



SUBJECT AREAS:

DNA

BIOPHYSICAL CHEMISTRY

MASS SPECTROMETRY

PREDICTIVE MARKERS

# Potent DNA damage by polyhalogenated quinones and H<sub>2</sub>O<sub>2</sub> via a metal-independent and Intercalation-enhanced oxidation mechanism

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Polyhalogenated quinones are a class of carcinogenic intermediates. We found recently that the highly reactive and biologically/environmentally important •OH can be produced by polyhalogenated quinones and H<sub>2</sub>O<sub>2</sub> independent of transition metal ions. However, it is not clear whether this unusual metal-independent •OH producing system can induce potent oxidative DNA damage. Here we show that TCBQ and H<sub>2</sub>O<sub>2</sub> can induce oxidative damage to both dG and dsDNA; but surprisingly, it was more efficient to induce oxidative damage in dsDNA than in dG. We found that this is probably due to its strong intercalating ability to dsDNA through competitive intercalation assays. The intercalation of TCBQ in dsDNA may lead to •OH generation more adjacent to DNA. This is the first report that polyhalogenated quinoid carcinogens and H<sub>2</sub>O<sub>2</sub> can induce potent DNA damage via a metal-independent and intercalation-enhanced oxidation mechanism, which may partly explain their potential genotoxicity, mutagenesis, and carcinogenicity.

Reactive oxygen species (ROS) are unavoidably produced due to cellular aerobic metabolism and exposure to natural and synthetic agents<sup>1</sup>. ROS can cause oxidative damage to genomic DNA and has been implicated in cancer and aging processes<sup>2</sup>. The hydroxyl radical (•OH) is recognized as the most reactive and harmful ROS and is an extremely reactive oxidant, which can directly attack the nucleophilic sites in DNA<sup>3</sup>. However, highly reactive •OH diffuses in very short distance (no more than one or two molecular diameters) before reacting with a cellular target<sup>4,5</sup>. Consequently, to oxidize DNA, •OH must be generated immediately adjacent to a nucleic acid molecule in cellular nuclei. Therefore, it is a key step to *in situ* decompose the •OH precursor hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), which is less reactive but can be delivered by diffusion to nuclei, and be further converted into highly reactive •OH. *In vitro* study showed a number of transition-metal ions (e.g., Fe<sup>2+</sup> and Cu<sup>+</sup>) might decompose H<sub>2</sub>O<sub>2</sub> to form •OH through Fenton reactions<sup>6,7</sup>. However, no free copper is available in eukaryotic cells<sup>8</sup>, and Cu<sup>2+</sup> displays much higher affinity ( $K_d = 9.1 \times 10^{-12}$  M –  $2.3 \times 10^{-16}$  M) to glutathione and copper proteins in living cells<sup>9</sup> than that to DNA ( $K_d = 10^{-8}$  M). This is consistent with the observation that intracellular copper does not catalyze the oxidation of DNA in *Escherichia coli* and mammalian cells<sup>7,10</sup>. Instead, iron, the most abundant transition metal in cells, may mediate •OH formation and probably threaten DNA because the complexation of iron (Fe<sup>2+</sup>) with DNA may allow the generation of •OH immediately adjacent to DNA. This is supported by recent studies, showing that H<sub>2</sub>O<sub>2</sub>-induced toxicity is mediated by intracellular labile iron<sup>11,12</sup>, and iron-catalyzed Fenton reaction is one of the most widely accepted mechanisms for •OH production in cells<sup>13–16</sup>.

Recently we found that polyhalogenated quinones (XBQs) could cause a homolytic decomposition of H<sub>2</sub>O<sub>2</sub> in a novel metal independent manner and lead to •OH production<sup>17,18</sup>. XBQs are a class of toxicological intermediates which can cause acute hepatotoxicity, nephrotoxicity, and carcinogenesis<sup>19,20</sup>. More recently, several XBQs, which are suspected bladder carcinogens, were identified as new chlorination disinfection byproducts in drinking water<sup>21,22</sup>. However, it is not clear whether •OH thus produced by XBQs and H<sub>2</sub>O<sub>2</sub> can induce potent oxidative DNA damage; and if so, what are the differences in oxidation between the single nucleosides and dsDNA.



Furthermore, what are the unique characteristics for the XBQ-mediated  $\bullet\text{OH}$  producing system compared with the classic iron-mediated  $\bullet\text{OH}$  producing system?

To answer these questions, it is critical to know whether and how  $\bullet\text{OH}$  can be generated immediately adjacent to DNA via the decomposition of  $\text{H}_2\text{O}_2$  by XBQs. It is apparent that XBQs do not act like transition metal ions (e.g.  $\text{Fe}^{2+}$  and  $\text{Cu}^{2+}$ ) to chelate with DNA. Rather than through the well-known metal-like chelation, we speculate that these hydrophobic XBQ molecules may intercalate with DNA and therefore potentiate DNA oxidation.

To test our hypothesis, we examined the oxidation of nucleoside deoxyguanosine (dG) and dG in dsDNA by XBQs and  $\text{H}_2\text{O}_2$ , the intercalation of XBQs with DNA and its effects on DNA oxidation. 8-Oxodeoxyguanosine (8-oxodG), a well-known biomarker for oxidative DNA damage<sup>23</sup>, was chosen to study DNA oxidation. 8-OxodG was characterized and accurately quantified using highly sensitive and specific ultra-performance liquid chromatography–electrospray ionization–tandem mass spectrometry (UPLC-ESI-MS/MS). Here we demonstrate, for the first time, a potent DNA damage by the widely distributed XBQs with  $\text{H}_2\text{O}_2$ , via a metal-independent and intercalation-enhanced oxidation mechanism, exerting even more potent oxidative DNA damage than the classic iron-mediated Fenton system.

## Results

**The oxidation of dG to 8-oxodG by TCBQ and other XBQs with  $\text{H}_2\text{O}_2$ .** In this work, we examined the oxidation of nucleoside dG and dsDNA by XBQs/ $\text{H}_2\text{O}_2$  as measured by the formation of 8-oxodG, the well-known biomarker for oxidation DNA damage<sup>23</sup>. 8-OxodG was characterized and accurately quantified using highly sensitive and specific UPLC-ESI-MS/MS. A major oxidation product was observed when dG was incubated with both TCBQ and  $\text{H}_2\text{O}_2$  for 2 h (trace a, Fig. 1A). This product showed not only the same chromatographic retention time (8.40 min) but also the same transition pair of multiple reaction monitoring ( $m/z$  284.1 $\rightarrow$ 168.0) as that of the standard 8-oxodG, and therefore was identified as 8-oxodG. MS fragmentation analysis of the identified 8-oxodG showed the fragmentation pattern of  $m/z$  284.1 $\rightarrow$ 168.0 $\rightarrow$ 140.0, which further confirmed this assignment (Supplementary Fig. S1). The frequency of 8-oxodG induced by TCBQ/ $\text{H}_2\text{O}_2$  (164 per  $10^6$  dG) was found to be 13 times higher than that of the untreated dG (12.5 per  $10^6$  dG). Only minor oxidation of dG was observed by  $\text{H}_2\text{O}_2$  alone (25.9 per

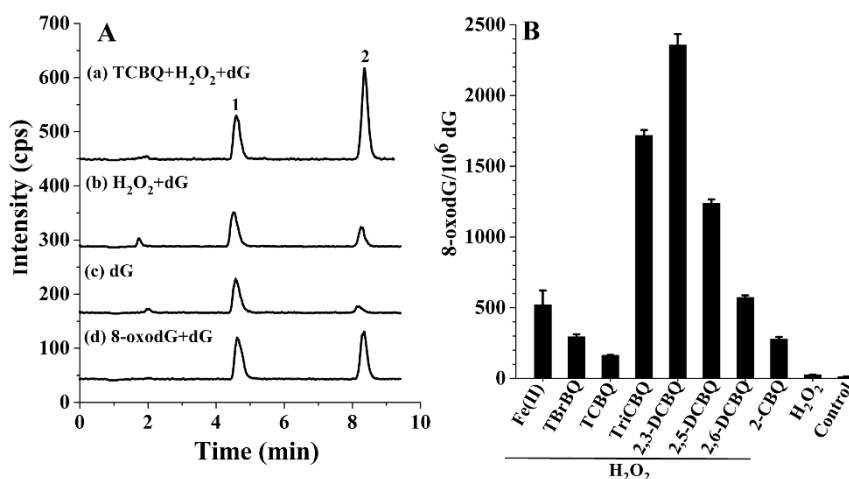
$10^6$  dG, trace b in Fig. 1). These results suggest that dG can be effectively oxidized to 8-oxodG by TCBQ/ $\text{H}_2\text{O}_2$ .

Similar effects were also observed when TCBQ was substituted by other XBQs. These include less chlorinated quinones such as 2-chloro-1,4-benzoquinone (2-CBQ), 2,3-dichloro-1,4-benzoquinone (2,3-DCBQ), 2,5-dichloro-1,4-benzoquinone (2,5-DCBQ), 2,6-dichloro-1,4-benzoquinone (2,6-DCBQ), trichloro-1,4-benzoquinone (TriCBQ), and other halogenated quinones, e.g., tetrabromo-1,4-benzoquinone (TBrBQ) (See chemical structures, Supplementary Scheme S1) (Fig. 1B). The frequency of 8-oxodG ranged from 164 to 2360 lesions per  $10^6$  dG. Among XBQs tested, 2,3-DCBQ is most potent, which can induce 14.3 times higher 8-oxodG (2.36 lesions per  $10^3$  dG) than TCBQ, and 4.5 times higher than Fe(II)-mediated Fenton system (521 lesions per  $10^6$  dG), under the same experimental conditions.

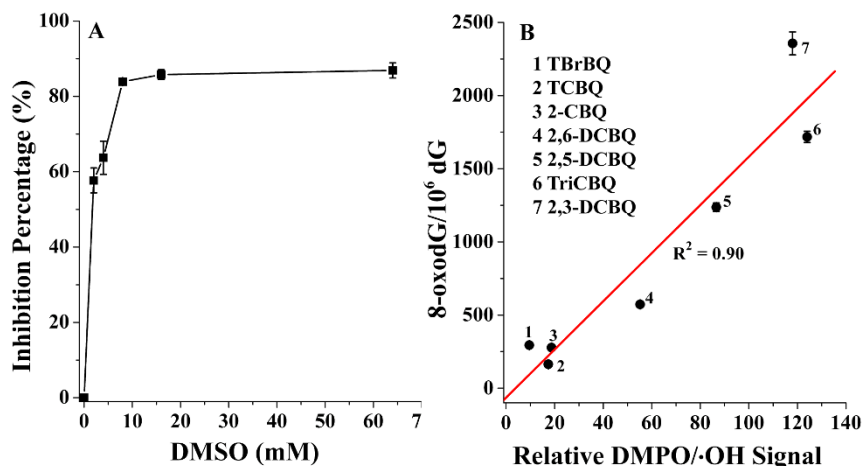
**The 8-oxodG formation was found to be mainly due to metal-independent  $\bullet\text{OH}$  production by XBQs/ $\text{H}_2\text{O}_2$ .** The oxidation of dG to 8-oxodG by TCBQ/ $\text{H}_2\text{O}_2$  was found to be efficiently inhibited by the typical  $\bullet\text{OH}$  scavenger dimethyl sulfoxide (DMSO) in a concentration-dependent manner (Fig. 2A). These results suggest that  $\bullet\text{OH}$  may play a critical role in 8-oxodG formation from dG oxidation by XBQs/ $\text{H}_2\text{O}_2$ . However, a minor fraction of the formed 8-oxodG (< 20%) could not be inhibited by DMSO. This is probably due to a small fraction of  $\bullet\text{OH}$  presented in bound form.

The contribution of  $\bullet\text{OH}$  was further validated by ESR spin-trapping results. We found that, consistent with our previous findings<sup>18</sup>, the short-lived  $\bullet\text{OH}$  could be trapped by the spin-trapping agent DMPO (5,5-dimethyl-1-pyrroline *N*-oxide) and form detectable long-lived DMPO/ $\bullet\text{OH}$  (Supplementary Fig. S2). Further correlation analysis revealed that the level of 8-oxodG is proportional to the signal intensity of DMPO/ $\bullet\text{OH}$  (at G3873), showing a linear correlation with a correlation coefficient of  $R^2 = 0.90$  ( $P < 0.005$ ,  $n = 7$ ) (Fig. 2B). Interestingly, the semiquinone radical could be observed, but its signal did not correlate with the signal intensity for the hydroxyl radical (Supplementary Fig. S2).

To exclude the possible role of trace amount of transition metal ions (e.g., iron, copper) contaminated in the buffer which were considered to be essential for  $\bullet\text{OH}$  formation<sup>12,24</sup>, all the buffers and water used for the reactions were pre-treated with excess chelex-100 resin overnight. Furthermore, no significant decrease in the level of 8-oxodG induced by TCBQ/ $\text{H}_2\text{O}_2$  was observed with metal-che-



**Figure 1** | (A) UPLC-ESI-MS/MS analysis of 8-oxodG produced during the oxidation of dG by TCBQ/ $\text{H}_2\text{O}_2$ . The addition of TCBQ (10  $\mu\text{M}$ ) and  $\text{H}_2\text{O}_2$  (50  $\mu\text{M}$ ), and dG (0.2 mM) was indicated as in traces a-c. Trace (d) was obtained from the analysis of the mixture of dG (0.2 mM) and 8-oxodG (10 nM). Peak 1 is unidentified peak, and peak 2 represents 8-oxodG. (B) The frequency of 8-oxodG generated from the oxidation of dG by XBQs/ $\text{H}_2\text{O}_2$ . All the reactions contained 0.2 mM dG, 50  $\mu\text{M}$   $\text{H}_2\text{O}_2$  and 10  $\mu\text{M}$  XBQs with an exception of the Fenton system, which contained 10  $\mu\text{M}$   $\text{FeCl}_2$  and 50  $\mu\text{M}$   $\text{H}_2\text{O}_2$ . Each sample was analyzed in triplicate.



**Figure 2** | The association of the DNA oxidation with the generated  $\bullet\text{OH}$  by XBQs/ $\text{H}_2\text{O}_2$ . (A) Inhibitive effect of DMSO on the production of 8-oxodG from the oxidation of dG. (B) by both TCBQ and  $\text{H}_2\text{O}_2$ . (B) Linear correlation of DMPO/ $\bullet\text{OH}$  signal intensity (at G value 3473) produced by XBQs/ $\text{H}_2\text{O}_2$  with the level of 8-oxodG from dG oxidation by XBQs/ $\text{H}_2\text{O}_2$ . Each reaction was conducted in triplicate.

lating agent DTPA (0.1–1 mM), a well-known inhibitor of the metal-mediated Fenton reactions<sup>13</sup> (Supplementary Fig. S3).

The above results clearly showed that  $\bullet\text{OH}$  produced in metal-independent manner makes a predominant contribution to the formation of 8-oxodG from the dG oxidation by XBQs/ $\text{H}_2\text{O}_2$ .

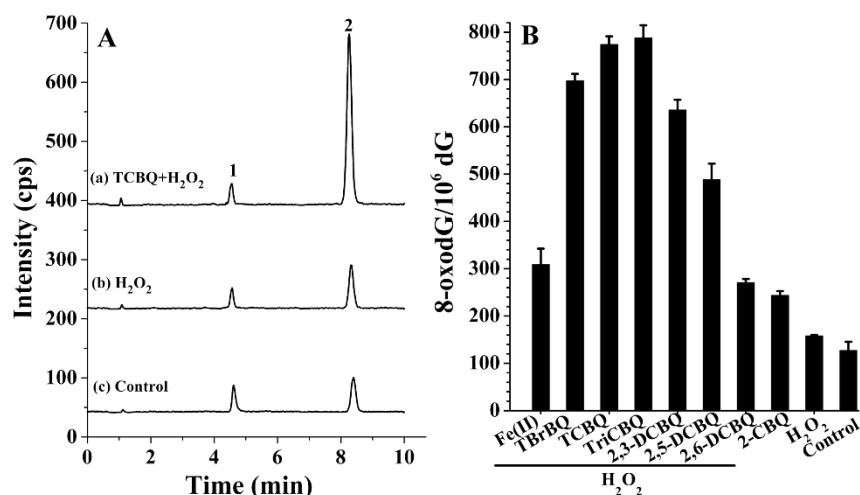
**Metal-independent oxidation of dsDNA to 8-oxodG by XBQs/ $\text{H}_2\text{O}_2$ .** Being different from single nucleosides, dsDNA molecules have unique double helix structure, which may change the oxidation potency of XBQs/ $\text{H}_2\text{O}_2$ . Therefore, we further tested the oxidation of dsDNA by TCBQ/ $\text{H}_2\text{O}_2$  using calf thymus dsDNA (ctDNA) as a model. As shown in Figure 3A, the frequency of 8-oxodG significantly increased up to 774.2 lesions per  $10^6$  dG from the control level (127.4 lesions per  $10^6$  dG) when ctDNA was treated by TCBQ/ $\text{H}_2\text{O}_2$ , with net increase of 8-oxodG about 646.8 lesions per  $10^6$  dG. In contrast,  $\text{H}_2\text{O}_2$  alone caused only minor net increase in the frequency (30.8 lesions per  $10^6$  dG). The oxidation of dsDNA was found to be dose-dependent on both TCBQ and  $\text{H}_2\text{O}_2$  (Supplementary Fig. S4), and such reactions were complete in 7.5 min (Supplementary Fig. S5).

Similar to the case of dG oxidation, the oxidation of dsDNA by TCBQ/ $\text{H}_2\text{O}_2$  was found to be markedly inhibited by  $\bullet\text{OH}$  scavenger

DMSO (Supplementary Fig. S6), but not by the general metal chelating agent DTPA (Supplementary Fig. S7), which further confirmed that the oxidation was mainly due to the metal-independent  $\bullet\text{OH}$  producing mechanism.

In addition to TCBQ, other XBQs can also effectively oxidize dsDNA in the presence of  $\text{H}_2\text{O}_2$  (Fig. 3B). The estimated frequency of 8-oxodG generated from XBQs/ $\text{H}_2\text{O}_2$  ranged from 116.4 to 660.6 lesions per  $10^6$  dG. When compared with iron(II)-mediated Fenton reaction, which generated 8-oxodG with a frequency of only 180.6 lesions per  $10^6$  dG, most XBQs/ $\text{H}_2\text{O}_2$  systems (except 2-CBQ and 2,6-DCBQ) showed a 2.0–3.7 times higher potency for the oxidation of dsDNA.

**XBQs/ $\text{H}_2\text{O}_2$  was more efficient to induce 8-oxodG formation in dsDNA than in dG.** As described above, both dsDNA and the nucleoside dG can be effectively oxidized to 8-oxodG by XBQs/ $\text{H}_2\text{O}_2$ . To our surprise, we found that the 8-oxodG yield of dsDNA vs the nucleoside dG varies significantly (Figs. 1B and 3B). The potency of oxidative damage to dsDNA ranks as a decreasing order: TriCBQ > TCBQ > TBrBQ > 2,3-DCBQ > 2,5-DCBQ > 2,6-DCBQ > 2-CBQ, which is quite different from that for the



**Figure 3** | (A) UPLC-ESI-MS/MS analysis of 8-oxodG in control (a),  $\text{H}_2\text{O}_2$ -treated (b), and TCBQ/ $\text{H}_2\text{O}_2$ -treated (c) calf thymus dsDNA. The reactions involved 400  $\mu\text{g}/\text{mL}$  ctDNA, 10  $\mu\text{M}$  TCBQ, and 50  $\mu\text{M}$   $\text{H}_2\text{O}_2$ . Peak 1 is unidentified peak, and peak 2 represents 8-oxodG. (B) The frequency of 8-oxodG generated from the oxidation of dsDNA by XBQs/ $\text{H}_2\text{O}_2$ . All the XBQs/ $\text{H}_2\text{O}_2$  reactions contained 400  $\mu\text{g}/\text{mL}$  ctDNA, 50  $\mu\text{M}$   $\text{H}_2\text{O}_2$  and 10  $\mu\text{M}$  XBQs. The Fenton system contained 400  $\mu\text{g}/\text{mL}$  ctDNA, 10  $\mu\text{M}$   $\text{FeCl}_2$ , and 50  $\mu\text{M}$   $\text{H}_2\text{O}_2$ . Each sample was analyzed at least in triplicate.



nucleoside dG (2,3-DCBQ > TriCBQ > 2,5-DCBQ > 2,6-DCBQ > TBrBQ > 2-CBQ ≈ TCBQ). Most notably, the two highly halogenated quinones TCBQ and TBrBQ display much higher oxidation potency toward dsDNA vs dG. Then, the question is: Why is there such a dramatic difference?

Since most of XBQs are hydrophobic and have a planar structure, XBQs might be intercalated between two neighboring base pairs in dsDNA. The limited data from electrospray ionization-mass spectrometry study of the interactions of several XBQs and oligonucleotides in gas phase also implicated a possible intercalation of XBQs in dsDNA<sup>25</sup>. XBQs, if strongly intercalated in dsDNA, may produce •OH immediately adjacent to the dsDNA via the homolytic decomposition of H<sub>2</sub>O<sub>2</sub> without the need for diffusion, and thus may cause more efficient oxidation of dsDNA. Based on the above analysis, we hypothesized that XBQs might be strongly intercalated in dsDNA, which enable the generation of •OH near dsDNA and therefore enhance their oxidation efficiency. To test this hypothesis, we examined the possible intercalation of XBQs in dsDNA.

**The more efficient oxidation of dsDNA by XBQs/H<sub>2</sub>O<sub>2</sub> is possibly due to their high affinity intercalation into dsDNA.** The intercalation affinity of XBQs in dsDNA was measured by a series of competitive binding assays. A well-known DNA intercalating dye PO-PRO-3 with an inert chemical structure and a known *K<sub>d</sub>* of 14.7 nM (Supplementary Fig. S8) was used as a fluorescent indicator of intercalation<sup>26</sup>. To avoid the possible loss of the fluorescence signal due to the reaction of the intercalated dye with •OH, H<sub>2</sub>O<sub>2</sub> was not included during these measurements. Evidently, the fluorescence signal of the intercalated dye in dsDNA decreases upon addition of any tested XBQ (Supplementary Fig. S9), suggesting a clear displacement of the intercalated PO-PRO-3 from dsDNA by each XBQ. The intercalation affinity of XBQs (*K<sub>d</sub>*) was calculated from the curves of the fluorescence signal vs the concentration of XBQs (Supplementary Fig. S9), which ranges from 20.0 nM to 1.32 μM (Table S1), and ranks as a decreasing order: TBrBQ ≥ TCBQ > TriCBQ > 2,5-DCBQ > 2,3-DCBQ > 2,6-DCBQ > 2-CBQ. These data provide strong evidence that XBQs indeed can intercalate into dsDNA.

To further evaluate the effects of the intercalation of XBQs on DNA oxidation, we calculated the frequency ratio of increased 8-oxodG in dsDNA vs in dG induced by XBQs/H<sub>2</sub>O<sub>2</sub>. By plotting the frequency ratio of 8-oxodG against the intercalation affinity (Fig. 4A), we found that all XBQs, except 2,3-DCBQ, have higher frequency ratios (0.22 – 4.3) than Fe(II)-EDTA-mediated Fenton system (0.22) (Supplementary Fig. S10). The results indicate that

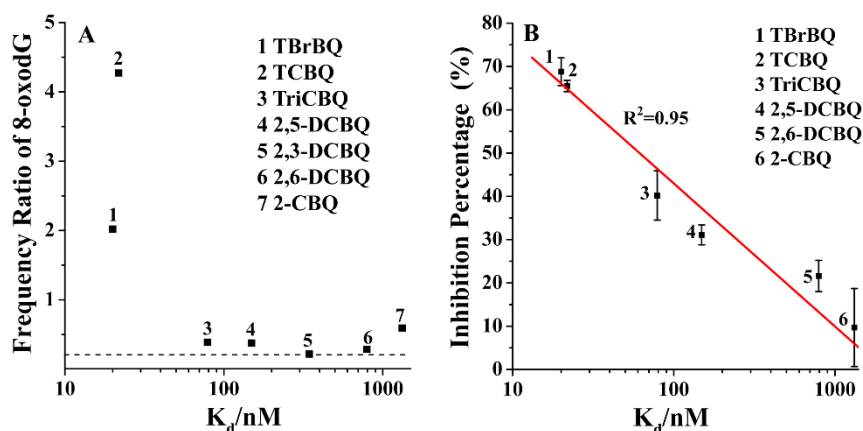
the intercalation of XBQs may facilitate their access to the nucleophilic sites in dsDNA and thus enhance their general damaging potency by generating •OH near the nucleophilic sites. Particularly, high affinity intercalation of TCBQ and TBrBQ in dsDNA significantly enhances their potency over one order of magnitude (9.2 – 20 times) as compared with the Fe(II)-EDTA mediated Fenton reaction.

To further validate the enhancement of the oxidation efficiency of XBQs by intercalation, a series of displacement experiments were conducted using ethidium homodimer-1 (EthD-1) as a displacing intercalator of dsDNA. EthD-1 was chosen because of its inert chemical structure<sup>27</sup>, high intercalation affinity, and ease-of-dissolution in water (Fig. S11). As shown in Fig. 4B, the oxidation of dsDNA by XBQs/H<sub>2</sub>O<sub>2</sub> was significantly inhibited by the intercalating displacement of EthD-1. The inhibition was dependent on the intercalation affinity of XBQs. The oxidation of dsDNA by highly, moderately and lowly halogen-substituted quinones (e.g. TBrBQ and TCBQ; 2,5-DCBQ and TriCBQ; and 2-CBQ) are correspondingly inhibited by EthD-1 over 65%, 31–40%, and 9.7–22%, respectively. These results clearly demonstrate that the displacement of XBQs from dsDNA by EthD-1 effectively reduces their DNA damaging potency, further supporting the important role of the intercalation of XBQs played in DNA oxidation by XBQs/H<sub>2</sub>O<sub>2</sub>. In conclusion, the more efficient oxidation of dG in dsDNA by XBQs/H<sub>2</sub>O<sub>2</sub> is due to their high affinity intercalation into dsDNA.

## Discussion

In this study, we demonstrate that dsDNA can be effectively oxidized by XBQs/H<sub>2</sub>O<sub>2</sub>, and the metal-independent generation of highly reactive •OH is found to be responsible for the direct oxidation of dsDNA. The oxidative potency of XBQs/H<sub>2</sub>O<sub>2</sub> is even higher than the classic iron-mediated Fenton system.

We show that 8-oxodG is one of major product for the oxidation of mononucleoside dG by TCBQ and H<sub>2</sub>O<sub>2</sub>. However, the formation of 8-oxodG in free nucleosides upon exposure to •OH requires the presence of reducing compounds, otherwise, due to the strong ability of the intermediate dG(–H)• oxidizing 8-oxodG, the overwhelming oxidation products in pure aerated aqueous solution are 2-amino-5-[2-deoxy-β-D-erythro-pentofuranosyl]amino]-4H-imidazol-4-one (dIz) and 2,2-diamino-4-[2-deoxy-β-D-erythro-pentofuranosyl]-5-(2H)-oxazolone (dOz), its hydrolytic decomposition product<sup>28–30</sup>. It is likely that the oxidizing XBQs/H<sub>2</sub>O<sub>2</sub> system contains a reducing species that could be the tetrachlorosemiquinone anion radical (TCSQ<sup>•–</sup>) that has been proposed to be generated by reduction of



**Figure 4** | (A) The correlation of the frequency ratio of 8-oxodG generated from oxidation of ctDNA vs dG with the intercalation affinity of XBQs with dsDNA. The dash line represents the value of the frequency ratio of 8-oxodG generated from oxidation of ctDNA vs dG Fe(II)-EDTA mediated Fenton reaction (ctDNA/dG = 0.22). (B) Inhibitory effect of EthD-1 on the formation of 8-oxodG from the oxidation of dsDNA by XBQs/H<sub>2</sub>O<sub>2</sub>. The concentrations for EthD-1, H<sub>2</sub>O<sub>2</sub>, XBQs, and ctDNA in the reaction systems are 50 μM, 50 μM, 10 μM, and 400 μg/mL, respectively. Each sample was determined by UPLC-ESI-MS/MS at least in triplicate.



TCBQ in aqueous solution<sup>17</sup>. The presence of TCSQ<sup>•-</sup> is indeed observed in XBQs/H<sub>2</sub>O<sub>2</sub> system (Supplementary Fig. S2). We also detected dlz in free nucleosides oxidized by XBQs/H<sub>2</sub>O<sub>2</sub>, and found the amount of the observed dlz is lower than that of the formed 8-oxodG (data not shown). These results clearly support the predominant formation of 8-oxodG. The situation is different in DNA since the ability for dG(−H)• to oxidize distant 8-oxodGuo is less probable in such an organized structure.

The potent oxidation of dsDNA by XBQs/H<sub>2</sub>O<sub>2</sub> demonstrated by this study may be associated with the genotoxicity, mutagenesis, and carcinogenicity of the ubiquitous halogenated phenols and their analogues, which are widely used as insecticides, herbicides, fungicides, and wood preservatives<sup>13,31</sup>. Pentachlorophenol (PCP) as a representative of halogenated phenols has been found in over one-fifth of the National Priorities List sites identified by the U.S. EPA and classified as a group 2B environmental carcinogen<sup>13</sup>. These phenols can be metabolized *in vivo* to form reactive XBQs by mammalian cytochrome P450 and peroxidases<sup>32–34</sup>. Although DNA adducts have been detected in model animals and in cultured mammalian cells treated by PCP or TCBQ, the level of DNA adducts is at least 10 times lower than that of oxidative DNA damage<sup>35–38</sup>. Since H<sub>2</sub>O<sub>2</sub> can be produced via the redox cycling of XBQs and their reduced forms<sup>36,39</sup>, it is possible that XBQs/H<sub>2</sub>O<sub>2</sub> could make an essential contribution to the genotoxicity of halogenated quinones through the generation of •OH. It should be noted that this metal-independent oxidation of dsDNA by TCBQ/H<sub>2</sub>O<sub>2</sub> can occur even at physiologically relevant concentrations of TCBQ (as low as 1 μM) (Supplementary Fig. S4A).

Interestingly, four XBQs (trichloro-, 2,6-dichloro-, 2,6-dibromo-, and 2,6-dichloro-3-methyl-1,4-benzoquinone), which are suspected as bladder carcinogens, have been recently identified in disinfected drinking water<sup>21,22</sup>, suggesting a pervasive and chronic exposure of XBQs to the public. Quantitative structure–toxicity relationship analysis indicated that XBQs are highly toxic, and the chronic lowest observed adverse effect levels (LOAELs) of XBQs are in the low μg/kg body weight per day range, which is 1000 times lower than most of the regulated disinfection by-products<sup>21</sup>. As shown in this study, TriCBQ could generate 3.7-fold more oxidative DNA lesions with H<sub>2</sub>O<sub>2</sub> than the classic iron-mediated Fenton system. Therefore, the oxidation of DNA by XBQs/H<sub>2</sub>O<sub>2</sub> may also be related to the potential nephro-carcinogenicity of the disinfection quinoid byproducts in drinking water.

In summary, here we demonstrated that XBQs/H<sub>2</sub>O<sub>2</sub> can effectively oxidize DNA independent of transition metal ions. Their oxidative potency to dsDNA is even higher than that of the classic iron-mediated Fenton reaction. We further proved that the high affinity intercalation of XBQs may greatly enhance their oxidative potency toward dsDNA. Regarding the wide distribution of XBQs in our environment, this study may have potential biological significance in future investigations on the genotoxicity of the ubiquitous halogenated quinoid carcinogens.

## Methods

The reactions of XBQs, H<sub>2</sub>O<sub>2</sub>, and dG/dsDNA were performed in phosphate buffer (PB, 20 mM, pH 7.4) at 37 °C for 2 h unless otherwise stated. All the PB buffers and water used for the reactions were pretreated by chelex 100 resins (Bio-Rad Laboratories, 5 g/L) to remove trace amount of transition metals. The oxidized dsDNA was enzymatically digested to nucleosides prior to UPLC-ESI-MS/MS analysis.

8-oxodG analysis was performed with a Zorbax Eclipse Plus C18 column (2.1 × 100 mm, 1.8 μm, Agilent) for separation, and electrospray MS/MS (Agilent 6410B, USA) for detection in the positive-ion mode.

ESR analysis of •OH was performed with a Bruker ESR300E spectrometer and a cavity equipped with liquid sample cell. The intercalation affinities of XBQs in dsDNA were measured using a series of competitive binding assays.

Details of the materials, methods, and experimental procedures are given in the Supporting Information.

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## Author contributions

H.L.W. and B.-Z.Z. designed research, R.C.Y. and Y.L.S. performed experiments on DNA oxidation, D.P.Z. performed experiments on intercalation assays, H.L.W., R.C.Y., D.P.Z. and Y.L.S. analyzed data. H.L.W., B.-Z.Z., R.C.Y., D.P.Z. and Y.L.S. wrote the paper.

## Additional information

Supplementary information accompanies this paper at <http://www.nature.com/scientificreports>

**Competing financial interests:** The authors declare no competing financial interests.

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