Received: 11 November 2016 | Revised: 20 December 2016 | Accepted: 22 December 2016

DOI: 10.1002/jbt.21898

WILEY

Mechanism of capsaicin inhibition of aldose reductase activity

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Abstract

Aldose reductase (AR) inhibitors play a vital importance as a potential therapeutic and preventive medicine when it comes to hyperglycemia associated diabetic complications. Additionally, capsaicin is used as a food additive and a drug in a number of diverse clinical trials. The aim of this study is to determine the in vitro inhibition behavior of capsaicin on AR enzyme activity, which was obtained from different rat tissues (heart, kidney, liver, and brain). We showed that AR was inhibited by capsaicin in the micromolar range and noncompetitive manner in all of the tissues. K_i values of capsaicin were found to be 8.87, 264, 535, and 597, respectively, in heart AR, kidney AR, liver AR, and brain AR. In conclusion, capsaicin may be an effective molecule when used in low concentrations to prevent diabetic complications associated with the polyol pathway.

KEYWORDS

aldose reductase, capsaicin, diabetic complications, inhibition

1 | INTRODUCTION

It is well known that hyperglycemia enhances glucose metabolism via the polyol pathway, and this has been implicated in the etiology of complications associated with diabetes such as neuropathy, nephropathy, retinopathy, and cardiopathy and cataract genesis.[1] This pathway involves two main enzymatic steps. Aldose reductase (AR: EC 1.1.1.21) is a key and rate-limiting enzyme of the polyol pathway. It is responsible for the reduction of the aldehyde group in the substrate: It uses Nicotinamide adenine dinucleotide phosphate reduced (NADPH) as a cofactor and catalyst for the conversion of glucose to sorbitol as the first step in the polyol pathway, and the second enzyme sorbitol dehydrogenase converts sorbitol to fructose with Nicotinamide adenine dinucleotide $(NAD)^+$ as a cofactor[2] (Figre 1). During diabetes, an abnormal activity of the polyol pathway leads to accumulation of sorbitol. Thus, water enters into the cells, where swelling takes place, which can cause a cataract. Cataract is one of many diabetic complications. Also, AR competes with Glutathione reduced (GR) for a Nicotinamide adenine dinucleotide reduced (NADPH) cofactor. This leads to a decrease in GSH level. An increase in NADH causes NADH oxidase (NOx) to produce reactive oxygen species (ROS), fructose-3-phosphate (F-3-P), and 3-deoxyglucosone (3-DG). Metabolites of fructose increase *advanced glycation end product* (AGE) and binding of AGE to receptor (RAGE), which increases oxidative stress[2] (Figure 2). So, the polyol route is an important metabolic pathway in the development of diabetic complications.

For this reason, reduction of polyol pathway activation by using AR inhibitors could be a potential therapeutic treatment or prevention medicine of diabetic complications. [3] A number of AR inhibitors have been found to delay diabetic complications in animal testing, and some have been evaluated in clinical experiments.[4] But, the use of these inhibitors has remained limited due to the undesirable side effects. [5,6] Even though it may have bad side effect, studies of the AR inhibitors are gaining importance every day. Particularly, studies which use natural compounds that have AR inhibitors are very popular. [7–10] There are many studies being done on isolating phenolic compounds from traditional plants and their effects on glucose metabolism particularly polyol pathway in recent years. [11–16] In this study, we examined the effect of capsaicin, which is a nonphenolic compound, on AR enzyme activity in different rat tissues.

Capsaicin (*trans*-8-methyl-*N*-vanillyl-6-nonenamide; CAP) is a naturally occurring alkaloid derived from the plants of genus Capsicum family, and it is a nonphenolic compound, which contains amide and lipophilic carbon chain on the one end and a hydrophilic ring on the other. [17] Capsaicin is the major pungent ingredient in chili peppers and is increasingly being used in the food and pharmaceutical industries. [18] Capsaicin exerts various pharmacologic and physiologic effects on a body, such as analgesia, anticancer, antiinflammation, antioxidant, and antiobesity. [19] Moreover, recent studies have shown that capsaicin may also help to reduce postprandial blood glucose and improve insulin resistance. [20] In addition, some studies have shown that capsaicin may be effective in reducing pain in patients with painful diabetic neuropathy. [21] However, effect of capsaicin on AR enzyme activity is unknown. Therefore, in this study, we aimed to investigate in vitro effect of capsaicin on AR enzyme obtained from different rat tissues (heart, kidney, liver, brain) and to identify potential inhibition profile

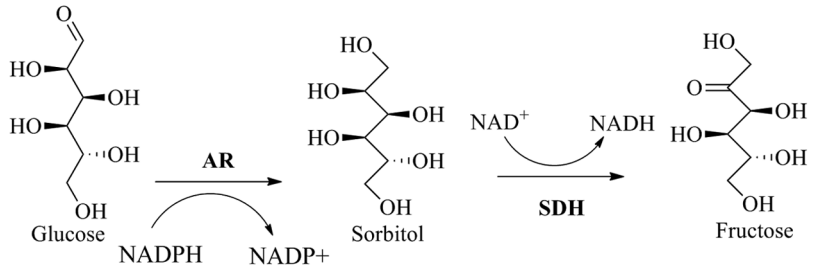

FIGURE 1 Mechanism of the polyol pathway

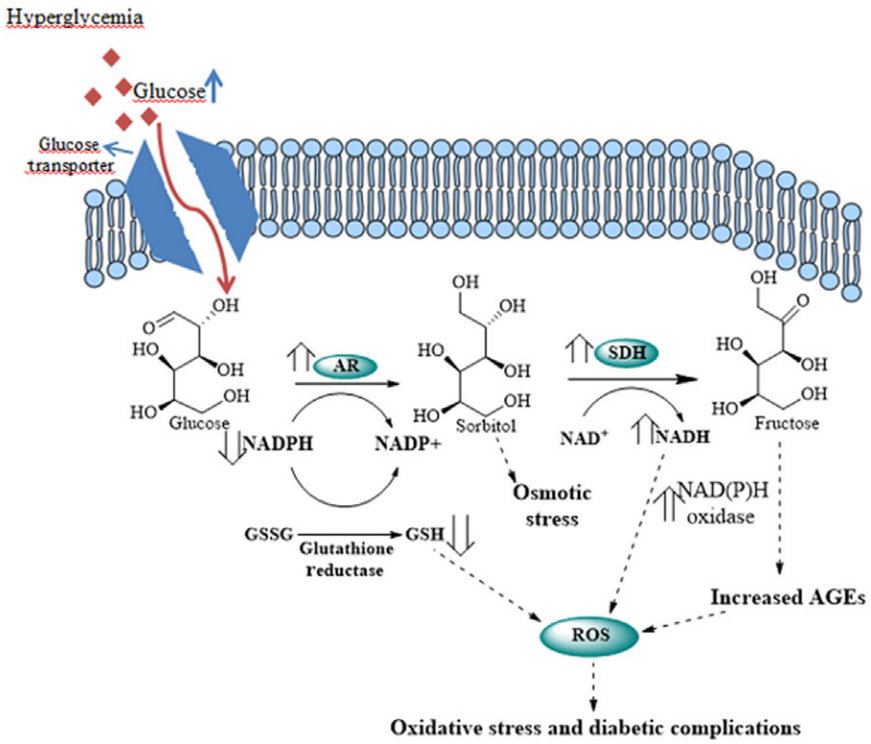

FIGURE 2 The relationship between oxidative stress and diabetic complications of the polyol pathway: The accelerated flux polyol pathway plays a critical role in the development of diabetic complications. Cataract is one of these diabetic complications. It is known that sorbitol accumulates in tissues and causes an increase in the osmotic pressure. Thus, water enters into the cells and swelling takes place, which can cause a cataract. Also, AR competes with GR for their cofactor NADPH. The activity of GR decreased when the activity of AR increased, and this leads to a decrease in the GSH level. Increased NADH caused NOx to produce ROS. F-3-P and 3-DG, and metabolites of fructose increased AGE, and binding oAGE to RAGE increases oxidative stress

and mechanisms of capsaicin for rat heart, kidney, liver, and brain AR enzyme.

2 | MATERIALS AND METHODS

2.1 | Chemicals and instruments

Capsaicin, protein assay reagents, NADPH, DL-glyceraldehyde, and all other chemicals were obtained from Sigma-Aldrich (Taufkirchen, Germany).

2.2 | Aldose reductase activity assay

AR activity was assessed by following the change in absorbance of NADPH at 340 nm spectrophotometrically. The 1-mL enzymatic reaction mixture contained 0.8 M Na-phosphate buffer (pH 5.5), 4.7 mM DL-glyceraldehyde, 0.11 mM NADPH, and enzyme solution.[22] One enzyme unit is defined as the amount of enzyme required to deplete 1 μmol of NADPH per minute.

2.3 | Partial purification of AR from rat tissues

Male Sprague–Dawley rats were bought from Atatürk University Medical Experimental Application and Research Center. The animals were housed in standard conditions. Rats were anesthetized with ether inhalation and then dissected to remove their hearts, kidneys, livers, and brains were removed. Afterward, they were washed with a cold isotonic saline solution to remove blood and stored at −80°C in a freezer. Rat tissues (heart, kidney, liver, and brain) were first cut into small pieces, cleaned with liquid nitrogen, and homogenized in 15 mL of 10 mM Na-phosphate buffer (pH 7.4). The homogenates

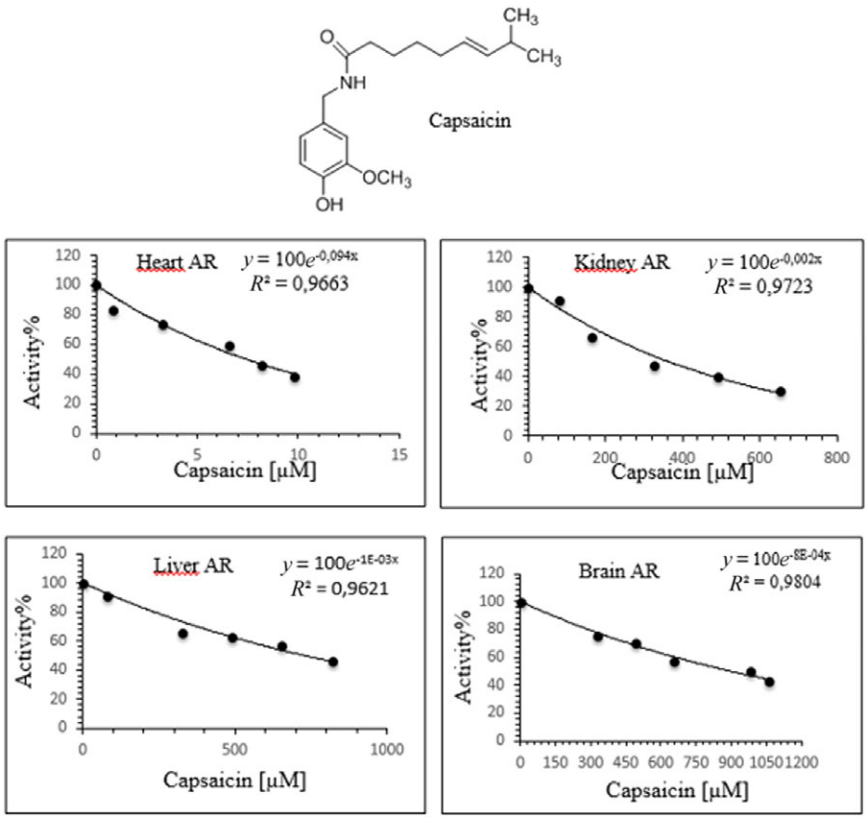

FIGURE 3 The molecular structure of capsaicin and activity%–[capsaicin] graphs, which were used in determination of capsaicin concentrations that cause 50% inhibition (IC_{50}) for each rat tissue's AR

were centrifuged 13.500×*g* for 60 min. Supernatants were used in the following study. The supernatant suspensions were precipitated with ammonium sulfate. The precipitation intervals were in the range 0%–70% for AR enzyme. The precipitates were collected by centrifugation at 13.500×*g* for 30 min and dissolved again in a 10 mM Na-phosphate buffer (pH 7.4). The solutions were dialyzed against 10 mM Na-phosphate buffer (pH 7.4) that contained 5 mM 2-mercaptoethanol. After dialyzing, the solutions were placed into eppendorf tubes and stored at −80°C. Kinetic experiments were conducted on freshly thawed samples kept at 4°C.

2.4 | In vitro inhibition studies

In inhibition studies, AR activity was examined by following the change in absorbance at 340 nm, spectrophotometrically. One milliliter of the reaction mix contains 0.2 M Na-phosphate buffer (pH 6.2), 10 mM DL-glyceraldehyde, 0.5 mM NADPH, and enzyme solution. [22] AR activity was measured using different concentrations of capsaicin. A control sample without inhibitor was also taken and for each tissue's AR enzyme activity%–[capsaicin] a graph was drawn. To determine the K_i constant, three different capsaicin concentrations were used. DL-glyceraldehyde was also used as a substrate at five different concentrations. K_i constant was obtained by using the Lineweaver–Burk graph (1/V−1/[S]), and inhibition type was found for capsaicin also. The obtained data were analyzed using the *t*-test, and they are given as $X \pm$ SD.

3 | RESULTS

In this study, we partially purified the AR enzyme from the rat's heart, kidney, liver, and brain and then investigated the in vitro inhibition effect of capsaicin on AR enzyme activity in these tissues. Both the K_i and IC_{50} parameters of the capsaicin were determined in this study via Lineweaver–Burk graphs (1/V−1/[S]) and activity%–[capsaicin] graphs, respectively. IC_{50} values of capsaicin were determined to be 7.5, 367, 734, and 904 μM for heart AR, kidney AR, liver AR, and brain AR, respectively. K_i values of capsaicin were determined to be 8.87 ± 2.45, 267 ± 30.1, 535 ± 114, and 597 ± 217 μM for heart AR, kidney AR, liver AR, and brain AR, respectively. Also, capsaicin showed no inhibition effect when it came to the heart AR, kidney AR, liver AR, and brain AR.

4 | DISCUSSION

Discoveries of new and natural AR inhibitors are required to improve the quality of life for diabetic patients by preventing diabetic complications. Particularly, studies using phenolic compounds as AR inhibitors have become very popular in recent years.[11–16] In this study, we examined the effect of capsaicin, which is a nonphenolic compound, on AR enzyme activity in different rat tissues.

Researchers have discovered that the use of capsaicin when it comes to diabetes can help to control blood sugar levels and also help to cure type I diabetes. [20,23] In addition, researchers have also

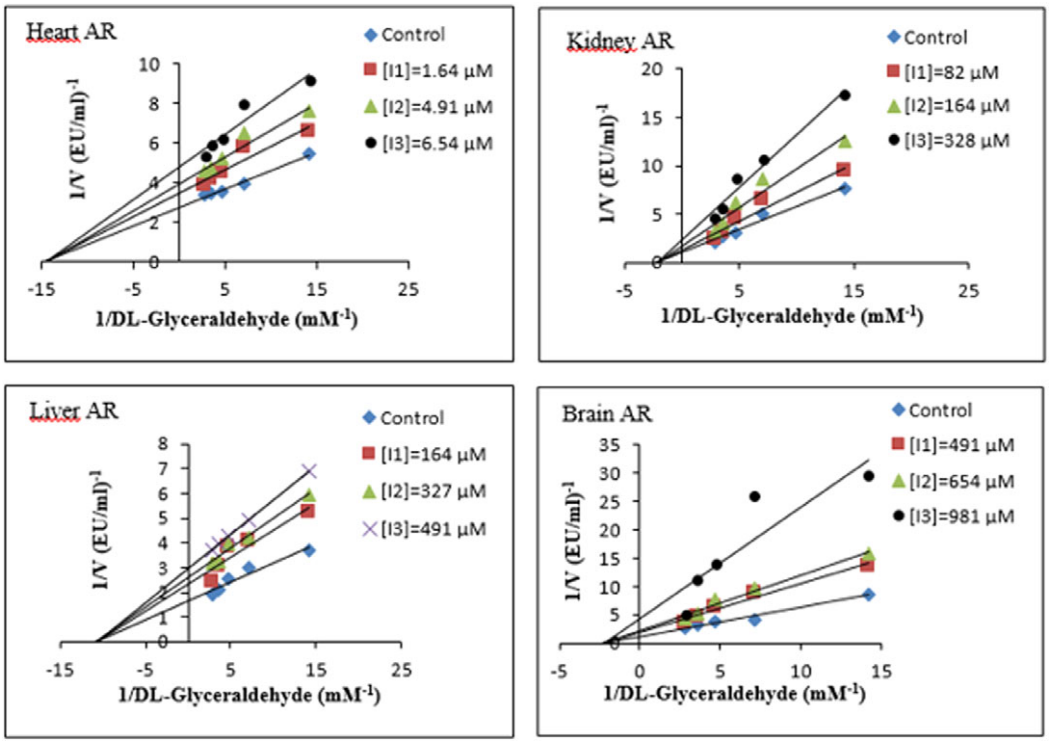

FIGURE 4 Lineweaver–Burk graphs of capsaicin using three different capsaicin and five different DL-glyceraldehyde concentrations for determination of K_i and inhibition type for each rat tissue's AR

reported that the use of capsaicin can be beneficial in the treatment of painful diabetic neuropathy. In a recent study, Yuan et al. examined the effect of capsaicin supplement on blood glucose, lipid metabolism, and pregnancy outcomes in women with gestational diabetes mellitus (GDM). The research suggests that capsaicin-containing chili supplement when used regularly improved postprandial hyperglycemia and hyperinsulinemia as well as fasting lipid metabolic disorders in women with GDM, and it decreased the incidence of large-for-gestational-age newborns. [23] But, there is no study examining the effect of capsaicin on AR enzyme activity in the literature.

In this study, we partially purified AR from rat heart, kidney, liver, and brain and then investigated the in vitro inhibition effect of capsaicin on AR enzyme activity in these tissues. Both the K_i and IC_{50} parameters of the capsaicin were determined in this study using Lineweaver–Burk graphs (1/V–1/[S]) and activity%–[capsaicin] graphs, respectively (Figures 3 and 4). For the rat heart, AR capsaicin had an IC_{50} value of 7.5 μM and K_i value of 8.87 ± 2.45 μM. For the rat kidney, AR, capsaicin had an IC_{50} value of 367 μM and K_i value of 264 ± 30.1 μM. For the rat liver AR, capsaicin had an IC_{50} value of 734 μM and K_i value of 535 ± 114 μM. For the rat brain AR, capsaicin had an IC_{50} value of 904 μM and K_i value of 597 ± 217 μM (Table 1). In addition, capsaicin showed noncompetitive inhibition effect on AR enzyme in heart, kidney, liver, and brain tissues. So, capsaicin can be bind to an AR enzyme somewhere other than the active site. This situation may change when it comes to the three-dimensional structure of the enzyme and may reduce the activity of the enzyme. Capsaicin showed inhibition effect at very low concentrations on rat tissue's AR enzyme activity, but capsaicin had a much stronger inhibitory effect on heart AR enzyme than the other tissues's AR. It was known that AR is associated with increased cardiac ischemic injury, and inhibition of AR can also protect against atherosclerosis. [24,25] These results suggest that pharmacological inhibition of AR with low concentration of capsaicin may be beneficial in treatment of cardiac diseases.

TABLE 1 IC_{50}, K_i Values and Inhibition Types of Capsaicin for Each Rat Tissue's AR

	Capsaicin		
Tissue	IC_{50} (μM)	K_i (μM)	Inhibition Type
Heart	7.5	8.87 ± 2.45	Noncompetitive
Kidney	367	264 ± 30.1	Noncompetitive
Liver	734	535 ± 114	Noncompetitive
Brain	904	597 ± 217	Noncompetitive

In conclusion, capsaicin exhibited a unique inhibition profile when we look at in a rat's heart, kidney, liver, and the brain AR enzyme. Therefore, capsaicin can be an effective molecule at low concentrations in preventing diabetic complications associated with the polyol pathway. These results clearly indicate that the development of capsaicin equivalents may be important in identifying new AR inhibitors, and this may also help to provide more effective and better therapeutic agents for diabetic complications in the future.

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How to cite this article: Alim Z, Kilinc N, Sengul B, Beydemir S. Mechanism of capsaicin inhibition of aldose reductase activity. *J Biochem Mol Toxicol*. 2017;31:e21898. https://doi.org/10.1002/jbt.21898