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Ischemic Preconditioning and the Role of Anti-Fibrinolytic Drugs: Translation from Bench to Bedside

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Preconditioning refers to a diverse group of biologic phenomena in which myocardial tissue is rendered ischemic for a short, non-lethal period, and subsequently exhibits reduced injury in response to ischemia.¹ This phenomenon, first discovered over 30 years ago², has become a major subject of laboratory research. Myocardial preconditioning can be induced with short periods of coronary occlusion known as ischemic preconditioning or by rendering a distant tissue ischemic, such as with an arm tourniquet, known as remote ischemic preconditioning. Preconditioning can also be induced pharmacologically by certain agents³ whereas others are known to interfere with preconditioning.^{4–6} A wealth of cellular mechanisms have been discovered that contribute to the development of preconditioning including changes in phosphoinositol-3, protein kinase C, reactive oxygen species, electron transport chain complex III, nitric oxide synthases, KATP channels, and the mitochondrial permeability transition pore.

Remote ischemic preconditioning is a reproducible phenomenon that has been observed and extensively studied in multiple species.^{7,8} In laboratory models, remote ischemic preconditioning can reliably decrease myocardial infarct size by 20–50%. Despite the ongoing interest in using remote ischemic preconditioning in cardiac surgery based on extensive animal data, only a small number of clinical trials have produced promising results.^{9–13} These preliminary trials prompted two large randomized clinical trials of remote ischemic preconditioning in cardiac surgical patients, RIPHeart and ERICCA, that were both completed in 2015 but to the disappointment of many, both trials were negative reporting no beneficial effect of remote ischemic preconditioning.^{14,15} How could such a robust laboratory effect be ineffective in a clinical trial?

Ischemic preconditioning is most commonly shown in laboratory models of ischemia-reperfusion where animals are anesthetized, usually with barbiturates, anesthetics which

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None - QJQ

None - JHL

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only weakly induce pharmacologic preconditioning in laboratory animals.^{16,17} In the cardiac operating room, patients are given a balanced anesthetic consisting of drugs known to more potently induce preconditioning such as volatile anesthetics and opioids, either of which may eclipse the protective effect of ischemic preconditioning by providing more robust pharmacologic preconditioning.^{18–22} Many of these drugs induce changes in gene and protein expression that are remarkably similar to those changes wrought by ischemic preconditioning.^{23,24} This knowledge led RIPHeart investigators to make the use of volatile anesthetics in patients enrolled in their study an exclusion criteria. Additionally, patients undergoing cardiac surgery may receive post-operative sedation, analgesics, beta-blockade, statins, and antifibrinolytic drugs, some of which are known to induce or interfere with preconditioning. As a large number of drugs have been noted in the laboratory to induce or modify preconditioning we have included Table 1 highlight some of the commonly used drugs that alter myocardial protection. While no large prospective randomized trials exist that clearly demonstrate a benefit to pharmacologic preconditioning two meta-analyses show a clinical benefit to cardiac anesthesia that incorporates volatile anesthetic known to induce preconditioning.^{19,20} Additionally, a review of Danish registry data showed a clinical benefit of exposure to volatile anesthetics in patients that did not experience preceding ischemia, reported as angina.²¹ Taken together these studies suggest that preconditioning can be induced by non-lethal ischemia, a host of pharmacologic agents, and can be enhanced or inhibited by a number of other drugs, however, it seems that pharmacologic preconditioning cannot be superimposed on ischemic preconditioning or *vice versa*. That brings us to the current study in the Journal of the carefully conducted experiments by van Caster *et al*²⁵, which have endeavored to clarify the role of the antifibrinolytic drug, tranexamic acid (TXA), in ischemic preconditioning and remote ischemic preconditioning.

There is a substantial precedent for the hypothesis that antifibrinolytic agents could interfere with remote ischemic preconditioning. Antifibrinolytic drugs are serine protease inhibitors that inhibit fibrinolysis, the cleavage of cross-linked fibrin by plasmin. This process, when uncontrolled, can deplete coagulation factors and lead to coagulopathy. Antifibrinolytics also have other indirect effects; by inhibiting the activity of plasmin, they can be anti-inflammatory. Plasmin, particularly when bound to the surface of macrophages, plays a critical role in monocyte activation and inflammation including activation of the complement cascade, exit from circulation into injured or infected tissue, cytokine production, and proteolytic activation of matrix-metalloproteases (MMP) with subsequent degradation and remodeling of extra-cellular matrix (ECM). Activated plasmin elicits chemotaxis and actin polymerization in monocytes including the release of cytokines downstream of the NF- κ B, AP-1, and STAT transcription factors.^{26,27} In cardiac surgery, TXA and aprotinin were studied using whole blood mRNA expression profiling of inflammatory genes. Of the 114 genes studied, eight produced less mRNA in the presence of aprotinin, while three genes were inhibited by TXA and aprotinin, the broader spectrum of aprotinin most likely owing to its broader inhibitory activity against serine proteases.²⁸ Antifibrinolytic use in clinical settings has anti-inflammatory effects,²⁹ and thus the potential to provide protection during ischemic insults. Several studies report that aprotinin is protective during ischemia.³⁰ While the anti-inflammatory effect of antifibrinolytics might enhance myocardial preconditioning

protecting heart tissue, there is also significant data suggesting that antifibrinolytics may impair preconditioning as reported in the current study in this journal by Van Caster et al.²⁵

In a series of carefully controlled animal experiments, Frässdorf *et al* demonstrated significant and reproducible inhibition of pharmacologic preconditioning by aprotinin.⁵ In a rat model of ischemia reperfusion aprotinin was able to eliminate the protective effects of sevoflurane preconditioning resulting in larger sized myocardial infarction. This was associated with a significant reduction in nitric oxide synthase phosphorylation, and has also been the subject of review.⁶ Figure 1 demonstrates aprotinin's inhibitory action on nitric oxide dependent preconditioning. This and other works^{31,32} have identified antifibrinolytic drugs as a potential inhibitor of preconditioning. Could the routine use of antifibrinolytic drugs be the reason why the large trials of remote ischemic preconditioning were negative? Preconditioning clinical trials to date have allowed for clinician usual practice in anesthetics and perioperative pharmacotherapy, making the rate of antifibrinolytic use unclear. Few studies have been conducted to examine the effect of antifibrinolytic drugs on preconditioning or remote ischemic preconditioning.

Although the study by Van Caster et al²⁵ in this journal is a negative study, it demonstrates a critical finding. TXA does not have an inhibitory effect on ischemic preconditioning or remote ischemic preconditioning in rats. This finding highlights the fact that small differences in TXA and aprotinin's activity, result in large differences in regard to its effects on ischemia reperfusion. While aprotinin potently blocks preconditioning, TXA seems to exert no effect on preconditioning. While this is not the same as a large trial in humans, it highlights a critical difference between TXA and aprotinin. While both molecules interact with the lysine binding site on plasmin, TXA is relatively specific for this while aprotinin retains broader inhibitory activity against multiple serine proteases. Aprotinin's broader inhibition of proteolytic activity including inhibition of PAR1 receptors is most-likely responsible for its blockade of the cascade of reactions that lead to preconditioning, pharmacologic preconditioning, and remote ischemic preconditioning.³³ TXA's specificity means that, in humans, it may not disturb preconditioning or remote ischemic preconditioning while it remains effective as an antifibrinolytic drug.³⁴

The work of van Caster *et al* provides additional information on the behavior of TXA in an animal model of preconditioning. This is key, as it may help us understand the interplay of the complex mix of drugs employed during a typical cardiac anesthetic. Given this complex interplay, it is possible that the reason two large randomized trials of remote ischemic preconditioning^{14,15} are negative has to do with a drug or drugs administered during surgery which could inhibit preconditioning, such as aprotinin. Alternatively, the mix of drugs given during a typical anesthetic could induce pharmacologic preconditioning so potently, that remote ischemic preconditioning has no further protection to give. While neither RIPHeart or ERICCA specifically mention antifibrinolytic use in their study protocol, animal data suggests that there may be significant differences in the behavior of antifibrinolytics as they relate to preconditioning. The increased specificity of TXA compared with aprotinin may mean that it can function effectively as an antifibrinolytic without inhibiting the protection granted by preconditioning, remote ischemic preconditioning, and pharmacologic preconditioning, at least in rats.

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Figure 1. Aprotinin inhibits endothelial nitric oxide synthase

eNOS – Endothelial Nitric Oxide Synthase

NO – Nitric Oxide

K_{ATP}- Mitochondrial ATP dependent potassium channel

Table 1

Physiologic and Pharmacologic Inducers of Preconditioning	Agents Known to Inhibit Preconditioning
Non-Lethal Periods of Ischemia Direct Ischemic Preconditioning ¹ Remote Ischemic Preconditioning ³	Adenosine Receptor Antagonists (Methylxanthines) Theophylline, Aminophylline ² Caffeine ²
Receptor and Plasma Membrane Interactions Volatile Anesthetics ⁴ Lidocaine ⁵ Adenosine and Adenosine Agonists ⁵ Opioids ⁶	KATP Channel Inhibitors (Sulfonylureas) Glipizide ² Glyburide ²
	Anti-Fibrinolytic Drugs Aprotinin ⁷
Phosphodiesterase Inhibitors Sildenafil ⁸	eNOS/iNOS inhibitors L-NAME ⁹
	Beta-Blockers Propranolol ¹⁰ Nipradolol ¹⁰

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