Preconditioning the diabetic human myocardium

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Abstract

Our objective was to determine whether human diabetic myocardium is amenable to the cardioprotective actions of ischaemic preconditioning. Human right atrial appendages were harvested from diabetic and non-diabetic patients undergoing elective coronary artery bypass graft surgery. The atrial trabeculae were isolated and subjected to 90 min. of hypoxia followed by 120 min. of reoxygenation, following which the percentage recovery of baseline contractile function was determined. The atrial trabeculae were randomized to: (i) controls (groups 1 and 3); (ii) standard hypoxic preconditioning (HPC) protocol consisting of 4 min. of hypoxia/16 min. of reoxygenation before the 90 min. index hypoxic period (groups 2 and 4); (iii) Prolonged HPC protocol consisting of: 7 min. of hypoxia/16 min. of reoxygenation before the index hypoxic period (group 5). In addition, basal levels of Akt phosphorylation were determined in right atrial appendages harvested from non-diabetic patients and diabetic patients to determine whether PI3K-Akt signalling is down-regulated in the diabetic heart. Standard HPC improved baseline contractile function in human atrial trabeculae harvested from non-diabetic patients (52.4 ± 3.8% with HPC versus 30.0 ± 3.2% in control: P = 0.001; N = 6/group), but not in atrial trabeculae isolated from diabetic patients (22.6 ± 3.3% with HPC versus 28.5 ± 1.9% in control: P > 0.05; N = 6/group). However, the prolonged HPC protocol did improve baseline contractile function in atrial trabeculae harvested from diabetic patients (42.0 ± 2.4% with HPC versus 28.5 ± 1.9% in control: P = 0.001; N = 6/group). Western blot analysis demonstrated lower levels of phosphorylated Akt in diabetic myocardium compared to non-diabetic myocardium (0.13 ± 0.03 arbitrary units versus 0.39 ± 0.11 arbitrary units; P = 0.047; N = 4/group). From the data obtained it appears that the threshold for preconditioning the diabetic myocardium is elevated which may be related to the down-regulation of the PI3K-Akt pathway.

Keywords: preconditioning • human • myocardium • diabetes mellitus • myocardial infarction

Introduction

Coronary heart disease (CHD) is a major cause of morbidity and mortality in patients with type 2 diabetes, with the incidence of cardiovascular disease in diabetic patients being twice that of non-diabetic men and three times that of non-diabetic women [1]. More recent estimates suggest that women in the age group of 35–54 years are five times more likely to have a myocardial infarction than women without diabetes [2]. Interventions such as a coronary artery bypass graft (CABG) surgery, are also associated with a significantly lower 5-year survival in diabetic patients than in non-diabetic with increased post-operative and long-term morbidity [3–5]. Furthermore, diabetic patients who survive an MI are more likely to develop heart failure than non-diabetic patients [6]. Therefore, new cardioprotective strategies which are capable of protecting the diabetic myocardium are required in order to improve clinical outcomes in diabetic patients with CHD.

In this respect, the endogenous cardioprotective phenomenon of ischaemic preconditioning (IPC) represents a powerful interventional strategy for protecting against myocardial injury arising from ischaemia-reperfusion injury. IPC is elicited by brief non-lethal episodes of myocardial ischaemia and reperfusion applied before the lethal ischaemic insult [7]. However, experimental animal studies suggest that the diabetic heart may be resistant to the cardioprotective effects of IPC [8], and this may be the case in diabetic patients [9, 10]. An experimental study from our laboratory suggests that a more robust IPC stimulus is used, the diabetic heart is still amenable to the infarct-limiting effects of IPC [11]. The apparent resistance of the diabetic heart to a standard IPC protocol may be attributed to the down-regulation of the PI3K-Akt pathway [11], a major component of the signal transduction pathway which underlies IPC [12, 13], as demonstrated in previous experimental animal studies [14]. In the current study we investigate whether myocardial tissue harvested from diabetic patients undergoing CABG is amenable to the cardioprotection elicited by IPC.
### Materials and methods

#### Study patients

Ethical approval for the study was obtained from the Research and Ethics Committee (REC), at the University College London/University College London Hospitals. Elective patients admitted for coronary artery bypass surgery or valve surgery, were consented. Patients over the age of 80, with a troponin positive event in the last 6 weeks, unstable angina, congestive cardiac failure (EF < 50%), renal failure, arrhythmias or on anti-arrhythmic medication were excluded. Patients diagnosed with type 2 diabetes on oral hypoglycaemic agents were consented for the study. The average duration of diabetes in these patients was 4.7 years. Type 2 diabetic patients who were either on insulin or glibenclamide were excluded. Patients diagnosed with type 2 diabetes on oral hypoglycaemic agents were consented for the study. The average duration of diabetes in these patients was 4.7 years. Type 2 diabetic patients who were either on insulin or glibenclamide were excluded. Patients diagnosed with type 2 diabetes on oral hypoglycaemic agents were consented for the study. The average duration of diabetes in these patients was 4.7 years. Type 2 diabetic patients who were either on insulin or glibenclamide were excluded.

During the surgery, and immediately prior to the insertion of the venous cannula of the bypass machine, a piece of the right atrial appendage was harvested. The appendage was transported to the laboratory in modified Tyrode’s buffer (comprising mmol/l 118.5 NaCl, 4.8 KCl, 24.8 NaHCO₃, 1.2 KH₂PO₄, 1.44 MgSO₄·7H₂O, 10.0 glucose and 10.0 pyruvic acid, oxygenated with a 95% O₂–5% CO₂ gas mixture, to maintain pH between 7.35 and 7.45, and maintain a pO₂ >35 kPa and a pCO₂ between 4.0 and 6.0 kPa) at less than 4°C in order to dissect atrial trabeculae, or snap frozen in liquid nitrogen within 5 sec. of harvesting from the patient, in order to conduct Western blot analysis to determine the levels of phosphorylated and total Akt. Of the non-diabetic patients, 10 were consented for the study of which six appendages were used for trabecula experiments and four were used for Western blot analysis. There were seven males and three females with ages ranging from 57 to 80 years. The average age was 71 ± 2.3 years. A profile of diabetic patients consented along with the HbA₁C levels and their oral hypoglycaemic therapy is shown in Table 1.

#### The human atrial trabecula model of simulated ischaemia-reperfusion injury

This experimental model was first established in our research laboratory and is both a robust and reproducible technique for assessing cardioprotective treatment strategies using human atrial trabeculae subjected to simulated ischaemia-reperfusion injury [17, 18]. In the laboratory, the human atrial trabeculae are dissected from the inner surface of the appendage and immersed in an organ bath containing modified Tyrode’s buffer and placed between two pacing electrodes. The temperature of the bath is kept at 37°C with a heat exchanger. A minimum of two trabeculae were dissected from each appendage as one of the trabeculae served as a control. The trabeculae were paced at 1 Hz for a period of 75 min. to allow it to stabilize. Ischaemia was simulated by replacing modified Tyrode’s buffer with a hypoxic glucose free buffer (containing in mmol/l 118.5NaCl, 24.8 KCl, 24.8 NaHCO₃, 1.2 KH₂PO₄, 1.44 MgSO₄·7H₂O, 1.8 CaCl₂·2H₂O, 10.0 glucose and 10.0 pyruvic acid, oxygenated with a 95% O₂–5% CO₂ gas mixture, to maintain pH between 7.30 and 7.35) and the pacing increased to 3 Hz. Reperfusion was simulated by replacing the organ bath with modified Tyrode’s buffer and reducing the pacing frequency to 1 Hz. The force of contraction of the trabeculae was calculated via a pacing transducer and software (Chart 5 for Windows). Trabecula diameter greater than 1.2 mm, damaged trabeculae determined visually, irregularly contracting trabeculae and is both a robust and reproducible technique for assessing cardioprotective treatment strategies using human atrial trabeculae subjected to simulated ischaemia-reperfusion injury [17, 18]. In the laboratory, the human atrial trabeculae are dissected from the inner surface of the appendage and immersed in an organ bath containing modified Tyrode’s buffer and placed between two pacing electrodes. The temperature of the bath is kept at 37°C with a heat exchanger. A minimum of two trabeculae were dissected from each appendage as one of the trabeculae served as a control. The trabeculae were paced at 1 Hz for a period of 75 min. to allow it to stabilize. Ischaemia was simulated by replacing modified Tyrode’s buffer with a hypoxic glucose free buffer (containing in mmol/l 118.5NaCl, 24.8 KCl, 24.8 NaHCO₃, 1.2 KH₂PO₄, 1.44 MgSO₄·7H₂O, 1.8 CaCl₂·2H₂O, 10.0 glucose and 10.0 pyruvic acid, oxygenated with a 95% O₂–5% CO₂ gas mixture, to maintain pH between 7.30 and 7.35) and the pacing increased to 3 Hz. Reperfusion was simulated by replacing the organ bath with modified Tyrode’s buffer and reducing the pacing frequency to 1 Hz. The force of contraction of the trabeculae was calculated via a pacing transducer and software (Chart 5 for Windows). Trabecula diameter greater than 1.2 mm, damaged trabeculae determined visually, irregularly contracting trabeculae either at baseline or at the end of reperfusion, or trabeculae with an amplitude of contraction less than 0.5 g were excluded. These exclusion criteria have been developed by our group following 12 years of experience with this model [17, 18].

The atrial trabeculae harvested from patients were randomized to the following groups (Fig. 1): (1) Non-diabetic control (n = 6); Atrial trabeculae harvested from non-diabetic patients were subjected to 75 min. of stabilization, 90 min. of index hypoxia and 120 min. reoxygenation; (2) Non-diabetic standard hypoxic preconditioning (HPC) (n = 6); Atrial trabeculae harvested from non-diabetic patients were subjected to a PC stimulus comprising 4 min. of hypoxia and 16 min. of reoxygenation.
prior to the 90 min. index hypoxia; (3) Diabetic control (n = 6): Atrial trabeculae harvested from diabetic patients were subjected to 75 min. of stabilization, 90 min. of hypoxia and 120 min. of reoxygenation; (4) Diabetic standard HPC (n = 6): Atrial trabeculae harvested from diabetic patients were subjected to a preconditioning stimulus comprising 4 min. hypoxia and 16 min. reoxygenation prior to the 90 min. index hypoxia; (5) Diabetic prolonged HPC (n = 7): Atrial trabeculae harvested from diabetic patients were subjected to a more robust preconditioning stimulus comprising 7 min. hypoxia and 16 min. reoxygenation prior to the 90 min. index hypoxia.

The recovery of baseline contractile function was calculated by dividing the force of contraction at the end of the protocol by the baseline, which was recorded at the end of the period of stabilization. This was expressed as a percentage of the baseline and is used as a surrogate marker of tissue injury.

**Western blot analysis**

Right atrial appendages from non diabetic (n = 4) and diabetic (n = 5) patients, were immediately frozen after being excised from the heart at the time of CABG surgery. They were stored in a -80°C freezer and were subsequently analysed for phosphorylation of Akt (serine 473) and normalized to the total Akt level. Equal loading was confirmed by probing for β-actin levels. SDS-PAGE immunoelectrophoresis was used to determine this along with primary and secondary antibodies obtained from New England Bio Labs (Ipswich, MA, USA) as previously described [12].

**Statistical analysis**

Data are presented as mean ± S.E.M. A factorial one-way ANOVA was used for comparison between more than two groups in the functional recovery experiments. Where a significant F-value was obtained, Fisher’s protected least significant difference post hoc to test for significance has been applied. A P-value of <0.05 has been taken to indicate significance. Protein band quantifications are expressed as mean value ± S.E.M. and differences between groups have been tested using a t-test. All data was analysed using Statview 4.5 (Abacus Concepts Inc., Berkley, CA, USA). Proteins were detected using chemiluminescence and bands visualized by exposure to photographic film and relative densitometry assessed using NIH Image-1.63 software.

**Results**

**Diabetic myocardium requires a stronger preconditioning stimulus**

There were no significant differences in the contractile functions of the atrial trabeculae at baseline (Table 2). Standard HPC of atrial trabeculae harvested from non-diabetic patients resulted in a significant improvement in recovery of baseline contractile function (52.4 ± 3.8% with HPC versus 30.0 ± 3.2% in control: P = 0.0011). This was not the case in atrial trabeculae isolated from diabetic patients where the recovery of baseline function was 22.6 ± 3.3% in the standard HPC group versus 28.5 ± 1.9% in the diabetic control group (P > 0.05). Interestingly, the more robust HPC protocol did manage to improve baseline contractile function in atrial trabeculae isolated from diabetic patients (42.0 ± 2.4% with prolonged HPC versus 28.5 ± 1.9% in diabetic control: P = 0.0014) (Fig. 2).

**Reduced levels of baseline Akt phosphorylation in human diabetic myocardium**

Compared to non-diabetic myocardium, the extent of phosphorylation of Akt in diabetic myocardium was significantly lower (0.39 ± 0.11 arbitrary units in non-diabetic versus 0.13 ± 0.03 arbitrary units in diabetic myocardium: (P = 0.047). In both non-diabetic and diabetic patients, there was no significant difference in the total Akt level when normalized against β-actin (1.37 ± 0.29 arbitrary units versus 0.84 ± 0.12 arbitrary units in non diabetic and diabetic myocardium, respectively) (Fig. 3).
Discussion

The major findings in this study are as follows: (i) Human atrial trabeculae harvested from diabetic patients undergoing CABG surgery are resistant to a standard HPC protocol; (ii) A more robust HPC stimulus is required in order to demonstrate a preconditioning effect in human atrial muscle isolated from diabetic patients; (iii) Baseline levels of Akt phosphorylation were significantly lower in right atrial appendages harvested from diabetic patients when compared to non-diabetic control patients. Therefore, we have demonstrated that the human diabetic myocardium is amenable to IPC provided that a sufficient stimulus is used. This apparent resistance to the cardioprotective effects of IPC were associated with lower levels of Akt phosphorylation at baseline in human diabetic hearts.

Our findings in the human heart are in keeping with our previous animal studies using type 2 diabetic rat hearts. Although a standard IPC protocol comprising one cycle of 5 min. ischaemia and reperfusion did not reduce myocardial infarct size in diabetic rat hearts, three cycles of IPC did result in a significantly smaller myocardial infarct size [11]. Interestingly, the resistance of the diabetic rat heart following the standard IPC protocol of one cycle was attributed to insufficient activation of the PI3K-Akt pathway, whereas the three cycles of IPC succeeded in limiting myocardial infarct size, as the PI3K-Akt pathway was sufficiently activated [11], suggesting a threshold level of kinase activity which needs to be achieved to confer cardioprotection in the setting of IPC. In contrast, Ghosh and colleagues [9] failed to precondition human right atrial appendages obtained from type 2 diabetic patients despite applying repeated cycles of hypoxia and reoxygenation. This discordant result may be attributed to that fact that the experimental model used in their study differs considerably from the one used by our laboratory. In their study the entire right atrial appendage sample is cut into thin slices and the whole specimen is immersed in hypoxic buffer and subjected to simulated ischaemia-reperfusion injury, at the end of which the amount of
hearts may have an altered KATP channel [21]; acute hypergly-
gusted that this may be due to a number of reasons: diabetic
change in the three mentioned parameters [10]. The authors sug-
other hand, did not benefit from the onset of angina, with no
atal mortality in non-diabetic patients. Diabetic patients, on the
dure, higher left ventricular ejection fractions and lower in hospi-
diabetes. They noted that the onset of prodromal angina 24 hrs
611 patients presenting with their first AMI, of whom 121 had
betic patients than in non-diabetic patients. Ishihara et al. studied
an aggressive hypoxic insult by using a slightly prolonged proto-
period of 15 min. during preconditioning may have initi-
ated necrosis in the atrial appendages. We have tried to avoid such
an aggressive hypoxic insult by using a slightly prolonged proto-
HPC instead and using a different end-point of injury.

Early experimental animal studies examining IPC in diabetic animal hearts produced contradictory data. Many groups used streptozotocin (STZ) or alloxan to induce a diabetic state. Although some reported additional protection, others noted no protection against end-points such as arrhythmias and myocardial stunning with the amount of protection achieved varying accord-
ing to the age of the animal [8]. This has led to criticism of mod-
els that use chemicals to induce diabetes as the effect of the
chemical could be widespread and non specific. Both these sub-
stances are highly toxic and induce diabetes by destroying islet
cells in the pancreas and their effect on the cardiovascular system
is unpredictable. Moreover, most STZ-induced mice models are
male, as female mice appear to be less sensitive to the effects of
STZ [19]. In other more reliable models of type 2 diabetes such as
the lean Goto–Kakizaki (GK) and the Zucker diabetic fatty rats, IPC
did not offer any protection [20], just as in diabetic animal models
of the dog, the rabbit and the sheep. If anything, outcome seemed
to worsen in the larger animal models [8].

In the clinical setting, IPC is known to be more difficult in dia-etic patients than in non-diabetic patients. Ishihara et al. studied
611 patients presenting with their first AMI, of whom 121 had
diabetes. They noted that the onset of prodomal angina 24 hrs
prior to the AMI was associated with a lower CK level after pro-

dure, higher left ventricular ejection fractions and lower in hospi-
tal mortality in non-diabetic patients. Diabetic patients, on the
other hand, did not benefit from the onset of angina, with no
change in the three mentioned parameters [10]. The authors sug-
gested that this may be due to a number of reasons: diabetic
hearts may have an altered KATP channel [21]; acute hypergly-
caemia has been shown to abolish IPC [22] and oral hypogly-
caemic agents like glibenclamide which block KATP channel opening
can prevent IPC [16]. In addition, it is also known that the initial
steps of insulin signalling and glucose transport are defective in
the type 2 diabetic heart [23], steps which may be inherent to
insulin mediated IPC [24]. Furthermore, basal protein kinase B
levels and insulin stimulated Akt, ERK and P13-K have been
shown to be defective in diabetic animals [14, 25]. Mitochondrial
dysfunction has also been suggested as a potential reason for
failure to precondition the human diabetic myocardium [26].
Hassoua et al. harvested appendages from non-diabetic patients
and diabetic patients who were insulin dependent, or were being

treated with metformin and glibenclamide. They noted that
although diazoxide depolarized mitochondrial membrane poten-
tial in isolates from non-diabetic patients, it failed to do the same
in diabetic mitochondrial isolates. They also reported that
although ischemia, phenylephrine, adenosine and diazoxide failed
to precondition diabetic appendages, protein kinase C and
p38MAPK activators were able to precondition them. This group,
as discussed earlier, used CK release as an end-point as com-
pared to the recovery of function used by us [26].

In addition to the above, we have shown that the levels of
phosphorylated Akt at baseline are lower in atrial appendages har-
vested from diabetic patients, when compared to non-diabetic
patients, which, to our knowledge, has never been reported. This
may offer a mechanistic explanation as to why a prolonged or
more robust HPC stimulus is needed to cause protection in dia-
abetic trabeculae. However, it is also likely that the defect could be
at any level in the signalling pathway and a firm conclusion cannot
be drawn from this data. Animal studies suggest that levels of
phosphorylated Akt are lower in diabetic hearts whether or not an
intervention is applied [27, 28]. In addition, studies suggest that
standard stimuli such as insulin and isoproterenol, that cause
phosphorylation of Akt in control animal hearts, fail to cause the
same level of phosphorylation in diabetic models. Yet, stimuli
were additive, a finding that is in keeping with human hearts,
where an additional HPC stimulus resulted in a better recovery of
function [14]. We speculate that the additional HPC stimulus
crossed a threshold activating the signalling pathway resulting in
cardioprotection however additional experiments are necessary to
clarify his point.

Metformin had been prescribed to 9 of the 13 diabetic patients
recruited into the study. In the United Kingdom Prospective
Diabetes Study (UKPDS), there was a significant reduction in the
incidence of myocardial infarction in type 2 diabetic patients who
were treated with metformin, which additionally, has been shown
to be superior to sulphonylurea therapy in reducing both all cause
mortality and cardiovascular mortality [29, 30]. It has been sug-
gested that the cardioprotective effects of metformin could be
attributable to actions other than glucose lowering, specifically,
through the activation of AMP-activated protein kinase (AMPK), an
enzyme regulating lipid and glucose metabolism [31]. In fact, the
acute administration of metformin in low doses has been shown
to activate AMPK-eNOS in diabetic and non-diabetic murine
hearts, while causing infarct size reduction following ischaemia-
reperfusion injury [32]. However, the effect of chronic metformin
therapy on the activity of AMPK has not been demonstrated.

Of the 13 diabetic patients consented for our study, 6 patients
were on sulphonylureas, which are thought to inhibit mitochondr-
ial KATP channels that may inhibit the cardioprotection elicited by
preconditioning. However, the role of the mitochondrial KATP ch-
nel in IPC has not been established without doubt [33, 34].
Furthermore, some sulphonylureas such as gliclazide have been
shown to be more selective for pancreatic mitochondrial KATP
channels as opposed to myocardial channels [35]. Indeed it has
been shown that newer sulphonylureas, such as gliclazide and
glimepiride, do not abolish IPC in rats whereas glibenclamide does
[16, 36, 37]. Four patients were prescribed gliclazide which, inci-
dentally, has been shown to inhibit preconditioning using supra-
maximal doses in human atrial muscle [38]. Two patients were on
glipizide, which does not inhibit myocardial preconditioning in
rabbits in vivo [39]. Additionally, one patient each was prescribed
repaglinide and rosiglitazone. Although repaglinide has been
shown to have no selectivity for pancreatic or myocardial KATP
channels.
channels, the results were included because of the debatable role of KATP channel in preconditioning. Pioglitazone, a thiazolidinedione and PPAR-γ receptor agonist similar to rosiglitazone, has been shown to activate PI3K-Akt and induce cardioprotection when administered acutely. However, it is likely that chronic administration of any PI3K-Akt activators would result in negative inhibition via activation of phosphatase and tensin homolog [40], as has shown to be the case with chronic atorvastatin administration by our group [41, 42].

A major limitation of our study is that it does not provide direct evidence that the prolonged HPC stimulus would result in greater phosphorylation of Akt. The evidence is not causal but only an association. To prove a causal relationship, it would be necessary to precondition trabeculae with the standard and prolonged HPC stimulus and compare the levels of Akt phosphorylation between the two groups. However, in our experience trabeculae do not provide sufficient tissue to be able to blot proteins by current methods. It may have been possible to subject an entire appendage to the standard and prolonged HPC protocol but difficulty in recruiting, collecting and handling human material have limited our options for additional measurements. Another limitation of our study was the unfortunate lack of HbA1C levels in some patients to co-relate with our findings. In the trabeculae experimental group, eight diabetic patients were included of which six had HbA1C levels. The mean HbA1C in the trabeculae group was 7.6% (7.0–8.2). Despite this, it was possible to precondition this group with a prolonged HPC stimulus. Thus, it is likely that diabetes associated with higher HbA1C levels may result in glycation of many cellular signalling proteins but it is still possible to activate these enzymes by using a stronger than normal preconditioning stimulus. A further drawback of our study is the use of human atrial tissue as opposed to ventricular tissue. This is based on the easier availability of atrial tissue.

In the UKPDS, intensive treatment with a target HbA1C of 7.0% was associated with no reduction in macrovascular complications as compared to the conventional treatment group [43]. Additionally, it has been suggested that despite intensive treatment in diabetic patients, mortality following AMI reduces only in the short term and in the long term the trend is maintained only in the non-diabetic patients [44, 45]. It may be possible, however, to improve both mortality and morbidity in both diabetic and non-diabetic patients if we could pharmacologically or mechanically activate the protective signalling pathway associated with pre- or post-conditioning and reduce infarct size following an AMI. Clinical studies are needed to evaluate whether this is possible and a better understanding of the pathology of the diabetic myocardium will perhaps improve long-term morbidity and mortality rates in this large subset of patients.

In conclusion, the present study demonstrates for the first time that myocardium from diabetic patients can be preconditioned ex vivo; however, the threshold for protection is raised. The findings from the current study suggest that diabetic patients are still amenable to the benefits of IPC but that they may require a stronger preconditioning stimulus compared to non-diabetic patients.

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References

1. Kannel WB, McGee DL. Diabetes and cardiovascular risk factors: the Framingham study. Circulation. 1979; 59: 8–13.
2. Mulnier HE, Seaman HE, Raleigh VS et al. Risk of myocardial infarction in men and women with type 2 diabetes in the UK: a cohort study using the General Practice Research Database. Diabetologia. 2008; 51: 1639–45.
3. Herlitz J, Wognsen GB, Emanuelsson H et al. Mortality and morbidity in diabetic and nondiabetic patients during a 2-year period after coronary artery bypass grafting. Diabetes Care. 1996; 19: 698–703.
4. Thourani VH, Weintraub WS, Stein B et al. Influence of diabetes mellitus on early and late outcome after coronary artery bypass grafting. Ann Thorac Surg. 1999; 67: 1045–52.
5. Szabo Z, Hakansson E, Svedjeholm R. Early postoperative outcome and medium-term survival in 540 diabetic and 2239 nondiabetic patients undergoing coronary artery bypass grafting. Ann Thorac Surg. 2002; 74: 712–9.
6. Abbott RD, Donahue RP, Kannel WB et al. The impact of diabetes on survival following myocardial infarction in men vs women. The Framingham Study. Jama. 1988; 260: 3456–60.
7. Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. Circulation. 1986; 74: 1124–36.
8. Ferdinandy P, Schulz R, Baxter GF. Interaction of cardiovascular risk factors with myocardial ischemia/reperfusion injury, preconditioning, and postconditioning. Pharmacol Rev. 2007; 59: 418–58.
9. Ghosh S, Staden NB, Galinianes M. Failure to precondition pathological human myocardium. J Am Coll Cardiol. 2001; 37: 711–8.
10. Ishihara M, Inoue I, Kawagoe T et al. Diabetes mellitus prevents ischemic preconditioning in patients with a first acute anterior wall myocardial infarction. J Am Coll Cardiol. 2001; 38: 1007–11.
11. Trang A, Haasenloy DJ, Mocanu MM et al. Preconditioning the diabetic heart: the importance of Akt phosphorylation. Diabetes. 2005; 54: 2360–4.
12. Mocanu MM, Bell RM, Yellon DM. PI3 kinase and not p42/p44 appears to be implicated in the protection conferred by ischemic...
13. Tong H, Chen W, Steenberg C et al. Ischemic preconditioning activates phosphatidylinositrol-3-kinase upstream of protein kinase C. Circ Res. 2000; 87: 309–15.
14. Huisamen B. Protein kinase B in the diabetic heart. Mol Cell Biochem. 2003; 249: 31–8.
15. Jonassen AK, Sack MN, Mjos OD et al. Myocardial protection by insulin at reperfusion requires early administration and is mediated via Akt and p70S6 kinase cell-survival signaling. Circ Res. 2001; 89: 1191–8.
16. Tomai F, Crea F, Gaspardone A et al. Ischemic preconditioning during coronary angioplasty is prevented by glibenclamide, a selective ATP-sensitive K+ channel blocker. Circulation. 1994; 90: 700–5.
17. Walker DM, Walker JM, Pugsley WB et al. Preconditioning in isolated superfused human muscle. J Mol Cell Cardiol. 1995; 27: 1349–57.
18. Sivaraman V, Mudalagiri NR, Di Salvo C et al. Postconditioning protects human atrial muscle through the activation of the RISK pathway. Basic Res Cardiol. 2007; 102: 453–9.
19. Hsueh W, Abel ED, Breslow JL et al. Recipes for creating animal models of diabetic cardiovascular disease. Circ Res. 2007; 100: 1415–27.
20. Kristiansen SB, Lofgren B, Stottrup NB et al. Ischaemic preconditioning does not protect the heart in obese and lean animal models of type 2 diabetes. Diabetologia. 2004; 47: 1716–21.
21. Smith JM, Wahler GM. ATP-sensitive potassium channels are altered in ventricular myocytes from diabetic rats. Mol Cell Biochem. 1996; 158: 43–51.
22. Kersten JR, Schmeling TJ, Orth KG et al. Acute hyperglycaemia abolishes ischaemic preconditioning in vivo. Am J Physiol. 1998; 275: H721–5.
23. Desrois M, Sidell RJ, Gaugier D et al. Initial steps of insulin signaling and glucose transport are defective in the type 2 diabetic rat heart. Cardiovasc Res. 2004; 61: 288–96.
24. Jonassen AK, Brar BK, Mjos OD et al. Insulin administered at reoxygenation exerts a cardioprotective effect in myocytes by a possible anti-apoptotic mechanism. J Mol Cell Cardiol. 2000; 32: 757–64.
25. Steiler TL, Galuska D, Leng Y et al. Effect of hyperglycemia on signal transduction in skeletal muscle from diabetic Goto-Kakizaki rats. Endocrinology. 2003; 144: 5259–67.
26. Hassouna A, Loubani M, Mataza BM et al. Mitochondrial dysfunction as the cause of the failure to precondition the diabetic human myocardium. Cardiovasc Res. 2006; 69: 450–8.
27. Shinohara T, Takahashi N, Ooie T et al. Phosphatidylinositrol 3-kinase-dependent activation of akt, an essential signal for hyperthermia-induced heat-shock protein 72, is attenuated in streptozotocin-induced diabetic heart. Diabetes. 2006; 55: 1307–15.
28. Gross ER, Hsu AK, Gross GJ. Diabetes abolishes morphine-induced cardioprotection via multiple pathways upstream of glycosyn synthase kinase-3beta. Diabetes. 2007; 56: 127–36.
29. UK Prospective Diabetes Study (UKPDS) Group. Effect of intensive blood-glucose control with metformin on complications of type 2 diabetes (UKPDS 34). UK Prospective Diabetes Study (UKPDS) Group. Lancet. 1998; 352: 854–65.
30. Johnson JA, Majumdar SR, Simpson SH et al. Decreased mortality associated with the use of metformin compared with sulfonylurea monotherapy in type 2 diabetes. Diabetes Care. 2002; 25: 2244–6.
31. Towler MC, Hardie DG. AMP-activated protein kinase in metabolic control and insulin signaling. Circ Res. 2007; 100: 328–41.
32. Calvert JW, Gundewar S, Jha S et al. Acute metformin therapy confers cardioprotection against myocardial infarction via AMPK-eNOS-mediated signaling. Diabetes. 2008; 57: 696–705.
33. O’Rourke B. Myocardial K(ATP) channels in preconditioning. Circ Res. 2000; 87: 845–55.
34. Minners J, Lacerda L, Yellon DM et al. Diazoxide-induced respiratory inhibition - a putative mitochondrial K(ATP) channel independent mechanism of pharmacological preconditioning. Mol Cell Biochem. 2007; 294: 11–8.
35. Quast U, Stephan D, Bieger S et al. The impact of ATP-sensitive K+ channel subtype selectivity of insulin secretagogues for the coronary vasculature and the myocardium. Diabetes. 2004; 53: S156–64.
36. Mocanu MM, Maddock HL, Baxter GF et al. Glimepiride, a novel sulfonylurea, does not abolish myocardial protection afforded by either ischemic preconditioning or diazoxide. Circulation. 2001; 103: 3111–6.
37. Maddock HL, Siedlecka SM, Yellon DM. Myocardial protection from either ischemic preconditioning or nicorandil is not blocked by gliclazide. Cardiovasc Drugs Ther. 2004; 18: 113–9.
38. Loubani M, Fowler A, Standen NB et al. The effect of gliclazide and glibenclamide on preconditioning of the human myocardium. Eur J Pharmacol. 2005; 515: 142–9.
39. Fiyns DM, Smith AH, Treadway JL et al. The sulfonylurea gliclazide does not inhibit ischemic preconditioning in anesthetized rabbits. Cardiovasc Drugs Ther. 2005; 19: 337–46.
40. Siddall HK, Warrell CE, Yellon DM et al. Ischaemia-reperfusion injury and cardioprotection: investigating PTEN, the phosphatase that negatively regulates PI3K, using a congenital model of PTEN haploinsufficiency. Basic Res Cardiol. 2008; 103: 560–8.
41. Ethymiou CA, Mocanu MM, Yellon DM. Atorvastatin and myocardial reperfusion injury: new pleiotropic effect implicating multiple prosurvival signaling. J Cardiovasc Pharmacol. 2005; 45: 247–52.
42. Mensah K, Mocanu MM, Yellon DM. Failure to protect the myocardium against ischaemia/reperfusion injury after chronic atorvastatin treatment is recaptured by acute atorvastatin treatment: a potential role for phosphatase and tensin homolog deleted on chromosome ten? J Am Coll Cardiol. 2005; 45: 1287–91.
43. Stratton IM, Adler AI, Neil HA et al. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. Bmj. 2000; 321: 405–12.
44. Cubbon RM, Wheatcroft SB, Grant PJ et al. Temporal trends in mortality of patients with diabetes mellitus suffering acute myocardial infarction: a comparison of over 3000 patients between 1995 and 2003. Eur Heart J. 2007; 28: 540–5.
45. Norhammar A, Lindback J, Ryden L et al. Improved but still high short- and long-term mortality rates after myocardial infarction in patients with diabetes mellitus: a time-trend report from the Swedish Register of Information and Knowledge about Swedish Heart Intensive Care Admission. Heart. 2007; 93: 1577–83.