Arh. farm. 2024; 74: 689 – 697 *Review article/Pregledni rad*

Isosteviol: a potential cardioprotective agent

Aleksandar Jovanović[1,2]

[1]Department of Basic and Clinical Sciences, [2]Center for Neuroscience and Integrative Brain Research (CENIBRE), University of Nicosia Medical School, Nicosia, Cyprus

Corresponding author: Aleksandar Jovanović**,** e-mail: jovanovic.a@unic.ac.cy

Received: 8 August 2024; Received in revised form: 1 September 2024; Accepted: 1 September 2024

Abstract

Cardioprotection is a term describing the myocardial property to protect itself from injury, particularly in the context of ischemia-reperfusion injury and other metabolic stresses. Recently, isosteviol, a diterpene derived from the hydrolysis of stevioside, a natural sweetener found in the leaves of the *Stevia rebaudiana* plant, has emerged as a potential cardioprotective compound. In addition to the many therapeutic benefits of isosteviol, including antihyperglycemic, antihypertensive, and anti-inflammatory effects, recent studies have suggested that this compound might have cardioprotective properties as well. It has been demonstrated that isosteviol possesses antioxidant and anti-inflammatory activities, while also regulating ion channels and mitochondrial activity. The cardioprotective effects of isosteviol are mediated through its interaction with multiple signaling pathways. Pre-clinical work has demonstrated that isosteviol regulates NF-κB, phosphatidylinositol 3-kinase/Akt (PI3K/Akt) pathways, and adenosine monophosphate-activated protein kinase (AMPK), all of which are well-established cardioprotective signaling pathways. All these findings highlight isosteviol's potential as a cardioprotective therapeutic agent. However, this potential needs to be further tested in randomized controlled trials, along with examining isosteviol's possible value in clinical practice, defining optimal dosing strategies, and understanding its long-term effects.

Key words: cardioprotection, isosteviol

https://doi.org/10.5937/arhfarm74-52624

Introduction

Cardioprotection is a term referring to a range of physiological and pharmacological strategies able to increase the resistance of the myocardium to ischemia-reperfusion (I/R) injury and various metabolic stresses. It is a consensus view that therapeutic strategies based on cardioprotection would be useful in treating many cardiovascular diseases where increased myocardial resistance to stress would be beneficial. In the search for cardioprotective therapeutics, isosteviol, a natural sweetener found in the leaves of the *Stevia rebaudiana* plant, has emerged as a potential candidate for such a therapeutic. Studies up to date have reported antihyperglycemic, antihypertensive, and anti-inflammatory properties of isosteviol (reviewed in 1). More recent studies have revealed that this compound possesses cardioprotective properties (2, 3).

The objective of this review is to shed light on isosteviol as a potential cardioprotective agent that deserves to be further tested in that regard. Taking into consideration the importance of cardiovascular diseases globally and the absence of the use of cardioprotective agents in clinical practice, understanding the potential of isosteviol as a cardioprotective agent is of considerable importance.

Isosteviol: Chemical Properties and Pharmacokinetics

Isosteviol is a bioactive diterpene derived from stevioside and a natural sweetener extracted from the leaves of the *Stevia rebaudiana* plant. This compound has gained interest for its pharmacological potential. Chemically, isosteviol possesses unique structural features that are different from other terpenoids as it has a rigid backbone that contributes to its stability and affects its interaction with biological targets. The lipophilic nature of isosteviol allows its penetration through cellular membranes, which is important for its activity at various intracellular sites. Following oral administration, isosteviol is absorbed through the gastrointestinal tract, but with a variable bioavailability influenced by various factors, such as formulation and the presence of other dietary components. Once absorbed, isosteviol is widely distributed throughout the body, with a particular affinity for tissues with high lipid content. The metabolism of isosteviol primarily occurs in the liver, where it undergoes conjugation reactions that increase its solubility. These metabolic transformations are mainly the addition of glucuronic acid or sulfate groups, which makes isosteviol more suitable for renal excretion. The metabolites of isosteviol are not particularly well-studied, but it seems that they retain some of the biological activities of the parent compound. The excretion of isosteviol and its metabolites occurs mainly through the kidneys and, to a lesser extent, through the biliary route. The reported elimination half-life of isosteviol varies and it is species-dependent (4-6).

Evidence supporting the notion that isosteviol is cardioprotective

So far, many pre-clinical studies have provided evidence in favor of the cardioprotective properties of isosteviol. The first piece of evidence was provided using

a whole heart I/R experimental model, where it has been shown that intravenous isosteviol significantly decreases the size of myocardial infarction and improves left ventricular function following I/R challenge (2, 3). It has been also shown that isosteviol prevents the development of myocardial hypertrophy induced by isoprenaline (7). The cardioprotection afforded by isosteviol was also confirmed at the cellular level, where isosteviol promoted the survival of cardiomyocytes subjected to hypoxia and hypoxia-reoxygenation (8-10; Figure 1). Cardioprotective effects were also described by various isosteviol analogs (11-15).

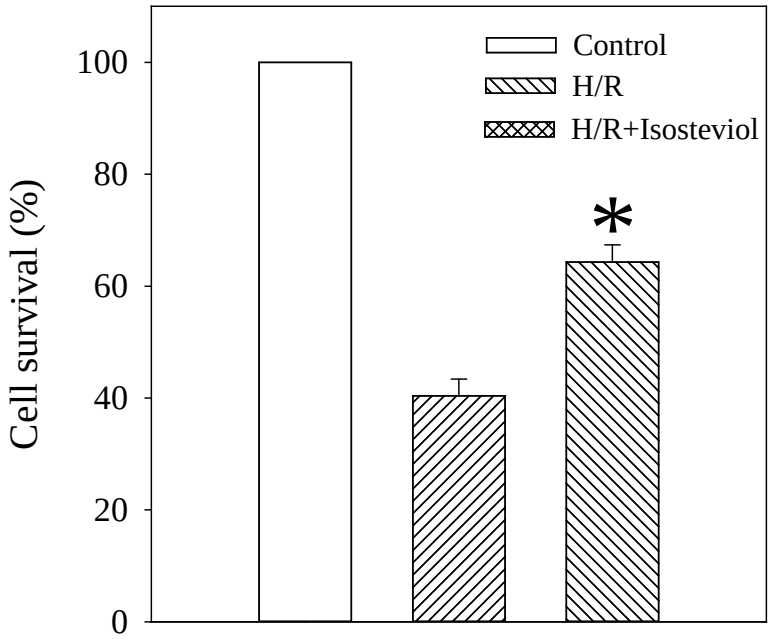

Figure 1. Isosteviol protects rat heart embryonic H9c2 cells against hypoxia-reoxygenation. Bar graph depicting cell survival under normoxic conditions (control) and hypoxia-reoxygenation in the absence (H/R) and presence of isosteviol (10 μM). Each bar represents mean ± SEM (n=3-6). $^{*}P < 0.01$. The graph is made based on data published in reference 10, where corresponding methods are also described in detail.

Slika 1. Izosteviol štiti embriogene H9c2 ćelije srčanih miševa od hipoksije-reoksigenacije. Na grafikonu je prikazano preživljavanje ćelija pod normoksičnim uslovima (kontrolna grupa) i hipoksija-reoksigenacija u odsustvu (H/R) i prisustvu izosteviola (10 μM). Svaka kolona predstavlja srednju vrednost ± SEM (n=3-6). *P < 0,01. Grafikon je napravljen na osnovu podataka objavljenih u referenci 10, gde su odgovarajuće metode takođe detaljno opisane.

Mechanisms underlying isosteviol-induced cardioprotection

The first report revealing cardioprotective properties of isosteviol suggested that isosteviol-induced cardioprotection is mediated via mitochondrial ATP-sensitive (K_{ATP}) channels (2). It has also been reported that isosteviol restores mitochondrial membrane potential, which would be in accord with its effect on mitochondrial K_{ATP} channels. In addition, it has been also suggested that isosteviol inhibits mitochondrial abnormal proteins dynamin-related protein 1 (Drp1), and mitochondrial fission 1 (Fis1), which seem to play key roles in cardioprotection (16). In addition to the mitochondrial K_{ATP} channels, reports have also suggested the potential involvement of sarcolemmal K_{ATP} channels (17), as well as a rapid component of delayed rectifier K^+ current (I_{Kr}) (18). It has been suggested that isosteviol reduces the over-production of reactive oxygen species (ROS) during I/R, and that this is the mechanism underlying the activation of I_{Kr} by isosteviol (18), as well as mitochondrial effects. The protective effect of isosteviol against myocardial hypertrophy has also been associated with the reduction in ROS production, stabilization of mitochondrial membrane potential, and maintaining intracellular Ca^{2+} homeostasis (7). Further studies have demonstrated that isosteviol also activates the PI3K/Akt/GSK-3β signaling pathway (8), which is a well-established cardioprotective pathway (19). It has been also reported that this compound activates ERK1/2 and AMPK (3, 10), which are well-established cardioprotective kinases that target multiple targets, including K_{ATP} channels (20-22). Finally, the protective effects of isosteviol on cardiomyocytes have been associated with the regulation of the SIRT1/PGC-1α signaling pathway, which is known to be involved in DNA repair, apoptosis, and inflammation (9).

All things considered, it is clear that isosteviol activates multiple well-established cardioprotective intracellular factors, which is similar to some other compounds that trigger cardioprotection (19). However, what is a unique property of isosteviol that has never been described for any other agent is its property to activate some cardioprotective signaling factors, such as ERK1/2, only in adverse, but not in physiological, conditions (10). As the main issue in introducing cardioprotective agents into clinical practice are their potential adverse effects, this feature of isosteviol could overcome that. All signaling pathways mentioned above have multiple intracellular effects, many of which are not desirable for therapeutics. If isosteviol, as it currently seems, activated those pathways only in challenged/diseased cells, healthy cells that are not challenged would likely not be affected. Thus, this unique property of isosteviol makes this compound a potential candidate that could be introduced into clinical practice. The suggested mechanisms underlying isosteviol-mediated cardioprotection are depicted in Table I.

Table I The mechanisms proposed to underlie isosteviol-induced cardioprotection

Tabela I Predloženi mehanizmi koji stoje u osnovi kardioprotekcije indukovane izostevilom

Mechanism	Description	References
Mitochondrial ATP-sensitive (KATP) channels	Isosteviol-induced cardioprotection is mediated via mitochondrial KATP channels.	(2)
Mitochondrial membrane potential	Isosteviol restores mitochondrial membrane potential, aligning with its effect on mitochondrial KATP channels.	(2)
Inhibition of mitochondrial abnormal proteins	Isosteviol inhibits dynamin-related protein 1 (Drp1) and mitochondrial fission 1 (Fis1), which play key roles in cardioprotection.	(16)
Sarcolemmal KATP channels	Potential involvement of sarcolemmal KATP channels in isosteviol-induced cardioprotection.	(17)
Rapid component of delayed rectifier K+ current (IKr)	Isosteviol activates IKr, reducing the over-production of reactive oxygen species (ROS) during I/R.	(18)
Reduction in ROS production	Isosteviol reduces ROS production, stabilizes mitochondrial membrane potential, and maintains intracellular Ca2+ homeostasis, protecting against myocardial hypertrophy.	(7)
PI3K/Akt/GSK-3β signaling pathway	Isosteviol activates the PI3K/Akt/GSK-3β signaling pathway, a well-established cardioprotective pathway.	(8), (19)
ERK1/2 and AMPK activation	Isosteviol activates ERK1/2 and AMPK, which are cardioprotective kinases targeting multiple intracellular targets including KATP channels.	(3), (10), (20-22)
SIRT1/PGC-1α signaling pathway	Isosteviol regulates the SIRT1/PGC-1α signaling pathway, involved in DNA repair, apoptosis, and inflammation.	(9)
Selective activation in hypoxia	Unique property of isosteviol to activate certain signaling factors, such as ERK1/2, only in hypoxic conditions, potentially reducing adverse effects in normoxic cells.	(10)

Clinical potential of isosteviol in cardioprotection

Although some clinical studies have been done with stevia-based sweeteners (1, 23), so far no proper clinical trials have been done to elucidate the therapeutic potential of isosteviol that would be based on its cardioprotective properties that are so well documented in preclinical studies (see above). Thus, there are still major limitations regarding potential isosteviol clinical use. The following is required to be done before seriously considering introducing isosteviol into clinical practice:

1. Dose-Response Relationship: The optimal dosage of isosteviol for cardioprotection needs to be established. Determining the most effective and safe dosage requires further research.
2. Pharmacokinetics Variability: Individual differences in the absorption, distribution, metabolism, and excretion of isosteviol may affect its efficacy and safety profile across different populations. Understanding these pharmacokinetic variabilities is essential for personalized treatment approaches.
3. Long-Term Effects: The long-term effects of isosteviol consumption, particularly at higher doses or in specific patient populations, need to be determined. Longitudinal studies are needed to assess the safety of prolonged isosteviol use.
4. Clinical efficacy: The clinical efficacy of isosteviol and comparisons with other cardioprotective agents need to be elucidated.

Conclusion

Isosteviol presents a promising natural compound with potential cardioprotective and therapeutic benefits. The preclinical studies have provided ample evidence that isosteviol possesses cardioprotective properties. In addition, there are some indications that isosteviol could selectively activate cardioprotective signaling pathways in cardiomyocytes exposed to stress, which, if confirmed in future studies, would be a unique property and a great advantage over other potential cardioprotective agents. However, no clinical studies have tested the clinical potential of isosteviol in the context of cardiovascular diseases. Thus, what is required in the future are 1) further preclinical studies addressing mechanisms underlying isosteviol-induced cardioprotection and 2) clinical studies that will test clinical safety and efficacy in the appropriate population of patients.

Declaration of Competing Interest

The author declares that he has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contributions

AJ wrote, reviewed, and edited the original draft.

References

1. Orellana-Paucar AM. Steviol glycosides from *Stevia rebaudiana*: An updated overview of their sweetening activity, pharmacological properties, and safety aspects. Molecules. 2023;28:1258.
2. Xu D, Li Y, Wang J, Davey AK, Zhang S, Evans AM. The cardioprotective effect of isosteviol on rats with heart ischemia-reperfusion injury. Life Sci. 2007;80:269-274.
3. Cao Y, Lu Z, Wang D, Tan KS, Liu W, Wu Q, et al. Therapeutic evaluation and metabolic reprograming of isosteviol sodium in a rat model of ischemic cardiomyopathy. Eur J Pharmacol. 2021;911:174539.
4. Jin H, Gerber JP, Wang J, Ji M, Davey AK. Oral and i.v. pharmacokinetics of isosteviol in rats as assessed by a new sensitive LC-MS/MS method. J Pharm Biomed Anal. 2008;48:986-990.
5. Adehin A, Tan KS, Lu Z, Cheng Q, Tan W. In vitro metabolic stability and biotransformation of isosteviol in human and rat liver fractions. Drug Metab Pharmacokinet. 2019;34:194-200.
6. Adehin A, Tan KS, Zou C, Lu Z, Lin Y, Wang D, et al. A compartmental approach to isosteviol's disposition in Sprague-Dawley rats. Naunyn Schmiedebergs Arch Pharmacol. 2020;393:1003-1011.
7. Chen Y, Beng H, Su H, Han F, Fan Z, Lv N, Jovanović A, Tan W. Isosteviol prevents the development of isoprenaline-induced myocardial hypertrophy. Int J Mol Med. 2019;44:1932-1942.
8. Zhang X, Lu Z, Abdul KSM, Changping MA, Tan KS, Jovanović A, Tan W. Isosteviol sodium protects heart embryonic H9c2 cells against oxidative stress by activating Akt/GSK-3β signaling pathway. Pharmazie. 2020;75:36-40.
9. Mei Y, Liu B, Su H, Zhang H, Liu F, Ke Q, et al. Isosteviol sodium protects the cardiomyocyte response associated with the SIRT1/PGC-1α pathway. J Cell Mol Med. 2020;24:10866-10875.
10. Abdul KSM, Faiz N, Jovanović A, Tan W. Isosteviol protects H9c2 cells against hypoxia-reoxygenation by activating ERK1/2. Cardiovasc Hematol Disord Drug Targets. 2021;21:73-77.
11. Jayachandra R, Zhao H, Cheng Z, Luo L, Sun T, Tan W. Synthesis of Isosteviol analogues as potential protective agents against Doxorubicin-induced cardiomyopathy in zebrafish embryos. Bioorg Med Chem Lett. 2019;29:1705-1709.
12. Mohammed Abdul KS, Rayadurgam J, Faiz N, Jovanović A, Tan W. Cardioprotection by isosteviol derivate JC105: A unique drug property to activate ERK1/2 only when cells are exposed to hypoxia-reoxygenation. J Cell Mol Med. 2020;24:10924-10934.
13. Zhang H, Liu B, Xu G, Xu C, Ou E, Liu J, et al. Synthesis and in vivo screening of isosteviol derivatives as new cardioprotective agents. Eur J Med Chem. 2021;219:113396.
14. Chen Z, Xu R, Jia Q, Xu X, Li D, Li Z, et al. Discovery of new D-ring modified isosteviol derivatives as potent cardioprotective agents against oxidative stress-trigged damage. Chem Biodivers. 2023;20:e202300085.
15. Chen Z, Li Z, Xu R, Xie Y, Li D, Zhao Y. Design, synthesis, and in vivo evaluation of isosteviol derivatives as new SIRT3 activators with highly potent cardioprotective effects. J Med Chem. 2024;67:6749-6768.
16. Sun X, Yang Y, Xie Y, Shi X, Huang L, Tan W. Protective role of STVNa in myocardial ischemia reperfusion injury by inhibiting mitochondrial fission. Oncotarget. 2017;9:1898-1905.
17. Fan Z, Wen T, Chen Y, Huang L, Lin W, Yin C, Tan W. Isosteviol sensitizes sarcKATP channels towards pinacidil and potentiates mitochondrial uncoupling of diazoxide in guinea pig ventricular myocytes. Oxid Med Cell Longev. 2016;2016:6362812.

18. Yin C, Chen Y, Wu H, Xu D, Tan W. Attenuation of ischemia/reperfusion-induced inhibition of the rapid component of delayed rectifier potassium current by isosteviol through scavenging reactive oxygen species. Biochim Biophys Acta Biomembr. 2017;1859:2447-2453.
19. Jovanović A. Cardioprotective signalling: Past, present and future. Eur J Pharmacol. 2018;833:314-319.
20. Sukhodub A, Jovanović S, Du Q, Budas G, Clelland AK, Shen M, et al. AMP-activated protein kinase mediates preconditioning in cardiomyocytes by regulating activity and trafficking of sarcolemmal ATP-sensitive K(+) channels. J Cell Physiol. 2007;210:224-236.
21. Mohammed Abdul KS, Jovanović S, Sukhodub A, Du Q, Jovanović A. Upregulation of cardioprotective SUR2A by sub-hypoxic drop in oxygen. Biochim Biophys Acta. 2014;1843:2424-2431.
22. Mohammed Abdul KS, Jovanović S, Jovanović A. Exposure to 15% oxygen in vivo up-regulates cardioprotective SUR2A without affecting ERK1/2 and AKT: a crucial role for AMPK. J Cell Mol Med. 2017;21:1342-1350.
23. Raghavan G, Bapna A, Mehta A, Shah A, Vyas T. Effect of sugar replacement with stevia-based tabletop sweetener on weight and cardiometabolic health among Indian adults. Nutrients. 2023;15:1744.

Izosteviol kao potencijalni kardioprotektivni agens

Aleksandar Jovanović[1,2]

[1]Department of Basic and Clinical Sciences, [2]Center for Neuroscience and Integrative Brain Research (CENIBRE), University of Nicosia Medical School, Nicosia, Cyprus

Autor za korespondenciju: Aleksandar Jovanović, e-mail: jovanovic.a@unic.ac.cy

Kratak sadržaj

Kardioprotekcija je termin kojim se opisuje sposobnost miokarda da se zaštiti od povreda, posebno u kontekstu ishemijsko-reperfuzionih povreda i drugih metaboličkih stresova. Odnedavno se izosteviol, diterpen dobijen hidrolizom steviosida, prirodnog zaslađivača koji se nalazi u listovima biljke *Stevia rebaudiana*, razmatra kao jedinjenje sa potencijalno kardioprotektivnim dejstvom. Pored brojnih terapijskih benefita izosteviola, uključujući njegovo antihiperglikemijsko, antihipertenzivno i antiinflamatorno dejstvo, nedavne studije su sugerisale da ovo jedinjenje može imati i kardioprotektivna svojstva. Pokazalo se da izosteviol deluje antioksidativno i antiinflamatorno, kao i da reguliše jonske kanale i mitohondrijsku aktivnost. Kardioprotektivni efekti izosteviola posredovani su njegovom interakcijom sa više signalnih puteva. Preklinička istraživanja pokazala su da izosteviol reguliše NF-κB, putanje fosfatidilinozitol 3-kinaze/Akt (PI3K/Akt) i protein kinazu aktiviranu adenozin monofosfatom (AMPK), što su sve potvrđeni kardioprotektivni signalni putevi. Svi ovi nalazi ističu potencijal izosteviola kao kardioprotektivnog terapijskog agensa. Međutim, ovaj potencijal je potrebno dalje testirati u randomizovanim kontrolisanim ispitivanjima, uz ispitivanje moguće vrednosti izosteviola u kliničkoj praksi, definisanje optimalnih strategija doziranja i razumevanje njegovih dugoročnih efekata.

Ključne reči: kardioprotekcija, izosteviol