THE EFFECTS OF CICLETANINE, A NEW ANTIHYPERTENSIVE AGENT ON INSULIN RELEASE IN RAT ISOLATED PANCREAS BY THE PERFUSION TECHNIQUE

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ABSTRACT

Treatment of hypertension has reduced the incidence of stroke, heart failure and renal failure. However, the incidence of coronary heart disease is not reduced to the same degree. Many of the drugs advocated as first-line drugs in the step-wise therapy have been shown to cause carbohydrate intolerance and it is an independent risk factor in the development of coronary heart disease. It is thus important to identify the antihypertensive drugs that may cause deterioration in glucose tolerance. Cicletanine, the first derivative of the furopyridines, is a new class of antihypertensive agents. It acts directly on vascular endothelium cells by increasing prostacyclin synthesis. It also decreases intracytosolic calcium levels in smooth muscles. The purpose of this study is to evaluate the effects of Cicletanine on insulin release in rat isolated pancreas by the perfusion technique adapted from Loubatieres and co-workers (1972). Doses used were based on therapeutic peak plasma concentration. Diazoxide was used as a positive control ie a known insulin suppressant. Cicletanine at 1/10 and equivalent therapeutic concentrations (0.5μg/mL and 5.0μg/mL) did not suppress insulin release. However, at concentration exceeding 10X its therapeutic levels (50μg/mL) it begins to suppress insulin release. In conclusion, Cicletanine did not inhibit insulin release at concentrations within the therapeutic range.

Keywords: *cicletanine, hypertension, insulin release*

SINGAPORE MED J 1996; Vol 37: 278-281

INTRODUCTION

It is estimated that the incidence of hypertension ranges from 10%-25% of the adult population in most countries[1]. The meta-analysis of Mac Mahon and colleagues[1] demonstrated that the control of high blood pressure effectively prevents complications. Pooled data from a number of trials support the conclusion that control of hypertension with the usual stepped-care drugs is very effective in reducing the incidence of stroke by approximately 40%. In sharp contrast, coronary heart disease, a manifestation of the atherosclerotic complications of hypertension has not been completely successful[2,3]. Although the reasons for this is still unclear and controversial, the metabolic side-effects of these drugs are a strong possibility.

Many of the drugs advocated as first-line drugs in the step-wise therapy have been shown to cause glucose intolerance[4] which is one of the independent risk factor for coronary heart disease[4,5]. It has been reported that the antihypertensive agents most commonly associated with deterioration in glucose tolerance were thiazide and thiazide-like diuretics[6-9], and the beta-blockers[10-12]. Diazoxide[8,13] and loop diuretics[9,14] have been shown to have similar effects as well. Diazoxide and certain calcium antagonists have been shown to effectively inhibit glucose induced insulin secretion *in vitro*[15,16]. Both non-selective and cardio-selective beta blockers, if used in high doses, also inhibit insulin secretions with increase in serum glucose, growth hormones and decrease in fat metabolism[17,18]. Insulin plays a key role in glucose metabolism. A higher percentage of diabetics are also seen amongst hypertensives[19].

With the advocation of the individualised therapy whereby antihypertensive drugs chosen as first-line therapy will depend on the individual's disease state and biochemical profile, more appropriate use of antihypertensive drugs based on their pharmacological profile will be encouraged and these may include newer antihypertensive drugs such as cicletanine. It is crucial to the management of the hypertensive patients that antihypertensive drug selection does not exacerbate glucose intolerance or other coronary heart disease risk factors.

Cicletanine is the first member of a new class of antihypertensive agent, the furopyridine. It acts directly on the vascular endothelium cells by increasing prostacyclin synthesis[20] and decreases the cytosolic calcium levels in the vascular smooth muscle cells by affecting cellular Ca^{++} binding[21]. Controlled therapeutic studies on cicletanine have shown it to be efficacious and is readily accepted by hypertensive patients[22].

The purpose of this experiment is to study the effects of cicletanine on insulin release in the rat isolated pancreas by the perfusion technique. Studies on isolated organ is expected to show a direct effect of the drug on the organ concerned as opposed to *in vivo* studies.

METHODS

The method used in this study were adapted from Loubatieres and co-workers[33]. A total of 30 male albino rats of Sprague Dawley strain weighing 200-300g were used. The rats fasted for twelve hours (water ad libitum) and were anaesthetised with pentobarbitone (30mg/kg). Their abdomen were opened wide by a mid-line and transverse incision on both sides. The colon and the lower intestine up to the level of the duodenum were

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isolated from the pancreas taking care to ligate all vessels going to and coming from the pancreas. The aorta with its mesentric branch and the coeliac axis, was identified. Loose ligatures were placed above, below and around the coeliac axis. The stomach was then dissected out, starting from the oesophageal junction, the lesser curvature and towards the duodenum where it was ligated and gently lifted out to separate them from the fragile pancreas. All tissues separated from the pancreas were tied proximally to the pancreas before ligating to minimise leakage when perfusing later. The aorta was tied off, first distal, then proximal to the coeliac axis. The coeliac axis was cannulated through the wall of the aorta and the cannula safely secured with cotton threads. The pancreas and duodenum was then perfused through the coeliac axis. Perfusion should be carried out immediately after the proximal aortic ligation to avoid anoxia to the pancreas. Next, the portal vein of animals was cannulated as near to the liver as possible. The pancreas was then gently lifted out by cutting the tissue underneath it and holding by the duodenum and the spleen to avoid injury to the pancreas. The pancreas was placed on a plastic net which was secured over the mouth of a beaker by a rubber band so that any oozing of the perfusing fluid from the pancreas drains directly into the beaker. The pancreas was perfused with Kreb's solution containing 2mg/mL of bovine serum albumin, initially with low glucose concentration (60mg%) for 5 to 10 minutes to stabilise. This was followed by taking five samples during low glucose perfusion. The tissue was then perfused with a high glucose concentration (300mg%) for 20 minutes, carried out in the presence or absence of the tested compounds. Samples were collected for 15 seconds of every minute. Aliquots from each collection was stored in deepfreezer for future assay. The solutions were bubbled with a mixture of 95% oxygen and 5% carbon dioxide. The perfusion was carried out using a roller pump at a constant rate of 4 mL/minute. The perfusing pressure was recorded by means of a mercury manometer connected to a side arm. The perfusion pressure ranges from 60 mmHg to 160 mmHg. The perfusion fluid was warmed by means of warming chamber at 36°C to 37°C before perfusing the tissue.

Three concentrations of cicletanine were chosen, based on the peak plasma/serum concentration achieved in humans when they were given a single standard 200 mg oral dose of cicletanine. this value was noted to be approximately 5.0µg/mL[23,24]. Diazoxide (10µg/mL) was used as a positive control which have been shown to decrease insulin release by approximately 50%[25-28].

The following treatment groups were studied:

Group I	Control	perfused with low glucose, then high glucose solution.
Group II	Cicletanine	0.5µg/mL in low glucose, then in high glucose solution.
Group III	Cicletanine	5.0µg/mL in low glucose, then in high glucose solution.
Group IV	Cicletanine	50.0µg/mL in low glucose, then in high glucose solution.
Group V	Diazoxide	10.0µg/mL in low glucose, then in high glucose solution.

Six pancreas were used in each group.

Preparation of drug solutions

Cicletanine is soluble in dimethyl sulphoxide (DMSO). It has minimal solubility in water. 50mg of cicletanine (powder) was dissolved in 1 mL of DMSO, to which 1 mL of water was added to make a 2 mL of stock solution. This was subsequently added drop by drop (to prevent precipitation if any) to the Kreb's solution and the volume was raised to one litre (1000 mL). This will give a concentration of 50µg/mL of cicletanine. The above volume was diluted 10 and 100 times to give concentrations of 5µg/mL and 0.5µg/mL of cicletanine respectively.

Diazoxide solution (10µg/mL) was prepared by a similar procedure.

Insulin assay

Immuno-reactive insulin was assayed by using Coat-A-Count Insulin Kit[34]. In this procedure, ^{125}I-labeled insulin competes with insulin in the unknown samples for sites on insulin-specific antibody immobilised to the wall of polypropylene tubes. After incubation, isolation of the antibody-bound fraction was achieved simply by decanting the tubes. The tubes were then counted in a gamma counter. Significant difference was calculated at $p \leq 0.05$ by the one-way analysis of variance (ANOVA).

RESULTS

The normal response of insulin release to high glucose concentration and the effects of diazoxide 10µg/mL on the insulin release pattern is shown in Fig 1. At low glucose concentration (equivalent to fasting level) the rate of insulin secretion remains at approximately 25µIU/mL. When changed to high glucose concentration (300mg/dL) the response by the beta-islet cells were almost immediate peaking within a couple of minutes to approximately 400µIU/minute of insulin. This concentration decreases sharply afterwards and level off at approximately 250µIU/mL and increases gradually again till the end of the experiment. Diazoxide 10µg/mL reduced the basal insulin secretion to 10µIU/minute at low glucose concentration (60mg%) and with high glucose concentration (300mg%) insulin release was significantly reduced to 172µUI/minute by diazoxide. The biphasic response was lost and insulin secretion was significantly ($p<0.05$) diminished to approximately 40% of the control value.

The insulin release pattern to high glucose concentration with 0.5µg/mL, 5µg/mL and 50µg/mL concentration of cicletanine added to the perfusion fluid is shown in Fig 2. At 0.5µg/mL and 5µg/mL the rates of insulin secretion were not significantly different from that of the control values (Fig 3). However, at 50µg/mL cicletanine significantly ($p<0.05$) reduced insulin release in response to high glucose concentration. At this concentration the rate of insulin secretion was decreased to approximately 50.8% during high glucose perfusion.

DISCUSSION

The rat isolated pancreas technique used in the study is suitable

Fig 1 – The normal response of insulin release to high glucose concentration the effects of diazoxide on the insulin release in isolated rat pancreas.

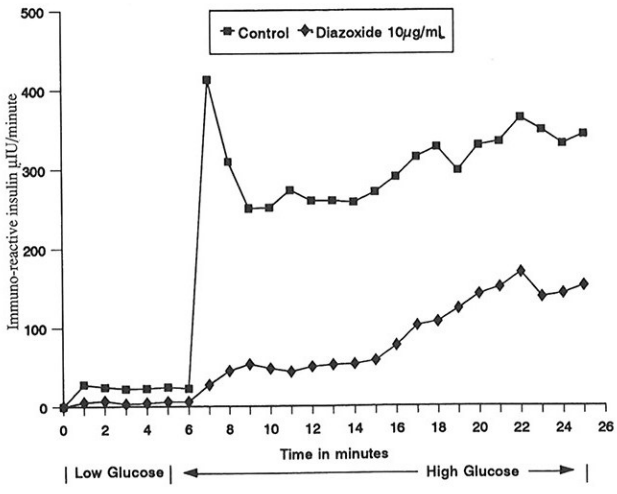
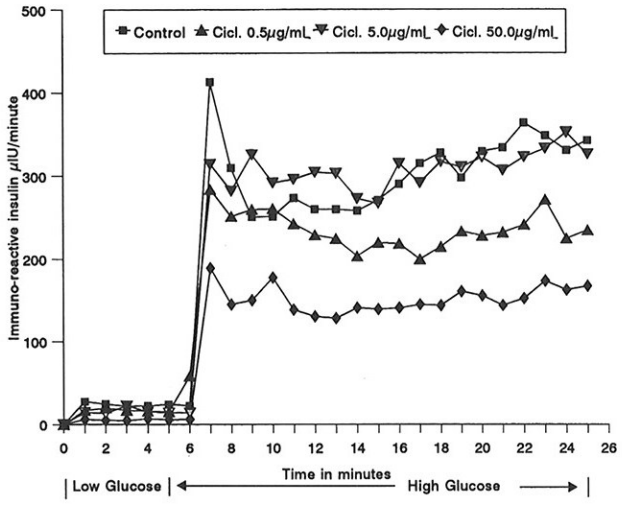


Fig 2 – Effects of cicletanine on insulin release in isolated rat pancreas.



for assaying the direct effects of drugs on insulin secretion in response to high glucose stimulation. Diazoxide used as the positive control effectively and predictably reduces the insulin secretion in response to high glucose stimulation by approximately 40%. This has also been shown by other workers[25-28].

Intracellular Ca^{++} plays an important role not only in the excitation-contraction coupling but also in the excitation-secretion coupling of the beta-islet cells that produces insulin[29]. Beside diazoxide, certain calcium antagonists[30,31] have been shown to be effective inhibitors of insulin secretion, presumably by affecting the cytosolic Ca^{++} levels.

Cicletanine has been shown to decrease the cytosolic Ca^{++} levels in vascular smooth muscle cells[32] and in so doing, contributing to its vasodilatory effect. A similar mechanism may be involved in the islet cells whereby at a higher concentration. (50µg/mL), the cytosolic Ca^{++} of the pancreatic beta islet cells may be reduced, thus decreasing the secretion of insulin to high glucose stimulation.

The peak plasma concentration of cicletanine after a single standard oral dose of 200mg was found within the range 6.17µg/mL to 9.24µg/mL[23]. It was shown that 90% of cicletanine is bound to plasma/serum proteins. Therefore the free form (or active form) is approximately 10% of 6.17µg/mL to 9.2µg/mL which is 0.617µg/mL to 0.924µg/mL. In this study, most of the cicletanine would be in the free form (active form) since the albumin (bovine) added in the perfusing fluid was very small (2gm/L). In this study, cicletanine begins to inhibit insulin secretion at 50µg/mL which is about 55 times higher than the unbound form of cicletanine encountered at therapeutic drug concentration. It is concluded that cicletanine at equivalent therapeutic concentrations (up to 5µg/mL) did not inhibit insulin secretion.

ACKNOWLEDGEMENTS

We wish to thank all the staff of Department of Pharmacology, Faculty of Medicine, UKM, for their assistance. Authors are also thankful to Beaufour Ipsen International, Malaysia for supplying cicletanine. The research is supported by Universiti Kebangsaan Malaysia grant No. 69/91

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