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Review

Silymarin and its constituents in cardiac preconditioning

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ABSTRACT

Silymarin, a standardised extract of *Silybum marianum* (milk thistle), comprises mainly of silybin, with dehydrosilybin (DHSB), quercetin, taxifolin, silychristin and a number of other compounds which are known to possess a range of salutary effects. Indeed, there is evidence for their role in reducing tumour growth, preventing liver toxicity, and protecting a number of organs against ischemic damage. The hepatoprotective effects of silymarin, especially in preventing *Amanita* and alcohol intoxication induced damage to the liver, are a well established fact. Likewise, there is weighty evidence that silymarin possesses antimicrobial and anticancer activities. Additionally, it has emerged that in animal models, silymarin can protect the heart, brain, liver and kidneys against ischemia reperfusion injury, probably by preconditioning. The mechanisms of preconditioning are, in general, well studied, especially in the heart. On the other hand, the mechanism by which silymarin protects the heart from ischemia remains largely unexplored. This review, therefore, focuses on evaluating existing studies on silymarin induced cardioprotection in the context of the established mechanisms of preconditioning.

Contents

Abbreviations: AC, adenylyl cyclase; ALDH, aldehyde dehydrogenase; ANT, adenine nucleotide transporter; AR, aderenergic receptor; ARE, aryl hydrocarbon receptor; ATP, adenosine triphosphate; cAMP, cyclic adenosine monophosphate; COX, cyclo-oxygenase; CsA, cyclosporine A; DAG, diacylglycerol; DHSB, dehydrosilybin; EGF, endothelial growth factor; EGFR, EGF receptor; FGF, fibroblast growth factor; GSK, glycogen synthase kinase; HIF, hypoxia induced factor; HUVEC, human umbilical vein endothelial cell; IP3K, inositol phosphate 3 kinase; IPC, ischemic preconditioning; IR, ischemia reperfusion; MMP, matrix metaloprotease; mPTP, mitochondrial permeability transition pore; mTOR, mitochondrial target of rapamycin; PDE, phosphodiestrase; PLC, phospholipase C; PKA, protein kinase A; PKC, protein kinase C; PKG, protein kinase G; ROS, reactive oxygen species; SIRT, silent information regulator two ortholog; VDAC, voltage dependent anion channel; VEGF, vascular endothelial growth factor.

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1. Introduction

Silymarin, a well known, multicomponent extract from the seeds of the milk thistle (*Sylibum marianum*), has been used for the treatment of various ailments, mainly those of the liver, for over two thousand years [1]. Interest in this venerable remedy has not been lost with the advent of the systematic scientific approach and modern biochemical methods, and there are now over four hundred clinical trials using silymarin or its components for liver related diseases alone [2]. In this day and age, silymarin is available as an extract from several major suppliers, each with its own standard composition, which varies dramatically between suppliers and appears to depend on variety and growing condition of the crop [3–5]. Typically, silymarin contains around 50% silybin, 20% silychristin, 10% silydianin, 5% isosilybin and between 10 and 30% of a typically unidentified organic polymer fraction formed from the above compounds. Additionally, a minor fraction of other flavanols including 2,3-dehydrosilybin (DHSB), quercetin, taxifolin, kaempferol and others is present [5,6]. Some of the constituents, including silybin, are present as a mixture of stereoisomers with contrasting biological activities [7,8]. It is understandable therefore, that small changes in the chemical composition of the extract can have a strong influence on its biological activity. On the other hand, this is largely irrelevant when working with the purified, individual components of silymarin. It should be noted that as a consequence of consisting of a number of bioactive compounds, silymarin does not have a single molecular target. Indeed, many of its components, as will become apparent from the discussion below, target more than one enzyme or process. Whilst this can be viewed as a pharmacologist's nightmare, the same pharmacologist may find that it can also become a treasure trove of interesting medicinal compounds and precursors. The milk thistle would serve well for this purpose, owing partially due to its wide range and ease of cultivation.

It is understandable, therefore, that more and more attention is being devoted to the possible protective effects of silymarin on organs besides the liver. As such studies examining protection by silymarin against ischemic damage to kidney, liver, brain and heart have emerged. This is most likely tied to the discovery, and more recently improved understanding, of pre- and post-conditioning. Applicable to tissue that has been subject to ischemia, these closely related biological phenomena prevent a large part of the damage that occurs upon its reperfusion. Whilst preconditioning must be applied during the early window, at least 24 h prior to ischemia, or the late window around 30 min prior to ischemia, post-conditioning can be applied immediately upon reperfusion. Given the unpredictable nature of infarcts, post-conditioning is undoubtedly more valuable as a treatment. Preconditioning, on the other hand, could be availed of when ischemia can be anticipated, for example during surgery or transport of organs [9,10]. The most common, and most clinically relevant, examples of this kind of injury are the heart and brain, where ischemic events manifest themselves as heart attacks and strokes respectively. Arguably, due to the increased window for treatment, pre- and post-conditioning of the heart makes a better example. Both pre- and post-conditioning can be induced either by a series of brief ischemia-reperfusion cycles, in which case they are known as ischemic pre- or post-conditioning (IPC), or by pharmacological agents, in which case they are known as pharmacological pre- or post-conditioning. The former was discovered in 1986 [11] using an open chest dog model, whilst the later arguably in 1984 [12]. Whilst IPC is the better known of the two, pharmacological preconditioning is probably more applicable in practice, as well as serving as a useful tool for the study of the mechanisms involved in IPC.

2. Preconditioning and silymarin

Following occlusion of the blood supply, ischemic tissue will eventually die by necrosis (curiously the 1986 study had already established a limit for the length of ischemia which preconditioning can protect against [11]). It follows that reperfusion became the main form of intervention for myocardial infarction. This led to the discovery of ischemia reperfusion (IR) injury of the heart, which occurs, as the name suggests, when following a prolonged period of ischemia, blood supply is restored to the ischemic tissue, paradoxically causing a rise in cell death. This is proposed to occur because the kick-starting of respiration, in cells where most of the ion gradients have all but collapsed, sets up the perfect conditions for the opening of the mitochondrial permeability transition pore (mPTP) and the subsequent induction of apoptosis. In accordance with this model, ischemic cells rapidly become hypoxic and switch to glycolysis for their source of adenosine triphosphate (ATP) hence becoming acidified. At the same time, levels of reactive oxygen species (ROS) increase and levels of ATP drop along with the activity of the Na^+/K^+ ATPase. Due to the increased proton concentration, i.e. intracellular acidification and reduced activity of the Na^+/K^+ ATPase, the Na^+/H^+ exchanger causes an influx of Na^+. This reverses ion-flux through the Na^+/Ca^{2+} antiporter, increasing the intracellular concentration of Ca^{2+}. Under normal conditions this increase in ROS and Ca^{2+} would be sufficient to open the mPTP and induce apoptosis, however, as low pH inhibits mPTP opening, apoptosis does not occur in ischemic cells. Instead the damage occurs upon reperfusion, when the mitochondrial pH begins to normalise with the restoration of the mitochondrial H^+ gradient and all the conditions for the opening of the mPTP have been met [13,14].

Pre- and post-conditioning, must therefore function either by reducing calcium concentrations in the cells [15–17], limiting over-production or accumulation of ROS or increasing the mPTP threshold [13,18]. In fact it has been shown that pharmacological opening of mPTP with atractyloside prevents preconditioning [19–21], whilst preventing this opening with cyclosporin A (CsA) induces preconditioning in rabbit hearts [20]. The latter prevents the binding of Cyclophilin D [22], whilst the former is a direct inhibitor of the adenine nucleotide transporter (ANT) [23]. Rasola et al. [18] suggest that glycogen synthase kinase 3β (GSK3β) and protein kinase Cε (PKCε) may be responsible for the phosphorylation and hence modulation of mPTP components. It appears that when phosphorylated and hence inhibited by PKCε, GSK3β shifts from the voltage dependent anion channels (VDAC) to ANT binding [24].

This coincides with reduced VDAC phosphorylation and may be central to pre- and post-conditioning [25] as there is evidence that GSK3β inhibition is a central and crucial step in

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