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Research Article

Complex factors in preconditioning a microarray gene

K. Schembri[1], **G. Grech**[2], **C. Saliba**[3], **C. Scerri**[4], **J. Galea**[*1]
[1] *Department of Cardiothoracic Surgery, Mater Dei Hospital, Msida, Malta*
[2] *Department of Pathology, Faculty of Medicine and Surgery, University of Malta, Msida, Malta*
[3] *Centre of Molecular Medicine and Bio-banking, University of Malta, Msida, Malta*
[4] *Department of Physiology, Faculty of Medicine and Surgery, University of Malta, Msida, Malta*

Abstract. Preconditioning is complex, strong, evolutionary conserved cellular survival mechanism that is exhibited by different species as well as in different organs. A focused approach on microarray evaluation of preconditioning will be used to highlight the lack of clarity in investigating this complex phenomenon, exacerbated by the absence of a standardised terminology. This paper is an extensive review of the scientific literature on the investigation of preconditioning by means of a microarray approach. It dissects the design of the experiments used to investigate such phenomenon and classifies the complex factors in investigating preconditioning. It presents an attention to detail to the lexicon with a suggested classification and terminology that describes preconditioning that may help stratify and clarify research in this field.

Keywords: preconditioning, microarray, complex factors, classification

1 Introduction

Preconditioning is a complex, evolutionary conserved, cellular survival phenomenon. The protective effect of preconditioning is described in different species as well as in different organs of the same species such as the heart (Correa-Costa et al., 2012; Jassem et al., 2009; Jun et al., 2011), the lung (Jun et al., 2011), the kidney (Correa-Costa et al., 2012), the liver (Jassem et al., 2009), the intestines (Wang et al., 2009), the retina (Kamphuis et al., 2007), the spinal cord (Carmel et al., 2004; Kim et al., 2008), the brain (Hirata et al., 2007; Kawahara et al., 2004) and skeletal muscle (Harralson et al., 2005; Moses et al., 2005). It is also possible to transfer this protective effect from a preconditioned rat heart to that of a naive rat heart using the coronary effluent (Serejo et al., 2007). The same effect has been shown in rabbits (Dickson et al., 1999; Leung et al., 2014).

This phenomenon falls under the wider term of hormesis. This term was first described by Southam et al. (1943) and recently revived by Calabrese (2004). Hormesis refers to a pattern of cellular responses to stressors whereby a beneficial effect results from exposure to low doses of agents or intensities of environmental factors that are otherwise toxic or lethal when given at higher concentration or intensities (Krenz et al., 2013). Murry et al. (1986) were among the first to report a type of preconditioning known as 'ischemic preconditioning' and referred to it as a 'rapid, adaptive response to a brief ischaemic insult, which slowed the rate of cell death during a subsequent prolonged period of ischemia'. In a dog heart model, they were able to prove that this phenomenon could reduce the infarct size by 75%. Four years later Kitagawa et al. described ischaemic preconditioning in the brain of gerbils (Kitagawa et al., 1990).

Understanding one of the strongest cellular defence mechanisms is challenging for many reasons. A focused approach on microarray evaluation will be used to highlight the lack of clarity in investigating this complex phenomenon, exacerbated by the absence of a standardised terminology. The following is a review of the scientific literature investigating preconditioning by means of a microarray approach and presents an attention to detail to the lexicon with a suggested terminology describing preconditioning that may help stratify and clarify research in this field.

Correspondence to: J. Galea (joseph.f.galea@um.edu.mt)

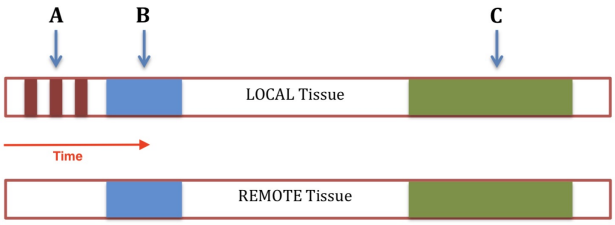

Figure 1: Showing: A (red) – Challenge. (Single or separated by two reperfusion episodes) B (blue) – Early Protection from Insult C (green) – Late protection from Insult

2 Preconditioning, gene expression and microarrays

Preconditioning is triggered by a stimulus, which will be forthwith referred as the 'challenge'. This challenge will protect the cell from a more potent ensuing event from now on referred to as the 'insult'. The protective effect is bi-temporal and is exhibited in local as well as in remote tissue (figure 1). Thus, preconditioning can be described as having four phases of protection. The first phase of protection occurs within minutes of the insult A (Red in figure 1), lasts 2 to 3 hours (B) (Blue in figure 1) and is commonly referred to as classic preconditioning (Bolli, 2000). The second phase of protection comes on at about 24 hours after the challenge and lasts up to 72 hours (C) (Green in figure 1) and is commonly known as the second window of protection (SWOP) (X. M. Yang et al., 1996). The above two phases describe local protection. The protective effect is transmitted to remote organs giving rise to two other phases commonly known as early and late remote preconditioning (Leung et al., 2014; Przyklenk et al., 1993). Towards the beginning of the 1990's the main focus of investigation was on the classic phase of preconditioning. Thornton et al. (1990) reinforced this drive by showing that the inhibition of protein synthesis did not alter myocardial protection afforded by preconditioning. The receptor-based response seen in classic preconditioning is a vital rapid response to the stressor (challenge) and can be considered a 'knee jerk or reflex' response that is not dependent on gene transcription. The delayed response which was described in 1995 by Yellon et al. is however a complex gene expression response, possibly an 'intelligent' response with the capability of anticipating potential ensuing threats (insults). Two main genetic approaches have been used in studying this aspect of preconditioning; candidate gene approach and genome wide analysis. The former is built around a hypothesis about the role of particular pathways such as inflammation, followed by a search for changes in specific gene expression levels related to inflammation by means of tools such as the polymerase chain reaction (PCR). These are in vitro models that are very useful because biochemical molecules can be used to alter the pathway under investigation and study its effect and relevance. A top to bottom approach using genome wide analysis interrogating the expression of thousands of genes in a single experiment such as in microarray analysis or next generation sequencing (NGS) as compared to the latter reductionist approach is an important tool in uncovering key molecular events in the cell's response to preconditioning. Limitations of these gene expression studies include, restricted time points, limited and biased transcripts represented on the array, the nature of the sample analysed, as well as an absence of clearly defined models (Kawahara et al., 2004).

3 Complex factors in preconditioning

Several factors that make preconditioning a complex process to study can be identified. These can be broadly subdivided into intrinsic and extrinsic factors. Intrinsic factors relate to the nature of the phenomenon and include; a wide spectrum of challenges, a bi-temporal nature and a spatial element. The extrinsic factors relate to the diverse experimental designs adopted by researchers, terminology and semantics used in describing the phenomenon and the overwhelming information generated by recent technology.

3.1 Intrinsic Factors

The intrinsic factors are inherent to the phenomenon and thus are not amenable to alteration however when identified they can be approached systematically facilitating a holistic approach in researching the phenomenon.

3.1.1 Wide spectrum of challenges

The first intrinsic factor is the wide spectrum of challenges that can induce preconditioning leading to the activation of diverse complex networks culminating to a common effect of enhanced cellular tolerance. These challenges include; hypoxia (Bernaudin et al., 2002), hyperthermia (Du et al., 2010), hypothermia (Nishio et al., 2000), epileptic fits (Sasahira et al., 1995) and drugs such as acetylsalicylic acid (Riepe et al., 1997). These are not simply challenges exclusive to the laboratory but have also been studied in the natural setting in humans. This is thought to occur in patients with ischaemic heart disease who exhibits recurrent anginal chest pain (Costa et al., 2005; Wall et al., 1994) or in patients with cerebrovascular disease who exhibit transient ischemic attacks (Moncayo et al., 2000). The different challenges studied in different species under different experimental conditions looking at different phases of preconditioning leads to a multiplier effect on the number of complex variables.

3.1.2 Bi-temporal nature

The second intrinsic complex factor is the bi-temporal nature creating three scenarios for investigation the third being the processes happening between the first and second instance. Each scenario is a complex event to study in its own merit. The preconditioning challenge invokes an early response with a very rapid manifestation of cellular protection that lasts a few hours and a late response or second wave becoming active at 24 hours from the initial challenge and lasting 72 to 96 hours (figure 1). The first wave is dependent on preformed molecules and activation of receptors such as adenosine A1/A3 (Murphy et al., 2008; Tsukamoto et al., 2005), opioid receptor activation (Schultz et al., 1995) and to a lesser degree bradykinin B2 (Wall et al., 1994). These molecules in turn activate downstream signalling cascades the earliest being protein kinase C (PKC). Downstream targets of PKC activation include 5'-nucleotidase, glycogen synthase kinase-3B (GSK-3B), mitochondrial permeability transition pore (mPTP), ATP-sensitive potassium channels in plasma membranes and mitochondria, proteins involved in apoptosis (Bax/Bad and Bcl-2), and adenosine A2b receptors (Costa et al., 2005; Hausenloy et al., 2003; Murphy et al., 2008; Tsukamoto et al., 2005; X. Yang et al., 2011). This does not exclude the possibility of an early genomic response but this is overshadowed by studies looking at the biochemical response. This is in contrast to the exponential increase in the literature regarding the extensive genetic response in the SWOP. A highlight of this response is the very important up-regulation of an intrinsic pro-survival genetic program that has been shown to attenuate apoptosis (Stein et al., 2007).

Intuitively the bi-temporal nature of the phenomenon drives research into these two time points potentially undermining inquiry into the interim period that possibly involves cellular memory. It is very plausible that the second wave involves the activation of a cellular memory mechanism allowing the cell to mount the response 24 hours after the initial preconditioning stimulus. This hypothesis is supported by evidence of cellular memory from studies of a similar phenomenon described in plants known as 'priming' (Pastor et al., 2013).

3.1.3 Spatial element

The third intrinsic factor is the spatial element. The protective effects are noted both locally at the site of preconditioning as well as in remote organs involving complex processes of cell signalling (Guo et al., 2019). Thus distant organs are somehow receiving the preconditioning trigger allowing them to respond effectively to the challenge, another mechanism that is still not fully understood (Billah et al., 2018). The remote organ protection effect is manifested for both temporal events that is the classic and SWOP. This remote effect introduces three scenarios with their own complex factors. The first is the local mechanism by which a challenged organ creates a signal that conditioning has happened. The second scenario is the transmission of this signal that is thought to involve both neural and humoral factors (Lim et al., 2010; Shimizu et al., 2009) and the third is the interpretation of this signal by distant organs and the mounting of a protective response.

3.2 Extrinsic factors

Studies of preconditioning using microarray techniques were chosen as the main criterion in order to simplify and focus analysis on a specific manageable scenario. Twenty-eight studies from PubMed satisfied the criterion of microarray and preconditioning and are shown in table 1. The extrinsic factors will be explored further by using the 28 studies referred to in table 1.

3.2.1 Classification and terminology

The first extrinsic factor is process classification and terminology. Using gene expression as the scenario it is clear from the literature that it is challenging to the unfamiliar reader to understand what phase of the preconditioning phenomenon is under investigation. This is confounded by the fact that different authors use different terminology to describe the same phases of preconditioning. In order to clarify and simplify this issue of preconditioning phases it is suggested that they are classified into four different phases based on temporal and spatial factors and that a standard terminology with an abbreviation system is adopted. The temporal response can be subdivided into two parts, early and late. The early response, referred to as 'classical', 'immediate', 'acute' or 'early phase' preconditioning is very rapid and confers tolerance lasting between 2 to 3 hours (figure 1). This is followed by an interim period where tolerance is not exhibited. The late phase is referred to as the second window of protection 'SWOP', 'delayed' preconditioning and 'late phase' preconditioning sets in at around 24 hours after the initial challenge and lasts up to 72 hours. Both the early and the late responses have a spatial component that is local and remote, transmitting the signal to distant cells and organs. The remote component is known as remote preconditioning. Remote preconditioning is expressed in both temporal aspects and thus the distant cells exhibit both early remote preconditioning as well as late remote preconditioning. Table 2 illustrates current terms used in describing preconditioning and a proposed classification and nomenclature shown in italics that integrates the different aspects of preconditioning.

The proposed terms and abbreviations would thus be local early preconditioning (LEPC), local late preconditioning (LLPC), remote early preconditioning (REPC),

	Spatial – Local	Spatial – Remote
Temporal – Early (2 to 3 hours)	Local early PC (LEPC) *Classical PC* *Immediate PC* *Acute PC* *Early phase PC*	Remote early PC (REPC) *Remote PC* *Remote ischemic PC*
Temporal – Late (24 to 72 hours)	Local late PC (LLPC) *Second window of protection* *Delayed PC* *Late Phase PC*	Remote late PC (RLPC) *Remote delayed PC*

Table 2: Current and proposed terms describing preconditioning (PC). The terms in italics are alternative terms that are found in the literature.

System	Species	Type of challenge	Challenge protocol	Challenge - insult interval	Type of insult	PC phase	Genes, Fold Change & Platform Setting	Experimental Design	First author, Journal & Year
Brain									
Cerebral cortex	*Mus musculus* 8-10 weeks male C57BL/6J	MCAO	Single 15-minute episode of MCAO	3 days	60 minutes MCAO	Second phase/ SWOP	7500 genes 2.2 fold Affymetrix MG_U74AV1	- Challenge at 24 hours - Insult at 24 hours - Insult altered by challenge 3 days before	Stenzel-Poore, *Lancet*, 2003
Hippocampus (CA1 cells)	*Rattus norvegicus* male Wistar SPF	BCAO	Single 2-minute episode of BCAO	3 days	6 minutes BCAO	Second phase/ SWOP	7000 genes 2 fold Affymetrix RG_U34A	- Challenge at 1, 3, 12, 24 & 48 hours - Insult at 1, 3, 12, 24 & 48 hours - Insult altered by challenge 3 days before	Kawahara, *J Cereb Blood Flow Metab*, 2004
Frontoparietal cortex	*Rattus norvegicus* adult male SHR **kwaa**	MCAO	Single 10-minute episode of MCAO	3 days	60 minutes MCAO	Second phase/ SWOP	1263 genes 2 fold Affymetrix RG_U34	- Challenge at 3, 6, 12, 24 & 72 hours - Insult at 6 hours - Insult altered by challenge 3 days before	Dhodda, *J Neurochem*, 2004
Cerebral cortex	*Mus musculus* adult male Swiss(not starved)	Hypoxia by 8% oxygen in nitrogen	Single 1 or 6 hour episode of hypoxia	12,18 & 24 hours	Permanent MCAO	Second phase/ SWOP	6000 genes 1.5 fold Affymetrix MG_U17A	- 2 challenges at 12, 18, 24 & 72 hours - Insult at 6 hours - Insult altered by challenge 12, 18 & 24 hours before	Tang, *Neurobiol Dis*, 2006
Hippocampus (CA3 sector)	*Mus musculus* C57BL/6	Seizure by intra-peritoneal kainic acid	Single episode of seizures	1 day	Status epilepticus by intra-amygdala kainic acid	Second phase/ SWOP	39000 genes 1.8 fold Affymetrix 430 2.0	- Challenge at 24 hours - NA - Insult altered by challenge 1 day	Hatazaki, *Neuroscience*, 2007

Table 1: Showing a description of preconditioning microarray experiments according to system, species, type of challenge, challenge protocol, challenge-insult interval, type of insult, preconditioning phase, genes, fold change and microarray platform setting, gene expression profile analysis and fist author, journal, year and reference number. (SHR – spontaneously hypertensive; MCAO – middle cerebral artery occlusion; BCAO bilateral cerebral artery occlusion ; HBO – hyperbaric oxygen; CpG – cytosine-guanine; LPS - lipopolysaccharide; OGD – oxygen-glucose deprivation; CAO – coronary artery occlusion; IPC – ischemic preconditioning; APC – anesthetic preconditioning; MAC – minimum alveolar concentration: SMAO – ; BP – blood pressure; NA – Not Available; PC – Preconditioning and SWOP – second window of protection (table continues overleaf, 1/6)

Cerebral cortex	*Rattus norvegicus* neonatal Sprague Dawley	Hypoxia by 8% oxygen in nitrogen (36°C)	Single 3 hour episode of hypoxia	2, 8 & 24 hours	Killed	NA	chip 30000 genes 1.2 fold Affymetrix Rat230_2	before - Challenge at 2, 8 & 24 hours - NA - NA	Gustavsson, *Pediatr Res*, 2007
Hippocampus (CA1 cells)	*Rattus norvegicus* neonatal male Wistar	HBO (3.5 atmosphere absolute)	Single 1 hour episode of HBO each day for 5 consecutive days	6, 12, 24 & 72 hours	8 minutes forebrain ischemia	First/ classic & second phase/ SWOP	20500 genes Fold change NA AgilentDNA Oligo	- NA - NA - Insult altered by challenge 6, 12, 24 & 72 hours before	Hirata, *Brain Res*, 2007
Forebrain (global ischemia)	*Rattus norvegicus* male Wistar (fasted)	BCAO	Single 3 minute episode of BCAO	3 days	6 minutes BCAO	Second phase/ SWOP	23060 genes 1.25 fold Affymetrix Rat230_2	- NA - Insult at 1, 4 & 24 hours - Insult altered by challenge 3 days before	Feng, *Brain Res*, 2007
Hippocampus (CA1 & CA3 sectors)	*Rattus norvegicus* adult male Sprague Dawley	Seizures by intraperitoneal Kainic acid	Single 20 minute episode of seizures on day 1 & day 2	1 day	Status epilepticus by intra-peritoneal kainate or pilocarpine	Second phase/ SWOP	10179 genes 1.25 fold Affymetrix RAE230A	- Challenge at 24 hours - Insult at 1 & 3 days - Insult altered by challenge 1 day before	Borges, *Neurobiol Dis*, 2007
Frontal cortex	*Mus musculus* 8-10 weeks C57BL/6	CpG oligodeoxynucleotide intraperitoneal injection	Single episode of CpG	3 days	MCAO (time not specified)	Second phase/ SWOP	NA 1.5 fold Affymetrix MOE430 2.0	- NA - Insult at 24 hours -Insult altered by challenge 3 days before	Marsh,*Stroke*, 2009
Frontal cortex	*Mus musculus*	LPS intraperitoneal injection	Single episode of LPS	3 days	45 minutes MCAO	Second phase/ SWOP	NA	- Challenge at 3, 24 & 72 hours - Insult at 3 & 24	Marsh, *J Neurosci*, 2009

Table 1: Continuation of table 1 (2/6)

	C57BL/6						1.5 fold Affymetrix MOE430 2.0	hours - Insult altered by challenge 3 days before	
Hippocampus	*Rattusnorvegicus* adult Sprague Dawley	OGD	Single 5 minute episode of OGD	NA	10 minutes OGD	NA	NA 1.3 fold Affymetrix Rat230_2	- Challenge at 3, 6 & 12 hours - NA - NA	Benardete, *Brain Res*, 2009
Cerebral cortex	*Mus musculus* adult male C57BL/6J	MCAO	Single 15 minute episode of MCAO	1 day	60 minutes MCAO	Second phase/ SWOP	NA	- Challenge at 24 hours - Insult at 24 hours - Insult altered by challenge 1day before	Lusardi, *J Cereb Blood Flow Metab*, 2010
Cortical neurons (*in vitro*)	*Rattus norvegicus* 18 day embryonic Wistar	OGD	Single 15 minute episode of OGD alternating with 15 minute reperfusion for 3 cyles	1 day	120 minutes OGD	Second phase/ SWOP	NA 1.5 fold Agilent G413 60mer 4x44	- Challenge at 3 hours - NA - Insult altered by challenge 1 day before	Prasad, *J Mol Neurosci*, 2012
Heart									
Heart (*in vivo*)	*Oryctolagus cuniculus* New Zealand white rabbit	Circumflex branch CAO	Single 5 minute episode of CAO alternating with 5 minute reperfusion for 2 cycles	NA	NA	NA	18376 genes 5fold NA	- Challenge at 5 hours - NA - NA	Simkhovich, *Heart Dis*, 2002
Heart (Langendorf isolated & perfused)	*Rattus norvegicus* male Wistar	Langendorff heart no flow ischemia	Single 5 minute episode of ischemiaalternating with 5 minutereperfusion for 3 cycles	NA	30 minutes no flow myocardial ischemia	NA	3200 genes NA NA	- NA - Insult at 2 hours -NA	Onody, *FEBS Lett*, 2003

Table 1: Continuation of table 1 (3/6)

Heart (Langendorf isolated & perfused)	*Rattus norvegicus* male Wistar	IPC by no flow ischemia & APC by isoflurance (1.5 MAC)	IPC - Single 5 minute episode of ischemia alternating with 5 minute reperfusion for 3 cycles APC – Single 110 minute episode of isoflurance	NA	NA	NA	8800 genes 2 fold Affymetrix RG_U34A	- Challenge at 110 minutes - NA - NA	Sergeev, *Anesthesiology*, 2004
Heart (*in vivo*)	*Mus musculus* 10-12 weeks C57BL/6	Hind limb ischemia by occlusion of femoral artery	Single 4 minute episode of occlusion alternating with 4 minute reperfusion for 6 cycles	15 minutes & 24 hours	Killed	NA	NA 1.5 fold AffymetrixMG_430 A	- Challenge at 15 minutes & 24 hours - NA - NA	Konstantinov, *J Thorac Cardiovasc Surg*, 2005
Heart (*in vivo*)	*Mus musculus*/ *Rattus norvegicus* male ICR/ Wistar	Hypoxia by a high-altitude chamber (380 Torr)	Single 15 hour episode of hypoxia for 2, 4 & 8 weeks	NA	NA	NA	6144 genes 2 fold NA	– Challenge at 2, 4 & 8 weeks - NA - NA	Chen, *Shock*, 2005
Myocardial, renal, intestinal, & lung	*Mus musculus* adult male Swiss Webster	SMAO	Single 2-minute episode of SMAO alternating with 2- minute reperfusion for 2 cycles	1 day	Killed	NA	1176 genes 1.7 fold NA	- Challenge at 24 hours - NA - NA	Huda, *Heart Lung Circ*, 2005
Heart (*in vivo*)	*Rattus norvegicus* male Wistar	NA	Single 5 minute episode of ischemia alternating with 10 minute reperfusion for 2	10 minutes	40 minutes ischemia (type not specified)	First/ classic phase	NA NA	- NA - Insult at 30 minutes - Insult altered by challenge 10 minutes before	Canatan, *Cell Biochem Funct*, 2008

Table 1: Continuation of table 1 (4/6)

			cycles				CodeLink bioarrays		
Blood									
Leukocytes	*Homo sapiens* adult male and female	Forearm ischemia by BP cuff inflation (2 00mmHg)	Single 5 minute episode of ischemia alternating with 5 minute reperfusionfor 3 cycles	NA	NA	NA	NA 1.5 fold Affymetrix HG_U133A	- Challenge at 24 hours - NA - NA	Konstantinov, *Physiol Genomics*, 2004
Retina									
Retina	*Rattus norvegicus* Male Wistar	Eye anterior chamber induced pressure by a 1.7m head	Single 5 minute episode of ischemia alternating with 24 hour reperfusion	1 day	60 minutes of anterior chamber raised pressure	Second phase/ SWOP	NA NA AgilentG4130A	- NA - Insult at 1, 2, 6 & 12 hours – Insult altered by challenge 1 day before	Kamphuis, *Mol Vis*, 2007
Lung									
Lung	*Rattus norvegicus*	Cessation of ventilation and perfusion by clamping of pulmonary vessels	Single 5 minute episode of ischemia alternating with5 minute reperfusion for 3 cycles	NA	2 hours of cold ischemia	NA	22226 genes 2 fold Illumina Rat Ref-12 expression beadchip	- NA - Insult at 1, 3, 6 & 24 hours - NA	Jun, *J Surg Res*, 2011
Intestines									
Small intestine (transplant)	*Rattus norvegicus* adult male Sprague Dawley	SMAO	Single 10 minute episode of ischemia alternating with 10 minute reperfusion	10 minutes	Transplantation	First/ classic phase	4096 genes NA NA	- NA - Insult at 1 hour - Insult altered by challenge 10 minutes before	Wang, *J Surg Res*, 2009

Table 1: Continuation of table 1 (5/6)

Kidney									
Kidney (*in vivo*)	*Mus musculus* male C57BL/6	No flow ischemia by clamping of both renal pedicles	Single 15 minute episode of ischemia	1 week	45 minute renal pedicle cross clamp	Second phase/ SWOP	NA 2/3 fold Agilent 4x44 K whole genome microarray	- NA - Insult at 6 hours - Insult altered by challenge 1 week before	Correa-Costa, *PLoS One*, 2012
Liver									
Liver (*in vivo*)	*Homo sapiens*	Pringle's manoeuvre occluding porta hepatis by a tourniquet	Single 10 minute episode of porta hepatis clamping	30 minutes	Transplantation	First/ classic phase	NA NA Affymetrix HG_U133A	- NA - Insult at 2 hours - Insult altered by challenge 30 minutes before	Jassem, *Liver Transpl*, 2009
Liver (*in vivo*)	*Homo sapiens*	Pringle's manoeuvre occluding porta hepatis by a tourniquet	Single 10 minute episode of porta hepatis clamping	NA	Transplantation	First/ classic phase NA?	NA NA NA	- NA - Insult at 90 minutes - NA	Raza, *Liver Transpl*, 2010

Table 1: Continuation of table 1 (6/6)

and remote late preconditioning (RLPC). Another terminological issue is the terms used when referring to the stimuli used to trigger preconditioning; the main stressor that is modified by preconditioning. Since many stimuli can trigger preconditioning and there are different methods for achieving this, it would be helpful if the stimuli used to trigger preconditioning are always referred to as the 'challenge' and the main stressor that is modified by preconditioning is referred to as the 'insult'.

3.2.2 Diverse investigational design

The second extrinsic complexity factor refers to the diverse investigational design adopted by researchers. In such a complex process, structure of design using standard protocols, classification and nomenclature is of utmost importance. Different preconditioning challenges elicit different biochemical and genetic response pathways limiting the significance of comparisons between studies. The different challenges in microarray studies utilized to induce preconditioning include hypoxia (MCAO, BCAO, hypoxia chamber, CAO, NFI, HLI, SMA, FAI, EAIP, CPV and OPH) (Bernaudin et al., 2002), hyperbaric oxygen (HBO), high altitude (Ostrowski et al., 2008), oxygen glucose deprivation (OGD) (Himori et al., 1991; Ito et al., 2000), hyperthermia (Du et al., 2010), hypothermia (Nishio et al., 2000), lipopolysaccharides (LPS) and oligodeoxynucleotide (Huang et al., 2013; Yu et al., 1999). Other challenges used in preconditioning experiments but not in these microarray studies are epileptic seizures (Belosjorow et al., 1999; Rosenzweig et al., 2007; Sasahira et al., 1995), cortical spreading depression (Kobayashi et al., 1995), chemical preconditioning with compounds such as 3-nitropropionic acid (3-NP) (Riepe et al., 1996), antibiotics such as erythromycin and kanamycin (Huber et al., 1999), acetylsalicylic acid (Riepe et al., 1997), N-methyl-D-aspartate (NMDA) (Himori et al., 1991), doxorubicin (Ito et al., 2000), 2-deoxyglucose (Yu et al., 1999) and sulfur dioxide (Huang et al., 2013). Microarrays interrogate a huge number of genes that may vary from one thousand to thirty-five thousand genes generating a huge amount of data. A comparison of different microarrays would be a good approach to understand the genomic response in preconditioning but the diversity of challenges used in this field is an important limiting factor.

Another important factor in design apart from the type of challenge is the duration and frequency of the challenge. The duration of the hypoxic challenge used by different investigators varies from 5 to 15 minutes of bilateral cerebral artery occlusion (BCAO). Another issue in structure is defining clearly what phase of the preconditioning is under investigation; local or remote, classic or SWOP. Gene expression investigations can focus on the effect of challenge on gene expression in local or remote tissue when compared to controls as well as the altering effect of the challenge on the insult expression profile. Out of the 7 studies on the heart, 6 looked at the effect of the challenge on gene expression whilst only one investigation looked at the effect of challenge in altering gene expression profile during the insult. In the latter study the time period between the challenge and the insult is not specified leaving unanswered the issue of whether classic or second window of protection was under study. Therefore, when studying the effect of the challenge on the insult gene expression profile, the timing between the challenge and the insult needs to be clearly defined. There is also large variability in the insult methods used. In the neuro studies the middle cerebral artery occlusion (MCAO) occlusion challenge varied from 45 to 60 minutes to permanent. Given the limited number of microarray studies in preconditioning it would be useful to focus on specific organs. Most of the work has been carried out on the brain (14 studies) and the heart (7 studies), undoubtedly due to the clinical importance of myocardial infarction and stroke, the commonest causes of death in western countries. Single studies investigated blood, retina, lung, small intestines, kidney and liver. Species variability included 14 studies in rats, 10 in mice, 2 in humans and 1 in the rabbit. Only two studies looked at the gene expression changes secondary to preconditioning at a remote site.

3.2.3 Overwhelming information

The final complex factor is the inevitable information overload generated by the advent of an ever-increasing array of powerful data generating investigational tools available for the researcher and which are constantly evolving. Tools such as microarrays have generated terabytes of data and as can be seen from table 1 the types of arrays used and the number of genes investigated has varied over time. This complicates the issue of data integration as well as the comparison of data with earlier investigations. Another new technology, which is bound to generate an even greater load of information, is next generation sequencing. New discoveries such as epigenetics can become a potential contributor to information overload. In fact, research into the epigenetics of preconditioning has started to be published from 2013 (Thompson et al., 2013).

4 Conclusion

Complex systems in biology such as preconditioning need a concerted effort in order to be deciphered. Scientific approaches, such as systems biology, an interdisciplinary field of study that focuses on complex interactions within biological systems, using a holistic approach as opposed to the more traditional reductionism are essential. The study of preconditioning needs

a 'systems thinking' approach based on a set of habits or practices facilitating the research in this field. This review proposes a basic practice, that of a standardized nomenclature and classification. The terms defined included 'phases' of preconditioning, 'challenge' and 'insult'. Table 2 is a proposal of terms used to describe the four phases of preconditioning. A clearer description of the investigation should also be taken into consideration. A definition from the outset of the model, whether in vitro or in vivo, the species studied, the type of challenge and the challenge protocol whether it is single or multiple episodes, form part of an essential approach in understanding preconditioning. When it comes to microarrays other essential issues include the design of an experiment that looks into the genetic expression response to the challenge, the genetic expression response to the insult and how the challenge alters the genetic response of the insult. Finally, time points such as the interval between the challenge and the insult and the interval between the challenge or insult and the gene expression investigation should be clearly defined from the outset.

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