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VASCULAR BIOLOGY (RS ROSENSON, SECTION EDITOR)

8-Hydroxy-2′-Deoxyguanosine and Cardiovascular Disease: a Systematic Review

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Abstract Oxidative stress due to an excess of reactive oxygen species (ROS) may play a role in the development and progression of cardiovascular disease (CVD). 8-hydroxy-2′-deoxyguanosine (8-OHdG) is a marker of oxidative DNA damage caused by ROS. This review aimed to assess the association between 8-OHdG and CVD by reviewing the literature. Studies in human subjects using either plasma or urine to determine 8-OHdG concentrations were surveyed. Eighteen relevant studies were found, of which 13 were case-control studies and five had a prospective design. Without exception, the case-control studies showed significant positive associations between 8-OHdG and CVD. In agreement, two prospective studies showed a significant association of 8-OHdG and heart failure. Furthermore, two prospective studies found a significant association between 8-OHdG and stroke, and finally, one prospective study showed a borderline significant (p=0.08) association between coronary artery disease (CAD) patients developing a cardiac event and 8-OHdG concentrations. In conclusion, high levels of 8-OHdG in blood and urine are associated with atherosclerosis and heart failure, but further large prospective studies are needed to investigate 8-OHdG as a predictor for cardiovascular diseases.

Keywords 8-OHdG · Cardiovascular disease · ROS · Oxidative stress

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Introduction

Reactive oxygen species (ROS) are oxygen-containing molecules that play an important role in cell signalling [1]. ROS contain unpaired electrons rendering them highly reactive and potentially dangerous oxidants. Under physiological conditions, ROS are scavenged by the antioxidant defences. If the balance shifts in favour of ROS, a state of oxidative stress arises [2]. Excessive ROS can cause oxidative damage to cellular components, including proteins, lipids and DNA. Damage to DNA is the most detrimental, since replication of damaged DNA can lead to genetic mutations or apoptosis [3, 4]. Damaged DNA bases can be excised and replaced by the DNA repair system. Following excision, the products of oxidative DNA damage enter the bloodstream and are excreted into the urine without being further metabolized [5]. Oxidatively generated DNA products can be found at low levels in the absence of oxidative stress, whereas increased concentrations might indicate or predict a pathological condition.

ROS are produced in several compartments of a cell, but the major source of endogenous ROS production is the mitochondria [6]. Mitochondria consume oxygen, converting it through the electron transport chain into superoxide anions, hydroxyl radicals and hydrogen peroxide [7]. Furthermore, superoxide anions might combine with nitric oxide (NO) to produce the strong reactive oxidant peroxynitrite which also decreases the availability of NO for vasoregulation. Other sources of ROS production are nicotinamide adenine dinucleotide phosphate (NADPH) oxidases in the plasma membrane and lipid metabolism in peroxisomes [8]. Hydroxyl radicals may be considered as the main endogenous contributor to oxidation of cellular DNA, because of its high reactivity [9]. A high reactivity is required to reach the DNA bases within the DNA helix and still possess enough energy to add to it [10]. However, because of its high reactivity, hydroxyl radicals can only diffuse short distances before it reacts with an

organic compound. Therefore, it can only damage DNA if it is produced in its close vicinity [10]. This can be facilitated by hydrogen peroxide which can be converted into hydroxyl radicals in the presence of reduced transition metal ions through the Fenton reaction [11, 12]. Hydrogen peroxide can diffuse relatively long distances and cross biological membranes to reach the DNA helix [13–15]. The DNA helix consists of four different nucleobases, namely, adenine, thymine, cytosine and guanine. Because the guanine base has the lowest ionization potential, this nucleobase is the most easily oxidized [16]. As illustrated in Fig. 1, the interaction of hydroxyl radicals with the double bond at the C-8 position of the guanine base leads to the production of 8-hydroxy-2′-deoxyguanosine (8-OHdG). 8-OHdG is the most abundant oxidative DNA repair product found in humans [7]. This stable oxidative modified DNA product has extensively been used to reflect the degree of oxidative damage to DNA. Increased levels of 8-OHdG have been linked to carcinogenesis [17], but might also be associated to other pathological conditions, like cardiovascular disease (CVD). This review evaluated the literature on the association of the oxidative DNA product 8-OHdG and CVD and discusses potential biologic mechanism.

Measurement of 8-OHdG

The concentration of 8-OHdG can be determined in different specimens, including blood, urine, cerebrospinal fluid, saliva, leukocytes and tissues. Leukocytes and tissue require DNA extraction and enzymatic digestion before the concentration of 8-OHdG can be determined [18]. These processes may lead to artificial induction of oxidation, resulting in an overestimation of 8-OHdG concentrations [19]. In addition, measurement of 8-OHdG in leukocytes and tissue is a measure of the steady state between damage and repair, so only the damaged bases at that moment are being measured. Therefore, studies using either tissue or leukocytes for the measurement of 8-OHdG concentrations are excluded from the present review. This review was restricted to urine and plasma, the most widely used specimens for the measurement of 8-OHdG concentrations that probably best reflect the total body DNA modification. The stability of 8-OHdG in plasma has not been investigated extensively, but plasma samples to be analysed for 8-OHdG should either be processed immediately or stored at −80 °C. It has been shown that urine samples are stable and can be stored at room temperature for 1 day and at least over 2 years when stored at −80 °C [20, 21••]. Moreover, urine has the advantages of being noninvasive and easily collected in contrast to plasma.

A variety of methods have been used to quantify 8-OHdG concentrations, such as enzyme-linked immunosorbent assay (ELISA), gas chromatography-mass spectrometry (GC-MS), high-performance liquid chromatography with electrochemical detection (HPLC-ECD) and liquid chromatography with tandem mass-spectrometry (LC-MS/MS). GC-MS, HPLC-ECD and LC-MS/MS are highly specific and sensitive methods [22, 23]. Several antibody-based ELISAs are commercially available, of which the ELISA with the 8-OHdG-specific N45.1 antibody is the most widely used [24]. Notably, 8-OHdG concentrations measured in urine by ELISA were in general twofold higher compared to chromatographic methods. Hu et al. [25] suggested that this overestimation is attributed to the cross-reaction of the N45.1 antibody with urea in urine. Although ELISAs overestimate 8-OHdG concentrations, significant positive correlations between 8-OHdG measured with ELISA and chromatographic methods have been shown in several studies [24–26]. Consequently, when accurate analysis is required, the chromatographic methods are preferable, but ELISAs can be used adequately to compare 8-OHdG concentrations within one study.

Study Selection

A literature search was performed using PubMed from 1966 to July 2014. Search terms included 8-OHdG, 8OHdG, 8-OH-dG, 8-OHG, 8-oxo-G, 8-oxo-dG, 8-hydroxydeoxyguanosine, 8-oxo-

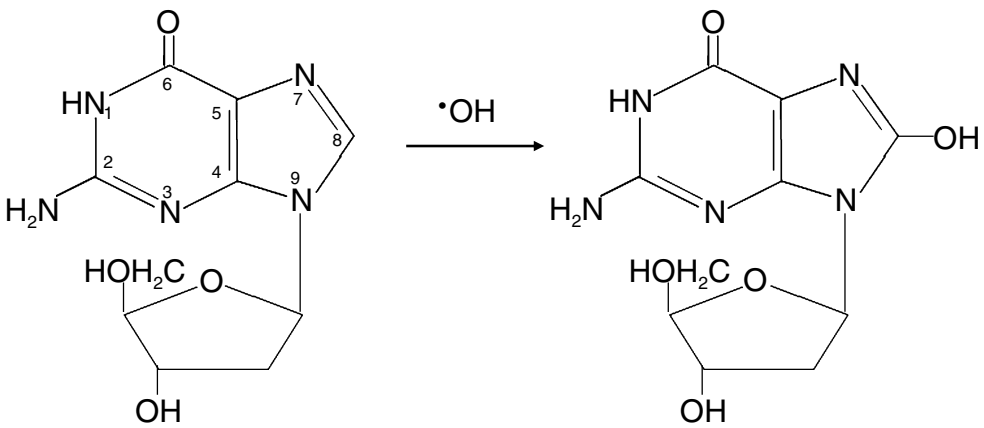

Fig. 1 Hydroxylation of deoxyguanosine by the addition of an OH group at the C-8 position leads to 8-hydroxy-2′-deoxyguanosine formation

guanine, 8-hydroxy-2-deoxyguanosine, 8-hydroxyguanine, 8-hydroxyguanosine, 8-oxo-2-deoxyguanosine and 8-oxo-7,8-dihydro-2′-deoxyguanosine, in combination with coronary artery disease, atherosclerosis, heart failure, ischaemic heart disease, stroke, myocardial infarction and cardiovascular disease. The search was restricted to articles written in the English language and studies conducted in human subjects using plasma and/or urine to determine 8-OHdG concentrations. As we were interested in studies containing the association between 8-OHdG and CVD, we excluded studies which only considered CVD risk factors.

The search yielded 18 relevant studies including one on cardiovascular disease [27], four on coronary artery disease (CAD) [28–31], two on myocardial infarction (MI) [32, 33], three on ischaemic stroke [34, 35••, 36•] and the remaining on heart failure (HF) [37–42, 43••, 44••]. All studies were published between 2005 and 2012. We divided the studies in an atherosclerosis group (n=10) and a heart failure group (n=8) as illustrated in Fig. 2. Data on the first author, publication year, disease, study design, number of cases and controls, specimen, laboratory methods, main results, measuring units and significance are presented in Tables 1 and 2.

Studies on the Association Between 8-OHdG and Atherosclerosis

In total, ten studies were found on atherosclerosis (Table 1), of which three were prospective and the remaining were case-control studies. One study, the Boston Puerto Rican Health Study by Lai et al. [27], did not distinguish between different types of CVD. This study showed significantly higher concentrations of 8-OHdG in subjects with self-reported CVD. Of the four studies found on CAD, three were of case-control design and one of prospective design. All three case-control studies on CAD observed statistically significant higher concentrations of 8-OHdG in patients compared to healthy control subjects [28–30]. Furthermore, Xiang et al. [29] found a positive association between the increase in 8-OHdG concentration and the number of diseased vessels. In addition, they showed that 8-OHdG levels were independently associated with the presence of CAD using a multivariable analysis including sex, smoking hypertension, hyperlipidemia, diabetes mellitus and age. A prospective study (median follow-up duration 7.8 years) [31] on CAD showed higher concentrations of 8-OHdG at baseline for CAD patients who experienced a cardiovascular event during follow-up compared to CAD patients who did not. However, this difference was borderline significant (p=0.08).

Two case-control studies on MI found increased 8-OHdG concentrations in MI patients compared to healthy controls 3–4 h after reperfusion therapy [32, 33]. One of these studies measured 8-OHdG levels before reperfusion therapy and found increased concentrations of 8-OHdG in MI patients compared to control subjects (p<0.05) [33].

So far, one case-control and two prospective studies about 8-OHdG and ischaemic stroke have been published. The case-control study showed that urinary 8-OHdG levels were significantly higher in stroke patients than in nonstroke patients and peaked at day 3 to 5 after the onset of stroke [34]. Moreover, 8-OHdG showed a significant correlation with the serum level of S100β, an indicator of the volume of cerebral infarction [45]. The prospective study of Brea et al. [35••] with a follow-up duration of 12 months showed that

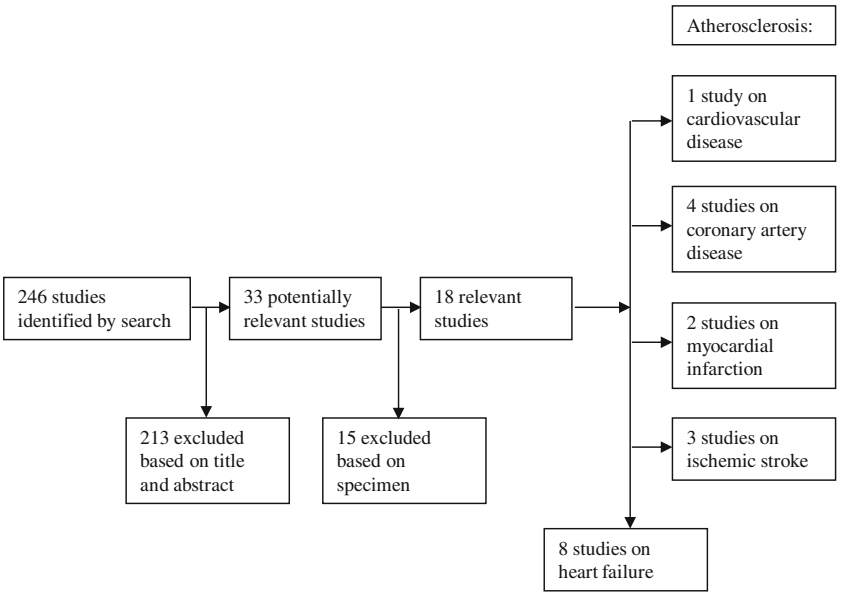

Fig. 2 Flowchart of the selection of relevant studies to examine the association between 8-OHdG and CVD

Table 1 Characteristics of the included studies on the association between 8-OHdG and cardiovascular disease, coronary artery disease, myocardial infarction and stroke

First author	Year	Disease	Study design	Cases	Controls	Specimen	Method	Main results[a]	Unit	*p* value
Lai	2008	CVD	Case-control	214	745	Urine	ELISA	CVD 156[b] Control 144[b]	ng/μg creatinine	0.035
Jaruga	2012	CAD	Case-control	22	22	Urine	LC-MS/MS	CAD 2.99±1.18 Control 1.93±0.56	nmol/mmol creatinine	<0.001
Xiang	2011	CAD	Case-control	74	53	Serum	ELISA	CAD 0.41 (0.30–0.57) Control 0.32 (0.25–0.43)	ng/ml	0.001
Arao	2009	CAD	Case-control	16	6	Urine	ELISA	CAD 16.9±7.6 Control 6.6±1.4[b]	ng/ml creatinine	<0.001
Arca	2008	CAD	Prospective	86	151	EDTA-plasma	ELISA	Event 4.86±1.48 No event 4.48±1.11	ng/ml	0.084
Himmetoglu	2009	MI	Case-control	28	27	Heparin-plasma	ELISA	MI 12.65±6.59 Control 3.08±1.06	ng/ml	<0.001
Nagayoshi	2005	MI	Case-control	62	48	Urine	ELISA	MI 24.4±3.8 Control 13.9±0.6	ng/mg creatinine	<0.05
Mizukoshi	2005	Stroke	Case-control	7	6	Urine	ELISA	Stroke 39,000 Control 14,000	ng/3 days	<0.01
Brea	2012	Stroke	Prospective	68	409	Blood	ELISA	Event 40.06±24.70 No event 33.11±15.18	ng/ml	0.003
Nakajima	2012	Stroke	Prospective	25	19	Urine	ELISA	Poor outcome 39.44 Good outcome 2.54	Δ ng/ml creatinine	0.004

CAD coronary artery disease, *CVD* cardiovascular disease, *MI* myocardial infarction

[a] 8-OHdG levels are presented as mean±standard deviation or median with interquartile range

[b] Retrieved from graph

patients who suffered vascular recurrence or vascular-origin death had higher levels of 8-OHdG. In addition, they found an association between 8-OHdG and both intima media thickness, a surrogate marker of atherosclerosis, and the presence of ipsilateral stenosis. Nakajima et al. [36•] divided 44 stroke patients according to their clinical outcomes based on the modified Rankin Scale (mRS), scaling patients from zero to six, from having no symptoms to being dead. The study found no significant difference in 8-OHdG concentration at admission between stroke patients with a good (mRS≤2) or poor (mRS≥3) outcome. However, the study showed a significantly higher increase in 8-OHdG concentrations at day 7 for patients with a poor outcome compared to those with a good outcome.

Studies on the Association Between 8-OHdG and Heart Failure

In total, eight studies were found on HF (Table 2), of which two were prospective and six were case-control studies. All case-control studies reported statistically significant higher 8-OHdG concentrations in HF patients compared to control subjects [37–42]. Three studies divided their HF group according to the New York Heart Association (NYHA) functional classification, which separates HF into four groups according to the severity of the symptoms. These studies found that 8-OHdG concentrations increased with NYHA class [38, 40, 42]. The study of Kobayashi et al. [42] showed that the correlation between 8-OHdG and NYHA was independent of age, gender, 8-isoprostane, interleukin-6 and angiotensin-II.

Some studies made a distinction in aetiology of HF, such as ischaemia, hypertension or cardiomyopathy. Rivera et al. divided the HF group in dilated cardiomyopathy (DCM), ischaemia and hypertension [39]. This study showed significantly higher 8-OHdG concentrations in the hypertensive-HF group compared to DCM-HF and ischaemic-HF ($p<0.05$). Two other studies compared DCM-HF patients with healthy subjects [38, 41], in which DCM-HF patients showed higher concentrations of 8-OHdG compared to control subjects. Nagoyoshi et al. compared ischaemic-HF caused by CAD with nonischaemic HF and controls [37] and found no difference between the two HF groups. However, both HF groups had higher 8-OHdG concentrations compared to healthy controls ($p<0.05$). Notably, the 8-OHdG concentrations in the ischaemic-HF group increased significantly with NYHA class and the number of diseased vessels ($p<0.05$). The prospective study of Susa et al. [43••] showed significantly higher 8-OHdG concentrations at baseline among HF patients who

Table 2 Characteristics of the included studies on the association between 8-OHdG and heart failure

First author	Year	Study design	Cases	Controls	Specimen	Method	Main results[a]	Unit	*p* value
Kobayashi	2010	Case-control	194	31	Urine	ELISA	HF 13.0±5.7 Control 8.2±1.9	ng/mg creatinine	<0.0001
Nagayoshi	2009	Case-control	164	33	Urine	ELISA	CAD 24.0±1.9 Non-CAD 19.7±4.1 Control 12.6±0.9	ng/mg creatinine	<0.05
Pignatelli	2008	Case-control	86	43	Citrate-plasma	ELISA	NYHA I–II 5.2[b] NYHA III–IV 6.7[b] Control 3.7[b]	ng/ml	<0.001
Rivera	2006	Case-control	78	12	Serum	ELISA	HF 0.34 (0.54) Control 0.04 (0.07)	ng/ml	<0.05
Kono	2006	Case-control	56	20	Serum	ELISA	HF 5.2±2.9 Control 3.0±1.5	ng/ml	0.0018
Watanabe	2006	Case-control	32	14	Serum	ELISA	NYHA I 0.24±0.08 NYHA II 0.32±0.11 NYHA III 0.75±0.57 Control 0.23±0.07	ng/ml	NYHA III vs. I—p=0.04; III vs. II—p=0.003 Control vs. I—NS Control vs. II—p=0.02 Control vs. III—p=0.003
Susa	2012	Prospective	123	63	Urine	ELISA	Event 15.7±6.5 No event 11.8±5.2	ng/mg creatinine	<0.0001
Suzuki	2011	Prospective	230	42	Serum	ELISA	Event 0.41 (0.25–0.63) No event 0.32 (0.23–0.46) Control 0.22±0.09	ng/ml	<0.001

CAD coronary artery disease, *HF* heart failure, *NYHA* New York Heart Association

[a] 8-OHdG levels are presented as mean±standard deviation or median with interquartile range

[b] Retrieved from graph

developed a cardiac event during follow-up (median follow-up duration of 649 days) compared to HF patients who had no event ($p<0.0001$). In addition, the group with a fatal event had higher levels of 8-OHdG than the nonfatal event group ($p<0.05$). Furthermore, multivariate analysis including 8-OHdG levels, age, gender, heart rate, NYHA, interleukin-6 and angiotensin-II and sodium identified 8-OHdG as a significant independent predictor for cardiac events in HF patients ($p<0.0001$). Moreover, Kaplan-Meier analysis found that patients with high 8-OHdG levels had higher cardiac event rates than those with low 8-OHdG levels. The prospective study of Suzuki et al. found significantly higher 8-OHdG concentrations in HF patients at baseline compared to healthy subjects, with 8-OHdG concentrations increasing with NYHA class [44••]. In addition, this study showed significantly higher 8-OHdG concentrations at baseline among HF patients who developed a cardiac event during follow-up (median follow-up period of 472 days) compared to HF patients who did not develop a cardiac event ($p<0.001$). Furthermore, Kaplan-Meier analysis illustrated that the cardiac event-free rate was significantly lower in the high 8-OHdG group compared to the normal 8-OHdG group ($p=0.0007$).

8-OHdG and Atherosclerosis, Implications and Possible Mechanism

Several studies showed higher 8-OHdG concentrations in CAD patients compared to control subjects, and one study found that 8-OHdG concentration increased with the number of diseased vessels. These findings suggest that 8-OHdG concentrations are associated with CAD and might indicate the severity of CAD. Since most studies did not perform multivariable analysis, associations could be due to other factors affecting both 8-OHdG concentrations and CAD. In addition, it was not possible to identify 8-OHdG as a cause or indicator of CAD because the majority of these studies had a case-control design. One prospective study showed a borderline, but not significant, difference between CAD patients developing a cardiovascular event or not [31]. These findings suggest that 8-OHdG concentrations cannot be used as a predictor for an acute cardiovascular event, but larger population-based prospective studies are needed to confirm this. Two studies that examined MI patients showed increased 8-OHdG levels after reperfusion therapy. Of note, the study of Nagayoshi et al. [33] observed that the 8-OHdG concentration was already increased in MI patients before reperfusion

therapy. This may indicate that the increased 8-OHdG levels are not just a cause of reperfusion injury, but are at least partly due to the MI itself or the preceding pathological disease. In addition, a high correlation (r=0.87, p<0.01) was found between the total urinary 8-OHdG contents and serum S100β values, suggesting a role for 8-OHdG as an indicator of infarction size [34]. Interestingly, one prospective study found 8-OHdG concentrations correlated with recurrent stroke and cardiac death in patients not receiving statins but not in the general stroke group [35••], suggesting that statins affect 8-OHdG concentrations and might reduce oxidative stress. Further research is needed to indicate whether 8-OHdG can be used as a predictor for stroke because prospective studies investigating 8-OHdG concentrations before the onset of stroke are still lacking.

How DNA Damage Might Initiate Atherosclerosis

Atherosclerosis is a pathological dysfunction that could lead to CAD, MI and ischaemic stroke. Atherosclerosis is thought to start with endothelial dysfunction, including impaired vasoregulation, impaired barrier function and adhesion of molecules to the endothelium. Vasoregulation can be impaired by an excess release of ROS, like superoxide anions, which can reduce the vasoregulator NO to peroxynitrite [46, 47]. Peroxynitrite itself can further impair vascular reactivity [47]. An impaired barrier function allows low-density lipoproteins (LDLs) and monocytes to cross the endothelium to the intima. In addition, LDLs and monocytes can adhere to the endothelium through LDL receptors and VCAM-1 receptors, respectively. These receptors are upregulated, caused by increased DNA expression which might be mediated by ROS [48–50]. In the intima, the monocytes migrate into macrophages and the LDL particles become oxidized by ROS [47, 51]. Interestingly, ox-LDL downregulates enzymes that take part in base excision repair [52], which is responsible for removal of 8-OH-Gua from DNA [53]. This may contribute to the development of the disease by increasing DNA damage [54]. Subsequently, ox-LDL is taken up by macrophages through scavenger receptors to form foam cells [48, 55]. Foam cells produce various growth factors and cytokines stimulating smooth muscle cells (SMCs) to migrate from the media into the intima. Thus, ROS might play a role in initiation and progression of an atherosclerotic plaque. The observed increase in 8-OHdG concentrations in CAD and MI could be attributed to increased DNA damage caused by ROS. Evidence for DNA damage in atherosclerosis can be found in the plaque which consists of a monoclonal population of SMCs. This monoclonal population could arise from a new lineage of SMCs, created by mutations. These mutations could be induced by oxidative DNA damage [56, 57] which is not properly repaired. In addition, the plaque contains a large amount of cells in apoptosis [58] or cell senescence [59]. Cell death and cell senescence can be caused by cell cycle arrest, which is an effect of nonrepaired DNA damage [60]. Another cause of senescent cells is shorting of telomeres which is accelerated by DNA damage [61••]. These findings support the idea of the contribution of DNA damage to the development of atherosclerosis. However, more research on this topic should be undertaken before the association between 8-OHdG concentrations and atherosclerosis is fully elucidated.

8-OHdG and Heart Failure, Implications and Possible Mechanism

Studies in HF patients showed consistently higher 8-OHdG concentrations in patients compared to healthy control subjects. Importantly, multivariable analysis in several studies showed that 8-OHdG is independently associated to HF. Moreover, an association between oxidatively damaged DNA and NYHA class has been observed in various studies [37, 40, 43••, 44••]. Taken together, this indicates a possible role for 8-OHdG as a potential independent marker for heart failure and its severity. In agreement, prospective studies showed higher 8-OHdG levels in the group who developed a cardiac event [43••, 44••]. This suggests that 8-OHdG may also be used as a useful biomarker for cardiovascular events in heart failure patients.

How DNA Damage Might Initiate Heart Failure

The mechanism between damaged DNA and HF is not completely understood, but it has been shown that ROS might cause contractile failure and structural damage in the myocardium by inducing changes in structure and function of myocytes and extracellular matrix [62•, 63]. Cardiac myocytes contain the highest volume density of mitochondria, where ROS formation takes place. ROS produced in mitochondria can easily damage mitochondrial DNA (mtDNA), firstly because ROS is produced in its close vicinity. Secondly, mtDNA is not in a complex chromatin structure with histones and has limited repair, leaving it vulnerable for ROS attack [64]. Finally, the superoxide radicals formed in mitochondria cannot cross the membranes and consequently accumulate within mitochondria were they could react with mtDNA. Mutations in mtDNA are associated with dilated cardiomyopathy [65, 66] and ischaemic heart disease [67] which can both lead to HF. Dysfunctional mitochondria might lead to a cycle of further ROS generation and loss of myocyte contractility and increased cell death [68, 69]. In HF, a decreased number of mtDNA as well as a decline in its protein expression is

associated with oxidative DNA damage [70]. These findings are in agreement with the increased 8-OHdG concentrations found in HF patients.

Conclusion

Elevated levels of 8-OHdG are clearly associated with CVD, supporting the hypothesis that DNA damage plays an important role in the (early) development of atherosclerosis. Although evidence is gathering that high levels of 8-OHdG in urine or blood may be a nonspecific indicator for CVD, further large population-based prospective studies are required to examine the utility of 8-OHdG to predict prognosis or response to treatment in CVD.

Compliance with Ethics Guidelines

Conflict of Interest Lona J. Kroese and Peter G. Scheffer declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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