Title: PYRUVATE CARDIOPLEGIA SOLUTIONS FOR ADMINISTRATION TO THE HEART DURING CARDIOPULMONARY SURGERY AND METHODS OF USE THEREOF

Abstract: The invention includes a novel cardioplegia solution in which pyruvate serves as the primary energy source. The solution may be used in any surgery where the heart must be arrested, but it is particularly useful in cardiopulmonary bypass surgery. Because the solution relies primarily upon pyruvate to protect the heart from damage during and immediately after arrest, other additives are not as necessary as with current cardioplegia solutions or are not necessary at all. The invention also includes a method for making the cardioplegia solution and a process of performing cardiopulmonary bypass surgery using the novel cardioplegia solution.
PYRUVATE CARDIOPLEGIA SOLUTIONS FOR ADMINISTRATION TO THE HEART DURING CARDIOPULMONARY SURGERY AND METHODS OF USE THEREOF

BACKGROUND OF THE INVENTION

This invention concerns a novel pyruvate-containing cardioplegia solution and the use of pyruvate in its manufacture. The cardioplegia is used to arrest the heart when the solution is introduced into the heart's coronary blood vessels. Chemical components of the solution protect the heart from injury and preserve heart muscle tissue throughout the period of cardiac arrest, allowing for improved post-surgical recovery of the heart's contractile performance as compared to lactate or glucose-based solutions currently used in the art.

Heart surgeries, such as coronary artery bypass grafting, valve replacement and repair of structural defects of the heart, are delicate procedures demanding a high level of surgical precision. It is typically necessary to temporarily stop the heart beat during these procedures so that the organ remains motionless, thereby facilitating the surgeon's work. The heart beat is arrested by introducing into the heart's coronary blood vessels special aqueous solutions, termed cardioplegia solutions, which contain chemicals that interrupt the physiological processes that cause the heart to beat.

A mechanical pump assumes the heart's role of supplying blood to the rest of the body while the heart is arrested. However, the heart's own coronary blood flow is interrupted during cardiac arrest and is not restored by the mechanical pump; the heart becomes ischemic. Coronary blood flow is critical because it supplies the heart with the fuels and oxygen it needs to generate ATP, the heart's main source of chemical energy. ATP supplies energy for numerous cellular processes that enable the heart to pump blood and which sustain the cells of the heart muscle. Cardiac arrest minimizes the heart's energy demands by interrupting the major energy-consuming process, that of pumping blood, but some energy is still required to support other cellular functions. Consequently the heart's energy reserves are depleted, albeit slowly, during cardiac arrest, due to the lack of coronary blood flow.

The gradual expenditure of energy reserves during cardiac arrest threatens to injure the heart's muscle tissue. Attempts have been made to minimize this energy depletion by
adding energy-yielding fuels to cardioplegia solutions. Thus, cardioplegia solutions have utilized glucose, lactic acid and amino acids to support energy production in the arrested heart.

Glucose and lactate are readily metabolized by the normal, healthy heart muscle, i.e. that with normal coronary blood supply. However, these compounds are ineffective fuels when coronary blood flow and delivery of oxygen is interrupted. The lack of oxygen causes massive accumulation of the metabolic cofactor NADH. As NADH accumulates within the heart muscle, it restrains metabolic activities of the enzymes glyceraldehyde 3-phosphate dehydrogenase and lactate dehydrogenase, which are required to consume glucose and lactate, respectively. See Kobayashi and Neely, 1979. Eventually energy production from glucose and lactate ceases. Moreover, NADH is harmful to the arrested heart because it serves as a precursor of harmful oxygen free radical compounds which injure the heart muscle cells. See Mohazzab-H et al. 1997 and Vandeplassoche et al., 1989.

Amino acids, particularly glutamate and aspartate, also have been tested as components of cardioplegia. See U.S. Pat. Nos. 4,988,515 to Buckberg and 5,290,766 to Choong. It has been proposed that these compounds could bolster the heart's energy-generating capacity by increasing activity of the Krebs cycle, the main energy-producing metabolic pathway in the heart cells. However, this pathway cannot generate energy when coronary blood flow and oxygen supply are interrupted, as during cardiac arrest.

Aside from energy depletion, oxygen-centered free radical compounds are another potential cause of injury to the heart muscle during and following cardiac arrest. See Bolli and Marbán, 1999; Bolli, 1991. During cardiac arrest, changes in the metabolic composition of the heart muscle, including accumulations of calcium ions and the metabolic cofactor NADH, set the stage for formation of massive amounts of free radicals when oxygenated blood is reintroduced into the coronary blood vessels after grafting is complete. Free radical compounds readily react with the normal constituents of cells, leading to chemical modifications of numerous proteins and membrane phospholipids that adversely affect the function of these biomolecules. By these mechanisms, free radicals injure or even kill heart muscle cells, leading to loss of functioning heart tissue and myocardial infarction. Cells are equipped with antioxidant molecules that defend the cells from free radical attack, but these endogenous antioxidant reserves are limited and are
inadequate to protect the heart from the enormous free radical assault triggered upon resumption of coronary blood flow. Conceivably, addition of antioxidant chemicals to cardioplegia solutions could help protect the heart by neutralizing harmful free radicals as they are generated. Neither glucose, lactate nor amino acids are antioxidants.

The addition of pyruvate to cardioplegia solutions overcomes the disadvantages of other fuels because of its unique effects on the energy metabolism and antioxidant biochemistry of the heart muscle cells. Unlike glucose and lactate, pyruvate does not generate NADH but instead lowers NADH concentration within the cell's cytoplasm, enabling glucose metabolism to continue during cardiac arrest or following restoration of coronary blood flow (Bünger et al., 1989). Pyruvate produces a substantially higher energy state in the heart muscle than do other fuels (Bünger et al., 1989; Tejero-Taldo et al., 1998; Mallet and Sun, 1999), thereby providing more chemical energy to be used by the heart to pump blood. These metabolic actions enable the pyruvate-treated heart muscle to recover its pumping performance more rapidly following surgery than heart muscle treated with other fuels (Bünger et al., 1989; Mallet, 2000).

In addition to serving as an excellent fuel for energy production, pyruvate is a powerful antioxidant in the heart. Pyruvate's unusual \( \alpha \)-keto carboxylic acid chemical structure enables it to directly react with and neutralize harmful oxygen free radical compounds (Constantopoulos and Barranger, 1984). In addition, metabolic conversion of pyruvate to citrate (Mallet and Sun, 1999; Tejero-Taldo et al., 1999) indirectly increases the amount of glutathione, the main antioxidant chemical in heart muscle cells. Glutathione is maintained by the metabolic cofactor NADPH. Citrate generated from pyruvate increases the amount of NADPH in the heart cells by increasing the metabolic activity of two processes that produce NADPH, namely, isocitrate dehydrogenase and the hexose monophosphate pathway. NADPH is used to restore glutathione consumed to neutralize free radicals. By this mechanism, pyruvate bolsters the heart muscle's natural defenses against free radicals.

Previous researchers have sometimes included pyruvate as an energy source in cardioplegia solutions, but they have failed to recognize its protective and other properties and have therefore additionally included other protective components. These additional components render the solution more costly than need be and the unnecessarily complex
formulation provides greater chance for errors in preparation and increases the chance for adverse reaction to the cardioplegia solution. For instance in U.S. Pat. No. 4,988,515 to Buckberg et al., pyruvate is a potential energy source for the claimed cardioplegic solution, as are glucose, fructose and malate. Because Buckberg failed to recognize the favorable actions of pyruvate, other modifications of the solution were made to avoid damage to the heart. Primarily these modifications include limiting the calcium ion concentration of solution to between 50 and 300 mol and providing a mechanism to maintain the metabolizable substrate concentration at 400-1000 mg% and osmolarity at an increased level of 400-500 mOsmol. Because Buckberg added these protective measures, he clearly failed to recognize the protective potential of pyruvate as the primary metabolite alone, resulting in an overly complicated cardioplegia solution.

In another patent, U.S. Pat. No. 6,153,647 to Mallet et al., the use of pyruvate to treat cardiac trauma is disclosed. However, the pyruvate is used to increase the inotropic effects of a β-adrenergic agonist which is co-administered, i.e., the desired effect is to increase the heart's contractions. Thus, the composition would not be used during surgery where it is undesirable to stimulate the heart's function due to the primary objective of arresting the heart. Similar effects using the same type of composition are described in isolated guinea-pig hearts in Tejero-Taldo, et al., 1998 and 1999. The applicability, if any, of these results to in situ guinea pig hearts of human patients is also not clear.

Similarly, in Mallet, 2000, the use of pyruvate to increase contractile properties of stunned heart tissue is discussed. Based upon this information, one would administer a pyruvate solution to a heart which one wishes to resume beating, but not to an arrested heart which is to remain quiescent for surgery. Four additional studies, Bünger and Mallet, 1994 and 1993, Mallet et al., 1990 and Bünger et al., 1989, address the actions of pyruvate in non-ischemic or in stunned hearts, but fail to address its uses in an arrested heart.

Additionally, these studies in isolated guinea pig heart are of uncertain relevance to human heart surgery. Although such studies are useful in ruling out immediate harmful effects and indicating that some positive effect may be possible, because of physiological differences between mammals, and especially between small mammals such as guinea pigs and large mammals such as humans, it is far from certain that any useful effect seen
in a guinea pig heart will be duplicated in a human heart. Also the extent of any useful effect is likely to vary a great deal among species.

Furthermore, in these guinea pig studies, the objective was to increase post-ischemic function of guinea pig hearts, whereas the in the present invention the cardioplegia is applied during ischemia to increase cardiac function at that time so the heart can recover more completely after the ischemia. The correlation between an increase in post-ischemic cardiac function and an increase in cardiac function during ischemia is not to such a degree that predictions of one effect may be based upon experiments to study the other.

Finally, there are obvious differences between using an isolated heart and a heart still located in a living organism which is intended to continue to function after the test. These differences alone make it nearly impossible to predict whether useful results will be obtained in a human surgery without doing such experiments in a human. Thus, guinea pig studies primarily serve to rule out severe safety implications and to indicate whether further experimentation using human patients is warranted.

Others have addressed the use of solutions containing chemical derivatives of pyruvate in post-ischemic events. For example, in three patents to Brunengraber et al., U.S. Patent Nos. 5,667,962, 5,876,916, and 5,968,727, a pyruvate solution is used to treat reperfusion injury following ischemia. However, these patents use a pyruvate thiolester, which is chemically and functionally different from unmodified pyruvate. Additionally, in the Brunengraber patents the pyruvate derivative is administered only after, not during an ischemic event.

The capacity of pyruvate to act as an energy source for and/or to prevent free-radical damage in organs, in some cases including the heart, which require additional energy (due to disease or because they have been removed for transplant) or are in unusual danger of free-radical damage is discussed in several other patents. These include U.S. Patent Nos. 6,143,784 to Greenhaff et al., 5,480,909 and 5,294,641 to Stanko, 6,086,789 to Brunengraber et al., 5,066,578 and 5,075,210 to Wikman-Coffelt et al., and 4,663,166, 5,100,677, and 6,020,007 to Veech. These patents disclose administration of pyruvate in a number of manners, many of which are topical, oral or intravenous. None of the patents disclose or suggest use of pyruvate in a carioplectic solution.
Thus, a need exists for a cardioplegia solution in which pyruvate is the major energy source and acts to prevent damage to the heart muscle.

SUMMARY OF THE INVENTION

The invention provides a novel cardioplegia solution which may be used in cardiopulmonary bypass surgery. The cardioplegia solution comprises pyruvate in a concentration of 0.2-50 mM. Preferably, the solution is made of water with the following chemicals dissolved therein in the following concentrations:

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyruvate (mM)</td>
<td>0.2-50</td>
</tr>
<tr>
<td>NaCl (mM)</td>
<td>0-250</td>
</tr>
<tr>
<td>KCl (mM)</td>
<td>0-250</td>
</tr>
<tr>
<td>Glucose (mM)</td>
<td>0-200</td>
</tr>
<tr>
<td>Insulin (U/l)</td>
<td>0-200</td>
</tr>
<tr>
<td>CaCl₂ (mM)</td>
<td>0-20</td>
</tr>
<tr>
<td>Lidocaine (g/l)</td>
<td>0-2</td>
</tr>
</tbody>
</table>

In preferred embodiments, the pyruvate and/or any of these additional components may be present in a variety of combinations of subrange of concentration set forth in greater detail in the detailed description which follows.

The pyruvate may be provided in the chemical form of a free acid, as a salt in which the metal cation is sodium, calcium, or potassium, or as a salt in which the cation is an organic compound, for example, creatine. The cardioplegia solution may include additives to prevent bacterial growth or pyruvate breakdown. It may also include amino acids and/or vitamins. Although one benefit of the solution is that it provides protection to the heart through use of pyruvate as the principle energy source and without the need for other agents, at times it may be desirable to obtain added protection by including a pharmacological agent in the cardioplegia solution. Preferred pharmacological agents are β-adrenergic receptor antagonists, Ca²⁺ channel antagonists, and antioxidants.

The invention also includes a method of preparing the cardioplegia solution described above. The method consists primarily of dissolving medical-grade electrolyte reagents in water then filtering the solution through a filter with a pore size between of between 0.05 and 1 μm. Filtration removes contaminants and sterilizes the solution.
Another aspect of the invention relates to use of pyruvate in the preparation of a cardioplegia solution for use in cardiopulmonary bypass surgery. Such a cardioplegia protects the heart from injury resulting from ischemia, thereby allowing rapid and robust recovery of heart mechanical function after bypass surgery. These beneficial effects result from a number of causes. For instance, the cardioplegia solution stabilizes the heart's energy reserves during the cardiopulmonary bypass surgery. Additionally, metabolism of the cardioplegia solution by the heart produces compounds which neutralize prooxidant compounds during and immediately after the period of arrest. Metabolism of the cardioplegia solution by the heart also maintains the heart's antioxidant components during cardiopulmonary bypass surgery.

Because of these beneficial effects, in many bypass procedures pharmacological inotropic support need not be administered following completion of the surgical procedure. Otherwise, it may be administered in reduced amounts or frequency as compared with patients in which a lactate-based cardioplegia is used.

The cardioplegia solution is administered to the heart after mixing with whole blood prior. The ratio of blood volume to cardioplegia solution volume may be between 0.1:1 and 20:1. It is preferably between 1:1 and 10:1 and more preferably between 2:1 and 8:1.

The above summary provides a general outline of the invention. For a better understanding of the invention and its advantages, reference may be made to the following description of exemplary embodiments, taken in conjunction with the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 presents data comparing the effects of a pyruvate-fortified cardioplegia solution of the present invention with those of a standard, lactate-fortified cardioplegia solution on hemoglobin oxygen saturation in coronary sinus blood (sampled as venous blood draining from the heart muscle). Data was obtained from a cohort of patients undergoing bypass surgery. In the graphs data from patients treated with the standard cardioplegia solution is shown in the left graph as circles while data from patients treated with the pyruvate-fortified cardioplegia solution is shown in the right graph as squares.
The filled symbols represent individual patients; the open symbols represent mean values. "Pre-CPB" indicates measurements taken prior to cardiopulmonary bypass surgery, while "Post-CPB" indicates measurements taken after surgery. Hemoglobin oxygen saturation indicates the concentration of oxygen in the heart muscle (Tenney, 1974, incorporated by reference herein).

Figure 2 presents data comparing the effects of a pyruvate-fortified cardioplegia solution with those of a standard lactate-based cardioplegia solution on release of proteins (CPK-MB in Panel A and Troponin-1 in Panel B) from injured heart muscle cells. Samples were taken from venous blood draining from the heart muscle. Data was obtained from a cohort of patients undergoing bypass surgery. In the graphs data from patients treated with the standard cardioplegia solution is shown in the left graph as circles while data from patients treated with the pyruvate-fortified cardioplegia solution is shown in the right graph as squares. The filled symbols represent individual patients; the open symbols represent mean values. "Pre-CPB" indicates measurements taken prior to cardiopulmonary bypass surgery, while "Post-CPB" indicates measurements taken after surgery. Release of heart muscle proteins increased after cardiac arrest with the standard cardioplegia solution but did not increase after arrest with the pyruvate-fortified cardioplegia solution.

Figure 3 presents data comparing the effects of a pyruvate-fortified cardioplegia solution with those of a standard lactate-based cardioplegia solution on post-surgical recovery of left ventricular contractile function following cardioplegia arrest. In the graphs data obtained using standard cardioplegia solution is shown as circles while data obtained using pyruvate-fortified cardioplegic solution is shown as squares. Data was obtained from a cohort of patients undergoing bypass surgery.

Figure 4 presents the number of patients requiring pharmacological treatments to stimulate the heart muscle following bypass with a pyruvate-fortified cardioplegia solution or a standard lactate-based cardioplegia solution. Data was obtained from a cohort of patients undergoing bypass surgery.

Figure 5 presents data regarding the stability of a pyruvate-fortified cardioplegia solution (square symbols) and a standard lactate-based cardioplegia solution (round symbols). The solutions were prepared from standard electrolytes and fortified with
lactate or pyruvate. Solutions were filtered (0.22 μm pore) to remove contaminants, then maintained at room temperature (c. 22 °C; shown by filled symbols) or refrigerated (c. 4 °C; shown by open symbols). Acetate was monitored as a degradation product of the carbohydrates. Lactate, pyruvate and acetate were measured by colorimetric assays at the indicated intervals following preparation of the solutions. Values are the mean of three measurements. RT = Room Temperature.

DETAILED DESCRIPTION OF THE INVENTION

The present invention includes a pyruvate-fortified cardioplegia solution for protecting the heart during cardiopulmonary bypass surgical procedures and its use in such procedures. The solution contains pyruvate anion at a concentration between 0.2 and 50 mM, and is administered into the heart's coronary blood vessels via the aorta and coronary sinus. This novel solution is superior to existing cardioplegia solutions because it both preserves the heart's energy resources, enabling the heart to pump blood more effectively following surgery, and it bolsters the heart's antioxidant defenses, providing better protection from harmful free radicals generated in the heart during surgery. Compared to other cardioplegia solutions, the use of this invention improves recovery of cardiac function following surgery.

The present invention achieves these favorable effects primarily through the use of pyruvate as the primary energy source in the cardioplegia solution. Although the invention may be employed in any type of surgery requiring cardioplegia solution, it is preferably used during cardiopulmonary bypass operations. Although the patients are preferably human patients, the invention may be employed in non-human animals, particularly in other large mammals.

The cardioplegia solution of the invention may contain other additives to prevent damage to the heart, but a main advantage of the invention is that it provides protection via the energy source, eliminating or reducing the need for any additives or other changes in formulation to prevent heart damage.

This invention will allow safer use of cardioplegia solution with improvement in early and presumably long-term functional recovery of the myocardium in patients. (See Figure 3.) It should be noted that the invention has nearly eliminated the need for post-
operative pharmacological inotropic support (See Figure 4), presumably by preventing damage to the heart as a result of arrest and ischemia. Consequently, in tests this has shortened each patient's stay in the intensive care unit by an average of one full day, with a savings of approximately $3000 per patient. As healthcare costs continue to rise, even greater savings may later be achieved.

Furthermore, the cardioplegia solution of the present invention is no more difficult to make than currently used solutions. In fact, is is simpler than many solutions which attempt to achieve pyruvate's protective effects through careful control of solution concentrations or osmolarity, or by limitation or addition of ingredients. Additionally, it is at least as stable as a lactate-based solution (See Figure 5), therefore it should have a shelf life similar to that of current solutions. Should a longer shelf-life be required, additives known to the art and acceptable for cardioplegia solutions may be included. Thus, the added benefits of the cardioplegia solution of this invention may be obtained without added production costs and possibly with a reduction of costs. Because it is used in the same manner as other cardioplegia solutions, it adds no use costs.

The invention may be better understood through reference to the specific examples which follow. Although only preferred embodiments of the invention are specifically described above and in the examples, it will be appreciated that modifications and variations of the invention are possible without departing from the spirit and intended scope of the invention.

EXAMPLES

Example 1: Preparation of Lactate and Pyruvate-Based Cardioplegia Solutions

Cardioplegia Solutions were prepared using standard electrolyte reagents suitable for medical formulations. After preparation, the solutions were sterilized and contaminants removed by filtration through a 0.22 μm pore filter.

The components of the lactate-based solution are as follows:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactated Ringers</td>
<td>800 ml (3140 mg Na lactate)</td>
</tr>
<tr>
<td>KCl (2mEq/ml)</td>
<td>108 mEq (54 ml)</td>
</tr>
<tr>
<td>NaHCO₃ (1 mEq/ml)</td>
<td>160 mEq (160 ml)</td>
</tr>
</tbody>
</table>
Dextrose, 50% 80 ml
Regular Insulin 80 units (0.8 ml)
CaCl₂ injection, 10% 800 mg (8 ml)
Lidocaine 1% 800 mg (80 ml)

Total volume 1182.8 ml

The components of the pyruvate-based solution are as follows:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyruvated Ringers</td>
<td>800 ml (3080 mg Na pyruvate)</td>
</tr>
<tr>
<td>KCl (2mEq/ml)</td>
<td>108 mEq (54 ml)</td>
</tr>
<tr>
<td>NaHCO₃ (1 mEq/ml)</td>
<td>160 mEq (160 ml)</td>
</tr>
<tr>
<td>Dextrose, 50%</td>
<td>80 ml</td>
</tr>
<tr>
<td>Regular Insulin</td>
<td>80 units (0.8 ml)</td>
</tr>
<tr>
<td>CaCl₂ injection, 10%</td>
<td>800 mg (8 ml)</td>
</tr>
<tr>
<td>Lidocaine 1%</td>
<td>800 mg (80 ml)</td>
</tr>
</tbody>
</table>

Total volume 1182.8 ml

These concentrations of lactate and pyruvate are 35 mM in the Ringers solution, 23.7 mM in the cardioplegia solution.

Example 2: Alternative Cardioplegia Solutions

Cardioplegia solutions were prepared on the day of surgery as described in Example 1. The control solution contained lactated rings as the main energy source. In the study solution, pyruvated rings was substituted for lactated rings. Pyruvated rings was prepared by dissolution of sodium pyruvate powder of highest commercially available purity (tissue culture grade, Sigma Chemical Co., St. Louis, MO) in sterile 0.9% NaCl. Both cardioplegia solutions contained 104 mM NaCl, 135 mM NaHCO₃, 91 mM KCl, 6 mM CaCl₂, 188 mM glucose, 68 U/l insulin, and 676 g/l lidocaine. Sodium lactate concentration in the control solution was 23.8 mM, and the study solution contained 10
mM sodium pyruvate. After adjusting the pH to 7.8 at 37 °C, particulate contaminants were removed by filtering the cardioplegia solutions through 0.5 m cellulose nitrate filters.

Although the above solutions in Examples 1 and 2 represent specific preferred embodiments of the invention, it will be understood that alternative embodiments with different concentrations and/or ingredients remain within the scope of the invention.

Specifically, other embodiments may have the following concentrations of ingredients, wherein each of the concentration ranges is a preferred embodiment:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Conc. 1</th>
<th>Conc. 2</th>
<th>Conc. 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyruvate (mM)</td>
<td>0.2 – 50</td>
<td>1-30</td>
<td>30-45</td>
</tr>
<tr>
<td>NaCl (mM)</td>
<td>0-250</td>
<td>10-100</td>
<td>100-200</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>0-250</td>
<td>10-100</td>
<td>100-200</td>
</tr>
<tr>
<td>KCl (mM)</td>
<td>0-250</td>
<td>10-100</td>
<td>100-200</td>
</tr>
<tr>
<td>Glucose (mM)</td>
<td>0-200</td>
<td>10-80</td>
<td>80-170</td>
</tr>
<tr>
<td>Insulin (U/I)</td>
<td>0-200</td>
<td>10-80</td>
<td>80-170</td>
</tr>
<tr>
<td>CaCl₂ (mM)</td>
<td>0-20</td>
<td>0.5-5</td>
<td>5-15</td>
</tr>
<tr>
<td>Lidocaine (g/I)</td>
<td>0-2</td>
<td>0.25-1</td>
<td>1-1.75</td>
</tr>
</tbody>
</table>

It will be understood to one skilled in the art that each concentration of each ingredient in the above list is a separate preferred embodiment. For instance, a solution may lie within the ranges of the third concentration for each ingredient except Lidocaine, which may be present in the concentration of the second column, 0.25-1. The appropriate combination of ranges and specific concentrations within each range will vary depending upon a variety of factors. These may include whether and which other pharmaceutical compositions are administered to the patient before, during, or after the surgery or the age and condition of the patient. Each component other than pyruvate indicated above may be substituted by any chemical known to have similar effects on the heart. In a specific preferred embodiment, the concentration of CaCl₂ is between 0.5 and 20 mM.

Preferred additives to the cardioplegia solution of the invention include vitamins and amino acids, β-Adrenergic receptor antagonists, Ca²⁺ channel antagonists, and
antioxidants. The pyruvate used to create the solution of the invention may be provided in the chemical form of a free acid, a salt in which the metal cation is sodium, calcium, or potassium, or as a salt in which the cation is an organic compound such as creatine.

Example 3: Chemical Stability of the Cardioplegia Solution

Solutions prepared as described in Example 1 except with a pyruvate or lactate concentration of 30 mM were maintained at room temperature or at 4 °C for up to 72 days. Samples were tested at days 1, 3, 7, 14, 21 and 72. Concentration of acetate was measured. Acetate is a degradation product of carbohydrates and its presence indicates breakdown of pyruvate or lactate. Lactate and pyruvate concentrations were also measured as an indicator of carbohydrate stability. Acetate, lactate and pyruvate concentration were measured using colorimetric assays standard in the art. The results of these assays are presented in Figure 5. The results indicate that the novel pyruvate-based cardioplegia solution is at least as stable as the standard lactate-based solution. Should greater carbohydrate stability or greater sterility be desired, pharmaceutically acceptable additives known to the art may be included in the solution. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopoeia or other generally recognized pharmacopoeia for use in animals, and more particularly in humans. The term "additive" refers to a diluent or other chemical with which the therapeutic is administered. The additive may be added to the composition at the time it is made or at a later time. Examples of suitable pharmaceutical additives are described in "Remington: The Science and Practice of Pharmacy", formerly "Remington's Pharmaceutical Sciences" by E.W. Martin.

Example 4: Human Studies

To demonstrate the utility and effectiveness of the invention for its intended application, the novel pyruvate-fortified cardioplegia solution was tested in patients undergoing open heart surgery for coronary bypass grafting. Cardioplegia solutions were prepared as described in Example 2. Patients were randomly assigned to one of two treatment groups: in one group, hearts were arrested with standard cardioplegia containing
lactate and glucose as fuels, and in a second group cardiac arrest was induced with the novel pyruvate-enhanced cardioplegia. In all surgeries the cardioplegia was mixed with patient blood in a ratio of blood volume:cardioplegia volume of 4:1 prior to administration to the heart. The mixture ratio may be anywhere between 0.1:1 and 20:1 and is preferably between 1:1 and 10:1 and more preferably between 2:1 and 8:1.

The protective effects of these solutions were compared by three criteria: coronary sinus hemoglobin oxygen saturation (Figure 1), a measure of coronary blood flow and oxygen delivery to the heart muscle; coronary sinus troponin-I and creatine phosphokinase-MB isoform (CPK-MB) release (Figure 2), which reflect the extent of injury to the heart muscle; and left ventricular stroke work index (Figure 3), a measure of the heart's contractile performance. Coronary sinus oxygen saturation was similar in the two groups before bypass surgery, but after surgery oxygen saturation increased sharply in the pyruvate group but not in the standard cardioplegia group (Figure 1). This indicates that use of the pyruvate-fortified cardioplegia solution improved the supply of oxygen to the heart muscle after surgery. Release of the cardiac muscle proteins troponin-I and CPK-MB increased in the standard group following surgery (Figure 2), indicating that the heart muscle was injured during the period of cardiac arrest. Pyruvate cardioplegia prevented the post-arrest increases in troponin-I and CPK-MB release. Thus, use of the novel pyruvate-fortified cardioplegia prevented cellular injury while the coronary blood supply was interrupted during cardiac arrest.

Cellular protection by pyruvate during cardiac arrest was manifest by improved contractile performance of the heart during the post-arrest recovery period (Figure 3). Cardiac function was similar in the two groups immediately after coronary blood supply was reinstituted and the heart weaned off of bypass, but function of the two groups differed remarkably by 4 hours post-bypass. Thus, left ventricular stroke work index declined between zero and four hours post-bypass in the standard cardioplegia group, but, in contrast, it increased considerably during the same period in the pyruvate cardioplegia group. Left ventricular function remained robust at least until 12 hours post-bypass in the pyruvate cardioplegia group, in contrast to the weak contractile function of hearts treated with standard cardioplegia. Thus, superior protection of the heart by pyruvate-fortified cardioplegia during cardiac arrest led to dramatically improved cardiac function post-
arrest. This improvement in cardiac recovery obviated the use of dobutamine to pharmacologically stimulate cardiac function (Figure 4). Thus, only 4 of the 15 pyruvate trials required any dobutamine to stimulate the heart post-bypass, but 10 of the 15 standard cardioplegia patients required pharmacological support. Dobutamine could potentially deplete the heart's energy reserves and thereby worsen injury to the heart, so the reduction in its use afforded by pyruvate is desirable.

It is important to note that in the method of this invention pyruvate is being given during the period of myocardial ischemia created by surgical arrest, as opposed to administration after the period of ischemia has ended (the so-called 'reperfusion' period). Ischemia and reperfusion are very different situations for the heart muscle. During ischemia, the coronary blood supply is interrupted, and, consequently, the heart muscle is deprived of its fuel and oxygen supplies. This is the situation the heart is subjected to during coronary bypass surgery. During reperfusion, the heart's blood supply has been restored, so the heart once again receives the fuel and oxygen needed to make energy. The intent of administering pyruvate during reperfusion is to stimulate the heart's contractile performance, that is, to help the heart beat stronger and pump more blood. The aim of this invention is to protect the heart during cardiac arrest, not to stimulate its function during that period which would interfere with surgery.

It should also be noted that the pyruvate-fortified cardioplegia is quickly washed out of the organ upon restoration of blood flow, so the improved function of the heart during the 12 hours following surgery is not due to a direct effect of pyruvate. Instead, the improved function is the result of excellent protection of the heart by pyruvate during the preceding ischemic period. Thus this mechanism is fundamentally different from pyruvate enhancement of cardiac performance in reperfusion.
WE CLAIM:

1. A cardioplegia solution comprising pyruvate in a concentration of 0.2 – 50 mM.

2. The cardioplegia solution of claim 1, wherein the pyruvate concentration is between 1-30 mM.

3. The cardioplegia solution of claim 1, wherein the pyruvate concentration is between 30-45 mM.

4. The cardioplegia solution of claim 1, wherein the solution comprises water with the following chemicals dissolved therein in the following concentrations:

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyruvate (mM)</td>
<td>0.2 -50</td>
</tr>
<tr>
<td>NaCl (mM)</td>
<td>0-250</td>
</tr>
<tr>
<td>KCl (mM)</td>
<td>0-250</td>
</tr>
<tr>
<td>Glucose (mM)</td>
<td>0-200</td>
</tr>
<tr>
<td>Insulin (U/l)</td>
<td>0-200</td>
</tr>
<tr>
<td>CaCl₂ (mM)</td>
<td>0-20</td>
</tr>
<tr>
<td>Lidocaine (g/l)</td>
<td>0-2.</td>
</tr>
</tbody>
</table>

5. The cardioplegia solution of claim 1, in which pyruvate is provided in the chemical form of a free acid, as a salt in which the metal cation is sodium, calcium, or potassium, or as a salt in which the cation is an organic compound.

6. The cardioplegia solution of claim 1, additionally comprising amino acids.

7. The cardioplegia solution of claim 1, additionally comprising vitamins.

8. The cardioplegia solution of claim 1, additionally comprising a pharmacological agent.
9. The cardioplegia solution of claim 8, wherein the pharmacological agent is selected from the group consisting of β-adrenergic receptor antagonists, $\text{Ca}^{2+}$ channel antagonists, and antioxidants.

10. Use of pyruvate in the manufacture of a cardioplegia solution for use in cardiopulmonary bypass surgery.

11. Use as claimed in claim 10, is which the cardioplegia solution is adapted to be diluted with whole blood prior to administration to the patient.

12. Use as claimed in claim 11, is which the cardioplegia solution is adapted to be diluted with whole blood in a volume ratio to cardioplegia solution volume between 0.1:1 and 20:1.

13. Use as claimed in claim 11, is which the cardioplegia solution is adapted to be diluted with whole blood in a volume ratio to cardioplegia solution volume between 1:1 and 10:1.

14. Use as claimed in claim 11, is which the cardioplegia solution is adapted to be diluted with whole blood in a volume ratio to cardioplegia solution volume between 2:1 and 8:1.

15. Use as claimed in claim 10, wherein the cardioplegia solution as used in cardiopulmonary bypass surgery protects the heart from injury resulting from ischemia.

16. Use as claimed in claim 10, wherein the cardioplegia solution as used in cardiopulmonary bypass surgery promotes recovery of heart mechanical function following surgery.

17. Use as claimed in claim 10, wherein the cardioplegia solution as used in cardiopulmonary bypass surgery stabilizes the heart's energy reserves during the surgery.
18. Use as claimed in claim 10, wherein the cardioplegia solution as used in cardiopulmonary bypass surgery is metabolized by the heart to produce compounds which neutralize prooxidant compounds during and immediately after a period of cardiac arrest.

19. Use as claimed in claim 10, wherein the cardioplegia solution as used in cardiopulmonary bypass surgery is metabolized by the heart and thereby maintains the heart's antioxidant components during cardiopulmonary bypass surgery.

20. Use as claimed in claim 10, wherein the cardioplegia solution as used in cardiopulmonary bypass surgery reduces or eliminates the need for pharmacological inotropic support following completion of the surgical procedure.

21. Use as claimed in claim 10, wherein the cardioplegia solution comprises pyruvate in a concentration of 0.2 – 50 mM.

22. Use as claimed in claim 10, wherein the pyruvate concentration is between 1-30 mM

23. Use as claimed in claim 10, wherein the pyruvate concentration is between 30-45 mM.

24. Use as claimed in claim 10, wherein the solution comprises water with the following chemicals dissolved therein in the following concentrations:
   
<table>
<thead>
<tr>
<th>Chemical</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyruvate (mM)</td>
<td>0.2 - 50</td>
</tr>
<tr>
<td>NaCl (mM)</td>
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</tr>
<tr>
<td>KCl (mM)</td>
<td>0 - 250</td>
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<tr>
<td>Glucose (mM)</td>
<td>0 - 200</td>
</tr>
<tr>
<td>Insulin (U/l)</td>
<td>0 - 200</td>
</tr>
<tr>
<td>CaCl₂ (mM)</td>
<td>0 - 20</td>
</tr>
<tr>
<td>Lidocaine (g/l)</td>
<td>0 - 2</td>
</tr>
</tbody>
</table>
25. A method of preparing a cardioplegia solution comprising the steps of:
   i) obtaining medical-grade electrolyte reagents and preparing in water a solution of the following reagents dissolved therein in the following concentrations:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyruvate (mM)</td>
<td>0.2 – 50</td>
</tr>
<tr>
<td>NaCl (mM)</td>
<td>0-250</td>
</tr>
<tr>
<td>KCl (mM)</td>
<td>0-250</td>
</tr>
<tr>
<td>Glucose (mM)</td>
<td>0-200</td>
</tr>
<tr>
<td>Insulin (U/l)</td>
<td>0-200</td>
</tr>
<tr>
<td>CaCl₂ (mM)</td>
<td>0-20</td>
</tr>
<tr>
<td>Lidocaine (g/l)</td>
<td>0-2;</td>
</tr>
</tbody>
</table>

   ii) filtering the solution through a filter with a pore size between of between 0.05 and 1 m.
FIG. 1

CORONARY SINUS O₂ SATURATION, %

Pre-CPB Post-CPB Pre-CPB Post-CPB
STANDARD PYRUVATE

FIG. 2A

TROPOinin I, ng/ml

Pre-CPB Post-CPB Pre-CPB Post-CPB
STANDARD PYRUVATE
FIG. 2B

FIG. 3
FIG. 4

SUBJECTS REQUIRING DOBUTAMINE, %

- 0-2 HOURS POST-BYPASS
- 2-4 HOURS POST-BYPASS
- TOTAL

FIG. 5A

LACTATE SOLUTION
- LACTATE RT
- LACTATE 4°C

PYRUVATE SOLUTION
- PYRUVATE RT
- PYRUVATE 4°C

FIG. 5B

LACTATE SOLUTION
- LACTATE RT
- LACTATE 4°C

PYRUVATE SOLUTION
- PYRUVATE RT
- PYRUVATE 4°C

SUBSTITUTE SHEET (RULE 26)
A. CLASSIFICATION OF SUBJECT MATTER

IPC 7    A61K31/167    A61K31/19    A61K33/00    A61K33/14    A61K47/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7    A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database consulted during the international search (name of database and, where practical, search terms used)

EPO-Internal, BIOSIS, WPI Data, PAJ, EMBASE, PASCAL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>WO 93 02653 A (SEGEL LEIGH D) 18 February 1993 (1993-02-18) page 18, line 12-15; table 1 page 15, line 6-20 page 12, line 5 page 7, line 3-8 example III</td>
<td>1-25</td>
</tr>
<tr>
<td>Y</td>
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</tr>
</tbody>
</table>

Further documents are listed in the continuation of box C. Patent family members are listed in annex.

* Special categories of cited documents:
  
  *A* document defining the general state of the art which is not considered to be of particular relevance
  
  *E* earlier document but published on or after the international filing date
  
  *L* document which may throw doubts on priority claim(s) or on how it is established the publication date of another citation or other special reason (as specified)
  
  *O* document referring to an oral disclosure, use, exhibition or other means
  
  *P* document published prior to the international filing date but later than the priority date claimed

* Other document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

*E* document member of the same patent family

Date of the actual completion of the international search

24 September 2002

Date of mailing of the international search report

10/10/2002

Name and mailing address of the ISA

European Patent Office, P.O. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 240-2040, Fax: 31 651 epo nl
Authorized officer

Blott, C
<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>RAO V ET AL: &quot;Insulin cardioplegia for elective coronary bypass surgery&quot; JOURNAL OF THORACIC AND CARDIOVASCULAR SURGERY, (JUN 2000) VOL. 119, NO. 6, PP. 1176-1184. PUBLISHER: MOSBY-YEAR BOOK INC, 11830 WESTLINE INDUSTRIAL DR, ST LOUIS, MO 63146-3318. ISSN: 0022-5223., XP001105068 TORONTO HOSP, DIV CARDIOVASC SURG, EN14-215, 200 ELIZABETH ST, TORONTO, ON M5G 2C4, CANADA (Reprint); TORONTO GEN HOSP, TORONTO, ON, CANADA; UNIV TORONTO, SUNNYBROOK HLTH SCI CTR, TORONTO, ON, CANADA abstract page 1178, column 1, line 7-12</td>
<td>1-25</td>
</tr>
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<td>X</td>
<td>WO 91 09520 A (UNIV CALIFORNIA) 11 July 1991 (1991-07-11) cited in the application claim 5; example 1</td>
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<td>X</td>
<td>US 4 988 515 A (BUCKBERG GERALD D) 29 January 1991 (1991-01-29) cited in the application column 1, line 10-38; claims 1-18 column 2, line 23-60 column 4, line 2-35 column 5, line 19-40 column 6, line 44-50</td>
<td>1-25</td>
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<tr>
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<td>abstract; table 1 page 799, column 1, line 6-page 799, column 2, line 18</td>
<td>1-25</td>
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</table>
Continuation of Box I.2

Claims Nos.: 15-20

Present claims 15-20 relate to cardioplegia solutions defined by reference to a desirable characteristic or property, namely:
claim 15: a cardioplegia solution as used in cardiopulmonary bypass surgery which protects the heart from injury resulting from ischemia
claim 16: a cardioplegia solution as used in cardiopulmonary bypass surgery which promotes recovery of heart mechanical function following surgery
claim 17: a cardioplegia solution as used in cardiopulmonary bypass surgery which stabilizes the heart’s energy reserves during surgery
claim 18: a cardioplegia solution as used in cardiopulmonary bypass surgery which is metabolized by the heart to produce compounds which neutralize prooxidant compounds during an immediately after a period of cardiac arrest
claim 19: a cardioplegia solution as used in cardiopulmonary bypass surgery which is metabolized by the heart and thereby maintains the heart’s antioxidant components during cardiopulmonary bypass surgery
claim 20: a cardioplegia solution as used in cardiopulmonary bypass surgery which reduces or eliminates the need for pharmacological inotropic support following completion of the surgical procedure.

The claims cover all cardioplegia solutions having these characteristics or properties, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a very limited number of such solutions. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the solution by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to solutions as defined in present claims 1-9.

The applicant’s attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.
INTERNATIONAL SEARCH REPORT

Box I  Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [ ] Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:

2. [X] Claims Nos.: 15–20
   because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
   see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II  Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.
<table>
<thead>
<tr>
<th>Patent document cited in search report</th>
<th>Publication date</th>
<th>Patent family member(s)</th>
<th>Publication date</th>
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</thead>
<tbody>
<tr>
<td>WO 9302653</td>
<td>18-02-1993</td>
<td>AU 2441592 A</td>
<td>02-03-1993</td>
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<td>US 5374624 A</td>
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