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Identification of the growth arrest and DNA damage protein GADD34 in the normal human heart and demonstration of alterations in expression following myocardial ischaemia

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Abstract

Growth arrest and DNA damage protein 34 (GADD34) is a multifunctional protein upregulated in response to cellular stress and is believed to mediate DNA repair and restore protein synthesis. In the present study we have examined GADD34 immunoreactivity in human myocardial tissue at defined survival times following cardiac arrest and determined alterations in expression following ischaemia. In the normal human heart, GADD34 immunoreactivity was generally intense and present within most cells. GADD34 immunoreactivity was downregulated in tissue displaying ischaemic damage and remained intense in adjacent non-infarcted tissue. Unlike brain, GADD34 was not found to be upregulated in the peri-infarct zone. Cells displaying apoptotic changes were located in regions displaying reduced GADD34 immunoreactivity. In the brain, it is thought that GADD34 supports re-initiation of protein synthesis following ischaemia. Similarly, GADD34 may perform important functions in cardiac tissue in response to ischaemia.

GADD34 is a member of a family of genes [1] known to be upregulated in response to cell cycle arrest and DNA damage [2]. We, and others have shown that GADD34 is also upregulated in response to ischaemic stress and is correlated with cell survival [3, 4, 5, 6]. The carboxyl region of GADD34 shares significant sequence homology with herpes simplex virus type 1 protein ICP34.5. One of the functions of ICP34.5 is to preclude host cell protein synthesis shutdown following viral infection (Fig 1)[7]. The conserved region of GADD34 has been shown to compensate for the corresponding region in ICP34.5 in modulating protein synthesis initiation [8]. Further, GADD34 knockout mice display irregularities in protein synthesis regulation [9]. GADD34 exerts its influence on protein synthesis via the unfolded protein response (UPR) pathway and dephosphorylates eukaryotic translation initiation factor 2 (eIf2alpha) (Figure 1). GADD34 has also been shown to interact with other cellular proteins involved in DNA repair i.e. proliferating cell nuclear antigen (PCNA) [10,11]. We are currently investigating GADD34 as a therapeutic target for cerebral ischaemic damage. However, little is known about GADD34 expression in the heart and if expression is altered with cardiac ischaemia. This study is the first to report the presence of GADD34 in human myocardial tissue and to examine the consequences of ischaemia on its expression.

Ethical approval for the study was granted by the local ethics committee. Cases of myocardial infarction were identified from post-mortem reports (n=30). Morphological features of infarction were identified in histologically stained (haematoxylin and eosin) sections and used to classify the cases into defined survival groups of <24hours (n=13) and 24hours-7days (n=17). Age matched control cases were also included (n=12) where sections displayed no histological evidence of recent acute infarction. GADD34 expression was assessed by immunohistochemistry using a rabbit polyclonal antibody for GADD34 (H-193, Santa Cruz, dilution 1:100). Labelling was visualised using the avidin-biotin complex method with diaminobenzadine as the chromogen. Negative controls were prepared by omitting the primary antibody. Images of histological sections were captured on an image analysis system (Leica IM500) and areas of infarction identified microscopically and mapped onto an outline image of the section. Images of GADD34 staining were then overlayed and random fields selected for scoring of GADD34 immunoreactivity. Two blinded investigators independently scored staining intensity within these fields as follows; 0= none, 1= minimal, 2= moderate and 3= maximal. Inter and intra investigator variability was found not to be significant. Double labelling of GADD34 and TUNEL (a marker of irreversible cell damage) was also carried out.

Strong GADD34 immunoreactivity was evident in normal, non-infarcted myocardial tissue and was localised to the cytoplasm of myocytes. There was no alteration in the cellular localisation of GADD34 with ischaemia and all cases showed cytoplasmic compartmentalisation of the protein. In the <24hr survival group, a moderate reduction in GADD34 staining intensity was observed in areas of infarction with immunoreactivity remaining relatively

intense in adjacent non-infarcted regions although staining was slightly reduced in the adjacent tissue (Figure 2(a)). In contrast, in the 24hour-7day survival group, GADD34 immunoreactivity was markedly reduced in infarcted tissue compared to adjacent non-infarcted tissue and that seen in control cases (Figure 2 (b,c,d) ($p<0.001$). Double labelling of tissue with TUNEL and GADD34 revealed TUNEL positive cells were present within the infarct. TUNEL positivity was generally associated with cells displaying a decrease in GADD34 immunoreactivity. Adjacent histologically non-infarcted and control case tissue displayed relatively few TUNEL positive cells (Figure 2(e) and (f)).

In rodent focal ischaemia (middle cerebral artery occlusion) our group has shown that GADD34 immunoreactivity is significantly reduced within the infarct and upregulated within cells of the peri-infarct zone [4]. Recently we have shown GADD34 upregulation in the peri-infarct zone in human ischaemic stroke [6] and also in various regions of the brain following cardiac arrest [5]. Protein synthesis inhibition is a major event following ischaemia. It was once thought that protein synthesis inhibition was a beneficial phenomenon protecting cells from misfolded proteins via the unfolded protein response pathway. It is now thought that long-term inhibition of protein synthesis may be maladaptive and following brain ischaemia, restoration of protein synthesis may promote cell survival. Unlike the brain, there is doubt whether increased or restored protein synthesis would be beneficial in the heart, due to the link between increased protein synthesis and hypertrophy of myocytes [12, 13]. Instead, a realistic therapeutic strategy in the heart may be to inhibit GADD34 expression to maintain protein synthesis inhibition and reduce hypertrophy as work rate increases. In this study we set out to investigate the consequences of ischaemia on cardiac GADD34 expression. We report strong GADD34 immunoreactivity in the normal human heart with a marked decrease in immunoreactivity within infarcted tissue. However, unlike the situation in the brain, there was no evidence of increased GADD34 immunoreactivity in peri-infarct zones in any of the cases examined.
In conclusion, we have observed for the first time that GADD34 is expressed in myocytes of the human heart and expression is altered in response to ischaemia.

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Figure 1 Schematic for the role of GADD34 in the initiation of protein synthesis and attenuation of programmed cell death as part of the integrated stress response pathway.

Figure 2(a) Boxplots of GADD34 immunoreactivity scores. Scores were analysed using a one-way ANOVA with post-test analysis of groups. The data are presented using median scores as a summary measure. (***$p<0.001$)
(b,c,d) Illustrations of GADD34 immunoreactivity in tissue following 24hrs-7days survival with the boundary between light and intense GADD34 staining demarcating infarcted and adjacent non-infarcted tissue (b) (Scale bar= 200μm) and at a high power showing staining in a non-infarcted (c) compared to that of infarcted regions (d) (Scale bar=100μm).
(e, f) Double labelling with Tunel and GADD34 immunostaining showing no Tunel positive cells and intense GADD34 immunoreactivity in tissue from a control case (e). Tunel positive cells were limited to regions of infarct (dark nuclear staining) (f) and regions where GADD34 immunoreactivity was reduced (light cytoplasmic) (Scale bar= 50μm).