Review

How does oxidative stress during diabetic pregnancy lead to cardiac ventricular septal defects?

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Abstract

Over 60 million women have diabetes worldwide, of which 1.9 million are in the United Kingdom. Pregestational diabetes mellitus (PGDM) related births are around 2% and have tripled in the last 15 years. PGDM can have a disastrous impact on the developing foetus, leading to a variety of birth defects including heart defects. The level of hyperglycaemia determines their prevalence and severity, with oxidative stress mediating the effects. This article seeks to understand how oxidative stress in PGDM can affect the development of the heart, causing ventricular septal defects. References were found using PubMed, with the following terms: "pregestational diabetes", "congenital malformations", "birth defects", "ventricular septal defects" and "oxidative stress". Only articles published in English and after 2007 to the present day were used. Oxidative stress (OS), caused by hyperglycaemia, has been shown to activate the ASK1/JNK1/2 pathway, which affects the development of the heart. It induces apoptosis in the migrating cardiac neural crest cells and decreases cell proliferation in the cardiac outflow tract cushions by targeting several important cyclins and cell cycle inhibitors. Both of these processes lead to the formation of ventricular septal defects due to decreased cellularisation of the cardiac jelly, a precursor of the outflow tract cushions. These findings highlight the importance of glycaemic control during pregnancy as these defects can be prevented by providing mothers with the correct care before and during pregnancy. However, future research is needed to identify potential therapeutic targets of the OS mediated pathways.

Abbreviations

ASK1 - Apoptosis regulating kinase 1
BMP4 - Bone morphogenic protein 4
CNCC - Cardiac neural crest cells
FoxO3a - Forkhead transcription factor 3a
JNK1/2 - c-Jun NH2-terminal kinase 1/2
OFT - Outflow tract
OS - Oxidative stress
PGDM - Pregestational diabetes
ROS - Reactive oxygen species
Trx - Thioredoxin
VSD - Ventricular septal defect

Introduction

Over 60 million women between the ages of 18 and 44 have diabetes worldwide, and around 1.9 million women have diabetes in the United Kingdom.[1,2] This number is all the more worrying when considering the devasting effects diabetes can have on the body and the developing foetus. Pregestational diabetes mellitus (PGDM) can be defined as when a women has type I or type II diabetes before conception.[3]

> *The prevalence of PGDM related births is around 2%, a figure that has almost tripled in the past 15 years.*[4]

Several studies agree that it is not the type of diabetes that mediates the degree of congenital defects but rather the degree of glycaemic control.[2,5] Indeed, a study found that hyperglycaemia in the 1st trimester due to poorly controlled PGDM resulted in major congenital defects in 5-10% of pregnancies and spontaneous abortions in 15-20% of pregnancies.[6] Population studies have shown that PGDM can increase the risk of various birth defects by up to five times, with congenital heart defects being the most frequent malformation due to PGDM, with a relative risk of 3.59 compared to the control population.[4,7] Many congenital heart defects have been linked with PGDM, some of the most prevalent being ventricular septal defects (VSDs) the risk of which are increased by twofold with PGDM.[2,4]

Oxidative stress (OS) has been found to be one of the most likely causes of diabetes related congenital heart disease, however, its mediating pathways are complex.[2] This article presents how OS leads to increased apoptosis and decreased cell proliferation and how this affects the development of the heart, ultimately leading to VSDs.

Methods

Relevant articles were found using the PubMed database (www.ncbi.nlm.nih.gov/pubmed). The following search terms were used in various combinations: "pregestational diabetes", "congenital malformations", "birth defects", "ventricular septal defects" and "oxidative stress". These keywords were considered most relevant to the scope of the article and enabled the identification of relevant papers for this review.

Only papers published in English and after 2007 to the present day were used, as these were deemed most up to date in current research. Once relevant papers were found, their abstracts were read and, if deemed suitable, the whole paper was analysed. Further papers were found by scanning the references of the original papers.

The embryological development of the heart

The heart is formed from the mesoderm 18 days after fertilisation, making it the first functional organ to be developed.[8] Heart development takes place in the cardiogenic area, where the primitive heart tube is formed. The primitive heart tube is made up of five distinct parts: the aortic sac, the outflow tract (OFT), the common primitive ventricle, the atrium and the sinus venosus.[9]

The OFT cushions are formed through a process of cellurisation of the cardiac jelly, an abundant extracellular matrix.[10,11] Cardiac neural crest cells (CNCC) from the dorsal neural tube migrate to the distal region of the OFT whereas, in the proximal region of the OFT, endothelial cells become mesenchymal cells in a process called epithelial to mesenchymal transition.[12] Both regions contribute cells to the cardiac jelly enabling the formation of the OFT cushions, a process that occurs under the influence of bone morphogenic protein 4 (BMP4). BMP4 is necessary for normal embryonic development, acting as a key protein for organogenesis, including CNCC migration as well as OFT septation and formation of membranous portion of ventricular septum.[13] During cardiac remodelling, the OFT cushions grow towards each other and fuse to form a continuous septum and then divide to form the semilunar valves.[11]

How does oxidative stress in women with PGDM affect embryonic development of the heart?

One of the most harmful effects of PGDM is that it induces OS, which has been shown to be responsible for congenital heart defects.[2,13] This is due to the fact that the concentration of reactive oxygen species (ROS) increases in conjunction with increasing glucose levels in PGDM.

> *ROS have been found to reduce the cells' antioxidant capacities, which leaves them vulnerable to increased apoptosis and decreased cell proliferation.*[6]

A study performed on chicks found that the OFT cushions were asymmetrical causing the lumen of the heart to be displaced in six out of the ten diabetic chicks compared to zero out of the ten control chicks.[5] This was hypothesised to be due to decreased cell proliferation in the OFT cushions as a result of decreased levels of cyclin D1 and increased levels of p21. Cyclins, such as cyclin D1, enable cell progression from the G1 phase to S phase, whereas cell cycle inhibitors, such as p21, cause cell cycle arrest at G1, therefore inhibiting DNA replication and mitosis.[14] Hyperglycaemia in PGDM has been found to decrease gene expression of cyclin D1 by threefold and increase gene expression of p21 by twofold, leading to decreased cell proliferation due to cell cycle arrest.[14]

A 2014 study evaluated the cellularisation of the cardiac jelly necessary for OFT development and found that the control group contained more than double the amount of mesenchymal cells compared to the diabetic group.[10] This was attributed to a decreased rate of cell mitosis in the diabetic group (17.93%) compared to the control group (35.85%). Furthermore, the study found elevated levels of OS localised to the OFT cushions.

A 2008 study that found that OS induced on E7.5 lead to a range of OFT defects in diabetic mice, despite the fact that OFT development occurs after E7.5.[15] They attributed the defects to apoptosis of CNCC, as apoptotic cells were found along the path of CNCC migration. This finding was also echoed by Zhao *et al*. (2012) who found decreased CNCC migration as a result of OS.[16]

Another study conducted in mice found that during embryonic day 10.5 of development (E10.5) diabetic mice were more susceptible to OFT stenosis resulting in smaller OFT cushions and a thinner right ventricle.[17] They also detected VSD at E15.5 due to suppressed development of OFT cushions at earlier stages of the embryonic development. They found decreased cell proliferation markers in the OFT cushions, but very few apoptotic cells were detected. This would indicate that decreased cell proliferation, as opposed to increased apoptosis was responsible for these VSDs.

A 2011 study made an interesting finding that hyperglycaemia affected OFT cushion development at E8.5 as opposed to OFT cushion remodelling at E11.5 as rates of VSDs were higher in mice made hyperglycaemic from E8.5 (49.2%) as opposed to mice made hyperglycaemic from E11.5 (6.2%).[16]

These studies all indicate that PGDM induces increased apoptosis in CNCC migration and decreases cell proliferation in OFT cushions, mediated by oxidative stress.

The role of ASK1 pathways in oxidative stress mediated VSDs

Despite oxidative stress being a commonly acknowledged cause of congenital heart defects, the pathways by which it affects embryonic development have been disputed. Earlier studies reported that oxidative stress inhibited the expression of the *pax3* gene, causing apoptosis through the expression of p53 (a tumour suppressor gene), which is normally suppressed by *pax3*.[15] However, recent studies have found a more likely pathway.[2,13,18] Apoptosis-signal-regulating kinase 1 (ASK1) was discovered as key in mediating the effects of OS on foetal cells (see **Figure 1**).[2]

ASK1 is part of the mitogen-activated protein kinase cascade, normally forming an inactivate complex with thioredoxin (Trx), which

plays a key role in cell growth, cell cycle regulation, and apoptosis.[13,19] A study measured the levels of ASK1/Trx complex, as well as the levels of oxidised Trx, and found lower levels of ASK1/Trx complex but much higher levels of oxidised Trx in hearts of diabetic mice compared to control mice.[13] This is due to oxidisation of Trx through ROS, hence dissociating Trx form the Ask1/Trx complex, enabling ASK1 activation.[13,18]

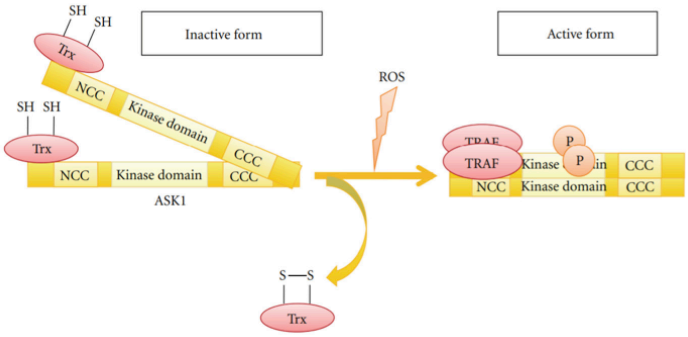

Figure 1. The role of ASK1 in OS. Trx retains ASK1 in its inactive form. However, ROS causes dissociation of Trx from ASK1, leading to activation of ASK1 by tumour necrosis factor receptor-associated factor (TRAF). CCC, C-terminal coiled-coil domain; NCC, N-terminal coiled-coil domain; P, phosphate. Figure adapted from Soga *et al*,[22] distributed under a CC BY 3.0 license, which permits unrestricted use, distribution, and reproduction in any medium.

Indeed, high levels of ASK1 were detected in embryonic hearts of diabetic mice at E12.5, a key stage of cardiogenesis. To prove that ASK1 mediates the teratogenic effects of OS on the embryo, the study examined the effects of ASK1 deletion on the prevalence of maternal diabetes induced congenital heart defects. The authors found that under diabetic conditions, one out of the 54 ASK1 negative diabetic mice showed congenital heart defects compared to 14 out of 55 control diabetic mice.[13]

ASK1 induces its effects through activation of the c-Jun NH2-terminal kinase 1/2 (JNK 1/2).[2,13,18] JNK1/2 has been shown to increase the activity of the Forkhead transcription factor 3a (FoxO3a) which up-regulates TNFR1 associated death domain (TRADD), triggering caspase 8 activation, causing apoptosis.[2,13] ASK1 deletion inactivates the JNK1/2 pathway by suppressing its phosphorylation.[13] This, in turn, was shown to abrogate the activation of FoxO3a as fewer apoptotic cells were detected in ASK1 negative diabetic mouse hearts compared to control diabetic mouse hearts.

However, apoptosis is not the only mechanism by which ASK1 disrupts normal embryonic development of the heart. It has previously been shown that decreased cell proliferation plays a critical role in OFT defects. ASK1 was found to mediate this effect as ASK1 deletion abrogated both decreases in cyclin D1 and increases in p21.[13] This, therefore, indicates that the ASK1-JNK1/2 pathway mediates the effects of oxidative stress on cell proliferation.

Furthermore, BMP4 plays a critical role in cardiac development.[13,20] The downregulation of BMP4 leads to decreased mitosis in OFT cushions, with a study showing deletion of BMP4 resulted in VSD in the membranous portion of the ventricular septum in 17 out of 19 BMP4 negative mice.[21] PGDM was found to significantly decrease levels of BMP4 in diabetic mice, but the effects of PGDM on BMP4 expression were diminished in ASK1 negative diabetic mice.[13]

It is, however, relevant to note that despite these findings being important, these studies are limited due to them being conducted in animals such as mice or chicks. Despite these acting as a useful comparison to human biology, they are not interchangeable, and therefore findings must be considered with this limitation in mind.

Conclusion

PGDM has damaging effects on the development of the foetal heart, in particular the OFT. Research has shown an important link between hyperglycaemia and OS, with there being a positive correlation between levels of ROS and glucose levels in PGDM. ROS leave cells vulnerable to increased apoptosis during cardiac neural crest cell migration to the cardiac jelly. ROS also causes decreased cell proliferation in the development of the OFT cushions.

These teratogenic effects are mediated through the ASK1-JNK1/2 pathway. This affects cyclins and cell cycle inhibitors leading to a decrease in cell proliferation and mitosis in embryonic hearts affected by PGDM. It also has an inhibitory effect on BMP4, leading to an increased risk of VSDs in the membranous portion of the ventricular septum. Finally, it initiates apoptosis through the FoxO3a-TRADD pathway. All of these culminate in reducing cell density in OFT cushions, leading to a variety of congenital heart diseases including VSDs.

These devasting congenital heart diseases are preventable through better glycaemic control during pregnancy, which can be achieved by ensuring that everyone receives the correct care before and during pregnancy. This key topic would benefit from additional research, exploring how the OS pathway could be used as a therapeutic target to prevent VSDs.

Contribution statement The author confirms that they have made substantial contributions to the conception or design of the work, drafting the work and final approval of the version to be included in Inspire.

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