EXTRACORPOREAL NEPHROLOGY GROUP [ECNG]

HTK SOLUTION

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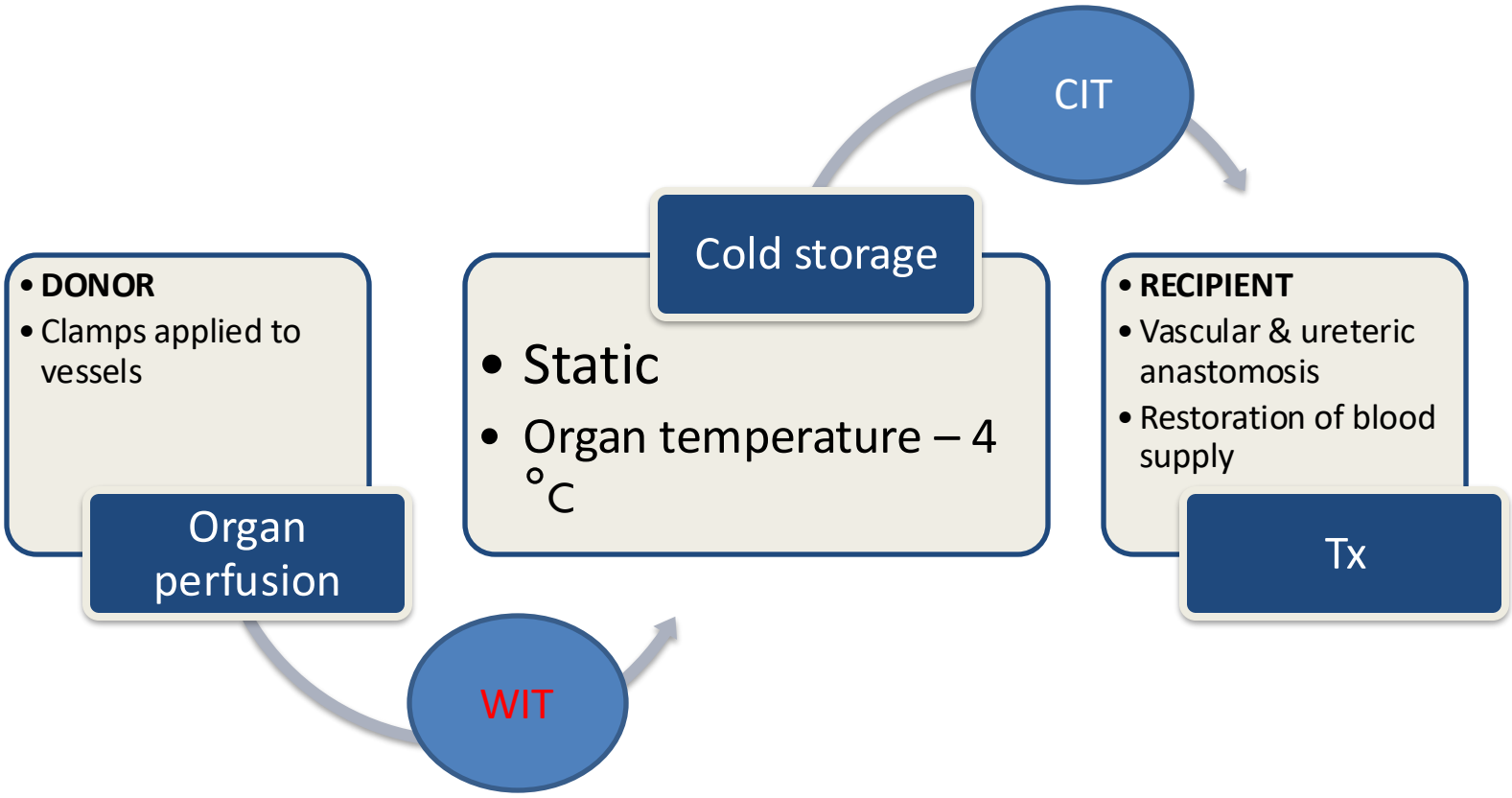
INTRODUCTION

- HTK solution is a specialised intracellular crystalloid solution used primarily for organ preservation.
- The acronym HTK stands for its three critical active ingredients: Histidine, Tryptophan, and Ketoglutarate.
- **Histidine:** Actively works as a powerful amino acid buffer. It maintains normal physiological pH and limits tissue damage caused by lactic acid buildup,
- **Tryptophan:** Functions as a membrane-protective stabilizer, preventing ion leakage across cellular barriers during transport.
- **Ketoglutarate:** Serves as a vital energy substrate that allows the tissue to restart internal ATP production quickly once blood flow is restored.
- **Mannitol:** Included as an osmotic agent to decrease risk of cell swelling and hunt down oxygen-free radicals.

Steps in kidney transplantation



1812 - Le Gallois
"if the place of the heart could be supplied by injection—and if, for the regular continuance of this injection, there could be furnished a quantity of arterial blood, whether natural or artificially formed, supposing such a formation possible—then life might be indefinitely maintained in any portion"

1849 - Loebell
first perfusion experiments on isolated pig kidneys

1885 - Von Frey and Gruber
First closed artificial circulation system similar to today's organ perfusion systems

1935 - Lindberg and Carrel
Pressurized Normothermic Oxygenated system organs viable 3-21 days

1953 - Lapchinsky
First animal models of hypothermic preservation of kidneys with successful transplantation

1954 - Murray
First successful kidney transplant between twins at Brigham and Women's Hospital

1968 - Belzer
Successful human kidney transpant after 17 hours preservation in static, hypothermic plasma perfusion

1969 - Collins
30 hours static cold storage with transplant in Collins solution

1986 – Wahlberg
UW solution

1975 – Bretschneider
HTK solution first described As a cardioplegia agent

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2011 - Hosgood
First normothermic machine perfusion renal transplant

1870 1880 1890 1900 1910 1920 1930 1940 1950 1960 1970 1980 1990 2000 2010 2020

Normothermia | Hypothermia | Hypothermic Machine Perfusion

Ischemia Reperfusion Injury (IRI)

ISCHEMIC INJURY

- **Metabolic Switch**: Deprived of oxygen, cells switch from aerobic to **anaerobic metabolism**, leading to rapid depletion of adenosine triphosphate (**ATP**).
- **Intracellular Acidosis**: Anaerobic glycolysis causes **lactate accumulation**, lowering intracellular pH.
- **Ionic Imbalance**: Failure of ATP-dependent pumps (Na+/K+ ATPase, Ca2+ ATPase) leads to an influx of sodium and water (**cellular oedema**) and a toxic build up of **cytosolic calcium**.
- **Priming for Damage**: ATP breakdown products like **hypoxanthine** accumulate, serving as precursors for reactive oxygen species (ROS) during the next phase.

REPERFUSION (PARADOXICAL INJURY)

Reperfusion restores oxygen and normalises pH, but this triggers a massive destructive cascade.

- **ROS Burst**: The sudden reintroduction of oxygen leads to a "burst" of **Reactive Oxygen Species (ROS)** production, causing extensive **lipid peroxidation** of cell membranes and DNA damage.
- **Mitochondrial Dysfunction**: ROS and calcium overload trigger the opening of **mitochondrial permeability transition pores (mPTP)**, leading to the release of pro-apoptotic factors like cytochrome C and succinate.
- **No-Reflow Phenomenon**: Despite clearing the physical obstruction, microvascular blood flow remains compromised due to endothelial cell swelling, microthrombi (clots), and intense vasoconstriction.

The Ideal Preservation Solution

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- Prevents cell swelling and tissue edema
  - Osmotic agents: hydroxy ethyl starch, lactobionate, mannitol, tryptophan
- Maintains intracellular pH
  - Histidine
- Provides substrates for generation of ATP
  - Adenosine, α-ketoglutarate, L-arginine
- Prevent oxygen free-radical injury
  - Glutathione, allopurinol, N-Acetylcysteine

(Jing 2018)

Composition

Component	University of Wisconsin	Histidine Tryptophan αKetoglutarate (HTK)
K+	125	10
Na+	25	15
Cl-	15	32
Ca2+	-	0.015
Mg2+	5	4
Colloid/Impermeant	Pentaraction Lactobionate Raffinose	Mannitol
Buffer	Phosphate	Histidine
Antioxidant	Glutathione Alloppurinol	Tryptophan αKetoglutarate
Aminoacids	-	Histidine Tryptophan
Other	Adenosine Sulfate	-

(Jing 201

RL is NOT an ideal perfusion solution

- High Na & Cl levels ; during CI ; increases fluid entry into cells ; membrane integrity / cell swelling , poor ischemic tolerance.
- Contains Ca ; causes Ca influx -> mitochondrial damage / activates apoptosis.
- Contains lactate as buffering agent ; poor maintenance of pH and biochemical stability.
- FDA – RL as resuscitation crystalloid.

Comparison of UW and HTK solution

Feature / Metric	University of Wisconsin (UW) Solution	Histidine-Tryptophan-Ketoglutarate (HTK) Solution
Solution Type	Intracellular-like (high K^+, low Na^+)	Extracellular-like (low K^+, high Na^+)
Viscosity	**High** (contains Hydroxyethyl starch colloid)	**Low** (no colloids, flows freely)
Primary Buffer	Phosphate	**Histidine** (excellent buffering capacity)
Potassium Level	High (≈ 125 mmol/L)	Low (≈ 9 mmol/L)
Flush Preparation	Requires mandatory **rinsing/flushing** before reperfusion to prevent hyperkalaemic cardiac arrest.	Can be reperfused with less risk of severe cardiac arrhthymias.
Required Volume	Low to moderate volume needed per organ.	**High volume** required to achieve deep cooling and complete clearing.
Ischemia Tolerance	Superior protection during **prolonged cold ischemia** (>8–15 hours).	Excellent for shorter times; slightly higher risk of tissue injury during long cold storage.
Primary Advantage	Gold standard for extended preservation and complex/marginal grafts.	Fast organ cooling, easier microvascular clearing, and **lower cost**.

HTK –N solution

- **HTK-N modifies the molecular baseline of traditional HTK to combat the specific cellular mechanisms of Ischemia-Reperfusion Injury.**

- **Dual Iron Chelators**: Includes **deferoxamine** (poorly membrane-permeable) and **LK 614** (highly membrane-permeable). Together, they bind "redox-active" iron released during cold storage, drastically reducing the generation of ROS.

- **Membrane-Stabilizing Amino Acids**: Supplemented with **glycine** and **alanine**. These inhibit the formation of hypoxia-induced plasma membrane pores, successfully preventing cell swelling and premature lysis.

- **Endothelial Function Boost**: Infused with **L-arginine** to act as a substrate for nitric oxide production, which minimizes microcirculatory vasospasms upon graft reperfusion.

- **Modified Buffering & Osmolyte Swap**: Standard histidine has been partially replaced with **N-acetyl-histidine** to lower cell toxicity.