in increased morbidity and mortality [39]. An often overlooked complication of cardiac arrest, which is defined partially by the complications arising from the metabolic derangements of the third phase of cardiac arrest, is post-cardiac arrest syndrome. This syndrome is complex and unique to each patient, as it results from the myocardial ischemia and metabolic derangements, as described above, superimposed upon the underlying etiology for the cardiac arrest and underlying patient co-morbidities. Classically, this syndrome is defined by post-cardiac arrest brain injury, post-cardiac arrest myocardial dysfunction, the systemic ischemia/reperfusion response, and the persistence of the precipitating pathology [40]. The management of post-cardiac arrest syndrome is typically approached similarly to septic shock, with emphasis on hemodynamic care in the form of fluid administration and inotropes in addition to therapeutic hypothermia for select patients [41]. These complications all arise due to the metabolic derangements that result from the decline in oxygen delivery. As previously stated, no therapeutic options that are currently employed address this pathophysiology directly.

8. Perfusion of Vital Organs

The myocardial complications of cardiac arrest are plentiful and, in combination with the large-scale systemic dysfunction that arises due to reperfusion injury, cause significant damage to numerous tissues and organs throughout the body. However, it is important to consider a more basic complication of cardiac arrest: the cessation or reduction of blood flow to vital organs. It is known that improved vital organ perfusion during resuscitation and following cardiac arrest is associated with improved outcomes [42]. Critical organs including the brain and myocardium are particularly susceptible to ischemia, and perfusion maintenance is vital to sustain their high metabolic activity [43].

The majority of the therapies utilized for cardiac arrest aim to maintain and restore vital organ perfusion. Optimal closed-chest CPR is known to generate circulation equivalent to 25–33% of normal cardiac output and as such is a major weak point in the resuscitation algorithm [44]. Percutaneous left ventricular assist devices (LVADs), such as Impella (Abiomed, Danvers, MA, USA) devices, have been applied in the acute setting to maximize cardiac output and improve vital organ perfusion. This therapy also has the benefit of continuing to augment cardiac output following ROSC and improving overall functionality after cardiac arrest [42]. These therapies have promise but are accessible only to select advanced medical centers. Meanwhile, therapy involving vasopressor medications, such as epinephrine, has been applied for cardiac arrest for the last 60 years. This therapy relies on its ability to combat the pathological vasodilation seen in the third phase of cardiac arrest and the vasoconstriction it causes diverts blood flow to maintain perfusion of vital organs [45]. While this therapy is known to result in improvements in outcomes, the high mortality associated with cardiac arrest shows that further therapies are required. The development of therapies that enhance oxygen delivery to vital organs is vital to the improvement of cardiac arrest resuscitation.

9. Limitations of Blood Transfusion Therapy in Cardiac Arrest

Thus far, this article has primarily discussed the metabolic complications associated with cardiac arrest in reference to an arrhythmic etiology. However, when considering the broad range of therapies offered for cardiac arrest, it is important to discuss blood and intravenous (IV) fluid administration, which is most commonly seen in cardiac arrest resulting from blunt trauma. This scenario often presents clinically with severe blood loss and hemorrhagic shock. In this setting, it has been shown that IV fluid administration decreases mortality by helping to prevent vascular collapse [46]. However, it is important to recognize that this therapy offers no benefit beyond volume replacement, while the use of blood products helps to restore intravascular volume while also repleting oxygen delivery capacity. This therapy comes with the risk of infection and adverse immunological reactions, while survival rates in patients treated with blood transfusion are not significantly improved [46]. Retrospective studies have shown that traumatic cardiac arrest patients treated with packed red blood cells (PRBCs) had improved likelihood of attaining ROSC (44.6% compared to 28% in control groups). However, there was minimal improvement between studied groups in survival until discharge (3.4% compared to 2.4% in control) [47]. With this in mind, it is clear that administration of PRBCs provides a benefit to patients, likely due to their capacity to support both circulation and oxygen delivery. It is also interesting to recognize that this therapeutic option theoretically has the potential to help target the underlying pathophysiology of the metabolic phase of cardiac arrest. It is unclear why an improvement in the rate of ROSC does not translate into improved survival, but an investigation into more efficient oxygen delivery methods is warranted to address this disparity.

It is important to recognize the shortcomings of red blood cell therapy when considering other oxygen delivery solutions for their potential application for cardiac arrest. During PRBC storage, potassium leakage through the red blood cell membrane generates a hyperkalemic supernatant solution, which explains the association between rapid blood transfusion and hyperkalemia in emergency settings [48]. Furthermore, it has been shown

that cardiac arrest is normally associated with a hyperkalemic state, with serum potassium levels of 5.63 ± 2.39 mmol/L [49]. As such, this population has a significant baseline risk for hyperkalemia and treatment with PRBCs exacerbates this issue, with measured potassium levels of 8.23 ± 1.99 mmol/L [49]. It has been suggested that washing of PRBC units prior to administration can help to prevent this complication, but this practice is not feasible in the emergency setting [48]. Currently, the electrolyte imbalances resulting from blood transfusion therapy generate the potential to exacerbate cardiac arrest and are major limitations to the application of this treatment.

10. Hemoglobin-Based Oxygen Carriers as a Potential Therapeutic Option for Cardiac Arrest

The use of hemoglobin-based oxygen carriers (HBOCs) is a novel method for systemic oxygen-delivery therapy that is actively being researched. This therapy, named "artificial blood", aims to emulate the function of hemoglobin in circulating red blood cells by delivering oxygen. HBOCs are classically solutions that are composed of acellular hemoglobin and deliver oxygen to tissues along the normal diffusion gradient [50]. This therapy option offers several advantages over PRBC transfusions including long shelf-life, the option for non-refrigerated storage conditions, the prevention of pathogenic agent transfer during blood transfusion, and the elimination of the need to cross-match blood types [51]. HBOCs can help to resolve the storage issues related to the use of PRBCs, including the potassium leakage that develops during storage discussed above. HBOCs also eliminate any immune reactions associated with transfusion procedures and can potentially also be used on patients who reject blood transfusion for religious reasons [50]. Nephrotoxicity is also a potential complication of PRBC transfusion therapy due to the release of unmodified free hemoglobin as red blood cell lyse. HBOCs are engineered with several modifications to help address the shortcomings of PRBC therapy and some have minimal renal excretion [52].

Earlier-generation HBOCs contained hemoglobins that were chemically modified using non-site-specific techniques, including conjugation and polymerization. These changes were made in an attempt to alter the physiological parameters of the hemoglobin molecules to increase their overall size and decrease their oxygen affinity and renal clearance [50,53]. It is important to note that, although the size of the hemoglobin molecules is increased, they are still significantly smaller compared to other blood components, such as red blood cells. As such, HBOCs have the potential to flow with plasma past areas of vessel occlusion, which normally prevents the passage of larger blood components, such as red blood cells.

One of these earliest HBOCs was a cross-linked hemoglobin tetramer known as diaspirin cross-linked hemoglobin. However, this product was found to decrease cellular perfusion and, as a result, drastically decreased the survival rate of study subjects [50,54]. This study and others similar to it demonstrated a need to further refine HBOCs before trials can be carried out.

Further chemical modifications, including conjugation to non-protein particles such as polyoxyethylene (POE) or polyethylene glycol (PEG), were introduced to diminish extravasation while retaining a low oxygen affinity [50,55]. Studies suggest that increasing the size of the HBOCs through polymerization or cross-linking diminishes many of the adverse effects associated with these products [50,55]. However, the methods by which these chemical modifications were performed were imprecise and resulted in non-homogeneous polymerization and conjugation products. This heterogeneity caused HBOCs produced by this methodology to have unforeseeable physiological and biochemical traits [50].

The proposed chemical modifications to hemoglobin produced a multitude of changes to its properties, including the alteration of its oxygen equilibrium curve, a decrease in cooperativity, the loss of the Bohr effect, and a decreased ability to bind CO_2. It was also noted that autoxidation of heme in most of these HBOCs occurred two to three times faster compared to the rate for unmodified hemoglobin, resulting in a decreased redox potential and the loss of heme [50]. Furthermore, the long half-lives of several HBOCs led to over-exposure of the products to the body's degradative and oxidative processes, causing the release of more free-heme into circulation. Aside from PolyHeme (Northfield

Laboratories Inc., Evanston, IL, USA) and pegylated human hemoglobin, it was found that most other HBOCs are highly susceptible to oxidative stress, leading to increased levels of ferryl hemoglobin in circulation [50]. On the other hand, these HBOCs did improve mitochondrial respiration significantly [50].

Despite many of these advancements having optimistic outcomes in initial preclinical and clinical studies, a JAMA (Journal of American Medical Association, US) meta-analysis by Natanson completely dismissed earlier-generation HBOCs as unsafe and too dangerous to be used in clinical settings, suggesting that all of the current HBOCs increase the risk of myocardial infarction and mortality. These outcomes were believed to be caused by the extravasation of HBOCs, leading to the depletion of NO and heightened vascular tone that hindered blood flow. The analysis also detailed further side effects, including the prevention of platelet inactivation and release of pro-inflammatory substances [51]. This analysis stifled the development of many HBOCs and slowed research within the field.

Currently, a novel generation of HBOCs (NG-HBOCs) is being developed to address the shortcomings brought forward by Natanson's meta-analysis. Research has been focused on finding solutions to circumvent the mentioned side effects, particularly extravasation and NO depletion. NG-HBOCs employ novel methods for the synthesis of hemoglobin polymers, such as a zero-linked polymerization process that helps to prevent extravasation [56].

Considering their functional similarity to PRBC transfusions as well as their many potential advantages, HBOCs are a logical candidate as a therapeutic option for cardiac arrest. Animal studies have been performed that reveal the potential benefits of these therapies. Manning et al. examined the effectiveness of HBOC-201 in promoting ROSC in cardiac arrest in a swine exsanguination model [57]. The addition of oxygenated HBOC-201 to selective aortic arch perfusion (SAAP) was evaluated and compared to oxygenated lactated Ringer's (LR) solution to determine the impact of HBOC-mediated oxygen delivery on circulation and survival rates. Of the six swine that received the HBOC-201 therapy, all six achieved ROSC at 1.9 ± 0.3 min, while five reached survival at one hour [57]. In comparison, only two of the six swine in the lactated Ringer's (LR) group reached ROSC with the addition of epinephrine, and none survived past the hour [57]. These results demonstrate the potential benefit of the early administration of HBOCs in the setting of cardiac arrest resuscitation. The benefit of the use of HBOCs over LR solution can be likely attributed to the ability of these solutions to effectively deliver oxygen to deprived tissues. The benefit seen in animal studies warrants continued research and development of these products.

11. Characteristics of Novel-Generation HBOCs (NG-HBOCs)

There are a small number of products that have been developed that are considered NG-HBOCs. This class of compounds employs new methods of synthesis and production, seeking to improve the biological efficacy and safety compared to previous generations. One NG-HBOC utilizes a zero-link polymerization process to produce ultra-high-molecular-weight hemoglobin polymers while having a viscosity similar to human plasma in solution [56,58]. Being larger than any vascular pore, NG-HBOCs with high molecular weights are incapable of extravasation and thus cannot deplete local NO to cause vasoconstriction, assisting in the maintenance of a suitable mean arterial pressure (MAP) [56,59,60]. One beneficial aspect of certain NG-HBOCs is their high oxygen affinity, ensuring that oxygen is offloaded primarily to severely hypoxic tissues [56,59,61]. Animal studies employing NG-HBOCs have been performed that have affirmed their ability to deliver oxygen and offload significant amounts of oxygen to hypoxic tissues when administered in low volumes, while not hindering the coagulation capacity of blood [54,59,62].

Since most acellular hemoglobins are prone to oxidation and denaturation, it is of utmost importance to minimize oxidative changes when introducing an acellular hemoglobin into the circulatory system. Furthermore, in order for an HBOC to work effectively as an oxygen carrier, it must remain in the reduced state (heme-Fe^{2+}) in the circulatory system. With regard to NG-HBOCs, it is known that ascorbic acid, an endogenous reducing agent

present in human plasma, is effectively able to maintain these products in their reduced state [58]. Furthermore, the stability of NG-HBOCs helps to ensure that minimal loss of heme iron occurs following infusion [63]. Some NG-HBOCs are available in both powder and liquid forms and have been shown to be resistant to oxidative changes, such as through exposure to urea, a strong denaturant [56,58]. This fact suggests that NG-HBOCs have the structural integrity and the redox stability required to emulate normal hemoglobin function.

Numerous animal studies have been performed employing NG-HBOCs. One study by Mito revealed that one NG-HBOC, when applied to an artificially induced stroke in an animal model, reduced the size of affected brain tissue by 40% [64]. Another experiment by Reynolds found that small-volume resuscitation of Long–Evans rats with an NG-HBOC improved survival rate [61]. A study by Ning revealed that poly-hemoglobin and purified stroma-free hemoglobin do not activate complement as complement system activation is attributed to the presence of cell membrane debris and endotoxins [65]. Therefore, it is logical that some NG-HBOCs should also not activate complement, as their structures are remarkably similar to the molecules examined by Ning. This would help to minimize reperfusion injury during the treatment of cardiac arrest.

The characteristics and successes of NG-HBOCs warrant further investigation into the application of these compounds for cardiac arrest resuscitation. These products could help to address the pathophysiology of the third phase of cardiac arrest by promoting oxygen delivery to tissues. Further trials are required to assess the application of these products in large animal models prior to their potential application in human clinical trials.

12. Conclusions

Existing therapeutic guidelines have proven inadequate in controlling the high mortality rate associated with cardiac arrest, and the development and improvement of these guidelines would greatly benefit medical care [1–3]. The current treatment paradigm involving defibrillation and chest compressions targets the pathophysiology of the first two phases of cardiac arrests but does not address the third phase, which results in metabolic derangement and organ injury [16]. Previously attempted but unsuccessful therapies addressing this third phase include blood transfusion therapy and first-generation HBOCs. Novel-generation HBOCs are being developed to address the shortcomings of first-generation HBOCs and have been shown in animal studies to be successful in resuscitation and reducing tissue injury [56,58,59,61,64]. Through a mechanism of oxygen delivery, it is possible that NG-HBOCs, if administered during the earlier stages of cardiac arrest, may offer a benefit to mortality.

Supplementary Materials: The following are available online at: https://www.mdpi.com/article/10.3390/cardiogenetics12010004/s1, Supplementary File 1: Methods.

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