Evaluation of DNA adduct damage using G-quadruplex-based DNAzyme

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Peer review under responsibility of KeAi Communications Co., Ltd.
Data availability.

ABSTRACT
Toxicity assessment is a major problem in pharmaceutical candidates and industry chemicals development. However, due to the lack of practical analytical methods for DNA adduct analysis, the safety evaluation of drug and industry chemicals was severely limited. Here, we develop a DNAzyme-based method to detect DNA adduct damage for toxicity assessment of drugs and chemicals. Among 18 structural variants of G4 DNAzyme, EA2 DNAzyme exhibits an obvious DNA damaging effect of styrene oxide (SO) due to its unstable structure. The covalent binding of SO to DNAzyme disrupts the Hoogsteen hydrogen bonding sites of G-plane guanines and affects the formation of the G4 quadruplex. DNA damage chemicals reduce the peroxidase activity of the G4 DNAzyme to monitor the DNA adduct damage by disrupting the structural integrity of the G4 DNAzyme. Our method for genotoxic assessment of pharmaceutical candidates and industrial chemicals can elucidate the complex chemical pathways leading to toxicity, predict toxic effects of chemicals, and evaluate possible risks to human health.

ARTICLE INFO
Keywords:
Toxicity assessment
DNAzyme
DNA adduct Damage
Styrene oxide
Pharmaceutical candidates

1. Introduction
Toxicity assessment is a major problem in pharmaceutical candidates and industry chemical development [1–3]. 1/3 of drug candidates fail due to toxicity issues until clinical testing, which gives rise to huge drug costs and human safety. Development of new chemicals for industries such as agriculture, cosmetics, or food processing have similar testing protocols. Toxicity bioassays or animal tests are commonly performed for toxicity assessment, but they rarely address specific chemical pathways of toxicity [4]. In vitro toxicity screening bioassays, such as cell-based toxicity assays, high-throughput screening approaches [7], and organ modeling approaches [4–6], supplement tools for predicting drug and chemical toxicity.

Toxic effects related to the DNA damages are referred to as genotoxicity, including oxidative damage and DNA adduct damage. DNA adducts could be considered as a biomarker of exposure, which leads to mutations and carcinogenesis [7]. Therefore, quantifying DNA adducts is valuable for assessing and evaluating the safety of exposure to drugs and industrial chemicals. Liquid chromatography-mass spectrometry (LC-MS) [8,9], immunoassay [10,11], radiolabeling techniques, and genomic sequencing [12] are widely used for DNA adduct analysis. However, they have some limitations, such as requirement for cumbersome sample preparation in LC-MS, the limited availability of antibodies in immunoassays, the risk of exposure to high radioactivity in radiolabeling techniques, and the limitation to certain lesions in sequencing-based analysis. A variety of electrochemiluminescence...
and to fully elucidate the pathways leading to toxicity. The lack of practical analytical methods for DNA adduct analysis, which are stabilized by Hoogsteen hydrogen bonds, G-stacking, and peroxidase-like activity [29] and catalyzes the oxidation of ABTS, activates DNA machines, and as building blocks for the assembly of nanostructures, and hemin/G-quadruplexes as mimicking horseradish peroxidase [27, 28]. The G-quadruplex/hemin horseradish peroxidase exhibits superior peroxidase-like activity [29] and catalyzes the oxidation of ABTS, amplex red or luminol with H$_2$O$_2$. The G-quadruplex/hemin horseradish peroxidase mimicking DNAzyme is widely used in biosensors. Oxidative damage of guanine to 8-oxoguanine by reactive oxygen species in genomic DNA has been implicated with various human diseases [20–25]. DNAzymes, which are DNA molecules with enzymatic activities [26], have emerged as an important class of nanostructures, and hemin/G-quadruplex DNAzyme exhibits superior peroxidase-like activity [29] and catalyzes the oxidation of ABTS, amplex red or luminol with H$_2$O$_2$. The G-quadruplex/hemin horseradish peroxidase mimicking DNAzyme is widely used in biosensors. Oxidative damage of guanine to 8-oxoguanine results in a partial and variable loss of peroxidase-like activity [30]. However, G-quadruplex DNAzyme has not been used in DNA adduct damage analysis for toxicity assessment of drugs and industrial chemicals.

Here, we develop a DNAzyme-based method to detect DNA adduct damage for toxicity assessment of drug and industrial chemicals. We investigate the mechanism of evaluation of DNAzyme-based DNA adduct damage system. As a proof of concept, we use styrene oxide (SO) as a model for DNA damage analysis based on the G4 DNAzyme-catalyzed peroxidation system. The covalent binding of SO to DNA disrupts the Hoogsteen hydrogen bonding sites of G-plane guanines and affects the formation of G4 structure. The change of G4 structure leads to the inhibition of DNAzyme peroxidase activity. G4 DNAzyme-catalyzed peroxidation system is feasible for DNA damage analysis, and applicable for a variety of methods. This methodology will provide a promising tool for genotoxic assessments of pharmaceutical candidates and industrial chemicals.

2. Materials and methods

2.1. Chemicals and materials

Styrene oxide (SO, 98%) is from Macklin. N-Nitrosodiethylylamine (ND, 99.0%), atropine sulfate hydroyde (AS, 98.5%), chloramphenicol (CP, 98.0%), N, N’-Dicyclohexylcarbodiimide (DCC, 99.0%), sodium chloride (NaCl, 99.5%), and sodium phosphate dibasic dodecahydrate (NaH$_2$PO$_4$·12H$_2$O, 99.0%), potassium chloride (KCl, 99.5%), sodium acetate trihydrate (CH$_3$COONa·3H$_2$O, 99%), acetic acid (CH$_3$COOH, 99.5%), and H$_2$O$_2$ (30%, v/v) are from Sinopharm Chemical Reagent Co., Ltd. Triton X-100, and 1 M Tris-HCl solution (pH 7.0 and 9.0) are from Sangon Biotech (Shanghai). Creatinine (Cr, 99%) is from Yuanye Bio-Technology Co., Ltd (Shanghai). Sterilized water is bathed for 5 min. The absorbance at 415 nm is measured.

5 μL different DNAzyme sequences (10 μM) and different concentrations of SO are mixed in 20 μL 5 × HEPES buffer. After incubation at 37 °C for 1 h, 10 μL hemin (10 μM) is added, and react for 0.5 h. The total amount of the system volume is 100 μL. The final concentration of G4 and hemin are 0.5 μM and 1 μM, respectively. The final concentrations of SO are 0, 0.1, 1, 5, 20, 50, 80, 100 μM, respectively. 10 μL ABTS (20 mM) and 2 μL H$_2$O$_2$ (0.1 M) are mixed in 88 μL NaAc/HAc solution (0.05 M, pH 5.0, containing 200 mM NaCl, 50 mM KCl, and 12.5 mM MgCl$_2$), and bathed for 5 min to initiate the ABTS$^-$ reaction. The absorbance at 415 nm is recorded by a Synergy HTX multifunctional enzyme marker at room temperature.

5 μL different DNAzyme sequences (10 μM) and 50 μL SO (100 mM) in 20 μL 5 × HEPES solution react for different time (0, 15, 30, 60, 90, 120, 180, 240 min) at 37 °C, then 10 μL hemin (10 μM) is added. The total amount of the system volume is 100 μL. The final concentration of G4 is 0.5 μM, hemin is 1 μM, and SO is 50 mM. Peroxidation reaction is performed after half an hour. 10 μL ABTS (20 mM), 2 μL H$_2$O$_2$ (0.1 M) and 88 μL NaAc/HAc solution (0.05 M) are mixed in the system, and bathed for 5 min. The absorbance at 415 nm is measured.

2.2. G4 DNAzyme-catalyzed peroxidation

Table 1

| Name       | Sequence (5’ to 3’) |
|------------|---------------------|
| T30695     | GGGTGGTGGTGGTGGTGGT |
| 17-DNAzyme | GGGTGGCGGGCGGGTGGG |
| PS2.M      | GTGGTAAACCCCGGGTGG |
| PS5.M      | TGGTGTACCTGTGGTGGTGGTGG |
| AGRO100    | GTGTGTTGGTTGGTGGTGG |
| EA2        | CGAGGGTGGTTGGTGG |
| EA3D       | GTGGTGGTGCTGGTGGTGG |
| EA4D       | CGAGGGTGGTTGGTGGTGG |
| EA6d       | GTGGTGGTGCTGGTGGTGG |
| VEGF       | GGGGCGGCGGGCGGCGG |
| c-Myc      | TGAGGGTGGTTGGTGGTGG |
| c-Ki21     | GGGGCGGCGGCGGCGG |
| Cat        | TGGTGGTGCTGGTGGTGG |
| T223       | GGGTGGTGCTGGTGGTGG |
| Telomere-G4 | GGGTGGTGGTGGTGGTGG |
2.3. Spectroscopic characterization

15 μL different DNAzyme sequences (100 μM) and different concentrations of SO are mixed in 60 μL 5 × HEPES buffer. After incubation at 37 °C for 1 h, 30 μL hemin (100 μM) is added. Half an hour after the reaction, the absorption spectra are measured at 300–500 nm by a Shimadzu UV-2501PC UV–Vis spectrophotometer. The total amount of the system volume is 300 μL. The final concentration of G4 is 5 μM, and hemin is 10 μM. The final concentrations of SO are 0, 0.1, 1, 5, 20, 50, 80, and 100 mM respectively.

Similarly, 15 μL different DNAzyme sequences (100 μM) and 150 μL SO (100 mM) in 60 μL 5 × HEPES solution react for different time (0, 15, 30, 60, 90, 120, 180, and 240 min) at 37 °C, then 30 μL hemin (100 μM) is added. The total amount of the system volume is 300 μL. The final concentration of G4 is 5 μM, and hemin is 10 μM, and SO is 50 mM.

2.4. Fluorescence measurement

15 μL EA2 DNAzyme (100 mM) and 150 μL SO (1 M) are added in 600 μL 5 × HEPES buffer, and incubated at 37 °C for different time (0, 15, 30, 60, 90, 120, 180, 240 min). Then 30 μL ThT (10 μM) is added to the mixture, and react for 20 min. Emission spectra at 450–600 nm are measured. The total amount of the system volume is 300 μL. The final concentration of G4 is 0.5 μM, and SO is 50 mM.

2.5. Circular dichroism (CD) spectroscopy

30 μL EA2 DNAzyme (100 mM) and 15 μL SO (1 M) are added in 60 μL 5 × HEPES buffer solution, and incubated at 37 °C for different time (0, 15, 30, 60, 90, 120, 180, and 240 min). After the incubation, the samples are measured by a MOS-500 circular dichroism spectrometer at 50 nm/ min from 230 to 320 nm with a slit width of 1 nm. Each spectrum obtained is corrected by subtracting the CD data of buffer. The total amount of the system volume is 300 μL. The final concentration of G4 is 10 μM, and SO is 50 mM.

3. Results and discussion

3.1. Activity analysis of structural variants of G-quadruplex DNAzyme

To investigate the mechanism of DNA damage, we use 18 structural variants of G4 DNAzyme (Table 1), which are widely used in previous work [31], to detect DNA damage. SO is the secondary metabolite, which is generated after styrene’s metabolism by the cytochrome P450 enzyme system [32]. As an active electrophilic reagent, it is carcinogenic to animals and humans [33], and binds covalently to DNA (Fig. 1). As a proof of concept, we use SO as a model for DNA damage analysis based on the G4 DNAzyme-catalyzed peroxidation system. The catalytic activity of G4 DNAzyme decreases with the increasing SO concentration and reaction time (Fig. 2 A and B), which indicates that G4 DNAzyme is applicable for DNA damage analysis.

Different G4 sequences exhibit different peroxidase catalytic activity to SO. We select sequence variants mentioned in previous literature [34–36]. Our strategy for designing sequence variants is illustrated as follows. The common feature of G-quadruplex sequences are guanine-rich single-stranded oligonucleotides. We make some hypotheses: does the guanine tetrad have to be exactly three guanines in a row? Could a random array of guanine-rich single-stranded DNA also form G-quadruplexes? If these G-quadruplexes can be formed, can these structures also be peroxidase-like normal G-quadruplexes? By summarizing the sequences of DNAzyme reported in the literature, we find that guanine accounted for more than 50% of the DNA strand of G-quadruplexes, and most of them take three consecutive guanines as the basic unit, separated by one or several other bases. Based on this, we would like to study randomly arranged guanine-rich DNA to investigate whether it has the DNAzyme property of fixed guanine quadruplexes.

Firstly, we explore the activity of 18 structural variants without adding SO. It shows that the initial activities of these 18 structural variants were different, due to their different structures. EA2, EAD3, EAD2, EAD, Cat, and EAD4 exhibit significant activity of DNAzyme.

Among 18 structural variants of G4 DNAzymes, EA2 DNAzyme [37] exhibits the most obvious DNA damage effect of SO (Fig. 1A, Tables S1 and S2). Because EA2 has three quartets isn’t as stable as G4 sequences with four quartets. The unstable EA2 is easily affected by SO, and results in a more obvious DNA damage effect. As shown in Fig. 2 A and B, the activity of EA2 DNAzyme correlates well with SO concentration of and reaction time.

We design different structural variants of EA2 DNAzyme. As shown in Fig. 2C and D, DNA damage effect increases with the increasing concentration and reaction time, indicating that SO affects the activity of DNAzyme. SO concentration correlates well with the reaction time and the absorbance intensity at 415 nm. As shown in Fig. 2 A and
Table S2, we calculate the ratio of the absorbance difference and the maximum absorbance of different variants after reaction at different concentrations. The results show that the ratio of EA2 is the biggest, indicating that EA2 exhibits a significant DNA damage effect. Therefore, we select EA2 sequence for the subsequent experiments. As shown in Fig. 2C and 2D, we calculate the ratio of the absorbance difference to the maximum absorbance after the reaction. Although EA2 does not exhibit the most obvious DNA damage effect, EA2 is a great choice with basic sequence structure. Thus, we select EA2 for the following experiments. As shown in Fig. 2C, Fig. 2D, Table S3, and Table S4, different structural variants of EA2 have different effects to DNA damage analysis. G4 DNAzyme-based peroxide-catalyzed system is suitable for DNA damage analysis.

3.2. Toxicity assessment by DNAzyme-based DNA adduct damage system

To investigate the possibility of G4 system for toxicity assessment, we investigate DNA damage of different chemicals [38–45]. As shown in Fig. 3A, 18 different chemicals (including 3-AB, 1,10-PM, ND, CP, AS, CTAC, SD, 2-HP, NBS, DCC, HN2, CTX, BU, BCNU, DDP, CBP, MS and SO) were tested. As shown in Fig. 3A, 18 different chemicals (including 3-AB, 1,10-PM, ND, CP, AS, CTAC, SD, 2-HP, NBS, DCC, HN2, CTX, BU, BCNU, DDP, CBP, MS and SO) were tested.
SO) exhibit different degrees of DNA damage effects.

The chemicals can be divided into 9 types: (1) Alkylating agent: HN2, CTX, BU, and BCNU. (2) Platinum complexes: DDP, and CBP. (3) Antioxidant: CP. (4) Catalyzer: 2-HP, SD, CTAC, and MS. (5) Oxidant: 3-AB. (6) Brominating agent: AS. (7) Dehydrating agent: DCC. (8) Redox indicator: 1,10-PM. (9) Ultraviolet absorbing agent: SO.

SO exhibits the most significant toxicity. CTX, HN2, 1,10-PM, AS, 3-AB, CBP, BCNU, BU, and CTAC exhibit obvious toxicity, while SD, ND, 2-HP, DDP, CP, CBP, and MS exhibit unobvious toxicity. The results consistent with the previous references, indicating that G4 DNAzyme system is successful for toxicity assessment of unknown chemicals. As shown in Fig. S4, the significant decrease of absorbance of DCC and CTAC is because they can react with substrate. It seems further to inhibit the activity of DNAzyme. 1 mM H₂O₂, 1 mM ABTS, and a small amount of HRP are added to the 0.05 M NaAc/HAc buffer solution. After the reaction, we add different concentrations of CTAC (0.1, 1, 5, 20, 50, 80, and 100 mM) and DCC (0, 0.1, 1, 5, 20, 50, 80, and 100 mM). The total volume is 200 μL, and the absorbance at 415 nm is measured.

The degrees of inhibition of peroxidase activity vary depending on the structure, molecular weight and plane structures of chemicals. It will affect the formation of DNA adduct. As shown in Fig. S5, we select AS as a proof of concept to investigate its inhibition mechanism. As shown in Fig. S5, the titration experiments indicate that AS affects the affinity of hemin to G4. With the addition of AS, the absorption of EA2-AS is lower than that of EA2. The binding of AS to G4-hemin affects the catalytic activity. The change of G4 structure leads to the inhibition of DNAzyme peroxidase activity. Fig. S5 shows the Soret band decreases with increasing SO concentrations and reaction times. It indicates that AS might occupy the position of H₂O at lower concentration, which increases the absorbance intensity at first. The results show that its inhibition mechanism is similar to that of SO. The results show that its inhibition mechanism is similar to that of SO. The results consistent with previous reference, indicating that G4 DNAzyme system is successful for toxicity assessment of chemicals.

As a proof of concept, we measure the absorbance at different SO concentration. As shown in Fig. S3 B, the absorbance displays an excellent linear relationship with the SO concentration in the range of 0.1–100 μM, where a linear regression equation is $Y = -0.0175X + 2.0896$, $R^2 = 0.9624$. The detection limit (LOD) of SO is defined as 3.21 μM with 3 σ/k (k is the slope of fitting lines).

### 3.3. Inhibition of DNAzyme activity with DNA adduct damage

To understand the mechanism of DNAzyme-based DNA adduct damage system, we study the topology, thermal stability, and heme binding affinity of DNAzyme. Different topologies of G4 have different characteristic peaks in CD spectra [46]. According to the orientation of the 5' to 3' ends, the topology of G4 can be divided into two types of structures, parallel and antiparallel. The parallel G4 structure exhibits a negative absorption peak at 240 nm and a positive absorption peak at 260 nm. And the anti-parallel structure of G4 exhibits a negative peak at 260 nm and a positive peak at 290 nm. The parallel G4 exhibits stronger peroxidase activity than anti-parallel G4. As shown in Fig. 4 A, EA2 DNAzyme exhibits a positive peak at 266 nm and a negative peak at 244 nm, which corresponds to the parallel G4 structure. CD spectra of EA2 exhibit a slight change with the reaction time from 0–180 min, indicating that the parallel structure of EA2 DNAzyme didn’t change when expose to SO. The peak intensity exhibits an obvious decrease up to 240 min, indicating that SO affects the folding of EA2 DNAzyme after a long incubation time.

We investigate the effect of different buffers on G4 DNAzyme-based DNA damage system. As shown in Fig. 4 B, the buffer solutions can affect the catalytic activity of G4, with the order of HEPES \(>\) MIES \(>\) PBS. As shown in Fig. 4 B, nitrogenous buffers can facilitate peroxidation reactions via exogenous general acid–base catalysis, and lead to more severe DNA damage [47,48]. As shown in Fig. 4C and D, the titration experiments indicate that SO affect the affinity of hemin to G4. With the addition of SO, the absorption of EA2-SO is lower than that of EA2. We conduct DNA damage detection at different temperatures. As shown in Fig. 3 D, the results show that the low temperature will inhibit the DNA damage of SO, while high temperature will damage the DNA structure, resulting in the failure of formation of a complex with heme.

E and D bands of hemin are observed in the high spin state of Fe (III) center [49]. As shown in Fig. 4 E and F, a strong Soret band is observed.

![Fig. 4. A. CD spectra of EA2 DNA with different reaction time. B. Effect of SO on EA2 catalytic activity in different buffers (5 \(\times\) HEPES, 5 \(\times\) PBS, and 5 \(\times\) MES solution, respectively). C. Spectrophotometric titration of hemin with different concentration of G4 DNAzyme. D. Spectrophotometric titration of DNA with different concentration of hemin. E. UV spectrum of different SO concentration in solution containing 10 μM hemin, 5 μM EA2 DNAzyme. F. UV spectrum of different reaction times between SO and EA2.](image-url)
at 404 nm in hemin-EA2 complex, which is a characteristic absorption peak of hemin ($\epsilon_{404} > 1.2 \times 10^5 \text{M}^{-1} \text{cm}^{-1}$) [50]. Fig. 4 E and F shows the effect of different concentrations and reaction time on the activity of G4 DNAzyme. When SO concentration and reaction time are up to 5 mM and 30 min, respectively, the Soret band decreases with the increasing SO concentration and reaction time. It indicates that SO might occupy the position of $\text{H}_2\text{O}$ at lower concentration, which increases the absorbance intensity at first. When SO replaces G4, and binds to hemin directly at high SO concentration or long reaction time, the absorbance decreases.

3.4. Potential mechanism of DNAzyme-based DNA damage analysis

The DNAzyme sequence folds to form the G4 structure in the presence of cations, which forms Hoogsteen hydrogen bonds between the guanines [51]. When SO binds covalently to DNA, it disrupts the Hoogsteen hydrogen bonding sites of G-plane guanines and affects the formation of G4 structure (Fig. 1). SO is one of the most versatile DNA binding agents, which binds to at least nine nucleic acid bases. The most common binding site is with guanine, mainly concentrated on the $N^6$, $N^7$ and $O^6$ atoms of guanine [52] (Fig. 1).

As shown in Fig. 1, the activity of G4 DNAzyme depends on three factors: topology, thermal stability, and affinity to heme [48,53]. Two longitudinal coordination bonds of hemin exhibit a significant effect to the G4-hemin catalytic peroxidation [54]. One binds to the G plane of G4, and the other one binds to $\text{H}_2\text{O}/\text{H}_2\text{O}_2$ to provide acid-base catalysis. The binding of SO with hemin occupies the $\text{H}_2\text{O}_2$ binding catalytic site, which leads to reduced catalytic activity. As shown in Fig. 4 E and F, Soret band of hemin exhibits a decrease after the addition of SO, which indicates that SO occupies the $\text{H}_2\text{O}_2$ binding catalytic site of hemin, and inhibits the peroxidase activity of DNAzyme [55].

To investigate the effect of uncombined SO, we used a biotin-labeled DNAzyme sequence (EA2-bio, 0.5 $\mu\text{M}$) for DNA damage analysis. EA2-bio folds into G4 structure, and binds with hemin to form a G4-complex. After the reaction with SO, the G4-complex is removed by streptavidin-modified magnetic beads. As shown in Fig. S1, the presence of SO results in the decrease of absorption, indicating that uncombined SO will react with ABTS.

3.5. The generality of DNAzyme-based DNA-adduct damage system

To test the generality of G4 system, UV, FL, CL and ECL are used for DNA damage analysis. We use ABTS, TMB, and OPD as substrates for G4 DNA adduct damage analysis. As shown in Fig. 5, different substrates exhibit different absorption intensities. The characteristic UV peaks for ABTS, TMB, and OPD are located at 415 nm, 650 nm, and 405 nm, respectively. The absorption intensity of ABTS is higher than TMB and OPD, which might be due to high affinity of the ABTS with the active site of hemin. UV spectra of different substrates depend on the conjugated structure and the rigid plane. The rigid structure of ABTS and G4 leads to a higher affinity and a higher absorption (Fig. 5 A). Firstly, the molar absorption coefficient of ABTS at 415 nm is $\epsilon = 3.6 \times 10^4 \text{L mol}^{-1} \text{cm}^{-1}$, and the molar absorption coefficient of TMB at 652 nm is $3.9 \times 10^4 \text{L mol}^{-1} \text{cm}^{-1}$. OPD can generate the colored DAP in the peroxide reaction with $\epsilon_{460}\text{nm}$ of $1.67 \times 10^4 \text{L mol}^{-1} \text{cm}^{-1}$ [56,57]. According to Beer-Lambert Law ($A = \epsilon \cdot c \cdot l$), the absorbance of ABTS and TMB with bigger $\epsilon$ will exhibit obvious change, which is more convenient for detection. Secondly, the hydrophilicity of three substrates is in the order of ABTS $>$ OPD $>$ TMB. The more hydrophobic of the substrate, the easier it is to attract the background molecules, which will reduce the chemical activity of the substrate and affect the enzyme activity [58]. Thirdly, cations can enhance the peroxidation reaction of G4, while ABTS is more sensitive to cations than TMB. Therefore, we chose ABTS as the hydrogen donor of our method [59].

The FL intensity of ThT greatly enhances when it binds to G4 [60]. We measure the FL spectra of different structural variants of G4 with SO (Fig. S3 A). FL of ThT greatly enhances with the addition of G4. As shown in Fig. S3 B, cations (such as $K^+$, $Mg^{2+}$, and $Na^+$) inhibit the FL intensity of G4-ThT complex, which lead to different degrees of reduction of FL intensity. We also investigate the effect of reaction time on G4-ThT without cations (Fig. S3 C). FL of the complex decreases with the increase of reaction time indicating that the binding of SO to G4 will affect the binding of ThT and G4 [61]. DNAzyme-based DNA adduct damage system can be used for FL analysis.

We investigate the effect of SO on luminol ECL. Nano-Au is used to catalyze $\text{H}_2\text{O}_2$, which reacts with luminol to emit light. As shown in Fig. S2, the ECL intensity decreases with the increase of SO concentrations, and exhibits a good linear relationship ($Y = -28.9338$}
X + 8491.6336, \( R^2 = 0.9586 \) with the SO concentration. G4 DNAzyme-based DNA adduct damage system is generally applicable for a variety of methods, such as CL, UV, FL, and ECL.

As shown in Fig. S6, we observe obvious inhibition effect to the activity of the DNAzyme of SO, indicating the method can be used to monitor the genotoxicity in cells. Proof-of-concept of our method provides an early screening tool for on-site environmental monitoring that specifically monitors the genotoxic reactivity of unknown chemicals and drug candidates.

4. Conclusion

We develop a DNAzyme-based method to detect DNA adduct damage for toxicity assessment of drug and chemical. Among 18 structural variants of G4 DNAzyme, EA2 DNAzyme exhibits obvious DNA damage effect of SO due to its unstable structure. As a proof of concept, we use SO as a model for DNA damage analysis based on the G4 DNAzyme-catalyzed peroxidation system, which exhibits pronounced effect due to its toxicity. The formation of G4 structure by SO leads to the inhibition of G4 DNAzyme peroxidase activity. The covalent binding of SO to DNA disrupts the Hoogsteen hydrogen bonding sites of G-plane guanine and affects the formation of G4 structure. The DNA damage chemicals affect the G4 DNAzyme peroxidase activity by disrupting the structural integrity of the G4 DNAzyme sequence. G4 DNAzyme-catalyzed peroxidation system is feasible for DNA damage analysis, and applicable for a variety of methods. This attributes to the assessment of possible genotoxicity of pharmaceutical candidates and industry chemicals. This methodology will provide a promising tool for genotoxic assessment of pharmaceutical candidates and industry chemicals. Proof-of-concept of this measurement provides an early screening tool for on-site environmental monitoring that specifically monitors the genotoxic reactivity of unknown chemicals and drug candidates. These features suggest future applications for toxicity screening as well as clinical and research measurements. Future progress would also benefit from miniaturization and simplification of the method and new protocols for faster, more convenient screening. These goals are currently being pursued in our laboratory.

Declaration of competing interest

All authors declared no competing interests.

Ethics approval and consent to participate

This manuscript reporting study does not involving human participants, human data or human tissues.

CRediT authorship contribution statement

Yi Xiao: Conceptualization, Methodology, Investigation, Writing – original draft, Writing – review & editing, Funding acquisition. Haomin Yi: Conceptualization, Methodology. Jingzhai Zhu: Conceptualization, Methodology. Shuhua Chen: Conceptualization, Methodology, Resources, Data curation, Writing – review & editing. Guofang Wang: Visualization, Software. Yilong Liao: Visualization, Software. Yuanjuan Lei: Software. Liyin Chen: Writing – review & editing. Xingcai Zhang: Conceptualization, Supervision, Writing – review & editing. Fangfu Ye: Conceptualization, Supervision, Funding acquisition.

Acknowledgments

This work was supported by National Natural Science Foundation of China (81803730), Natural Science Foundation of Hunan Province (2019JJ50383), Natural Science Foundation of Changsha (kq2022256), Huxiang High-Level Talent Innovation Team (2018RS3072), Scientific and Technological Projects for Collaborative Prevention and Control of Birth Defect in Hunan Province (2019SK1012), and Key Grant of Research and Development in Hunan Province (2020DK2002). Dr. Zhang acknowledges the support from Harvard/MIT.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioactmat.2022.10.002.

References

[1] S.J. Kwon, D.W. Lee, D.A. Shah, B. Ku, S.Y. Jeon, K. Solanki, J.D. Ryan, D.S. Clark, J.S. Dorcick, M.-Y. Lee, High-throughput and combinatorial gene expression on a chip for metabolism-induced toxicity screening, Nat. Commun. 5 (1) (2014) 3739.
[2] C. Uamsaky, A.E. Morellato, M. Bieckrer, M.A. Schiedegeger, M.R. Martinifk, G. A. Fernández, P. Ok, K. Kolesnikova, H. Reingruber, G. Bollini, G.P. Crossan, N. Sommer, M.E. Monge, B. Schumacher, L.B. Pontel, Endogenous formaldehyde scavenges cellular glutathione resulting in redox disruption and cytotoxicity, Nat. Commun. 13 (1) (2022) 745.
[3] A.A.-O. Cohen, K.A.-O. Ioannidis, A.A.-O. Ehrlich, S.A.-O. Regenbaum, M.A.-O. Cohen, M. Ayann, S.S. Tikva, Y.A.-O. Nahmias, Mechanism and reversal of drug-induced nephrotoxicity on a chip, Sci. Transl. Med. 13 (582) (2021) 1–11.
[4] C.A.-O. McAl eer, C.A.-O. Long, D.A.-O. Elbrecht, T. Sasserath, L.A.-O. Bridge, J.A.-O. Runsey, C. Martin, M. Schnepper, Y.A.-O. Wang, F. Schuler, A.A.-O. Roth, C. Funk, M.A.-O. Shuler, J.A.-O. Hickman, Multi-organ system for the evaluation of efficacy and off-target toxicity of anticancer therapeutics, Sci. Transl. Med. 11 (517) (2019) eaaw5516.
[5] K.-J. Jang, A. Otsino Monical, J. Ronxhi, H.-K. Lim, L. Ewart, R. Kolida Konstanza, P. Petropolis Deborha, G. Kulkami, E. Rubins Jonathan, D. Congelio, J. Narwroth, D. Simic, W. Lam, M. Singer, E. Barale, B. Singh, M. Bone, J. Streeter Anthony, C. Manthey, B. Jones, A. Srivastava, C. Anderson Linda, D. Williams, H. Park, R. Barrile, J. Sliz, A. Herland, S. Haney, K. Karalis, E. Inger Donald, A. Hamilton Geraldine, Reproducing human and cross-species drug toxicities using a Liver-Chip, Sci. Transl. Med. 11 (517) (2019) eaaw5516.
[6] D. Hah, B.D. Leslie De Fau - Mathews, J.P. Mathews Bld Fau - Fraser, S. Fraser Jp Fau - Jurek, G.A. Jurek S Fau - Hamilton, K.S. Hamilton Ga Fau - Thornelor, M. A. Thorneloe Kx Fau - McAlexander, D.E. McAlexander Ma Fau - Inger, D. E. Inger, A human disease model of drug toxicity-induced pulmonary edema in a lung-on-a-chip microdevice, Sci. Transl. Med. 4 (159) (2012) 1–8.
[7] P. Jangili, N. Kong, J.H. Kim, J. Zhou, H. Liu, X. Zhang, W. Tso, J.S. Kim, DNA-damage-response-targeting mitochondria-activated multifunctional prodrug strategy for self-defense tumor therapy, Angew. Chem. 134 (16) (2012), e202117075.
[8] J. Helbark Harold, B. Beckman Kenneth, K. Shigemasa Mark, B. Patrick, A. Woodall Alan, C. Yeo Helen, N. Ames Bruce, DNA oxidation matters: the HPLC-electrochemical detection assay of 8-oxo-deoxyguanosine and 8-oxo-guanine, Proc. Natl. Acad. Sci. USA 95 (1) (1998) 288–293.
[9] K. Taghizadeh, J.I. McFaf, B. Pang, M. Sullivan, M. Dong, E. Plummer, P. C. Dedon, Quantification of DNA damage products resulting from deamination, oxidation and reaction with products of lipid peroxidation by liquid chromatography isotope dilution tandem mass spectrometry, Nat. Proc. 3 (8) (2008) 1287–1298.
[10] X. Ji, L. Ge, C. Liu, Z. Tang, Y. Xiao, W. Chen, Z. Lei, W. Gao, S. Blake, D. De, B. Shi, Capturing functional two-dimensional nanosheets from sandwich-structure vermiculite for cancer theranostics, Nat. Commun. 12 (2021) 1124.
[11] C. Liu, S. Sun, Q. Feng, G. Wu, Y. Wu, N. Kong, Z. Yu, J. Yao, X. Zhang, W. Chen, Z. Tang, Arsenene nanodots with selective killing effects and their low-dose combination with 8-克莱米恩 for cancer therapy, Adv. Mater. 33 (37) (2021), 2102054.
[12] Y. Ding, A.M. Fleming, C.J. Burrows, Sequencing the mouse genome for the oxidatively modified base 8-oxo-7,8-dihydroguanine by Ox-seq, J. Am. Chem. Soc. 139 (2017) 2569–2572.
[13] I. Bist, S. Song, I.M. Mosa, T.E. Keyes, A. Martin, R.J. Forster, J.F. Rusling, Electrochemiluminescent array to detect oxidative damage in dsDNA using [O(3pp)][33phben-COOH][2-](γ-)Nafion/Graphene films, ACS Sens. 1 (2016) 272–278.
[14] D.P. Wasalathanthri, D. Li, D. Song, Z. Zheng, D. Choudhary, I. Jansson, J.F. Rusling, Electrifying organ-specific metabolic toxicity chemistry from electrochemiluminescent enzyme/DNA arrays and bioreactor bead-DLC-MS/MS, Chem. Sci. 6 (4) (2015) 2457–2468.
[15] D.P. Wasalathanthri, V. Mani, C.K. Tang, J.F. Rusling, Microfluidic electrochemical array for detection of reactive metabolites formed by cytochrome P450 enzymes, Anal. Chem. 83 (24) (2011) 9499–9506.
[16] D. Jiang, M. Shen, B. Abhaidu, J.F. Rusling, Organ-specific screening for protein damage using magnetic bead bioreactors and LC-MS/MS, Anal. Chem. 92 (7) (2020) 5337–5345.
[17] D. Jiang, S. Malla, V.J. Fu, D. Choudhary, J.F. Rusling, Direct LC-MS/MS detection of guanine oxidations in exon 7 of the p53 tumor suppressor gene, Anal. Chem. 89 (2017) 12872–12879.
[18] S. Malla, K. Kadiyamuri, T.-J. Fu, D. Choudhary, I. Jansson, J.B. Schenkman, J. F. Rusling, Chemical selectivity of nucleobase adduction relative to in vivo
