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DNA breakage induced by piceatannol and copper(II): Mechanism and anticancer properties

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Abstract. Piceatannol (3,3',4,5'-tetrahydroxy-*trans*-stilbene; Pice), found in a variety of plant sources including grapes, red wine, peanuts and rhubarb, is known as a metabolite and analog of Resveratrol (3,5,4'-trihydroxy-*trans*-stilbene; Res) and has higher bioactivity than Res. To explore the mechanism of DNA damage induced by Pice in the presence of copper (Cu) (II), gel electrophoresis, UV-visible spectroscopy, fluorescence spectroscopy and Fourier transform infrared spectroscopy were used. The results of gel electrophoresis demonstrated that the hydroxyl radical played a critical role in DNA cleavage. Spectroscopy confirmed that the mechanism of DNA cleavage induced by Pice-Cu(II) involves the Haber Weiss and Fenton reactions. Pice chelates with Cu(II) as a bidentate ligand, and the Pice-Cu(II) complex undergoes intramolecular electron transfer to form the semiquinone radical anion and Cu(I), which may be reoxidated by O_2 to form Cu(II) with hydroxyl radical generation. In brief, the formation of the hydroxyl radical and the Cu(II)/Cu(I) redox cycle play a key role in inducing DNA damage. In this process, Pice demonstrated pro-oxidant properties. Oxidative product(s) of Pice, semiquinone, was formed and Cu(I) was reoxidized to Cu(II). The redox cycling of copper generated reactive oxygen species, which induced DNA cleavage, the hallmark of cell apoptosis. The mechanism of DNA breakage induced by Pice-Cu(II) may be a significant pathway through which cancer cells are killed.

Introduction

Findings of previous studies showed that the antioxidant properties of plant polyphenols may not fully account for their chemopreventive and anticancer effects. It has been suggested that the cytotoxic activity of plant polyphenols, at least *in vitro*, may be related to their pro-oxidant activity (1,2). The majority of plant polyphenols possess both antioxidant and pro-oxidant activity. Several studies have also documented that plant polyphenol, a well-known antioxidant, exhibits pro-oxidant properties *in vitro*, particularly in the presence of transition metal ions such as copper (3-6), which accelerates lipid peroxidation and induces oxidative DNA damage.

Copper is an essential microelement in chromatin and is closely associated with bases of DNA; it is also one of the most redox-active metal ions present in cells (7). The concentration of copper has been found to be much higher in cancer cells than in normal cells (8), and the endogenous copper ions could be mobilized easily by polyphenols and the consequent pro-oxidant action (7). Therefore, Hadi *et al* proposed that the pro-oxidant action of polyphenolics would be an important mechanism underlying their anticancer and apoptosis-inducing properties and a better explanation for the anticancer effect of polyphenols as well as the preferential cytotoxicity towards cancer cells (9).

Resveratrol (3,5,4'-trihydroxy-*trans*-stilbene; Res) has been reported to have both anticarcinogenic and cardioprotective activity, which is mainly due to its antioxidant and anticoagulant properties (10-13). It is also involved in the inhibition of tumor initiation, promotion and progression. Previous reports on Res indicate that it may have a relatively low bioavailability due to its biotransformation and rapid elimination (14-16). Piceatannol (3,3',4,5'-tetrahydroxy-*trans*-stilbene; Pice) was first isolated from the seeds of *Euphorbia lagascae* (17), and is found in a variety of plant sources including grapes, red wine, peanuts, sugar cane and rhubarb (18-21). As with Res, Pice has been reported to have various pharmacological properties including anti-inflammatory (22,23) and immunosuppressive features, and is a promising chemopreventive agent with anti-cancer activity against various tumors (24,25).

Res is known to metabolize to Pice via specific human cytochrome P450 (CYP) enzymes, including CYP1A1, CYP1A2 and CYP1B1, which are overexpressed in a wide variety of human tumors (26,27). Thus, Res acts as a pro-drug and Res-hydroxylated derivative Pice is a compound responsible for the biological activity against tumors. In addition,

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due to the presence of *ortho*-dihydroxy, Pice exhibits a better biological activity than Res (28). However, detailed information regarding the properties of Pice remains largely unknown. If pro-drugs are converted to biologically 'active metabolites' by cytochrome P450 enzymes (P450s) during the drug development process, it is essential to study the properties of Pice to establish its efficacy, toxic effect, pharmacokinetics and anticancer mechanism in particular.

A growing number of studies have focused on molecules that may bind genetic materials, such as DNA (29,30). There has been an increasing demand for the investigation of DNA-targeting molecules and the mechanism of their activity towards DNA, and special attention has been paid to the intercalating agents as a class of anti-tumor compounds (31,32).

In this study, we investigated the mechanism of the Pice-Cu(II) reaction. In this reaction, Cu(II) was reduced to Cu(I) by Pice with the concomitant formation of reactive oxygen species (ROS), and Pice formed a complex with Cu(II). The reaction presumably led to the formation of 'oxidized product(s)' of Pice, which also catalyzed the reduction of Cu(II). In addition, the biological activity of the reaction system was studied. We found that the Pice-Cu(II)-mediated reaction induced pBR322 DNA breakage. Such studies are significant not only in designing novel anti-tumor agents but also in developing more effective approaches to prevent and/or control cancer through dietary intervention.

Materials and methods

Materials. Supercoiled plasmid pBR322 DNA was purchased from Bio Basic Inc. (Markham Ontario, Canada). Bathocuproine was purchased from Acros Organics (Pittsburgh, PA, USA). The Plasmid Maxi Preparation kit was purchased from Omega Biotek Inc. (Doraville, GA, USA). All the other chemicals were of analytical grade. Pice was dissolved in dehydrated alcohol as a 1 mM stock solution and stored at -20˚C in the dark; $CuSO_4$ was dissolved in ultrapure water as a 1 mM stock solution. Both Pice and $CuSO_4$ were diluted with 0.01 M phosphate-buffered saline (PBS) prior to use, and the diluted solution was used within 2 h.

DNA-cleaving activity. DNA strand breakage was measured in terms of conversion of supercoiled pBR322 plasmid DNAs to open circular or linear forms by gel electrophoresis. The reaction mixture (10 μl) contained 100 ng pBR322 DNA, 200 μM $CuSO_4$ and 200 μM Pice, equal molar antioxidants and metal ion chelating agents in PBS at pH 7.4. After being preincubated for 1 h at 37˚C, the reaction mixture was treated with 2 μl loading buffer (6X), and then immediately loaded onto a 1.2% agarose gel containing 1 μg/ml gelview. Horizontal gel electrophoresis was performed in 1X TBE buffer (pH 8.3) and photographed with ultraviolet-visible (UV-visible) transillumination.

UV-visible spectra measurements. UV-visible spectra were measured with a TU-1901 dual beam UV-visible spectrophotometer (Beijing Purkinje General Instrument Co., Ltd., Beijing, China).

Time-related UV-visible spectra of Pice-Cu(II). A reaction mixture (2 ml) containing 50 μM Pice in PBS (pH 7.4) was prepared and underwent spectral analysis. The spectral tracing was started by adding 1 ml of 50 μM $CuSO_4$. The spectra were recorded every 5 min after adding $CuSO_4$ until the variation was invisible.

Stoichiometry of Cu(I) production. Varying amounts of Pice and Cu(II) were added to the reaction mixture (2 ml) containing 0.01 M PBS (pH 7.4) and 300 μM bathocuproine, and the final reaction volume was maintained at 3 ml by adding PBS. The bathocuproine-Cu(I) complex was determined by measuring the absorbance at 480 nm following incubation for 10 min at room temperature (33).

Formation of Pice-Cu(II) complex. Ethylenediaminetetraacetic acid (EDTA), a well-known chelating agent for metal ions, was used to detect the formation of the Pice-Cu(II) complex. The reaction mixture (final volume of 3 ml) contained 50 μM Pice, EDTA and Cu(II) in PBS. The spectra of the reaction mixtures were measured when Cu(II) (50 μM) had reacted for 10 min before and after the addition of EDTA, respectively.

Interaction between Pice and pBR322 DNA. The interaction between Pice and pBR322 DNA was detected at 17˚C and 37˚C, respectively. The PBS reaction mixtures (3 ml) contained 50 μM Pice and 100, 200 and 400 μl pBR322 DNA (30 ng/μl), respectively. The spectra were measured after incubation for 1 h at 17˚C and 37˚C, respectively.

The interaction between Pice and pBR322 DNA in the presence of Cu(II) was also detected at 37˚C. The PBS reaction mixtures (3 ml) contained 50 μM Pice, Cu(II), and 100, 200 and 400 μl DNA, respectively. The spectra were measured following incubation for 1 h at 37˚C.

Fluorescence measurements. Fluorescence spectra were recorded on an LS-55 fluorescence spectrophotometer (Perkin-Elmer) in a 1-cm quartz cuvette using an excitation wavelength of 280 nm and an emission wavelength of 300-500 nm. The slit widths for excitation and emission were set at 10 nm. The PBS reaction mixture (final volume of 3 ml) consisted of 50 μM Pice, 0 or 400 μl pBR322 DNA (30 ng/μl) and 50 μM Pice. The spectra were measured following incubation for 1 h at 37˚C.

Fourier transform infrared (FTIR) measurements. The infrared spectra were recorded as KBr pellets on an FTS 3000 FTIR spectrometer, within a wavelength range of 650-4000 cm^{-1}, and captured at a spectral range by accumulating 32 scans with a resolution of 4 cm^{-1}. It should be noted that Pice is easily oxidized during the FTIR spectra measurement, which causes difficulty when measuring the spectra directly, whereas Res is stable and shares a similar structure with Pice. Therefore, Res was used as a control to determine the structure of the product(s) of Pice-Cu(II).

Results

Strand breakage of plasmid pBR322 DNA induced by Pice in the presence of Cu(II). The cleaved supercoiled pBR322 DNA and the open circular and linear DNA were used to assess DNA strand breakage (33). We found that at a concentration

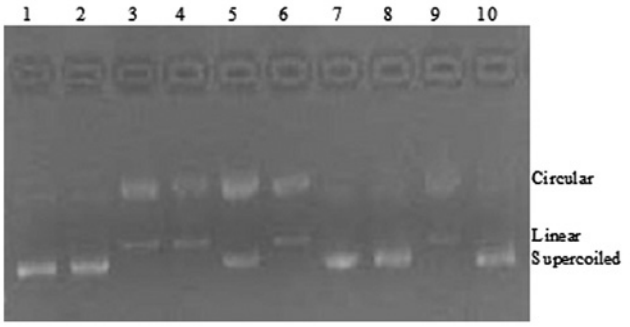

Figure 1. Mechanism of Pice-Cu(II)-mediated degradation of pBR322 DNA: lane 1, DNA; lane 2, DNA+Pice; lane 3, DNA+Pice+Cu(II); lane 4, DNA+Pice+Cu(II)+Vc; lane 5, DNA+Pice+Cu(II)+GSH (the type of reduction); lane 6, DNA+Pice+Cu(II)+Bath; lane 7, DNA+Pice+Cu(II)+mannitol; lane 8, DNA+Pice+Cu(II)+EDTA; lane 9, DNA+Pice+EDTA+Cu(II); lane 10, DNA +Cu(II). Pice, piceatannol; Bath, bathocuproine; GSH, glutathion.

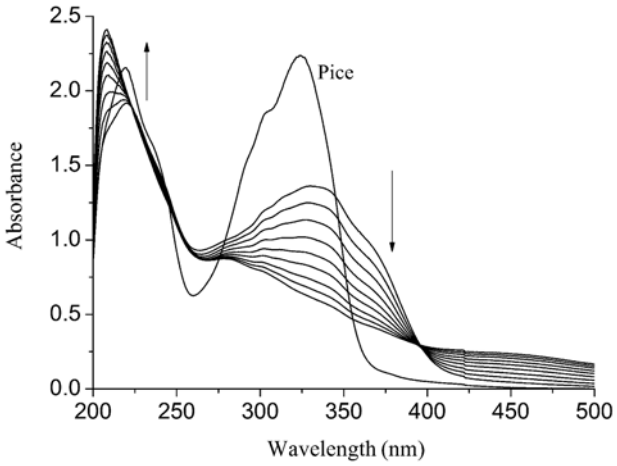

Figure 2. Time-related absorption spectra of Pice-Cu(II). Pice, piceatannol.

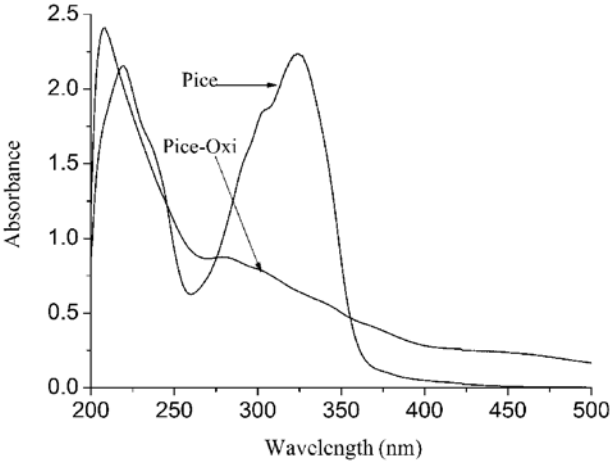

Figure 3. UV absorption spectra of Pice and its oxidative products. Pice, piceatannol.

of 200 μM, neither Pice nor Cu(II) alone showed the ability to damage DNA (Fig. 1, lanes 2 and 10). However, Cu(II) and Pice worked cooperatively to break the supercoiled DNA (Fig. 1, lane 3).

To clarify the roles of oxygen-derived active species in the DNA strand breakage induced by Pice in the presence of Cu(II), the free radical scavengers and the Cu(I)/Cu(II) chelator were used to prevent DNA strand breakage. Antiscorbic acid (antioxidant), glutathion (GSH, ROS scavenger), mannitol ($HO^{\bullet}$ scavenger), EDTA [the specific Cu(II) chelator] and bathocuproine (the specific Cu(I) chelator) were all provided to protect pBR322 plasmid DNA against strand breakage induced by Pice in the presence of Cu(II). It was found that antiscorbic acid did not prevent DNA strand breakage (Fig. 1, lane 4), whereas GSH and mannitol completely prevented DNA strand breakage (Fig. 1, lanes 5 and 7), indicating that ROS, particularly $HO^{\bullet}$, is important in inducing DNA breakage. Bathocuproine did not effectively protect DNA from breakage (Fig. 1, lane 6), whereas EDTA was able to protect DNA from breakage (Fig. 1, lane 8), although the protection was depressed if EDTA was added after Pice-Cu(II) had reacted with DNA for 15 min (Fig. 1, lane 9). These results suggest that both ROS($HO\cdot$) and Cu(II) are critical to DNA damage.

UV-visible spectral changes of Pice in the presence of Cu(II). To clarify the mechanism of DNA damage induced by the Pice-Cu(II) system, the UV-visible absorption changes of Pice in the presence of Cu(II) in PBS buffer (pH 7.4) under aerobic conditions were measured (Fig. 2). The peaks of Pice absorption were at 218 and 323 nm (Fig. 2). When Cu(II) was added to Pice, the absorbance at 323 nm disappeared rapidly, accompanied by a red shift at 332 nm, which provided evidence of the formation of the chelate complex Pice-Cu(II). The absorbance at 332 nm decreased with increasing time. A blue shift was also found from 218 to 208 nm with the increasing absorbance, which signaled the formation of new product(s). A new weak peak appeared near 459 nm, due to the formation of the *ortho*-semiquinone anion. In addition, the *ortho*-semiquinone radical anion was more easily oxidized to form the final product *ortho*-quinone (35). Two isosbestic points at 248 and 395 nm and the isosbestic point at 395 nm suggested a direct transition from one form [Pice-Cu(II) complex] to another (*ortho*-semiquinone anion).

To further understand the mechanism underlying the reaction of the Pice-Cu(II) system, the UV-visible spectrum of the oxidative product(s) of Pice was measured (Fig. 3). In Fig. 3, the spectrum of the oxidative product(s) is identical to that of the reaction product(s) of the Pice-Cu(II) system, indicating that the oxidative product(s) of Pice is one of the reaction product(s) of the Pice-Cu(II) system. Thus, Pice was oxidized and the reducing agent would be Cu(II) in this reaction, and the reaction mechanism was similar to that of other polyphenolic compounds (2).

If the reducing agent was Cu(II), Cu(I) would be formed. To detect Cu(I) in the reaction system, bathocuproine, a selectively trapping agent of Cu(I) was used. The absorbance at 480 nm provided evidence of the formation of the bathocuproine-Cu(I) complex (33). The result of the production of Cu(I) by stoichiometry is shown in Fig. 4. The absorbance at 480 nm was shown to vary with the molar ratio of Pice to Cu(II), indicating the existence of Cu(I) in the Pice-Cu(II) reaction system. No clear maximum was found where the absorption

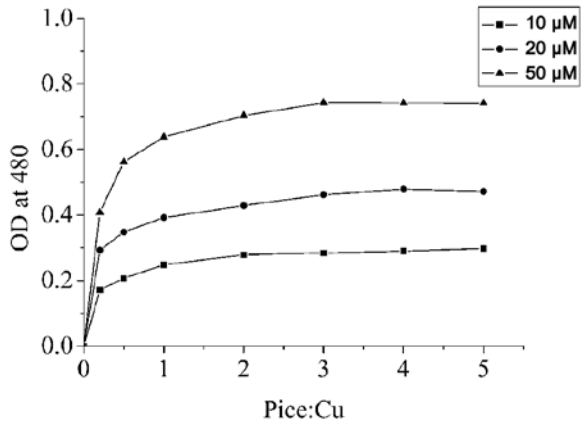

Figure 4. Differences in absorbance at 480 nm of samples with and without added Cu(II) is plotted vs. equivalents of Cu(II) per molar equivalent of Pice (concentration of bathocuproine used was 300 mM in all cases). Pice, piceatannol. OD, optical density.

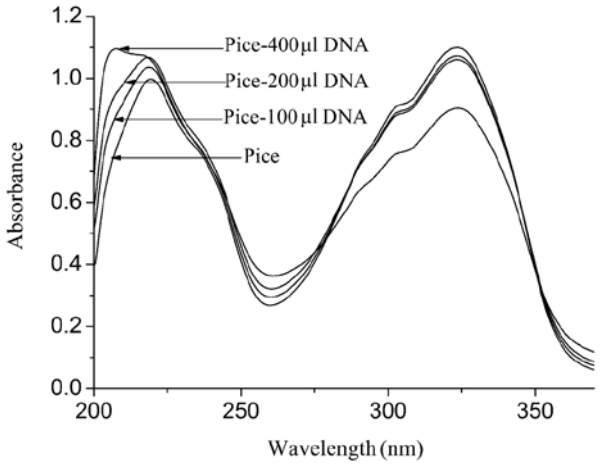

Figure 6. Spectra of Pice with different DNA levels at 17˚C. Pice, piceatannol.

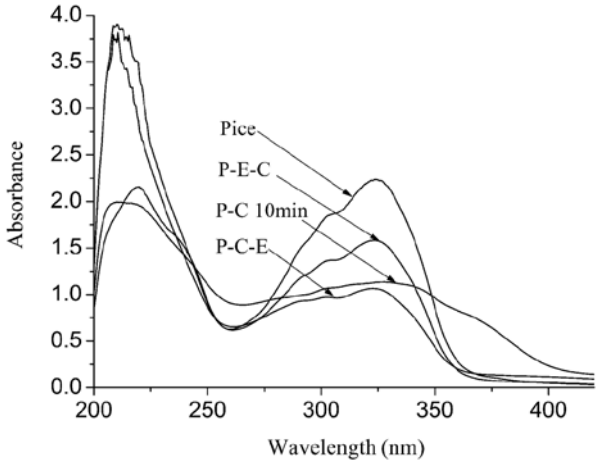

Figure 5. Confirmation of the complex compound between Cu(II) and Pice. Pice/P, piceatannol; E, EDTA; C, Cu(II).

reached a plateau, suggesting that copper ions may be recycled in this reaction (Fig. 4).

As Cu(II) was reduced to Cu(I) by Pice, it is essential to clarify whether the Pice-Cu(II) complex was formed. A well-known chelating agent for metal ions, EDTA, was used to confirm the formation of the Pice-Cu(II) complex (Fig. 5). When Cu(II) was added to Pice, the peak at 323 nm disappeared and the red-shifted peak appeared at 332 nm. Upon addition of EDTA after 10 min, the red-shifted band (332 nm) returned to its initial position (323 nm) with a decrease in absorbance. If EDTA was added to Pice before Cu(II), the spectrum of Pice changed more weakly than it did when EDTA was added after Cu(II), and there was no peak shift. The above phenomena therefore indicate that Pice chelates with Cu(II) as a bidentate ligand, thus facilitating intramolecular electron transfer to form the stable *ortho*-semiquinone anion (Fig. 2).

Mechanism of Pice-DNA and Pice-DNA-Cu(II). The absorption band of electrons is commonly used to study the interactions between micromolecules and DNA. In general, micromolecules possess electron absorption in the UV-visible region, and their electron atmosphere varies in the presence of DNA, which causes the variation of ligand circumstance. These phenomena may be demonstrated using the electron absorption spectrum.

When intercalation occurs between DNA and micromolecules, the absorption intensity of micromolecules degrades, and the absorption peak shows a red shift effect. π electron accumulation may occur between the intercalating ligand and base pairs of DNA, which may cause the conjugation between the π* vacant orbital of micromolecules and the π electron orbital of DNA base pairs. The decrease in the energy level induces π→π* transition energy decrease and generates the red shift effect, and the degree of the red shift reflects the ability of intercalation.

The bathochromic effect occurs following the micromolecule-DNA reaction, and the extent of the bathochromic shift increases with the increasing concentration of DNA, which indicates that the molecular aggregation and hydrogen bond damage cause interactions between micromolecules (36). If the electrostatic interaction and groove binding occur, the weak red shift and hypochromic effect may appear.

Fig. 6 shows the spectra of Pice with different DNA levels at 17˚C. Following the addition of pBR322 DNA, the absorbance of Pice at 323 nm decreased with the increasing DNA levels following the weak red shift (3 nm). These changes indicated that the interaction between Pice and DNA was groove binding. The absorption at 218 nm increased, and when the DNA level increased to 400 μl, the blue shift occurred from 218 to 205 nm, suggesting that the katogene in hydrogen bond and molecular aggregation of Pice also existed besides the intercalation (36).

The spectra of Pice with different DNA levels at 37˚C are shown in Fig. 7. In contrast to the situation at 17˚C, the hypochromic effect and the red shift were evident, indicating that intercalation also occurred between Pice and DNA. Similar to the situation at 218 nm, the hyperchromic effect was further elevated and the absorption peak moved to 208 nm instead of 205 nm with an increase in DNA, indicating that oxidization also occurred in the reaction system. All of these factors

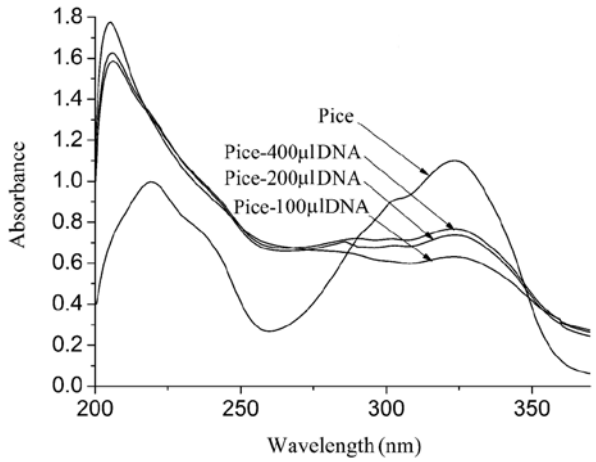

Figure 7. Spectra of Pice with different DNA levels at 37˚C. Pice, piceatannol.

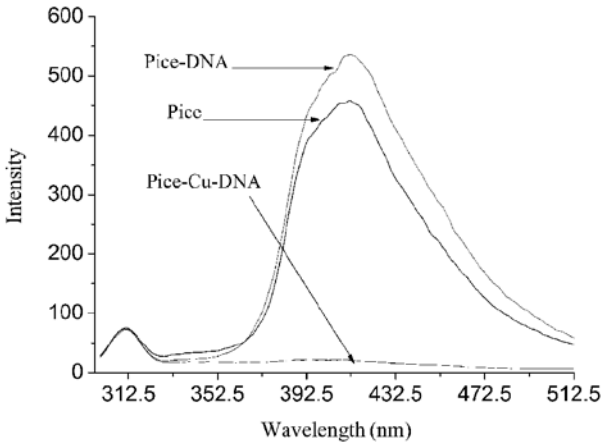

Figure 9. Fluorescence spectra of Pice, Pice-DNA, and Pice-Cu(II)-DNA at 37˚C. Pice, piceatannol.

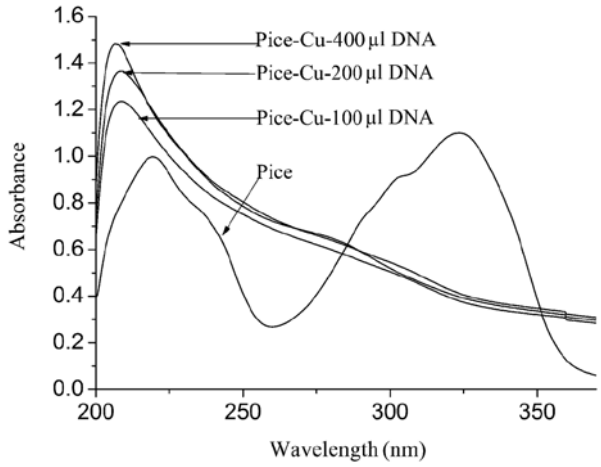

Figure 8. Spectra of Pice-Cu(II) with different DNA levels at 37˚C. Pice, piceatannol.

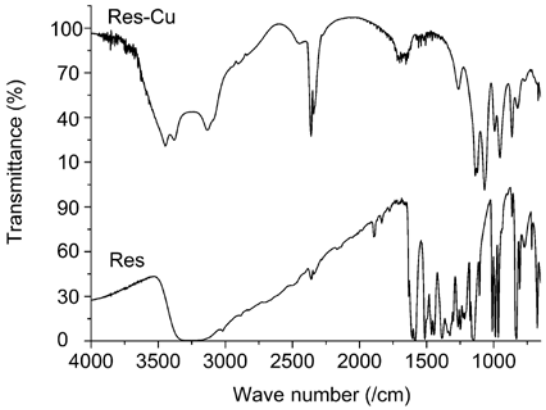

Figure 10. Fourier transform infrared (FTIR) spectra of Res and the reaction product of Res-Cu(II). Res, Resveratrol.

suggest that the degree of the interaction between Pice and DNA positively correlated with the temperature.

To further elucidate the mechanism of DNA breakage, the spectra of Pice with different DNA levels at 37˚C in the presence of Cu(II) were determined (Fig. 8). The absorption peak at 323 nm disappeared when Cu(II) was present in the reaction system, the blue shift occurred at 218 nm, and the peak moved from 218 to 208 nm accompanied by the increase in absorbance. These results combined with those obtained in the former sections indicate that Pice was oxidized completely in this reaction system. From these results, we hypothesized that the intermediate product mainly caused the damage to DNA.

Fluorescence spectra of Pice, Pice-DNA and Pice-DNA-Cu(II). DNA possessed no endogenous flourescence, although it affected the fluorescence character of the micromolecule through the interactions. In general, the fluorescence intensity was enhanced, and the degree of the enhancement was correlated with the degree of binding. Notably, the rigidity of the micromolecule was enhanced, whereas the micromolecule entered the hydrophobic environment from the hydrophilic environment, which also increased the fluorescence intensity.

The fluorescence spectra of Pice, Pice-DNA and Pice-Cu(II)-DNA at 37˚C were measured. As shown in Fig. 9, with the addition of DNA, the fluorescence intensity of Pice increased, mainly due to the hydrophobic protection of DNA. Simultaneously, the architecture of the DNA was damaged.

FTIR spectra. To determine the structure of Pice, the FTIR spectra of Res, Pice and the reaction product of Res-Cu(II) were measured (Figs. 10 and 11). We found that the medium intensity band emerged in the range 3000-3500 cm^{-1}, which was attributed to the phenolic hydroxyl group [ν(OH)] of Res; 1630 cm^{-1}, which was attributed to the stretching vibration of -C=C- outside the aromatic ring; 675 and 806 cm^{-1}, which was attributed to the out-of-plane bending vibration of =CH in the aromatic ring. The bands of 990-1300 cm^{-1} were attributed to the in-plane bending vibration of =C-H in the aromatic ring, and the several sharp bands of 1606, 1585, 1514, 1465 and 1384 cm^{-1} were attributed to the in-plane stretching vibration of -C=C- in the aromatic ring. In the produce(s) spectrum of Res,

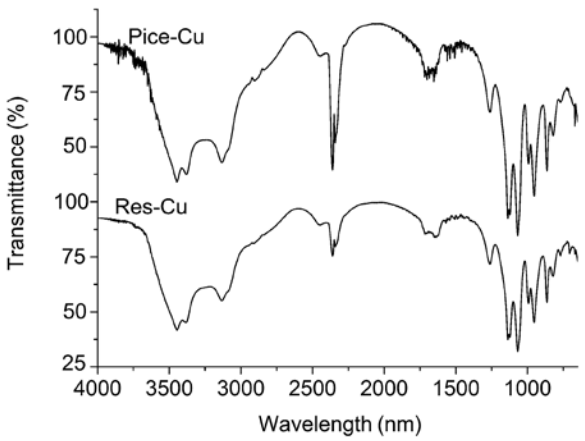

Figure 11. Fourier transform infrared (FTIR) spectra of the reaction product of Res-Cu(II) and Pice-Cu(II).

the out-of-plane bending vibration of =CH and the in-plane stretching vibration of -C=C- in the aromatic ring disappeared, and the new band at 1650-1680 cm^{-1} appeared, which was attributed to the stretching vibration of quinonyl. These results indicate that the aromatic ring in Res was destroyed when Res reacted with Cu(II). The product was semiquinone, which was the oxidative product of Res. It was also found that the bands of the in-plane bending vibration of =C-H in the aromatic ring varied, which further confirmed the conclusion.

From the FTIR spectra of the reaction product of Res-Cu(II) and Pice-Cu(II) in Fig. 11, two products were shown to have an almost identical spectrum structure, which suggested that the reaction mechanism of Res-Cu(II) and Pice-Cu(II) was identical. When Res was taken into account, oxidized forms of Pice were produced in the presence of Cu(II).

Discussion

In this study, the mechanism of oxidative DNA damage induced by Pice in the presence of Cu(II) was investigated in detail. Our results show that Pice is effective as an antioxidant, and it may become pro-oxidant in the presence of Cu(II) to induce DNA damage.

The Res-Cu(II)-induced oxidative DNA damage was previously studied (5,33,35). Similarly, although Pice is widely believed to be an antioxidant, our results support its pro-oxidant properties. According to the structure-activity relationship of the compounds in Res and its analogs, the compounds bearing *ortho*-dihydroxyl groups, followed by compounds bearing 4-hydroxyl groups, are the most active in inducing plasmid pBR322 DNA strand breakage and calf thymus DNA damage in the presence of Cu(II) (35). Obviously, the high activity of Pice is due to its *ortho*-dihydroxyl group which is capable of chelating with Cu(II) to form Pice-Cu(II) complexes. The Pice-Cu(II) complex is able to undergo intramolecular electron transfer to form semiquinone radical anion and Cu(I).

Possible mechanisms of DNA damage induced by Pice in the presence of Cu(II) are shown as follows:

$$Pice(OH)_4 \rightleftharpoons Pice(OH)_2O_2^- + 2H^+$$

$$Pice(OH)_2O_2^- + Cu(\mathrm{II}) \rightarrow Pice(OH)_2O_2^{\bullet -} + Cu(\mathrm{I})$$

$$Cu(\mathrm{I}) + O_2 \rightarrow Cu(\mathrm{II}) + O_2^{\bullet -}$$

$$2O_2^{\bullet -} + 2H^+ \rightarrow H_2O_2 + O_2$$

$$Cu(\mathrm{I}) + H_2O_2 \rightarrow Cu(\mathrm{II}) + {}^{\bullet}OH + OH^- \quad \text{(Fenton reaction)}$$

$$O_2^{\bullet -} + H_2O_2 \rightarrow {}^{\bullet}OH + OH^- + O_2 \quad \text{(Haber Weiss reaction)}$$

$$Pice(OH)_2O_2^{\bullet} + Cu(\mathrm{I}) + O_2 \rightarrow Cu(\mathrm{II}) + \text{ortho-quinone}$$

The initial electron transfer oxidation of Pice induced by Cu(II) generates the corresponding semiquinone radical anion. Reacting with O_2, the radical undergoes a second electron transfer to form *ortho*-quinone (and *para*-quinone) and $O_2^{\bullet -}$. At neutral pH, $O_2^{\bullet -}$ protonates and forms H_2O_2. H_2O_2 may also be formed by dismutation of $O_2^{\bullet -}$. Thus, H_2O_2 can immediately participate in both Haber Weiss and Fenton-type $^{\bullet}OH$ formation and DNA cleavage reaction. In addition, Cu(II) can be reduced to Cu(I) by Pice, and it is the reoxidation of Cu(I) to Cu(II) that gives rise to $^{\bullet}OH$, the main radical that induces DNA damage. Thus, presumably the mechanism of DNA cleavage induced by Pice-Cu(II) is a complex one involving the Haber Weiss and Fenton reactions and reduction of Cu(II). In addition, *ortho*-quinones are reportedly involved in DNA damage by forming covalent adducts with DNA (4,37,38). In brief, the formation of the hydroxyl radical and the Cu(II)/Cu(I) redox cycle play a key role in inducing DNA damage.

Among the oxygen radicals, the hydroxyl radical is the most electrophilic, with a high reactivity, and therefore possesses a small diffusion radius. Thus, to cleave DNA, the hydroxyl radical should be produced in the vicinity of DNA (7). Copper is one of the most redox-active metal ions present in cells and is closely associated with chromatin. Copper concentration in tissues increases in various malignancies (8). Copper ions from chromatin may be mobilized by Res and other polyphenols, resulting in DNA breakage in cells, the hallmark of cell apoptosis (39). Since Res has been detected in tissues such as the kidney and liver, it is reasonable to assume that Res is transported across cell membranes due to its lipophilic nature (40). Pice, as the metabolite of Res, is transported across cell membranes, thus becoming potentially available for cell uptake and intracellular signaling. In addition, the polyphenol curcumin-mediated apoptosis of HL60 cells has been shown to be closely related to the increase in concentration of reactive oxygen species, possibly through the reduction of transition metal ions in cells (41). Therefore, the mechanism of DNA breakage induced by Pice-Cu(II) may be an important pathway through which cancer cells are killed while normal cells survive.

The antineoplastic activity of Pice also involves the intercalation of drugs into DNA, which eventually leads to DNA damage (42). Our results suggest that the intercalation of Pice into DNA occurs, which may be another mechanism of its anticancer activity. The mechanism of DNA damage induced by Pice-Cu(II) is similar to that of known anticancer drugs, such as camptothecin, etoposide and γ-radiation (9). Therefore,

Pice-Cu(II)-mediated DNA cleavage is physiologically feasible and may have biological significance.

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