GENOTOXICITY AND CARCINOGENICITY

PON1 increases cellular DNA damage by lactone substrates

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Abstract

Paraoxonase 1 (PON1) is a high-density lipoprotein (HDL)-associated enzyme that by hydrolysing exogenous and endogenous substrates can provide protection against substrate induced toxicity. To investigate the extent to which PON1 provides protection against lactone induced DNA damage, DNA damage was measured in HepG2 cells using the neutral Comet assay following lactone treatment in the presence and absence of exogenous recombinant PON1 (rPON1). Low dose lactones (10 mM) caused little or no damage while high doses (100 mM) induced DNA damage in the following order of potency: α-angelica lactone > γ-butyrolactone ~ γ-hexalactone > γ-heptalactone ~ γ-octaclactone ~ γ-furanone ~ γ-valerolactone > γ-decalactone. Co-incubation of 100 mM lactone with rPON1, resulted in almost all cells showing extensive DNA damage, particularly with those lactones that decreased rPON1 activity by > 25%. In contrast, with the lactones that are poor rPON1 subtrates (γ-decalactone and γ-furanone), rPON1 did not increase DNA damage. DNA damage induced by a 1 h co-treatment with 10 mM α-angelica lactone and rPON1 was reduced when cells when incubated for a further 4 h in fresh medium suggesting break formation was due to induced DNA damage rather than apoptosis. Preincubation (1–6 h) of α-angelica lactone with rPON1 in the absence of cells, decreased cellular DNA damage by around 40% in comparison to cells treated without preincubation. These results suggest that in addition to its well-recognised detoxification effects, PON1 can increase genotoxicity potentially by hydrolysing certain lactones to reactive intermediates that increase DNA damage via the formation of DNA adducts.

Keywords PON1 · DNA damage · Lactones

Abbreviations

AL	α-Angelica lactone
BL	γ-Butyrolactone
DL	γ-Decalactone
FUR	γ-Furanone
HDL	High-density lipoprotein
HepL	γ-Heptalactone
HexL	γ-Hexalactone
OL	γ-Octaclactone
PON1	Paraoxonase 1
TMZ	Temozolomide
VL	γ-Valerolactone

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Introduction

Paraoxonase 1 (PON1) is an HDL associated enzyme that has been shown to hydrolyse a wide range of exogenous and endogenous substrates that include phosphotriesters such as organophosphate (OP) metabolites paraoxon and diazoxon, the nerve gases sarin and soman (Costa et al. 2003; Draganov and La Du 2004), arylesters such as phenylacetate and 2-naphthylacetate, and oxidised lipids and lactones (Billecke et al. 2000; Aharoni et al. 2004). Such hydrolyses are considered to reduce the toxic effects of these substrates.

Thus, PON1 can provide protection against diazoxon toxicity: PON1 knock-out mice showed more susceptibility to this agent and the administration of exogenous PON1 induced resistance (Shih et al. 1998; Li et al. 2000). PON1 also displays anti-oxidant and anti-atherogenic activities and can provide protection against, for example, lipid peroxidation and mitochondrial dysfunction (Bacchetti et al. 2017). If, as is suspected, the biological role of PON1 is to protect outer cell membranes against damage by the products of lipid peroxidation, e.g., fatty lactones (Moya and Manez 2018) and perhaps also by homocysteine, e.g., homocysteine thiolactone (Moya and Manez 2018) or glucose (Soran et al. 2009), its location on HDL particles would allow it to have the widest distribution and hence the greatest efficacy. However, though the full range of physiological, i.e., endogenous substrates of PON1 remains to be defined (Soran et al. 2009; Mackness and Mackness 2015; Bacchetti et al. 2017), structure-activity studies suggest that these substrates may be lactones (Khersonsky and Tawfik 2005).

Low serum PON1 activity has been associated with an increased risk of cardiovascular diseases (Soran et al. 2009), neurological disorders (Menini and Gugliucci 2014) and, increasingly, with certain types of cancer (Bacchetti et al. 2017). One possible mechanism for this association is that the metabolism of DNA damaging substrates by PON1 reduces the amount of DNA damage formed (and hence mutational risk) following exposure. Indeed, in some studies there is evidence that serum PON1 activity provides protection against the formation of oxidative DNA damage. Serum PON1 activity has been negatively correlated with both urinary 8-OHdG levels in patients diagnosed with moderate Alzheimer's disease (but not in healthy elderly volunteers: Zengi et al. 2011), and with serum 8-OHdG levels in patients with newly diagnosed laryngeal cancer (but not age and sex-matched healthy individuals: Karaman et al. 2010). Furthermore, serum PON1 activity was inversely correlated with DNA damage, as measured by the alkaline comet assay, in lymphocytes from workers that sprayed OPs (but not in healthy volunteers with no exposure: Singh et al. 2011) and in leukocytes from dyslipidemic patients with type two diabetes (Manfredini et al. 2010).

Single nucleotide polymorphisms of the PON1 gene (e.g., PON1-Q192R) can directly affect PON1 activity and hence may also be associated with DNA damage. Men with idiopathic infertility with the R isoform had higher levels of sperm DNA fragmentation and sperm 8OHdG (Ji et al. 2012). Furthermore, sperm DNA integrity (as measured using a nick translation assay) decreased with increasing OP exposure at the month of sampling only in healthy individuals with the RR genotype but not the QR or QQ genotype (Pérez-Herrera et al. 2008). In contrast, the Q allele was associated with higher lymphocyte DNA damage (measured using the alkaline comet assay) in workers

employed for spraying OPs but not healthy normal volunteers with no exposure history (Singh et al. 2011). Previously, we reported that N7-methyldeoxyguanosine levels in lymphocyte DNA were higher, but not significantly so, in pesticide-exposed individuals with the RR genotype in contrast to those with the QQ genotype (Gómez-Martín et al. 2014). In other studies, the PON1 Q192R genotype had no impact on DNA damage, as measured by alkaline comet assay of peripheral blood mononuclear cells (Liu et al. 2006) or leucocytes (da Silva et al. 2008) of pesticide-exposed workers and controls.

The effects of PON1 activity and PON1 genotype on DNA damage parameters are thus inconsistent and this may be due to a number of factors including study design and an inability to assess exposure to DNA damaging PON1 substrates. The nature of the relationship between DNA damage and genotype is further complicated by the observation that any effect of genotype on PON1 activity can vary with the PON1 substrate. For example, the R isoform of the PON1-Q192R SNP has been reported to hydrolyse paraoxon faster, but diazoxon slower, than the Q isoform (Davies et al. 1996; Richter et al. 2009). To investigate the associations between PON1 activity and DNA damage we have used a model system in which we exposed the liver cancer cell line, HepG2, to a series of lactones that were shown to have a range of abilities to inhibit the action of PON1 on its "standard" substrate, paraoxon, and hence potency as PON1 substrates. Given that AAL reacts with the DNA surrogate, 4-(*p*-nitrobenzyl)pyridine (Fernández-Rodríguez et al. 2007) and 2-furanone and 2-pyrone can induce DNA strand breaks and form γH2AX foci in K562 cells (Calderón-Montaño et al. 2013), we anticipated that PON1 would provide protection against lactone induced DNA damage. However, we found that for some but not all lactones, DNA damage was increased.

Materials and methods

Materials

All chemicals used were of the highest available grade. Unless otherwise specified chemicals were obtained from Sigma-Aldrich. Anhydrous sodium acetate, tris-base and sodium chloride (NaCl) were purchased from Fisher Company (UK). Normal melting point (NMP) agarose and phosphate buffer saline (PBS) were from SeaKem®LE and Disodium Ethylenediaminetetraacetic acid (Na$_2$EDTA) procured from AnalaR Company. SYBR gold was purchased from Invitrogen, UK. Recombinant PON1-G3C9 variant (rPON1), provided by Professor Dan Tawfik from the Department of Biochemistry, Weizmann Institute

of Science, Rehovot, Israel, was produced in *E. coli* by directed evolution (Aharoni et al. 2004).

Cell culture

Human hepatocellular carcinoma cells (HepG2) were purchased from Sigma-Aldrich and were maintained at 37 °C in an incubator at 5% CO_2. HepG2 cells were selected for the present studies based on their widespread use in toxicology studies, including COMET assays, and our familiarity with their culture and maintenance. Cells were cultured in Minimum Essential Medium (MEM; Gibco) containing 10% Foetal Bovine Serum, 2 mM L-glutamine, 0.105% sodium bicarbonate, 1% penicillin (10,000 units/ml) − streptomycin (10 mg/ml) and incubated at 37 °C, 5% CO_2, 3% O_2.

Measurement of rPON1 activity following incubation with lactones

100 mM lactone was incubated with 15 µg rPON1 in Tris HCl buffer pH8 at 37 °C. After 3 h, phosphotriesterase activity was determined spectrophotometrically using paraoxon [*O,O*-diethyl *O*-(4-nitrophenyl) phosphate] as the substrate and a COBAS MIRA semi-automated microtiter plate reader to quantify the absorbance at 405 nm of the liberated *p*-nitrophenol (Charlton-Menys et al. 2006). rPON1 activity is presented as mmol/ml/min. The activity in Tris–HCl buffer pH8 was taken as 100% and used to calculate the decrease in rPON1 activity in the presence of lactones.

Neutral Comet assay

At low levels of DNA damage, the neutral comet assay visualises the relaxation and electrophoretic migration of supercoiled DNA which can be the result of single strand nicks when the DNA remains attached to the nuclear matrix (Collins et al. 2008). As the extent of strand breaks increases, very large stretches of DNA can detach from the matrix and move out of the nucleus and into the gel (Afanasieva and Sivolob 2018). We refer to these events as DNA damage, recognising that the neutral Comet assay measures a mix of single and double strand breaks.

HepG2 cells, suspended in MEM, were incubated in the presence or absence of rPON1 with lactones (10 mM or 100 mM) or temozolomide (TMZ: 2.5 and 5 µM) and hydrogen peroxide (H_2O_2: 10 and 20 µM) as positive controls and DNA damage was assessed by a neutral comet assay according to previous studies but with minor modifications (Dumax-Vorzet et al. 2015; Dhawan et al. 2018). Cells ($\sim 10^4$) were suspended in LMP agarose at 37 °C and applied to a microscope slide pre-coated with 1% NMP agarose. After gel setting, the slides were immersed in freshly made precooled lysis buffer (2.5 M NaCl, 100 mM EDTA, 10 mM Tris-base, 10% DMSO and 1% Triton X-100, pH 10) for 100 min at 4 °C to lyse the cells. The slides were then equilibrated for 20 min in cold electrophoresis buffer (300 mM sodium acetate 100 mM Tris, pH9) then electrophoresis was carried out at 25 V (~ 300 mA) for 25 min. After neutralisation in cold neutralization buffer (0.4 M Tris-base pH 7.5), slides were stained with SYBR gold used according to the manufacturer's instructions and examined using a NIKON Fluorescent Microscope (excitation 465–495 nm, emission: 515–555 nm bandpass filter) provided with a Hitachi, HV, BCCD camera which was used to capture images.

Visual examination of the slides showed that, following treatment with high doses of some lactones in combination with rPON1, DNA damage occurred such that during electrophoresis, DNA detached from the nuclear matrix and migrated into the gel, with tails separated by some distance from the head. Freely available Comet software could not be used for these analyses. The range of DNA damage was, therefore, evaluated by visual scoring: images of cells were assigned to five DNA damage grade categories (0–4) according to the extent of DNA migration and tail length: (i) grade 0, cells with a head and no apparent tail; (ii) grade 1, cells with a head and a tail that was less than the diameter of the head; (iii) grade 2, cells with a head tail that was around twice the diameter of the head; (iv) grade 3, cells with a tail that was more than twice the diameter of the head and (v) grade 4, cells with a tail that was detached from the sometimes very faint head and several head diameters from it. The final score, expressed as genetic damage indicator (GDI), was calculated using the formula described previously (Collins 2004; Marques et al. 2016):

$$\begin{aligned} GDI = & (\% \text{ grade } 0 \text{ cells} \times 0) + (\% \text{ grade } 1 \text{ cells} \times 1) \\ & + (\% \text{ grade } 2 \text{ cells} \times 2) + (\% \text{ grade } 3 \text{ cells} \times 3) \\ & + (\% \text{ grade } 4 \text{ cells} \times 4). \end{aligned}$$

The GDI was calculated from the grade scores of 200–250 cells. All data are presented as mean ± standard error. To assess the significant differences in the means, data were subjected to one way ANOVA with the level of significance set at $p < 0.05$.

Results

Initial studies were carried out to determine the biologically effective dose of lactones needed to elicit responses in the COMET assay under the exposure times and culture conditions used in our studies. Our findings indicated that at 10 mM the tested lactones did not induce significant levels
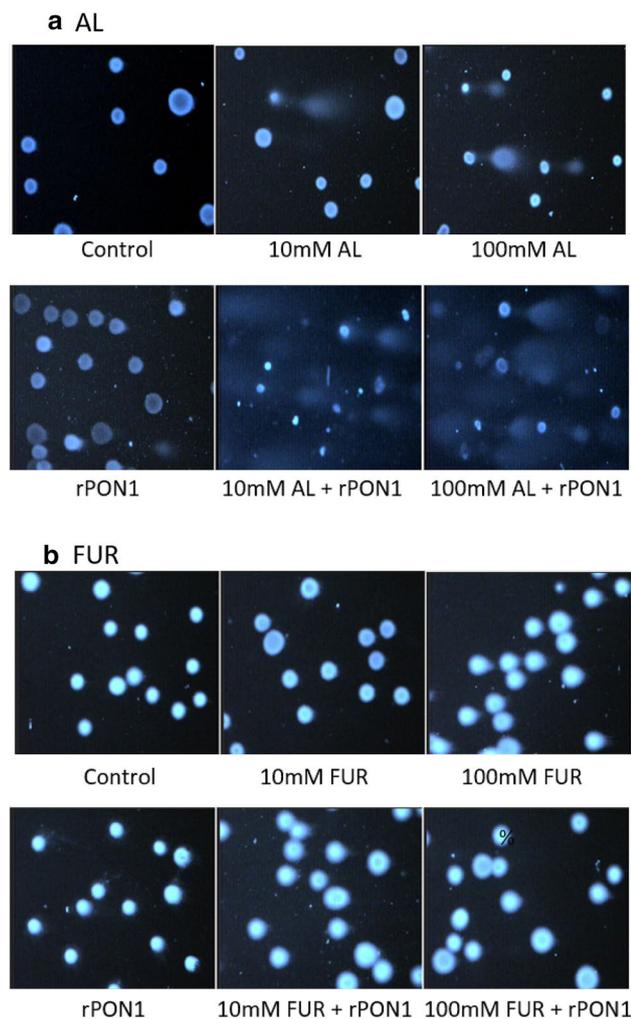
a AL

b FUR

Fig. 1 Neutral Comet analysis of HepG2 cells following exposure to AL and FUR in the presence or absence of exogenous rPON1. HepG2 cells were treated with AL (**a**) or FUR (**b**) in the presence or absence of 30 μg rPON1 for 3 h containing 10% DMSO and neutral Comet analysis undertaken

of DNA damage in HepG2 cells and that similar results were observed with 100 mM lactones except for AL and hence further work was carried out using these concentrations.
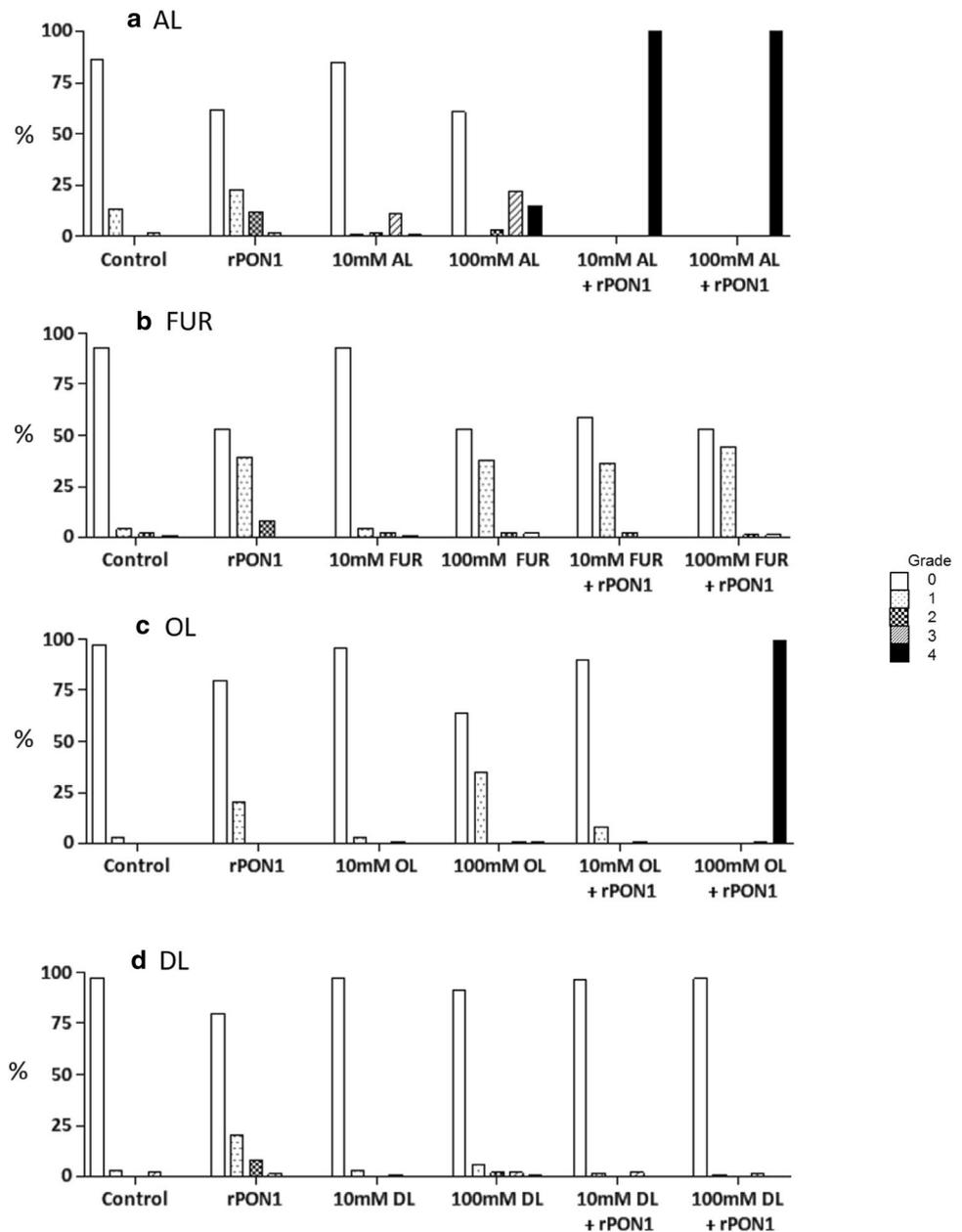
Co-incubation of lactones with rPON1 can increase DNA damage in HepG2 cells

Figure 1 shows representative neutral Comet images of untreated control HepG2 cells or HepG2 cells following treatment with rPON1 alone, AL or FUR, or a combination of rPON1 and AL or FUR. Co-incubation of rPON1 with AL but not FUR greatly increased the number of damage cells (Fig. 1). This can be seen more clearly in Fig. 2 which shows the spectrum of DNA damage induced by AL, FUR, OL and DL in the presence or absence of rPON1. In the

absence of exogenous rPON1 or lactones, 85–94% of cells showed no evidence of DNA damage with the damage that was present being predominantly grade 1 damage with very few or no cells with other grades of damage. The addition of rPON1 alone increased DNA damage levels, with up to 40% and 10% of cells with damage grades levels 1 and 2, respectively, and with only a few cells in any higher damage grades. Incubation of HepG2 cells with 10 mM FUR, OL and DL resulted in levels of DNA damage that were closely similar to those seen in untreated cells (Fig. 2). However, 10 mM AL alone increased DNA damage and ~ 10% of the cells had grade 3 damage. Incubation of HepG2 cells with 100 mM lactones increased DNA damage levels in those cells treated with AL, FUR, and OL but not with DL. With DL, the damage levels were closely similar to those seen at the lower dose. With FUR and OL, there was an increase in the number of cells with grade 1 damage. In contrast, with AL, the increases were seen predominantly in damage grades 3 and 4. The co-incubation of rPON1 with these lactones resulted in a very wide range of effects on DNA damage. Thus, little or no effect was seen with either dose of DL or the lower dose of OL, while there was a substantial increase in the number of damage level 1 cells at the lower dose of FUR that was not seen at the higher FUR dose. The most extensive effects of PON1 were seen with the higher doses of OL, and both doses of AL, where all of the cells had grade 4 damage.

The GDI in the presence and absence of rPON1 for all the lactones tested are shown in Table 1. In the absence of rPON1, lactones alone at 10 or 100 mM induced little DNA damage with GDI values being less than 50 except for 100 mM AL (GDI = 124) and BL (GDI = 55). In the presence of rPON1, treatment with 10 mM lactones increased DNA damage especially with AL, (GDI = 400); BL (GDI = 126) and VL (GDI = 69). In contrast, treatment with 100 mM lactones in the presence of rPON1 increased DNA damage for all lactones except DL (GDI = 0 both in the presence and absence of rPON1) and FUR (GDI = 40 with rPON1 and 37 in the absence of rPON1). Interestingly, both DL and FUR showed the lowest % decrease in rPON1 activity following co-incubation of the lactone with rPON1 (Table 1). In contrast, rPON1 reduced DNA damage induced by both temozolomide (a direct acting alkylating agent) and hydrogen peroxide (an inducer of reactive oxygen species formation). The GDI following treatment of HepG2 cells with 2.5 and 5 μM TMZ alone was 72 and 122, respectively, but 38 and 20 in the presence also of rPON1. The GDI following treatment of HepG2 cells with 10 and 20 μM H_2O_2 alone was 116 and 95, respectively, but 35 and 52 in the presence also of rPON1.

Fig. 2 Spectrum of DNA damage induced in HepG2 cells following exposure to lactones in the presence or absence of exogenous rPON1. HepG2 cells were treated with AL (**a**); FUR (**b**), DL (**c**), DL (**d**), in the presence and absence of 30 µg rPON1 for 3 h containing 10% DMSO, a neutral Comet analysis undertaken and DNA damage visually classified into five grades as described in the text

DNA damage induced by co-incubation of AL with rPON1 is reduced following cell recovery

To assess whether DNA damage induced by co-incubation of lactones with rPON1 reflected DNA damage per se (and hence potentially repairable) or an apoptotic mechanism, HepG2 cells were co-incubated with AL and rPON1 for 1 h alone or for 1 h followed by a 4 h recovery time after the culture media containing AL and rPON1 was replaced. Combined treatment with AL and rPON1 for 1 h resulted in very extensive DNA damage with almost all cells showing damage level 4 (Fig. 3). Following the recovery period of 4 h, the proportion of grade 4 cells was reduced from 86 to 24% in cells treated with 10 mM AL but there was no decrease in this proportion when cells were treated with 100 mM AL. The spectrum of DNA damage observed in cells treated with either TMZ or H_2O_2 also changed following a 4 h recovery period with an increase in cells with no damage being observed following recovery from ~2 to 30% with TMZ and ~5 to 55% with H_2O_2.

Table 1 Lactone induced decreases in PON1 activity and increased genomic damage levels

Lactone	Genomic damage indicator (GDI) +PON1/−PON1 (% +PON1[a])		% Decrease in PON1 activity
	10 mM lactone	100 mM lactone	
AL	400/31 (100)	400/124 (100)	91.6
OL	6/0 (1.5)	396/37 (99.0)	70.7
HepL	33/2 (8.3)	400/39 (100)	64.8
HexL	6/0 (1.5)	400/47 (100)	54.9
VL	69/10 (17.3)	265/30 (66.3)	44.6
BL	126/44 (31.5)	400/55 (100)	27.3
DL	0/0 (0)	0/0 (0)	9.4
FUR	32/0 (8)	40/37 (10)	3.7

Mean GDI ($\pm$ SD) was 12.2 ± 6.9 and 47.5 ± 13.9 for cells in the absence of both lactone and rPON1 and cells in the presence of rPON1 alone, respectively ($n = 6$)
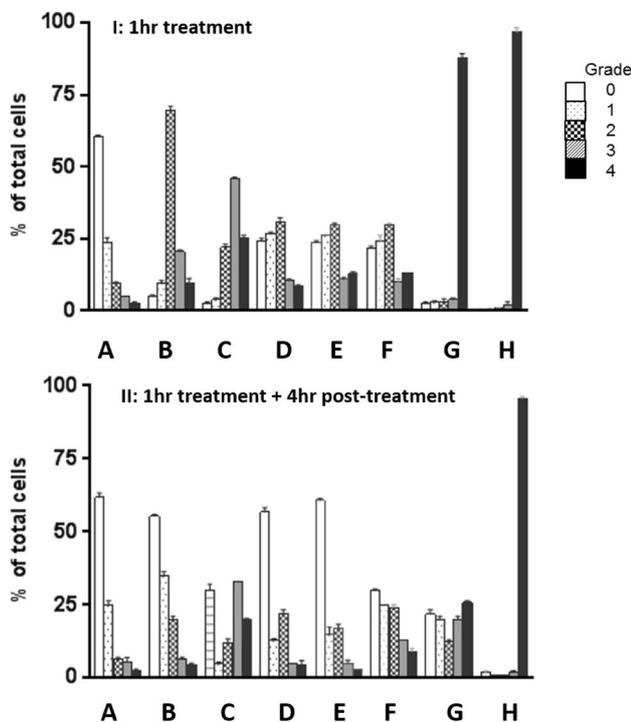
[a]% of maximum GDI score available (= 400)

Fig. 3 Repair of DNA damage in HepG2 cells following exposure to AL and exogenous rPON1. HepG2 cells were either (**I**) treated with AL in the presence of 30 µg rPON1 for 1 h or (**II**) treated with AL in the presence of 30 µg rPON1 for 1 h followed by 4 h exposure to medium free from test agents. The neutral Comet assay was undertaken and the DNA damage was visually classified into five grades as described in the text. A: Control cells (no treatment); B: 40 µM H$_2$O$_2$; C: 5 µM TMZ; D: 30 µg rPON1; E: 10 mM AL; F: 100 mM AL; G: 10 mM AL + 30 µg rPON1; H: 100 mM AL + 30 µg rPON1

Pre-incubation of AL with rPON1 reduces DNA damage formation

To assess whether DNA damage was induced by a short-lived reactive intermediate formed during rPON1 hydrolysis, AL was incubated with rPON1 for up to 6 h before addition of the mix to HepG2 cells. With a 1 h pre-incubation, DNA damage was reduced to ~60% of that damage induced without pre-incubation and this did not decrease with further pre-incubation (Fig. 4).

Discussion

In this study, we demonstrate for the first time that co-incubation of lactones with rPON1 can significantly increase DNA damage in HepG2 cells as determined in the neutral comet assay. Furthermore, DNA damage was reduced by (i) pre-incubation of AL with rPON1 and (ii) by allowing the cells to recover following treatment with AL and rPON1. This suggests that the action of rPON1 on these lactones results in reactive intermediates that damage DNA.

Exposure to 2-furanone and 2-pyrone increased DNA damage in K562 cells as assessed by the alkaline Comet assay and in both K562 and AA8 cells as detected by γ-H2AX foci formation (Calderón-Montaño et al. 2013), but this study did not assess the possible effect of PON1. PON1 displays both lactonase and lactonizing activity (Teiber et al. 2003) so an equilibrium is likely, depending on the lactone in question. This possibly explains our observation that pre-incubation of AL with PON1 causes an initial decrease in DNA damage in HepG2 cells, but with little effect on further
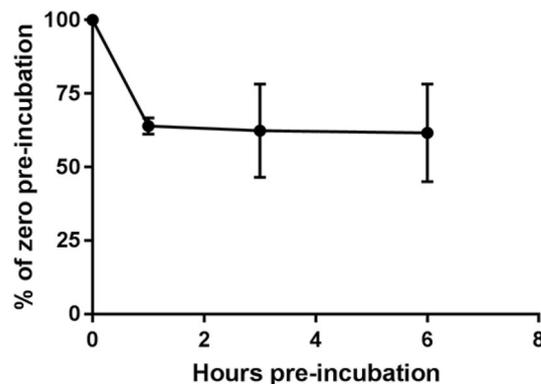

Fig. 4 DNA damage levels (GDI) in HepG2 cells following pre-incubation of AAL with rPON1 as % of no pre-treatment. AL was pre-incubated with 30 µg rPON1 for up to 6 h before adding the mix to the cells which were then left for a further 1 h prior to neutral Comet assay. The DNA damage was visually classified into five grades as described in the text and the GDI calculated and expressed as a % of the control damage (i.e., that damage induced without any pre-treatment)

preincubation. This might argue that the DNA damaging moiety is the intact lactone, and indeed a number of different lactones including AL have been shown to react with the DNA surrogate, 4-(*p*-nitrobenzyl)pyridine (Manso et al. 2005; Fernández-Rodríguez et al. 2007). However, lactones are hydrolysed by PON1 to the corresponding hydroxyl acid (Billecke et al. 2000; Teiber et al. 2003) and acyl glucuronides of carboxylic acid containing agents have been reported to be toxic and react with cellular macromolecules (Lassila et al. 2015; Van Vleet et al. 2017). The exact nature of the DNA damaging agent is thus currently unclear.

Extensive DNA damage as observed here and characterised by a small nucleoid head and a large detached tail have previously been ascribed to apoptosis (Choucroun et al. 2001). However, there is clear evidence that this type of damage is not specific to apoptosis and can be observed following exposure to certain genotoxic agents (Rundell et al. 2003; Lorenzo et al. 2013). In addition, the kinetics of induction of apoptosis do not support this as an explanation. Our results are consistent with a DNA damage mechanism given the rapid formation of the DNA damage and the repair of the damage observed at the lower AL dose when the cells were allowed to recover following AL treatment. Previous studies showed that cells deficient in BRCA2 are more sensitive to 2-pyrone (and to a lesser extent 2-furanone; Calderón-Montaño et al. 2013), suggesting a crucial role of homologous recombination (HR) mediated repair to prevent 2-pyrone induced cell cytotoxicity. However, given its requirement for DNA replication, HR seems unlikely to explain the repair of AL/rPON1 induced DNA damage in HepG2 cells.

rPON1 alone clearly demonstrated a consistent ability to introduce DNA damage in HepG2 cells. The mechanism of this induction of damage is currently unknown. While direct cellular uptake of PON1 seems unlikely, PON1 is known to interact with components of serum used in the cell culture media which might facilitate such uptake. If PON1 is taken up by cells, DNA damage might result from the phosphotriesterase function of PON1 (Bigley and Raushel 2013) acting on either pre-existing DNA phosphotriesters (Jones et al. 2010) or, non-specifically, on DNA phosphodiesters, i.e., an intrinsic endonuclease activity. An alternative explanation could be that the formation of DNA damage such as strand breaks after PON1 exposure alone might be due to an direct or indirect inhibition of topoisomerase-II (TopoII) as the lactones 2-pyrone and FUR are inhibitors of TopoII in A549 lung cancer cells and generate, what were referred to as, double-strand breaks (Calderón-Montaño et al. 2013). However, the possibility that PON1 interacts with serum components to generate DNA damaging agents that are taken up by the cells, cannot be excluded.

Previous studies have examined correlations between PON1 activity or genotype and measures of DNA damage

with inconsistent results. The strength of the present study is that we have directly examined in model systems the effect of PON1 on lactone-induced cellular DNA damage. The increased DNA damage that results from the interaction of PON1 with certain lactones is consistent with a previous hypothesis that that PON1 may be capable of converting acetoxy esters to hydroxylamine which may then form DNA damaging nitrenium ions in acidic environments such as the bladder epithelium (Oztürk et al. 2009). Other cellular mechanisms considered to be protective have similarly been shown to be able to increase toxicity with appropriate substrates. For example, the DNA repair protein, O^6-alkylguanine-DNA alkyltransferase, protects cells against the killing effects of a wide range of alkylating agents (Margison and Santibáñez-Koref 2002) but increases the toxic effects of dibromoalkanes (Abril et al. 1997). It is also well-recognised that PON1 provides protection against the toxic effects of other substrates such as OP oxons (Shih et al. 1998; Li et al. 2000). This suggests that any protective effect of PON1 and any PON1 gene-environmental interactions will depend, in part, on the relative balance of different substrates.

Lactones such as AL are considered relatively non-toxic with little evidence for genotoxicity and are widely used in foodstuffs and in fragrances with no safety concerns (Adams et al. 1998; Joint FAO/WHO Expert Committee on Food Additives 1998). The average total daily intake in Europe of all aliphatic lactones has been estimated to be ~ 30 mg/person based on the annual production volume of lactones and population numbers (Joint FAO/WHO Expert Committee on Food Additives 1998). Given our findings, it may be prudent to better establish levels of human exposure to lactones in the general population as a first step to establish if the toxicity of lactones needs to be re-evaluated.

In conclusion, we demonstrate that in a model system, PON1 does not provide protection against the DNA damaging effects of lactone substrates and indeed can greatly exacerbate DNA damage they induce. The extent to which this can occur directly in humans is currently unclear but warrants further investigation given the potential for human exposure to both exogenous and endogenous lactones.

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