Pharmacology & Therapeutics 140 (2013) 167–175

Contents lists available at ScienceDirect

Pharmacology & Therapeutics

journal homepage: www.elsevier.com/locate/pharmthera

Multiplicity of effectors of the cardioprotective agent, diazoxide

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ARTICLE INFO

Keywords:
Cardioprotection
Ischemia
Ischemic preconditioning
Diazoxide
K_{ATP} channels
Mitochondria

ABSTRACT

Diazoxide has been identified over the past 50 years to have a number of physiological effects, including lowering the blood pressure and rectifying hypoglycemia. Today it is used clinically to treat these conditions. More recently, another important mode of action emerged: diazoxide has powerful protective properties against cardiac ischemia. The heart has intrinsic protective mechanisms against ischemia injury; one of which is ischemic preconditioning. Diazoxide mimics ischemic preconditioning. The purpose of this treatise is to review the literature in an attempt to identify the many effectors of diazoxide and discuss how they may contribute to diazoxide's cardioprotective properties. Particular emphasis is placed on the concentration ranges in which diazoxide affects its different targets and how this compares with the concentrations commonly used to study cardioprotection. It is concluded that diazoxide may have several potential effectors that may potentially contribute to cardioprotection, including K_{ATP} channels in the pancreas, smooth muscle, endothelium, neurons and the mitochondrial inner membrane. Diazoxide may also affect other ion channels and ATPases and may directly regulate mitochondrial energetics. It is possible that the success of diazoxide lies in this promiscuity and that the compound acts to rebalance multiple physiological processes during cardiac ischemia.

Contents

Abbreviations: ΔΨm, mitochondrial membrane potential; 5HD, 5-hydroxydecanoate; DNP, 2,4-dinitrophenol; I/R injury, ischemia/reperfusion injury; IPC, ischemic preconditioning; K_{ATP} channel, ATP-sensitive K^+ channel; ROS, reactive oxygen species; SDH, succinate dehydrogenase.

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0163-7258/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.pharmthera.2013.06.007

1. Introduction

Diazoxide (CAS Number: 364-98-7; 7-chloro-3-methyl-2H-1,2,4-benzothiadiazine 1,1-dioxide; Fig. 1) has a molecular weight of 230.7 and a molecular formula of $C_8H_7ClN_2O_2S$. It is a white powder insoluble

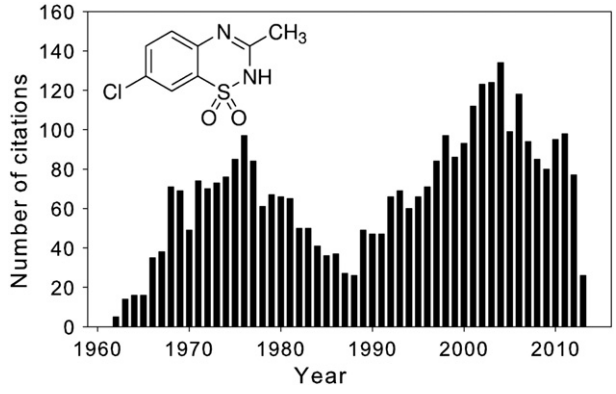

Fig. 1. The number of PubMed citations returned when searching with the keyword "diazoxide". The data are binned over the time period 1960–2013. The inset shows the structural formula for diazoxide.

in water, but soluble in organic solvents (e.g. 10 mg/ml in DMSO). The dogma has arisen in recent years (particularly in the cardioprotection literature) that diazoxide is an agent with a unique molecular target. This is not the case and the purpose of this literature review is to highlight the multiplicity of diazoxide effectors to assist in a better understanding of mechanisms involved in the established cardioprotective effects of this compound.

2. History

In the early 1960's, a study was designed to examine possible non-diuretic mechanisms by which benzothiadiazines lower blood pressure — diazoxide was found to directly cause vasodilation of blood vessels independent of diuretic actions (Rubin et al., 1962). Early reports, however, also demonstrated that some hypotensive drugs such as diazoxide led to elevated blood glucose levels (hyperglycemia) (Wolff, 1964; Okun et al., 1964). The following years saw a large rise in publications, mostly related to the hypotensive and hyperglycemic effects of diazoxide (Fig. 1). Other actions, including effects on renal excretory function, also started to emerge (Johnson, 1971; Rubin et al., 1968). Nevertheless, the compound became accepted for its oral use in the management of intractable hypoglycemia and intravenously in the management of hypertensive emergencies. The publication rate waned in the mid-1980s. Following the identification of diazoxide molecular effectors in pancreatic β-cells (Henquin & Meissner, 1982; Trube et al., 1986) and vascular smooth muscle cells (Standen et al., 1989), a secondary rise in diazoxide-related publications occurred (Fig. 1), which was further stimulated by the mid-1990's findings that diazoxide has powerful cardioprotective properties (Garlid et al., 1997; Nakai & Ichihara, 1994).

3. Clinical use

In tablet form (e.g. Proglycem, FDA approved in 1976) diazoxide is prescribed orally (usually 2 to 3 times daily) for the management of symptomatic hypoglycemia. Side effects include shortness of breath, swelling in extremities, tachycardia, chest pain, blurred vision, bruising or bleeding, unusual weakness; and decreased frequency of urination. Intravenously (e.g. Hyperstat) diazoxide is indicated as a peripheral vasodilator for short-term use in the emergency reduction of blood pressure in severe, nonmalignant and malignant hypertension in hospitalized adults; and in acute severe hypertension in hospitalized children, when prompt and urgent decrease of diastolic pressure is required. Diazoxide is also used to treat hypoglycemia that results from congenital hyperinsulinism of infancy (HI) (Hussain et al., 2004). The mechanisms of diazoxide's clinical action relate predominantly to the opening of pancreatic and smooth muscle K_{ATP} channels, as will be discussed in subsequent sections.

4. Diazoxide is cardioprotective against ischemic insults

During the treatment of patients with hypotension, early studies suggested an increase in myocardial injury with diazoxide (e.g. chest pain and ST elevation) (Kanada et al., 1976; O'Brien et al., 1975). These effects may have been related to the hypotensive action of the drug. Most controlled animal studies to date, however, as well as in vitro studies with human cardiac tissues, suggest that diazoxide has cardioprotective properties (Garlid et al., 1997; Nakai & Ichihara, 1994; Wang et al., 1999). Intrinsic adaptive physiological processes within the myocardium render the heart more resistant to potentially lethal ischemic injury. One of the protective phenomena is ischemic preconditioning (IPC) — the most powerful means of delaying myocardial injury that has been identified to date (Yellon & Downey, 2003). Diazoxide is powerfully anti-ischemic and/or recapitulates the cardioprotective effects of IPC. A full review of these studies is beyond the scope of this review and some of these studies are highlighted in Table 1. The cardioprotective effects are observed in a variety of species (rat, rabbit, dog and human), with ex vivo and in vivo methods and over a concentration range of ~10–100 μM (or 1–10 mg/kg intravenously). Most studies utilize a single dose of diazoxide. In one study, the EC_{25} for diazoxide's protective effect (measured as an increased time to onset of contracture) in isolated rat hearts subjected to 30 min global ischemia was reported to be ~10 μM (Garlid et al., 1997). The $K_{1/2}$ is likely to be higher. In another study, the optimal protective concentration in isolated rat hearts subjected to ischemia/reperfusion was reported to be 80 μM (Wang et al., 1999). With instrumented dogs, 80 μM (but not 8 μM) diazoxide was reported to provide partial protection against the development of a post-ischemic infarct (Sanada et al., 2001). The protective effect of diazoxide is equivalent to that of ischemic preconditioning (IPC) and diazoxide is often used as a pharmacological means to induce preconditioning. Moreover, both the IPC- and diazoxide-induced protection are minimized by tolbutamide, HMR-1883 or glibenclamide (sulfonylurea compounds that block various types of K_{ATP} channels (Trube et al., 1986; Escande, 1989; Faivre & Findlay, 1989; Quayle et al., 1995; Gogelein et al., 1998)) or 5HD (often used as a mitochondrial K_{ATP} blocker, but which also has other off-target effects; see later) (Birincioglu et al., 1999), suggesting a causative link between the diazoxide effector(s) and mechanism(s) involved in IPC. The purpose of this mini-review is to highlight the multiplicity of diazoxide effectors.

5. Diazoxide activates pancreatic β-cell K_{ATP} channels

Diazoxide has long been recognized to be hyperglycemic by inhibiting insulin release from pancreatic β-cells (Wolff et al., 1963; Loubatieres et al., 1966; Okun et al., 1964). The mechanism was found to be an increased membrane K^+ permeability (measured with Rb^+ flux assays), leading to membrane hyperpolarization, inhibition of Ca^{2+} influx (Henquin & Meissner, 1982) and diminished insulin secretory release. Shortly after the discovery of cardiac K_{ATP} channels (Noma, 1983; Trube & Hescheler, 1984), similar channels in the pancreatic β-cell were found to be responsible for the diazoxide-induced increase of K^+ permeability (Trube et al., 1986). The diazoxide-sensitivity of the pancreatic β-cell K_{ATP} channel is high ($K_{1/2}$ of 7–20 μM; Table 2), which is in the same concentration range as the drug's cardioprotective benefit (Table 1). Pancreatic β-cell K_{ATP} channels are unlikely to be involved in diazoxide's cardioprotective effects when using ex vivo preparations (e.g. isolated hearts). In patients, however, or when using diazoxide with in vivo experimental approaches, the possibility must be considered that diazoxide may elevate blood glucose levels, which in turn may influence the ischemic outcome. Provision of glucose, together with insulin and potassium (GIK), has clear beneficial effects during ischemia (Opie, 1975) but the benefit of increasing glucose levels alone is questionable (LaDisa et al., 2004; Spath et al., 1976).