FTIR ANALYSIS FOR RETINA ASSOCIATED WITH DIABETIC CHANGES AND TREATMENT WITH OAT

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ABSTRACT

Objective: Diabetes is known to induce oxidative stress along with deranging various metabolisms. One of the most serious complications of diabetes, a disease that has seen a worldwide increase in the prevalence, is diabetic retinopathy, which is a leading cause of acquired blindness. The aim of this study is to evaluate the effect of oat on the diabetic-induced oxidative stress and if this can attenuate the development of diabetic retinopathy.

Methods: Changes of retina structure were performed by using the application of Fourier transform infrared spectroscopy.

Results: The results demonstrated that diabetic retinopathy was associated with changes on the retina structure which appear after receiving a single dose of streptozotocin (STZ) 60 mg/kg. These changes clearly appeared in the NH-OH, CH and fingerprint regions. The use of oat in case of diabetic was associated with different beneficial effects on the retina constituents, as showed by the changes toward control of the same Fourier transform infrared spectroscopy bands.

Conclusion: Oat can be considered as a novel treatment modality for diabetic retinopathy and further studies is required to optimize dosing and formulations that are maximally effective.

Keywords: Rats, Diabetic, Streptozotocin, Retina, FTIR-Oat.

INTRODUCTION

Diabetes increases oxidative stress in the retina and in its capillary cells, which considered as one of the major metabolic abnormalities associated with the development of diabetic retinopathy (DR) [1-4]. Diabetic retinopathy is a classic chronic micro vascular complication of the retina caused by the deleterious metabolic effects of hyperglycemia, which results in extensive and early neuro degeneration. Neuroretinal degeneration initiates several metabolic and signaling pathways that participate in the microvasculopathy process as well as in disturbances of the blood-retinal barrier (BRB), a key phenomenon in the pathogenesis of DR [5]. In diabetes, retinal mitochondria become dysfunctional and mitochondrial DNA (mtDNA) is affected [6-8]. All the blood vessels of the retina have tight junctions that help to protect them against leaking, but prolonged high concentrations of glucose damage these tight junctions and the vessels become leaky allowing the fluid and/or blood to seep into the retina, which results in the swelling of the retina [9]. Due to progressive dysfunction, the capillaries die prematurely resulting in ischemia that can be followed by neovascularization and finally retinal detachment and blindness [10].

In the progress of DR, the basement membrane thickens and the blood pressure is altered. In addition pericytes and endothelial cells undergo accelerated apoptosis resulting in pericyte ghosts and acellular capillaries [11]. The leukocytes become less deformable, and retinal leukostasis is increased affecting the endothelial function [12, 13]. In addition to increase in reactive oxygen species (ROS) in the retina, the antioxidant defense system is also compromised in diabetes [14-16].

Cumulative studies demonstrated that dietary fiber can significantly reduce the risk of cardiovascular disease and diabetes [17]. This is due in part to the ability of fiber to reduce postprandial glycaemia and improve long-term glycemic control [18]. It was assumed that the rheological properties of soluble dietary fibers are highly related to their effects on control of the glucose concentration [19]. For instance, the ability of oat-derived β-glucan to reduce postprandial glycemia has been strongly correlated with its viscosity [20], demonstrating an inverse linear relationship between the logarithm of viscosity measures and peak postprandial plasma glucose and insulin responses after consuming various doses of purified oat. Despite these findings, the levels of viscosity required to achieve specific glucose-lowering effects are not well understood. Still, the majority of trials investigating dietary fiber have not represented the principles of polysaccharide solubility and viscosity as the main determinants of its physiological outcome. While a small number of studies have shown the effect of oat on diabetes [21, 22], none examine its effect on the development and progression of DR.

The aim of this study is to evaluate the effect of oat-meal supplementation (10 and 20% w/w) on the diabetic-induced oxidative stress and its potential effect to attenuate the development of DR. The results of this study may provide an alternative for enhancing nutrition and diabetic control during DR.

MATERIALS AND METHODS

Streptozotocin induced diabetic retinopathy and study design

Streptozotocin (STZ) induced DR according to the model previously suggested by Sayed (2012) [23]. Nine-week-old 200±20g male Albino rats were randomly selected from the animal house facility at the Research Institute of Ophthalmology, Giza, Egypt. The animals were kept separately under good ventilation and adequate standard diet. They were housed in specially designed cages and maintained under constant air flow and illumination during the experimental periods. The animals were handled according to the ARVO (The Association for Research in Vision and Ophthalmology) statements for the use of animals in research, and the research protocol was approved by the local ethical committee. The animals were kept under observation for one week prior to the start of the experiment. 10 rats were randomly selected as a control group (group 1), which received a single tail vein injection of 0.801 /L citrate buffer only. The other rats received a single dose of STZ (Sigma S-0130) in citrate buffer pH 4.5 through the indwelling catheters over 2 min, at a fixed dose of 60mg/kg. Only rats, with blood glucose higher than 250 mg/dL after two days were considered as being diabetic in the fasting state. Blood glucose was measured by using Accu-Chek Active GS392 (Germany). Rats with blood glucose levels, lower than 200 mg/dL were excluded from the study. All studies were carried out two days after STZ injection. Diabetic rats were classified to three groups each contains ten rats: group 2, untreated diabetic untreated rats and groups 3, 4 (10 rats each), oat treated diabetic rats, and untreated rats and groups 3, 4 (10 rats each), oat treated diabetic rats.
rats. Rats of these groups were supplemented with oat 10 and 20%, respectively, on the diet (W/W). Treatment was continued for 12 w starting from day two after STZ administration. At the end of the experiment, all groups were killed. Eyes were enucleated, and then opened by corneal section through the ora serrata where the anterior segment constituents can be removed so that the retina is exposed and can easily be obtained. Each retina was immediately processed for IR-characterization; if not, it was kept for 10 min (maximum) in a sterilized dark glass vial, flushed with dry nitrogen gas and stored at 20°C.

**Fourier transforms infrared spectroscopy (FTIR)**

Retinae were freeze-dried separately for 1 h, and then mixed with KBr powder (2 mg retina: 98 mg KBr) to prepare the KBr disks that will be used for the FTIR investigation. FTIR measurements were carried out using Nicolet-iS5 infrared spectrometer (Thermo Fisher Scientific Inc, Madison, USA) with effective resolution of 2 cm⁻¹. Each spectrum was taken from 100 sample interferograms. The spectrometer was subject to a continuous dry N₂ gas purge to remove interference from atmospheric CO₂ and H₂O vapor. The spectra were baseline corrected, then smoothed with Savitsky–Golay filter to remove the noise before Fourier transformation. Three spectra from each sample were obtained and averaged using OriginPro software (Origin Lab Corporation, Northampton, MA, USA) to obtain the final average group spectrum which was normalized according to certain peaks and used in the figures.

**Statistical evaluation**

Data was represented as the mean±SD For comparison between multiple groups the analysis of variance (ANOVA) procedure was used, where a commercially available software package (SPSS-11, for windows) was used and the significance level was set at P<0.05.

**RESULTS AND DISCUSSION**

FTIR spectroscopy is a non-destructive technique, which provides quantitative biochemical information about biological samples. It is a valuable technique due to its high sensitivity in detecting changes in the molecular constituents of tissues.

The FTIR spectra of retina for control, diabetic and diabetic rats supplemented oat 10 and 20%, on the diet (W/W) groups covering the range 4000–1000 cm⁻¹ was shown in fig. 1. The FTIR spectra of retina for control, diabetic and diabetic rats supplemented oat 10 and 20%, on the diet (W/W) groups covering the range 4000–1000 cm⁻¹ was shown in fig. 1. The FTIR spectra of retina for control, diabetic and diabetic rats supplemented oat 10 and 20%, on the diet (W/W) groups covering the range 4000–1000 cm⁻¹ was shown in fig. 1. The FTIR spectra of retina for control, diabetic and diabetic rats supplemented oat 10 and 20%, on the diet (W/W) groups covering the range 4000–1000 cm⁻¹ was shown in fig. 1. The FTIR spectra of retina for control, diabetic and diabetic rats supplemented oat 10 and 20%, on the diet (W/W) groups covering the range 4000–1000 cm⁻¹ was shown in fig. 1. The FTIR spectra of retinae were baseline corrected, then smoothed with Savitsky–Golay filter to remove the noise before Fourier transformation. Three spectra from each sample were obtained and averaged using OriginPro software (Origin Lab Corporation, Northampton, MA, USA) to obtain the final average group spectrum which was normalized according to certain peaks and used in the figures.

**NH-OH region**

Fig. 2 demonstrates that the main band of the control pattern was found at 3445±3 cm⁻¹. After deconvolution procedure this main band was resolved into three bands at 3553±3, 3412±5 and 3235±3 cm⁻¹ that corresponds to stretching OH (υOH) labeled as 1, stretching OH symmetric (υOHsym) labeled as 2 and stretching OH asymmetric (υOHasym) labeled as 3 respectively, as previously mentioned by Devbeshko et al. [24]. After induction of diabetes, there are detectable changes in the retinal structure appeared in NH-OH region. A significant decrease of band position and significant increase in bandwidth of υOH is observed in relation to control which indicates the formation of hydrogen bonds with different structural states. There is a shifting of υOHsym and υOHasymp mode band toward higher wave number, with changes in bandwidth, which indicates that the hydrogen bond has been destructed and/or weakened [25].

After treatment with oat, the υOH and υOHsym mode bands were mimicking the control with changes of bandwidth only as showed in table (1). But υOH sym is very sensitive to diabetic even after oat administration.

The above findings indicate that there are changes in the protein structure of the retina associated with diabetes and oat administration has protective effects on the retina. These findings were supported by the findings of Tapola et al. [21] and Kowluru et al. [26].
and \( \mathrm{\text{gmp}} \)PO\(_2\) in the diabetic group was observed. Also appear of three bands: CH\(_{\text{aldehyde}}\) (labeled as 6) and COC\(_{\text{ester}}\) (labeled as 8) in the diabetic group and 10\% oat and P-D-C \( \text{syn} \) (labeled as 9) in the diabetic group only. These changes confirm the change in the environment rather than a retina function disorder.

**Fig. 4:** Representative IR spectra in the fingerprint region (1800–1000 cm\(^{-1}\)). The numbers above the peaks are to facilitate their assignment and to identify their characteristic absorption bands. The use of oat in case of diabetes was associated with different beneficial effects on the retinal constituents, as showed by the changes toward control of the retina structure. These changes clearly appeared in the NH-OH, CH and fingerprint regions. Th e use of oat in case of diabetes was associated with different beneficial effects on the retinal constituents, as showed by the changes toward control of the retina structure.

In summary, our data demonstrate that the oat-meal nutritional supplementation, which is currently in preclinical trials, has potential effects to maintain the structure and function of the retina of human subjects with long-term diabetes. This is achieved, possibly, via changes in the protein structure of the retina. Supplementation with oat appears as an inexpensive adjunct therapy to inhibit retinal dysfunction.

**CONCLUSION**

STZ-induced DR (60 mg/kg, single dose) was associated with changes in the retina structure. These changes clearly appeared in the NH-OH, CH and fingerprint regions. The use of oat in case of diabetes was associated with different beneficial effects on the retinal constituents, as showed by the changes toward control of the same FTIR bands. In summary, oat can be considered as a novel treatment modality for DR and further studies are required to optimize dosing and formulations that are maximally effective.

**REFERENCES**

Ocular adverse effects of anti-cancer chemotherapy and targeted therapy

Parul Singh1* and Abhishek Singh2*

Abstract

Systemic anti-cancer therapies can produce acute and chronic organ damage. Ocular toxicity induced by anti-cancer chemotherapy is not uncommon, but underestimated and under-reported. The development of more aggressive regimens, use of newer agents and combination chemotherapies have resulted in a significant increase of reported cases of chemotherapies induced ocular side effects. Visual changes have been attributed to a number of chemotherapeutic agents such as antimetabolites, alkylating agents, taxanes and platinum agents. In addition to the eye itself, structures of the skin including the eye lids may be affected. Ocular toxicities induced by chemotherapeutic agents are generally not preventable; therefore clinicians must be aware of potential vision threatening complications. Prompt consultation with an ophthalmologist can lead to early detection, proper diagnosis and appropriate therapeutic measures. Dose reduction or discontinuation of incriminated drugs may help in reducing severity and duration of side effects. An ophthalmologist should be part of team caring for patients undergoing systemic chemotherapy for baseline examination and ongoing assessment. Baseline examination will help to diagnose adverse effects caused later due to chemotherapeutic agents and; diagnose any pre-existing conditions, especially in elderly patients. It is easy to miss association between chemotherapy and visual changes. This article document ocular changes that are believed to be related to the administration of certain chemotherapeutic agents.

1. Alkylating agents - Alkylating agents have the cytotoxic ability to substitute hydrogen atoms in certain organic compounds by alkyl groups. They include platinum complexes, nitrogen mustard derivatives, alkyl sulphonates and nitrosoureas.

A. Platinum complexes

(I) Cisplatin: Cisplatin, a heavy metal compound, is an established drug for treatment of head and neck, lung, cervical, ovarian and testicular cancer, upper gastrointestinal malignancies, osteogenic sarcoma, neuroblastoma, recurrent brain tumors in children, and urinary bladder cancer. Cisplatin is known to produce non-specific macular pigmentary changes more likely to occur at high-dose intravenous regimens. Wilding et al reported 13 women who were treated with high cumulative doses of cisplatin for ovarian tumors. Blurred vision in eight patients, decreased color vision in three patients, irregular pigmentation in the macula in six patients and central scotoma bilaterally [9]. Intra-carotid administration of cisplatin has led to severe ocular and orbital toxicity like ipsilateral retrobulbar blindness [3-5], temporary homonymous hemianopia [6,7] and macular pigmentary changes more likely to occur at high-dose intravenous regimens. Wilding et al reported 13 women who were treated with high cumulative doses of cisplatin for ovarian tumors. Blurred vision in eight patients, decreased color vision in three patients, irregular pigmentation in the macula in six patients and central scotoma bilaterally [9].
leukemia, advanced mycosis fungoides (cutaneous lymphoma) and head and neck cancer. The ocular toxicities caused by methotrexate consists of peri-orbital edema, ocular pain, blurred vision, photophobia, conjunctivitis, blepharitis and decreased reflex tear secretions [20]. When methotrexate is administered by intrathecal route as in acute leukemias, optic neuropathy and intranuclear ophthalmoplegia can develop and, this can be potentiated by concurrent cranial irradiation usually used in such cases [40, 41]. Intra-carotid administration of methotrexate in combination with intravenous cyclophosphamide, resulted in macular edema and retinal pigment epithelial changes in all patients, despite intra-carotid injection of mannitol [42]. A case was also reported by Penjavic et al, in which they described a reduced full field ERG in B-wave amplitude [43].

(II) Pentostatin: It is used for treatment of refractory B-cell chronic lymphocytic leukemia, advanced low grade non-hodgkin’s lymphoma, refractory or relapsed acute leukemias and mycosis fungoides. The ocular toxicities caused include diplopia, photophobia and, decreased visual acuity secondary to optic neuritis with or without disc edema or cortical blindness [44].

(II) Pentostatin: Pentostatin is used for treatment of hairy cell leukemia, cutaneous T-cell lymphoma and chronic lymphocytic leukemia. It has been found to be associated with abnormal vision, amblyopia, conjunctivitis, dry eye, problems with lacrimation, photophobia, retinopathy and watery eyes [45,46].

3. Mitotic inhibitors
A. Taxanes:
(I) Paclitaxel: Paclitaxel is known to produce neurotoxicity when used for its indications in metastatic or relapsed breast cancer and advanced ovarian cancer [47]. Both transient scintillating scotoma and visual impairment have been reported after its use [48]. Other ocular side-effects induced by paclitaxel include photosensitivity and possible ischemic optic neuropathy.

(II) Docetaxel: It is indicated in locally advanced, metastatic and refractory breast cancer, advanced gastric and gastro-esophageal adenocarcinoma, locally advanced head and neck cancer, metastatic prostate cancer and locally advanced or metastatic non-small cell lung carcinoma. S Kolink and Doughman reported a case of erosive conjunctivitis and punctal stenosis secondary to docetaxel administration [49]. Esmaeili et al reported canalicular narrowing and naso-lacrimal duct obstruction in three patients [50, 51].

B. Plant alkaloids:
(I) Vincristine, vinblastine, vindesine, vinorelbine: They are used for acute lymphoblastic leukemia, ewing’s sarcoma, hodgkin’s disease, non-hodgkin’s disease, lung cancer, breast cancer and soft tissue sarcomas. Cranial nerve palsies include ptosis, internal ophthalmoplegia, corneal hyperesthesia and lagophthalmos [10].

C. Topoisomerase Inhibitors:
(I) Topoisomerase inhibitor II: Etoposide is a topoisomerase-II inhibitor. Intra-arterially administered etoposide results in arterial thrombosis [59] which can affect the eye by occluding central retinal artery. Etoposide is used of treatment of retinoblastoma in combination with cisplatin or carboplatin. Due to synergistic effects with cisplatin two cases of retinal toxicities have been reported [60].

(II) Topoisomerase inhibitor I: Irinotecan and topotecan are topoisomerase-I inhibitors used for cytotoxic treatment of carcinoma. They do not have any significant ocular adverse effects reported yet.

4. Antibiotics
A. Anthracyclines:
(I) Doxorubicin: It is used in combination regimens in the treatment of breast cancer, ovarian cancer, non-hodgkin’s lymphoma, sarcoma and acute leukemia. Excessive lacrimation and conjunctivitis have been reported as its ocular adverse effects [61].

(II) Mitomycin-C: Mitomycin-C is used in combination chemotherapy regimens in gastric, pancreatic, colon, lung, urinary bladder, breast and cervical cancer. It is also used as hypoxic cell selective cytotoxic agent in combination with radiation therapy in anal and head and neck cancers. The only known ocular toxicity after systemic use of mitomycin-c is blurred vision [61]. All other severe ocular toxicities were reported after topical use in ophthalmologic surgeries.

(III) Mithramycin (Plicamycin): Mithramycin is used in treatment of hypercalcemia of malignancies and for testicular cancer. There was a single case reported of peri-orbital pallor in absence of anemia as ocular adverse effect [20].

5. Hormonal agents
A. Tamoxifen: It is used as adjuvant therapy of estrogen dependent breast carcinoma. In 1978, Kaiser-Kupfer and Lippman described four patients who developed keratopathy and retinopathy after tamoxifen use [62,63]. Decreased visual acuity, bilateral macular edema, retinal yellow-white dots and corneal opacities, bilateral optic neuritis and retinal hemorrhages has also been reported [64, 65]. Gorin et al described intra-retinal crystals and posterior sub-capsular opacities with tamoxifen usage [66]. On the whole, the most common tamoxifen induced ocular toxicities remain to be the retinopathy and cataract, lesions of cornea and optic nerve.

B. Anastrazole: The incidence of cataract with anastrazole usage was found to be lesser than with tamoxifen. The ATAC (Arimidex, Tamoxifen, alone or in combination) trial randomized patients with hormone receptor positive localized breast cancer to five years of anastrazole or tamoxifen use. Cataracts were described in 6% patients receiving anastrazole versus 7% receiving tamoxifen [67].
predisposed patients are screened and examined regularly during
and after chemotherapeutic therapy. Reporting of ocular adverse
effects of chemotherapy should be encouraged for definite analysis
of the burden. Anticipation of various treatment related toxicities
may also provide the opportunity for pharmacists to develop
intervention strategies that could minimize expected adverse
effects. On the whole, oncologist and ophthalmologist should
work together in order to prevent irreversible ocular toxicities of
chemotherapeutic agents and to determine true cause of visual
disturbance.

Table 1: Common ocular adverse effects and anti-cancer drugs
associated.

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FT-IR Spectroscopy in Medicine

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1. Introduction

Infrared spectroscopy has been widely applied for the characterisation of various substances. Due to its sensitivity to the chemical information and architecture of the molecule, infrared spectroscopy can play an important role in new applications such as in the life-science field and not only in the traditional fields of physics and chemistry. Spectroscopic techniques are simple, reproducible, non-destructive without particular sample preparation. As a result, they provide information for the functional groups, bonds and molecular structure.

Herschel discovered the existence of infrared radiation when he tried to measure the heat produced by separate colors of a rainbow spectrum in 1800. He noted that the highest temperature fell beyond the red end of the spectrum, implying the existence of invisible light beyond the red. Herschel termed this light calorific rays. Infrared spectra originate on the vibrational motions of atoms in chemical bonds within molecules. When a beam of light containing the IR radiation band is passed through a sample, light energy from the photons is absorbed by the chemical bonds and excites the vibrational motions. As a molecule absorbs radiation at a specific frequency, it produces a band in the infrared spectrum at the corresponding wavenumber. The approximate position of an infrared absorption band is determined by the vibrating masses and the chemical bonds (single, double, triple). The exact position of the band depends also on electron withdrawing or donating effects of the intra- and intermolecular environment and coupling with other vibrations. The strength of absorption increases with increasing polarity of the vibrating atoms. The modes of vibration in a molecule that can absorb infrared radiation are many and increase with increasing complexity of the molecule. The vibrations that contribute to the spectrum are bending and stretching vibrations between atoms and rocking, twisting and wagging of a functional group (Theophanides, 1984; Goormaghtigh et al., 1999).

Fourier transform infrared spectroscopy is preferred over dispersive or filter methods of infrared spectroscopy due to the sensitivity and the rapid data collection. The FT-IR spectrometer uses an interferometer to modulate the wavelength from a broadband infrared source. Light emitted from the infrared source is split by a beam splitter. Half of the light is reflected towards a fixed mirror and from there reflected back towards the beamsplitter where about 50% passes to reach the detector. The other half of the initial light intensity passes the beam splitter on its first encounter, is reflected by the moving mirror back to the beamsplitter where 50% of it is reflected towards the detector (Figure 1). When the two
chains (e.g. linolenic, arachidonic, etc.), and/or due to lipid peroxidation. For this reason, the intensity of this band can be used as a diagnostic band of LDL.

The $CH_2$ asymmetric ($2929 \text{ cm}^{-1}$) and symmetric ($2851 \text{ cm}^{-1}$) stretching vibrations give intense bands, while asymmetric $CH_3$ stretching at $2955 \text{ cm}^{-1}$ and symmetric stretching at $2865 \text{ cm}^{-1}$ bands are seen as shoulders. The bands arise from lipids, phospholipids and membranes. The intensity of symmetric and asymmetric stretching vibrations of $CH_2$ and $CH_3$ reflect lipid hyperoxidation (Liu et al., 2002). The increase in the intensity of the bands in coronary artery shows that the environment is less lipophilic due to fragmentation of the lipoproteins and accumulation of free cholesterol and cholesterol esters in the atheromatous core, as a result membrane fluidity changes significantly (Anastassopoulou and Theophanides, 1990).

Significant changes are also observed in the infrared absorption bands in the region $1800-1500 \text{ cm}^{-1}$, as it is shown in the spectra. The presence of cholesterol esters and other ester-containing compounds is also identified from the carboxyl ion ($-O-C=O$) stretching absorption at $1735 \text{ cm}^{-1}$ apart from the $C=C-H$ stretching band ($3077 \text{ cm}^{-1}$). This band confirms lipid hyperoxidation and the increased intensity of the band indicates increased LDL concentrations according to the blood analyses of the patient. All the patients who underwent coronary endarterectomy showed higher intensity in the specific band. The bands at $1735$ and $3077 \text{ cm}^{-1}$ can be used as indicators for LDL cholesterol of patients.

The Amide I absorption band, arises mainly from the $C=O$ stretching vibration with minor contributions from the out-of-phase CN stretching vibration, the CCN deformation and the NH in-plane bend. The Amide I band is down-shifted near $1635 \text{ cm}^{-1}$, approximately $20 \text{ cm}^{-1}$ difference compared to the absorption of a normal tissue ($1656 \text{ cm}^{-1}$), suggesting a
conformational change in α-helixes (Anastassopoulou et al., 2009). The shifting of the Amide I band suggests that proteins lose their structure from α-helix to random coil due to fragmentation induced from free radical reactions. The exposure of proteins to free radicals induces secondary structural changes, since secondary structure is stabilized by hydrogen bonding of peptide backbone. Proteins are organized into α-helixes, but the hydrogen bond is damaged, so the chains open and are more prone to free radicals, leading to the change of α-helix to random coil.

The change of dipole moment of peptide bond, as it is shown in equation [1] at resonance structures, leads to a change in the orientation of amino groups (NH) to the carbonyl group C=O, resulting in the destruction of alpha –helix and the secondary structure of proteins.

\[ \text{C}_{\alpha} \text{N} \leftrightarrow \text{C}_{\alpha} \text{O} \]  

(1)

The band at 1537 cm\(^{-1}\) is attributed to the vibrations of Amide II. The amide II mode is the out-of-phase combination of the NH in plane bend and the CN stretching vibration with smaller contributions from the CO in plane bend and the NC stretching vibrations. The bands of Amide I and Amide II are representative of –NH-CO- vibrations of proteins (Theophanides et al., 1988). The analysis of the spectra by Fourier self-deconvolution was used to enhance resolution in the region 1800-1500 cm\(^{-1}\) (figure 6 and 7).

![FT-IR spectra of coronary artery](image1)

Fig. 6. A: FT-IR spectra of coronary artery in the region 1800-1500 cm\(^{-1}\), B: Deconvolution of the spectra in the same region.

As it is determined from the deconvolution in the spectra of the coronary artery of a patient, the band at 1781 and 1768 cm\(^{-1}\) is attributed to the carboxyl anions –COO- of the
Large bilateral foveal cysts in the inner retina of a patient treated with tamoxifen, diagnosed with Fourier-domain optical coherence tomography

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Purpose: To report a case of a patient receiving tamoxifen with visual deterioration and describe the unusual optical coherence tomography (OCT) findings.

Method: Observational case report.

Results: A 55-year-old female patient was referred to our department complaining of gradual visual deterioration in both eyes. Medical history was unremarkable apart from breast cancer for which she had received tamoxifen for 10 years (mean dosage 20 mg/day). Best corrected visual acuity was 20/400 in her right eye and 20/40 in her left eye. Fundoscopy in both eyes was without any obvious signs of maculopathy. However, Fourier-domain OCT demonstrated bilateral extensive areas of disruption in inner retinal layers without any signs of crystalline retinopathy. Six months after the cessation of tamoxifen, the situation remains unchanged.

Conclusion: Patients receiving tamoxifen should be monitored with high-resolution OCT for fundoscopically invisible changes in the inner retinal layers, the progression of which may seriously affect the patient’s vision and subsequently their quality of life.

Keywords: maculopathy, OCT

Introduction
Optical coherence tomography (OCT) is an interferometric imaging technique which produces cross-sectional images of the retina and in recent years has been widely used in the study of macular diseases. Tamoxifen is a nonsteroidal antiestrogen, which belongs to the family of selective estrogen receptor (ER) modulators, drugs that have the ability to occupy ERs, acting as ER antagonists in breast tissue. As a result, tamoxifen is widely used as adjuvant endocrine therapy for women with hormone-responsive breast cancer.

Incidence of ocular toxicity among patients receiving tamoxifen is rare (0.6%); cataract, vortex keratopathy, optic neuritis and retinopathy are the most common manifestations. We report, herein, a case of a 55-year-old woman who presented with bilateral, severe visual deterioration after treatment with tamoxifen and describe the Fourier-domain OCT findings.

Case report
A 55-year-old female patient was referred to our department complaining of gradual visual deterioration in both eyes. She had been seen by her local optician and ophthalmologist who had failed to explain the reduction in her vision. Medical history was unremarkable apart from breast cancer, diagnosed in 2002, for which she received...
tamoxifen for 10 years (mean dosage 20 mg/day), which was withdrawn only 15 days prior to her visit. The reason for the prolonged reception of tamoxifen is not known, since her oncological medical record was not available. The patient’s best corrected visual acuity at presentation was 20/400 in her right eye and 20/40 in her left eye. Intraocular pressure was 17 and 13 mmHg in her right and left eye, respectively. Examination of the anterior segment was unremarkable. Fundoscopy in both eyes was without any obvious signs of maculopathy (Figure 1). Posterior vitreous was attached to the retina in both eyes. Fourier-domain OCT was used for examination of the fovea; it demonstrated bilateral extensive areas of disruption in inner retinal layers (Figure 2A and B). The OCT type that was used for the diagnosis was RTVue model RT-100 by Optovue (Fremont, CA, USA). Through the OCT, the dimensions of the foveal cystoid spaces were measured and found to be 78 µm × 823 µm in the right eye and 74 µm × 607 µm in the left eye. Measurement was achieved by using the manual program of the instrument. Fluorescein angiography did not show any leakage and excluded the diagnosis of type 2 retinal telangiectasia. Six months after the cessation of tamoxifen, the situation remains unchanged.

Discussion

OCT is an interferometric imaging technique which produces cross-sectional images by mapping the depth-wise reflections of low-coherence laser light from tissue. Fourier- or spectral-domain OCT refers to Fourier transformation of the optical spectrum of the low-coherence interferometer. Imaging of multilayer objects, such as retina, results in various modulation periodicities representing the depth of each layer, with amplitude of the spectrum modulation proportional to the reflectivity of the layer. The greatest advantage of spectral-domain OCT, over conventional time-domain OCT is the increase in scan speed. With the spectral-domain OCT, imaging with 25,000–100,000 axial scans per second is routinely possible. This is more than 100 times faster than the time-domain technique.1,2

Tamoxifen is a nonsteroidal antiestrogen, which belongs to the family of selective ER modulators, drugs that have the ability to occupy ERs (ERα and ERβ), acting as ER antagonists in breast tissue. As a result, tamoxifen is widely used as adjuvant endocrine therapy for women with breast cancer. ERα and ERβ are also present within the neural retina and the pigment epithelium of men and women, where tamoxifen is reported to decrease glutamate uptake.3

Incidence of ocular toxicity among patients receiving tamoxifen is rare, approximately 0.6%. Ocular manifestations of tamoxifen include tamoxifen retinopathy, corneal epithelial deposits which can lead to vortex keratopathies, induction of posterior subcapsular cataract, and clinically evident optic neuritis.

Tamoxifen retinopathy is typically characterized by the presence of small refractile deposits in the nerve fiber and inner plexiform layers, primarily in the perifoveal area. Refractile deposits do not cause significant visual impairment; histopathologically, they are suggested to represent axonal degeneration, mainly in the nerve fiber layer in the parafoveal region. Due to tamoxifen’s amphiphilic nature, it is suggested that it binds to polar lipids, accumulates in lysosomes, and causes cell oxidative damage.4 Numerous researchers have suggested that tamoxifen retinopathy is not caused by actions of tamoxifen on ERs, but stems instead from tamoxifen’s cationic amphiphilic properties, which resemble those of chloroquine.3

All cases of tamoxifen maculopathy studied with spectral-domain OCT in the literature include crystalline maculopathy

![Figure 1 Optical Coherence Tomography of the right eye.](image)

**Abbreviations:** ELM, External Limiting Membrane; GCL, Ganglion Cell Membrane; ILM, Internal Limiting Membrane; INL, Inner Nuclear Layer; IPL, Inner Plexiform Layer; IS/OS, Inner-Outer Segment Photoreceptors; NFL, Nerve Fiber Layer; OPL, Outer Plexiform Layer; ONL, Outer Nuclear Layer; PE, Retinal Pigment Epithelium.
with refractile deposits, accompanied by unilateral foveal cysts of small size. In all cases, the described lesions consisted of hyperreflective substances in combination with outer retinal atrophy, photoreceptor disruption, and defects in the outer retinal layers which caused the decrease in the visual acuity of the patients. In our case, interestingly, the retinal damage concerned only the inner layers, without any refractile deposits or outer retinal defects, giving the impression of a pseudohole from which tamoxifen maculopathy can be differentiated by the absence of posterior vitreous detachment and the normal foveal contour. The absence of refractile deposits, posterior vitreous detachment, and outer retinal defects was responsible for the almost “normal appearance” of the macula in the fundoscopy. However, the permanent bilateral reduction in the vision of our patient associated with subtle fundoscopic changes emphasizes the role of early detection of inner foveal damage with high-resolution OCT. Patients receiving tamoxifen should be monitored with high-resolution OCT for fundoscopically invisible changes in the inner retinal layers, the progression of which may seriously affect a patient’s vision and subsequently their quality of life.

**Disclosure**

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

**References**

Importance of Tissue Preparation Methods in FTIR Micro-Spectroscopical Analysis of Biological Tissues: ‘Traps for New Users’

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Abstract

Fourier Transform Infrared (FTIR) micro-spectroscopy is an emerging technique for the biochemical analysis of tissues and cellular materials. It provides objective information on the holistic biochemistry of a cell or tissue sample and has been applied in many areas of medical research. However, it has become apparent that how the tissue is handled prior to FTIR micro-spectroscopic imaging requires special consideration, particularly with regards to methods for preservation of the samples. We have performed FTIR micro-spectroscopy on rodent heart and liver tissue sections (two spectroscopically very different biological tissues) that were prepared by desiccation drying, ethanol substitution and formalin fixation and have compared the resulting spectra with that of fully hydrated freshly excised tissues. We have systematically examined the spectra for any biochemical changes to the native state of the tissue caused by the three methods of preparation and have detected changes in infrared (IR) absorption band intensities and peak positions. In particular, the position and profile of the amide I, key in assigning protein secondary structure, changes depending on preparation method and the lipid absorptions lose intensity drastically when these tissues are hydrated with ethanol. Indeed, we demonstrate that preserving samples through desiccation drying, ethanol substitution and formalin fixation and have compared the resulting spectra with that of fully hydrated freshly excised tissues. We have systematically examined the spectra for any biochemical changes to the native state of the tissue caused by the three methods of preparation and have detected changes in infrared (IR) absorption band intensities and peak positions. In particular, the position and profile of the amide I, key in assigning protein secondary structure, changes depending on preparation method and the lipid absorptions lose intensity drastically when these tissues are hydrated with ethanol. Indeed, we demonstrate that preserving samples through desiccation drying, ethanol substitution and formalin fixation significantly alters the biochemical information detected using spectroscopic methods when compared to spectra of fresh hydrated tissue. It is therefore imperative to consider tissue preparative effects when preparing, measuring, and analyzing samples using FTIR spectroscopy.

Introduction

Fourier transform infrared (FTIR) spectroscopy and imaging is an emerging technique in the field of ex vivo diagnostics. In the last twenty years, the infrared spectra of single cells and intact tissues originating from dozens of species and cell types have been analyzed by several groups.
These studies have not only provided important information regarding the macromolecular contents and their distribution in a cell or tissue sample but have also demonstrated the ability of FTIR spectroscopy to differentiate between diseased and non-diseased states [7–10], determine cell cycle stage [11] and monitor cell death [12]. By measuring the absorption of infrared light by a sample, the characteristic energies and intensities of absorbance bands of cellular macromolecules can be detected and assigned, including carbohydrates [13–15], lipids [16], proteoglycans [17,18], collagens [14,19,20], nucleic acids [21], and proteins [20]. Moreover, detail about the structure and local environment of these macromolecules can also be elucidated. FTIR spectroscopy requires no pre-analytical chemical modification and is relatively inexpensive, rapid and can operate in an automated fashion. This also allows for tissues to undergo pathological examination after infrared spectroscopy with no danger of the process compromising the tissue. Therefore, FTIR offers an extremely attractive complementary technique in many diagnostic settings.

However, it has become apparent that how the tissue is handled prior to FTIR imaging requires a number of considerations that are relatively unimportant when using conventional microscopy methods [22–24]. These include sample thickness, hydration and the interference caused by external substances such as dyes and culture media [3]. Moreover, infrared spectra of cells and tissue samples are prone to baseline distortions from Mie scattering which is a result of sample morphology [25,26]. FTIR spectroscopy is also limited to a penetrative depth of approximately 10 μm because of detector saturation and thus require either thin sectioning or the measurement performed using attenuated total reflectance (ATR) spectroscopy where the depth of penetration is approximately 3 μm depending on the wavelength, refractive index of the IR window and sample material and the angle of incidence of the IR beam into the crystal.

There is the potential to use this technique to retrospectively analyse archived biological tissues. However, over recent decades it has become apparent that sample collection, processing and preservation have the potential to influence the biochemical spectra of biological tissues (for example Mie scattering effects) [3,27]. Hence, in order to utilize FTIR micro-spectroscopy to accurately assess biological tissue samples, it is imperative to understand how different methods of preparation for analysis impact on the biochemical spectra and thus avoid misleading interpretation of data analysis.

The aim of the present study was to evaluate several different sample preparation techniques for FTIR examination of heart and liver tissue sections (two spectroscopically very different biological tissues) taken from healthy adult rats. The sections were prepared for FTIR analysis by four different methods typically used for histological preparation: A) desiccation dehydration, B) dehydration by ethanol substitution, C) formalin fixation and D) direct measurement of sectioned hydrated tissue.

Materials and Methods
Sample preparation for FTIR transflection spectroscopy of dried samples

The collection of organs for this study was harvested from an unrelated project utilising rats and the entire study was specifically approved by the Monash University, ‘School of Biomedical Sciences Animal Ethics Committee A’. The animal care and experimental procedures were carried out in accordance with the Australian ‘Code of Practice for the Care and Use of Animals for Scientific Purposes’. These animals were sacrificed by 100mg/kg pentobarbitone intraperitoneal injection and organs collected immediately afterwards.

At postmortem, liver and heart were immediately excised from adult rats, trimmed of connective tissue and sliced into pieces of approximately 1 mm in thickness. The tissue pieces were
the other two dried tissue preparations, significant wave number shifts and splitting of the CH₂ and CH₃ band features were observed (Table 1 and Table 2). In the case of the formalin fixed tissues, this can be attributed to the cross-linking action of the fixative on proteins. The spectra of the formalin fixed tissues also exhibited band shifts for many of the peaks in the spectral region from approximately 1330–1150 cm⁻¹ which can be associated with amide III modes and in particular with the spectrum of collagen.

In the heart tissue spectra two bands characteristic of collagen at 1205 and 1285 cm⁻¹ were observable in the wet tissue spectra but only weakly detected in the formalin fixed and ethanol dehydrated tissue spectra. These collagen marker bands were apparently absent from the desiccated sample spectra. Bands in the region 1160–950 cm⁻¹ are largely due to stretching vibrations of CO groups in carbohydrates although CO in the ribose backbone of nucleic acids and the νsPO₂⁻ (symmetric phosphate stretching) modes also contribute absorbance at around 1080 cm⁻¹. In the desiccated and ethanol treated liver tissue samples the triplet of bands due to glycogen (1154, 1080, 1035, 1022 cm⁻¹) appeared strong in the spectra but were weak in the wet spectra and altered to a broad band feature in the formalin fixed tissues. Establishment of glycogen levels is important for the diagnosis of many diseases [35,36]. Clearly the spectra indicate that formalin fixation has some effect on glycogen chemistry in tissues. In this regard, there are conflicting reports in the literature in relation to the effects of formalin on tissue glycogen. Formalin has been used as a method for assay extraction of glycogen from tissues [37], whereas others have reported that formalin is effective for localizing and preserving glycogen content in tissues [38]. Our findings support the latter; there was no evidence that formalin removes glycogen from either the heart or liver tissue.

Conclusions

To our knowledge, this is the first time in the FTIR micro-spectroscopic examination of mammalian tissues, that the spectroscopically detectable variations in sample chemistry arising from different preparation techniques have been described. The choice of sample preparation and fixation should be made carefully when commencing new studies on these tissue types to ensure that the macromolecular content of interest is not unduly perturbed by the sample preparation process. Prospectively, the best approach is to use freshly excised tissues for immediate analysis. This is not to say however that studies based on the extremely valuable and pre-existing collections of archived tissue should not be conducted. Rather, care must be taken to ensure that the biochemical alterations which have occurred in preparing these tissues for archiving are fully understood and considered.

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Author Contributions

Conceived and designed the experiments: VZ DRW KRB BRW MJB. Performed the experiments: VZ DRW. Analyzed the data: VZ DRW KRB. Contributed reagents/materials/analysis tools: JTP BRW. Wrote the paper: VZ DRW KRB MJB.

References

FTIR Assessment of the Effect of Ginkgo biloba Leave Extract (EGb 761) on Mammalian Retina

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Abstract  Ginkgo biloba extract has been therapeutically used for several decades to increase peripheral and cerebral blood flow as well as for the treatment of dementia. The extract contains multiple compounds such as flavonoids and terpenoids that are thought to contribute to its neuroprotective and vasotropic effects. In this study, we investigated the effect of prolonged administration of EGb 761, up to 10 weeks, on mammalian retina using Fourier transform infrared spectroscopy (FTIR). Two main groups were involved in this study: the normal group (n = 10); and EGb-administrated group (n = 50) that received—orally—a dose of 40 mg/kg/day EGb 761. The results demonstrated that EGb administration was associated with different beneficial effects on the retinal constituents especially the underlying amide I protein secondary structure components as well as the NH-OH region. It concluded that the optimum daily administration period of EGb (40 mg/kg) for ophthalmic applications that targeting the retina ranges from 5 to 8 weeks.

Keywords  Eye • Retina • Ginkgo biloba • Protein secondary structure • FTIR • Spectroscopy

Introduction

Ginkgo biloba has been used worldwide as herbal medicine, and is one of the most commonly prescribed botanical drugs [1]. Many clinical therapeutic applications have already been reported, including cerebrovascular insufficiency [2], cognitive speed [3], dementia and Alzheimer’s disease [4], and aging damages [5]. These useful effects are due to reduction of platelet-activating factor associated abnormalities [6]; scavenge against free radicals [7]; and improvement of peripheral circulation [8].

Standardized Ginkgo biloba extract (EGb 761) typically contains 24% Ginkgo flavone glycosides (flavonoids, Ginkgo heterosides), 6% terpenes [9], and controlled amounts of other substances, including proanthocyanidines and organic acids [10]. It has been proposed that antioxidant mechanisms underlay some of the therapeutic effects of EGb, with a growing body of evidences that point toward free radical and lipid peroxidation reactions as participants in peripheral and central vascular diseases and neuronal damage. Ginter et al. [11] and Maitra et al. [12] suggest that the flavonoids, particularly quercetin and myricetin, are the beneficial constituents of EGb in preventing free radical induced neuronal damage. Diabetes mellitus characteristically exhibits signs of oxidative stress in the retina, resulting in thickened basement membranes and altered retinal vessel permeability. In diabetic animal models, EGb improved retinal functioning by decreasing oxidative retinal stress [13]. In the same context, different animal studies considering the ischemic retina were demonstrated that pre-treatment with EGb761 was found to inhibit lipid peroxidation, and reduce the functional and morphological impairments when chronically applied for 10 days [14] or acute for 5 min [15]. Also, EGb prevents the inflammatory reaction associated with retinal detachment after induction of vitreoretinopathy leading to decrease the frequency of retinal detachment [7].

Other studies of antioxidant status and macular degeneration note that elderly subjects with a high antioxidant
1,800–1,000 cm\(^{-1}\) (fingerprint region). Within the fingerprint region, the amide I band which appears at 1,650 cm\(^{-1}\) will be considered separately since it consists of several underlying bands which correspond to different protein secondary structural components.

**NH-OH Region**

Figure 1 shows that the main band of the normal pattern was found at 3,297 ± 2 cm\(^{-1}\). The curve enhancement procedure resolved this band into four structural components (Table 1) that were centered at 3420 ± 3, 3290 ± 2, 3181 ± 2, and 3061 ± 3 cm\(^{-1}\), these bands correspond to stretching OH (labeled as 1), stretching OH symmetric (labeled as 3), stretching NH symmetric (labeled as 4), and CH ring (labeled as 5), respectively, as previously mentioned by Dovbeshko et al. [17]. From this figure, two different observations could be obtained; for the first two weeks of EGb administration, the contour of the NH-OH region was also resolved into four structural bands with different stretching NH asymmetric (labeled as 2) in W-1 and W-2 while, there is restriction in the symmetric stretching mode of NH bond in W-1 and that of OH bond in W-2. The second observation is related to the rest of the groups received EGb, where the number of the estimated structural bands was increased to five components with different characteristics. Stretching OH mode of vibration is sensitive to EGb administration periods from W-3 to W-9. Besides band splitting was noticed during these administration periods, the vibrational frequencies of the OH stretching bands were increased (Table 1). At W-10, although there was an increase in the vibrational frequency (3,545 ± 1 cm\(^{-1}\)), it is concomitant with reduced bandwidth (63 ± 3 cm\(^{-1}\)). In this range, as the frequency shift to the lower range, it indicates larger contributions of asymmetric O–H mode of vibration.

Stretcching NH asymmetric vibrational mode was observed in all groups which received EGb except at W-3 and W-9, with the highest vibrational frequency noticed at W-10. Moreover, its bandwidth was fluctuated with the administration periods. Stretching OH symmetric band that noticed at 3,290 ± 2 cm\(^{-1}\) in the normal with bandwidth of 141 ± 5 cm\(^{-1}\), was also observed at: W-1; with reduced frequency and higher bandwidth, W-3; mimicking the normal characteristics, W-9 and W-10; with reduced bandwidth. On the other hand, the stretching NH symmetric band shows three characteristics that can be related to EGb administration periods, at W-2 and W-3; this band has the same band position and bandwidth as the normal, at W-4 up to W-7 of EGb administration; there was significant increase in both band position and bandwidth. Finally, at W-8 till W-10 of administration there was significant increase in bandwidth only. The band position of the C–H ring was increased after different periods of EGb administration except for W-3 while the bandwidth was significantly increased for all administration periods (Table 1).

**C–H Stretching Region**

The infrared absorption pattern of normal retina that was shown in Fig. 2, was characterized by three absorption bands in the IR range 3,000–2,800 cm\(^{-1}\). The curve enhancement procedure that used to resolve any overlapping peaks confirms the presence of these three bands that were centered at 2,965 ± 2 cm\(^{-1}\) with bandwidth of 23 ± 2 cm\(^{-1}\), 2,926 ± 3 cm\(^{-1}\) and its bandwidth is 31 ± 2 cm\(^{-1}\) and 2,862 ± 3 cm\(^{-1}\) with corresponding bandwidth of 33 ± 2 cm\(^{-1}\). These bands can be assigned as CH\(_3\) asymmetric, CH\(_2\) asymmetric, and CH symmetric (due to CH\(_3\) of protein and CH\(_2\) of lipid), respectively, as previously mentioned by Severcan et al. [18].

The administration of EGb has no effect on this region either on band position or on bandwidth, where the average wavenumber and bandwidth (BW) of CH\(_3\) asymmetric, CH\(_2\) asymmetric and CH symmetric during EGb administration were 2,965 ± 1 cm\(^{-1}\) (BW = 22 ± 1 cm\(^{-1}\)), 2,926 ± 1 cm\(^{-1}\) (BW = 31 ± 2 cm\(^{-1}\)), and 2,862 ± 2 cm\(^{-1}\) (BW = 33 ± 2 cm\(^{-1}\)), respectively.

**Fingerprint Region**

In the wavenumber range 1,800–1,000 cm\(^{-1}\), the vibrational motion around the ester C=O (due to lipid, labeled as 1) of the normal retiniae is restricted and the band not
content was significantly increased at W-1 (transitionally) as well as during the administration interval from W-5 to W-8. On the other hand, the content of β-sheet was significantly reduced from W-1 to W-3 and from W-5 to W-8. β-turn structural content was significantly increased from W-2 to W-4 while reduced from W-7 till the end of the experiment.

**Discussion**

The results of this study revealed that the NH-OH region of FTIR spectra (Fig. 1) of the retinal tissue is sensitive to systemic EGb administration. As shown in Table 1, this region shows the same characteristics during the overall administration range from W-4 till W-8. During this administration interval, four conclusions were noticed and related to: stretching O–H band that was splitted into two bands with higher wavenumber, the stretching N–H asymmetric band was detected throughout this interval, broadening in the stretching N–H symmetric bandwidth and shifting of C–H ring band into higher wavenumber with broadening in its bandwidth. These findings indicated that there are different structural configurations and conformations that co-exist in the system which can be ascribed to the protective effects of EGb. These findings were supported by the findings of Barth [19] and Yang et al. [20] where the splitting of the band is indicative for the presence of several structural and/or conformational changes. The NH bond exists in several membrane constituents that include protein and lipid. The detection of NH asymmetric stretching mode from W-4 to W-8 give the impetus of the formation of hydrogen bond and this hydrogen bond most probable to stabilize protein structure [19]. Moreover, the administration of EGb has no effect on the C–H stretching region arising from lipid and protein either in band position or in bandwidth as demonstrated in Fig. 2.

EGb administration was associated with different distribution of protein secondary structural components as shown in (Fig. 4). Based on Table 3, the content of these structural components was characterized by four observations; firstly, transitional increase in protein solubility at W-1 due to the transitional increase in α-helix content and reduced β-sheet content. It was reported that the increased β-sheet content resulted in the more insoluble protein [21]; secondly, during the administration interval from W-2 to W-4, β-turn structure was increased. β-turns are the smallest type of protein secondary structure, joining other elements of secondary structure such as α-helix and β-sheets and abruptly change the direction of the polypeptide chain and may dictating the folding of longer polypeptide chains [22, 23]. β-turns are common conformations that enable protein to adopt globular structures, and may serve as a nucleation site for folding/refolding of proteins [24]. Their formation is often rate limiting for folding where protein stability is intimately connected with protein folding. Accordingly, retinal proteins are more folded/stable during the EGb administration interval W-2 to W-4. Thirdly, from W-5 to W-8, the protein solubility was increased, as in W-1, due to increased α-helix content that concomitant with the reduction in the β-sheet content. The final observation was noticed at W-9 to W-10 where α-helix and β-sheet contents were mimicking the normal value but decreased β-turn structure still propagating from W-7, this may be indicative that retinal proteins are become less folded. It is also interesting that in W-4 of EGb administration, the protein secondary structure components show the presence of a band near 1,665 cm−1 that assigned as turn structures. These turn structures favor the formation of hydrogen bonding network and contribute to the stability of β-sheet [25]. It can be concluded that EGb administration was associated with enhancement in the retinal protein solubility/stability from W-1 to W-8. It is also noticed that these variations in retinal protein characteristics were concomitant with reduced β-sheet content. Although β-sheets are important for normal biological activities, they are involved in many diseases including cancer and neurodegenerative diseases. There are two types of β-sheets, intramolecular (with vibrational frequency range 1,630–1,620 cm−1) and intermolecular (with vibrational frequency <1,620 cm−1). The former type is associated with protein folding and is detected in all groups received EGb as well as in the normal one, while the later type is associated with biomolecular recognition, protein quaternary structure, protein–protein interactions, and peptide and protein aggregation. This intermolecular β-sheet was found in the normal retinal proteins (at 1,619 cm−1) as well as at W-4 (at 1,618 cm−1) of EGb.

Table 3  Summary of curve fitting analysis of retinal amid I bands which show the individual protein secondary structure components that expressed as the area percentage of the total band areas

<table>
<thead>
<tr>
<th></th>
<th>α-helix (%)</th>
<th>β-sheet (%)</th>
<th>β-turn (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>48.7 ± 6</td>
<td>37.1 ± 4</td>
<td>14.2 ± 2</td>
</tr>
<tr>
<td>W-1</td>
<td>77.1 ± 5</td>
<td>11.3 ± 2</td>
<td>11.6 ± 2</td>
</tr>
<tr>
<td>W-2</td>
<td>43.9 ± 3</td>
<td>27.7 ± 2</td>
<td>28.4 ± 4</td>
</tr>
<tr>
<td>W-3</td>
<td>43.5 ± 4</td>
<td>24.8 ± 4</td>
<td>31.7 ± 3</td>
</tr>
<tr>
<td>W-4</td>
<td>42.4 ± 5</td>
<td>36.3 ± 6</td>
<td>21.3 ± 3</td>
</tr>
<tr>
<td>W-5</td>
<td>64.4 ± 3</td>
<td>23.5 ± 5</td>
<td>12.1 ± 3</td>
</tr>
<tr>
<td>W-6</td>
<td>79.3 ± 4</td>
<td>7.1 ± 2</td>
<td>13.6 ± 2</td>
</tr>
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<td>W-7</td>
<td>84.1 ± 3</td>
<td>11.5 ± 2</td>
<td>4.4 ± 2</td>
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<tr>
<td>W-8</td>
<td>64.5 ± 3</td>
<td>26.8 ± 3</td>
<td>8.7 ± 2</td>
</tr>
<tr>
<td>W-9</td>
<td>52.7 ± 2</td>
<td>40.2 ± 4</td>
<td>7.1 ± 3</td>
</tr>
<tr>
<td>W-10</td>
<td>53.5 ± 5</td>
<td>37.6 ± 6</td>
<td>8.9 ± 2</td>
</tr>
</tbody>
</table>

† Statistically significant


Fourier transform infrared study of the effect of diabetes on rat liver and heart tissues in the C–H region

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Abstract

Diabetes mellitus is characterized by hyperglycemia, a relative lack of insulin. The metabolic disturbances in diabetic patients are often associated with cardiac and liver dysfunctions. Generally, experimental diabetic models in animals have been used to study diabetes-related changes in organ function, but the complexity of intact tissues can cause contradictory results. For this reason, different techniques have been used to understand the mechanisms of these dysfunctions in diabetic organs. The purpose of the present study is to investigate the effects of streptozotocin (STZ)-induced diabetes on rat liver and heart tissues at the molecular level by Fourier Transform Infrared (FTIR) spectroscopy. Wistar rats of both sexes, weighing 200–250 g, were made diabetic by a single injection of 50 mg kg \(^{-1}\) intraperitoneal (i.p.) streptozotocin dissolved in 0.05 M citrate buffer (pH 4.5) and they were kept for 4–5 weeks. The diabetes status was checked by measuring the blood glucose level. In the complex FTIR spectra, the bands in the C–H region for example the CH\(_2\) antisymmetric and symmetric stretching, the CH\(_3\) symmetric and asymmetric stretching vibrations due to lipids and proteins in the 3000–2800 cm\(^{-1}\) region and CH\(_2\) scissoring around 1464 cm\(^{-1}\) and the CH\(_3\) scissoring at 1454 cm\(^{-1}\) were analyzed. Characteristic spectral bands of these diabetic samples were compared with those of control group to confirm the effect of diabetes on liver and heart tissues. The FTIR spectra revealed dramatic differences in the band positions and bandwidths, signal intensity values and signal intensity ratios between diabetic and control tissues. Similar differences were observed for diabetic liver and heart tissues. A significant increase in the bandwidth of the CH\(_2\) symmetric and antisymmetric stretching and the CH\(_3\) symmetric and asymmetric stretching bands has been observed for both tissue types. The wavenumber of the CH\(_3\) asymmetric stretching band shifts to lower values, indicating an increase in the order in the deep interior part of the lipid chains. The ratio of the CH\(_2\) symmetric to CH\(_3\) symmetric stretching band (lipid:protein ratio) decreases by 13\% for diabetic heart and liver tissues. A decrease is also detected in the ratio of the CH\(_2\) scissoring to the CH\(_3\) scissoring mode. The overall intensity of these bands is seen to increase for diabetic tissues. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Liver tissue; Heart tissue; FTIR; Diabetes

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Furthermore, the collagen accumulation in the interstitium has been observed in a canine model of mild diabetes and enhanced diastolic stiffness [14]. Collagen accumulation in the diabetic myocardium is mostly of the insoluble type, which is associated with a reduction of the soluble fraction [15]. Analogous to the diabetic heart, increased myocardial stiffness is present in the aging heart along with an increased collagen volume fraction in the interstitium [16]. Accumulation of lipid in the myocardium has been repeatedly observed in experimental and clinical diabetes. This effect was explained in part due to diversion of free fatty acids from phospholipid and incorporation into triglyceride [14]. In one of our previous studies, the impairment was observed in mechanical activity of diabetic rats which was correlated well with our histological findings [17]. These results were confirmed with the present FTIR study, where we have also observed structural and functional alterations in diabetic tissues.

In conclusion, FTIR spectroscopy is an excellent technique for the investigation of biological structures due to its sensitivity and ability to give valuable information about the functional groups, which might have diagnostic value for biological systems. The value of infrared spectral analysis comes from the fact that the modes of vibrations of each group are very sensitive to changes in chemical structures, conformation and environment. Disease-induced changes can easily be detected with this technique. The results of the present study show that FTIR spectroscopy is a very informative technique to differentiate diabetic tissues from healthy ones at the molecular level. We believe that the detailed analysis of other bands, which is in progress in our laboratory, may provide a more detailed picture of the effect of diabetes on tissue and may contribute to more effective use of the FTIR spectroscopic technique in biological tissue analysis.

Acknowledgements

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References

Induction of Autophagy and Cell Death by Tamoxifen in Cultured Retinal Pigment Epithelial and Photoreceptor Cells

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PURPOSE. We investigated the mechanism of tamoxifen (TAM) retinotoxicity using human retinal pigment epithelial (RPE)-derived (ARPE-19) and photoreceptor-derived (661W) cells.

METHODS. Cultured ARPE-19 and 661W cells were treated with 5 to 10 μM TAM, and the resultant cell death was quantified using lactate dehydrogenase (LDH) release assay. Cellular oxidative stress was determined by measuring 5-(and-6)-carboxy-2',7'-dichlorofluorescein diacetate (H2-DCFDA) fluorescence. Changes in intracellular free zinc levels were monitored using the zinc-specific fluorescent dye, FluoZin-5 AM. Autophagic vacuole formation was assessed morphologically in ARPE-19 and 661W cells transfected with the fluorescent protein-conjugated markers, RFP-LC3 or GFP-LC3.

RESULTS. Following exposure to TAM, both ARPE-19 and 661W cells had cytosolic vacuoles within 1 hour and underwent cell death within 18 hours. In both cell types, TAM-induced cell death was accompanied by increased oxidative stress and elevated zinc levels, and was attenuated by the antioxidant N-acetyl-L-cysteine (NAC) or the zinc chelator N,N,N',N'-tetraakis(2-pyridylmethyl)-ethylenediamine (TPEN). The levels of LC3-II as well as the number of autophagic vacuoles (AVs) increased after TAM treatment. Double staining for lysosomes and AVs showed that autolysosome formation proceeded normally. Consistent with this, autophagy flux was increased. Finally, as shown in other cases of autophagic cell death, lysosomal membrane permeabilization (LMP) as well as caspase-dependent apoptosis contributed to TAM-induced cell death.

CONCLUSIONS. ARPE-19 and 661W cells were vulnerable similarly to TAM-induced cytotoxicity. Increases in zinc levels and oxidative stress, excessive activation of autophagy flux, and ultimately the occurrence of LMP and consequent caspase activation may contribute to the well-established retinal cytotoxicity of TAM. (Invest Ophthalmol Vis Sci. 2012; 53:5344–5353) DOI:10.1167/iovs.12-9827

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TAMoxifen (TAM) is a nonsteroidal estrogen receptor (ER) antagonist used widely as a chemotherapeutic agent against breast cancer.1 However, in a subset of patients, TAM induces macula-involving retinopathy, which can compromise vision seriously.2,3 Especially in women treated with high daily or cumulative doses of TAM,4 RPE generally has been considered the primary target of TAM-induced retinotoxicity.5 However, recent clinical studies have reported that TAM also disrupts photoreceptors as well6–8. The RPE is a single layer of cells adjacent to and surrounding the photoreceptor outer segment (POS) of the retina. As such, the RPE has critical roles in the maintenance of the POS, in part by removing shed debris of rods and cones.5 On the other hand, photoreceptor cells are specialized type of neurons in the retina proper. The initial signal transduction for visual perception is the conversion of light energy to electrical signals in the outer segment of photoreceptor cells.10 The finding that RPE and photoreceptors are damaged in TAM retinotoxicity may reflect RPE damage by TAM, since the RPE is necessary for the maintenance of overlying photoreceptors. Alternatively, TAM might well induce cytotoxicity to RPE and photoreceptors.

Because TAM is used increasingly to treat breast cancer patients, the prevalence and clinical significance of TAM-induced retinopathy also are rising. However, although it is known that TAM penetrates the blood-retinal barrier11 and induces oxidative stress,12 the mechanism of TAM-induced retinotoxicity is not yet clear. Because TAM is known to induce autophagy in certain cancer cells,13 and since autophagy can cause cell death,14 it seems possible that an autophagic mechanism also may be involved in TAM-induced retinopathy.

Autophagy or macroautophagy is a lysosome-dependent bulk degradation system for recycling of long-lived proteins, protein aggregates, and organelles, such as mitochondria, the endoplasmic reticulum, and peroxisomes.15,16 It is activated under various cellular stress conditions, such as starvation and oxidative stress, and thus is essential for cellular viability. We have shown recently that TAM induces autophagic cell death in breast cancer cells independent of ERs.14 Hence, it seems possible that TAM-induced retinopathy also may be caused by a similar mechanism.

In our study, we compared the vulnerability of human RPE-derived ARPE-19 cells and mouse photoreceptor-derived 661W cells to TAM toxicity. In addition, we examined the possibility that TAM-induced cell death occurs by an autophagic mechanism.

MATERIALS AND METHODS

Cell Culture

ARPE-19 cells were obtained from the American Type Culture Collection (Cat #CRL-2502, Manassas, VA) and cultured in Dulbecco’s Modified Eagle Medium Nutrient Mixture F-12 (DMEM/F-12; Invitrogen, Carlsbad, CA)
Early Detection of Tamoxifen-induced Maculopathy in Patients With Low Cumulative Doses of Tamoxifen

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Ophthalmic Surgery, Lasers and Imaging Retina

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Abstract
Pathogenic macula determined by techniques including 3-dimensional optical coherence tomography OCT (3D-OCT), in six breast cancer patients who had received low cumulative doses (4.2 to 9.6 g) of tamoxifen is described. Fluorescein angiography showed varying amounts of foveolar hyperfluorescence. 3-dimensional OCT revealed one or several foveal cystoid spaces in 10 of 12 eyes with or without focal disruption of the photoreceptor transition zones. Time-domain OCT did not indicate cystoid spaces in two of the eyes that clearly showed intraretinal cysts on 3D-OCT. Tiny disruptions of photoreceptor transition zones were also more clearly visible on 3D-OCT. Previous studies have clearly shown retinopathy following long-term or high dosages of tamoxifen. Our results indicate that patients with low cumulative doses of tamoxifen can also suffer visual symptom-related foveal cystoid spaces and/or macular thinning. 3D-OCT is very effective in detecting early subtle changes in tamoxifen-induced maculopathy that can occur in asymptomatic patients.
Two-Year Carcinogenicity Study of Tamoxifen in Alderley Park Wistar-derived Rats

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ABSTRACT
Tamoxifen, a nonsteroidal antiestrogen used widely in the treatment of breast cancer, was tested in a conventional 2-year carcinogenicity bioassay in rats, a species in which tamoxifen acts variably as a partial agonist and antagonist on different target tissues. Groups of 51 males and 52 females were given 5, 20, and 35 mg/kg of tamoxifen/day by gastric intubation in 0.5% hydroxypropyl methylcellulose at 5 ml/kg dose volume. There were 102 male and 104 female controls dosed with vehicle alone.

Growth rate and food consumption were reduced in all treated groups. The major finding was a dose-related increase in the incidence of hepatocellular tumors which were first observed after 31 weeks of treatment in the top dose group. The majority of the neoplasms were hepatocellular carcinomas showing a well differentiated trabecular pattern. Some tumors were glandular in type. Mortality was increased in the 20 and 35 mg/kg dose groups compared with controls as a result of these tumors. By contrast, survival was greater than controls in rats given 5 mg/kg tamoxifen despite the presence of hepatocellular tumors due to a reduction in the number of pituitary tumors in females and less chronic renal disease in males.

The mechanism of hepatic tumor induction by tamoxifen in rats is unclear. In view of the lack of genotoxic activity in conventional genotoxicity studies and lack of similar effect in mice or in humans, the findings may relate to a particular constellation of effects in rats. All other drug-induced changes in this study were nonneoplastic in nature and most appeared to be the result of hormonal perturbation since they were confined to endocrine organs or have been seen previously in rats treated for long periods with tamoxifen.

INTRODUCTION
Tamoxifen (tamoxifen citrate or Nolvadex; ICI 46, 474) is a triphenylethylene nonsteroidal antiestrogen that has been used for the treatment of breast cancer since 1969. It represents a widely used endocrine treatment for patients with all stages of breast cancer (1, 2). It is a well-tolerated drug with a low level of adverse effects, particularly when compared with more conventional cytotoxic anticancer drugs which produce damage to rapidly proliferating cells in sensitive tissues. These were believed to be the consequence of long-term treatment with tamoxifen relate more to the potential adverse health consequences of hormonal manipulation than to effects on rapidly proliferating cells (4, 5).

This side effect profile of tamoxifen in female patients broadly reflects the low level of toxic effects observed in the early preclinical safety studies (6). Tamoxifen showed low acute and chronic toxicity in conventional studies performed in rats, mice, and dogs. Most of the pathological findings were found in the endocrine and reproductive systems in these species and appeared related to the exaggerated pharmacological activity at high doses.

Following long-term treatment of Alderley Park outbred mice with tamoxifen, treatment-related neoplasms were confined to hormone-sensitive tissues. These were believed to be the consequence of long-term derangement of endocrine status in this species in which tamoxifen is classified as a full estrogen (6, 7).

While the findings in these earlier preclinical studies fully support the use of tamoxifen in palliative treatment of breast cancer, consideration of tamoxifen for use in treating nonmalignant disease and breast cancer prophylaxis among women at high risk of developing mammary cancer led to the initiation of a conventional rat carcinogenicity study in 1986. Although some studies have addressed the carcinogenicity of tamoxifen in the rat after exposures up to 1 year (8, 9), this conventional 2-year rat carcinogenicity bioassay is now reported in view of the potential importance of tamoxifen in breast cancer prophylaxis.

MATERIALS AND METHODS
The study was designed as a conventional carcinogenicity study in accordance with Food and Drug Administration and Organization for Economic Cooperation and Development guidelines and full Good Laboratory Practice compliance. All animal work conducted in this study was performed in accordance with the principles of the United Kingdom Animal (Scientific Procedures) Act 1986 (10). Tamoxifen was administered by gastric intubation as a suspension in 0.5% hydroxypropyl methylcellulose in 0.1% aqueous polysorbate 80 at a dose volume of 5 ml/kg body weight to Alpk:APfSD, Wistar derived (Alderley Park) rats for a period of 2 years. A total of 255 male and 260 female rats was divided into control and three dose groups (Table 1). The animals were 35 to 36 days old at the start of dosing on October 1, 1986. The low dose level was chosen as a multiple (approximately 6-fold) of the maximum recommended human dose of 20 mg bid (i.e., 0.8 mg/kg/day for a 50-kg woman) and the high dose to produce some adverse effects based on findings in a previous 6-month study in JCL-SD rats (11).

Animals were housed as groups of 3 males or 4 females in stainless steel mesh cages (45 × 28 × 20 cm) under standard environmental conditions at 21°C ± 2°C, relative humidity 60 ± 15%, a 12 h light-12 h dark cycle; a conventional rodent diet (pelleted, γ-irradiated NDD diet from Special Diets Services Ltd.) and tap water from an automatic watering system were provided ad libitum.

Throughout the study all animals were inspected at least twice daily. Body weight and food consumption were measured at intervals throughout the study. Eyes of all rats were examined by direct ophthalmoscopy before the start of dosing and several times during the study at approximately 3-month intervals. Blood samples were taken at necropsy from the vena cava for RBC and WBC counts from all rats surviving to the end of the study and where possible also from rats requiring to be killed prematurely. Blood samples from tail veins were also analyzed for standard clinical chemistry during weeks 51–53 on 43 randomly selected male and 38 randomly selected female rats from each group. Drug levels were measured in blood from the vena cava at variable times after dosing from animals requiring to be killed prematurely and at the end of the study using chromatographic separation by high performance liquid chromatography followed by post-column irradiation and fluorescence detection. Animals were killed by inhalation of halothane (Fluothane; ICI Plc). All animals were given a full autopsy in which internal organs were carefully inspected. The livers from all animals were sectioned at intervals of 3–5 mm and cut surfaces carefully inspected for nodules. Sections from left and right lateral and median lobes were fixed in formol-saline, processed, and examined histologically using standard hematoxylin and eosin stained sections. A full range of other tissues and samples from all tumors were also processed in a similar manner and examined histologically. Live animal data were assessed by analysis of variance using a SAS computer system. Statistical analysis of each tumor type for each sex was performed using the method of Peto et al. (12).

RESULTS
The doses of 5, 20, and 35 mg/kg/day used in this study produced mean serum tamoxifen concentrations of 166 (range, 40–697), 664 (range, 57–1188), and 636 (range, 106–1230) ng/ml, respectively.
**Hormonal therapy**

Treatment that adds, blocks, or removes hormones. For certain conditions (such as diabetes or menopause), hormones are given to adjust low hormone levels. To slow or stop the growth of certain cancers (such as prostate and breast cancer), synthetic hormones or other drugs may be given to block the body’s natural hormones. Sometimes surgery is needed to remove the gland that makes a certain hormone. Also called endocrine therapy, hormone therapy, and hormone treatment. Hormones are chemicals substances that regulate specific body functions, such as metabolism, growth and reproduction. They travel in the blood and control the activity or growth of certain cells. For example, the hormones estrogen and testosterone control the growth, development and function of female and male reproductive organs. Some cancer cells need hormones to grow. For example, some breast cancer cells need estrogen to grow. Cancer cells that need hormones to grow are called hormone dependent. Drugs, surgery or radiation therapy to specific organs can be used to change hormone levels. Hormonal therapy affects hormone levels throughout the body, so it is considered a systemic therapy. **Hormone therapy is another form of systemic therapy. It is most often used as an adjuvant therapy to help reduce the risk of the cancer coming back after surgery, but it can be used as neoadjuvant treatment, as well. It is also used to treat cancer that has come back after treatment or has spread.** Estrogen promotes the growth of about 2 out of 3 of breast cancers—those having receptors for the hormones estrogen (ER-positive cancers) and/or progesterone (PR-positive cancers). Because of this, several approaches to blocking the effect of estrogen or lowering estrogen levels are used to treat hormone receptor-positive breast cancers. Hormone therapy does not help patients whose tumors are both ER- and PR-negative.
Multifocal electroretinography, color discrimination and ocular toxicity in tamoxifen use.

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ABSTRACT

PURPOSE:
To study prospectively retinal function, color discrimination, and ocular toxicity in women treated with standard-dosage tamoxifen for breast cancer.

METHODS:
Thirty visually asymptomatic patients with at least 2 years of continuous tamoxifen therapy underwent multifocal electroretinography (ERG), color discrimination testing, and ophthalmic examination. The results were compared with 17 patients who were not taking tamoxifen after breast cancer surgery and to an additional age-matched group of 21 healthy women.

RESULTS:
Multifocal electroretinogram amplitudes and latencies were comparable among the three studied groups, and individual recordings were within age norms from our own lab. In the treated group, mild diffuse color vision loss was found in two patients with normal fundi. Three other patients had ocular toxic effects, with two cases of refractile retinal crystals and one case of keratopathy.

CONCLUSIONS:
The aspects of central retinal function that are assessed by multifocal ERG were not affected even after at least 2 years of tamoxifen use, suggesting that the multifocal ERG is not sufficiently sensitive to detect tamoxifen-associated change that might occur. Some degree of color vision loss and ocular toxic effects were found in a few cases of this cohort, suggesting that women using tamoxifen should receive an eye exam at least as often as recommended for middle-aged people.
Oxidative Stress Plays an Important Role in the Pathogenesis of Drug-Induced Retinopathy

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Several pharmaceutical agents have been associated with rare but serious retinopathies, some resulting in blindness. Little is known of the mechanism(s) that produce these injuries. Mechanisms proposed thus far have not been embraced by the medical and scientific communities. However, preclinical and clinical data indicate that oxidative stress may contribute substantially to iatrogenic retinal disease. Retinal oxidative stress may be precipitated by the interaction of putative retinal toxins with the ocular redox system. The retina, replete with cytochromes P450 and myeloperoxidase, may serve to activate xenobiotics to oxidants, resulting in ocular injury. These activated agents may directly form retinal adducts or may diminish ocular reduced glutathione concentrations. Data are reviewed that suggest that indomethacin, tamoxifen, thioridazine, and chloroquine all produce retinopathies via a common mechanism—they produce ocular oxidative stress. Exp Biol Med 229:607–615, 2004

Key words: oxidative; stress; drug; retinopathy; myeloperoxidase

Drug-induced retinopathy is an infrequent but serious complication associated with the use of a number of pharmacologically and structurally diverse compounds. Though a number of potential mechanisms have been proposed to explain these iatrogenic injuries, the etiology of drug-induced retinopathy is largely unknown.

One theory contends that many retinal toxins are cationic amphiphilic drugs (CADs) that concentrate in lysosomes and produce phospholipidosis. Theoretically, these CADs could increase lysosomal pH, inhibiting enzymatic function within the organelle. Alternatively, such agents could disrupt the lysosomal membrane. Potentially, either mechanism could lead to the accumulation of phospholipids. Progressive phospholipidosis somehow impairs cellular function resulting in retinopathy. However, many pharmaceuticals commonly used today are CADs, and most do not produce retinopathy (Table 1; Ref. 1). In animal models, drug-induced retinal phospholipidosis has not been directly linked with diminished retinal function (1–4). In addition, only a few CADs have ever been shown to produce phospholipidosis in humans, and of those that do, there is little evidence that this produces significant clinical disease (1).

Another proposed theory asserts that retinal toxicity is related to binding of toxic agents to ocular melanin. However, many drugs on the market today bind to melanin and produce no ocular toxicity (Table 2; Ref. 5). Furthermore, chloroquine and chlorpromazine, two compounds classically associated with retinopathies, produce similar retinal lesions in both pigmented and nonpigmented animals (5). Toxicologists have noted the lack of causation between melanin binding and ocular toxicity for decades (5, 6). It is possible that a number of mechanisms may be operative in the development of drug-induced retinopathy. Currently, there is no general mechanism accepted for most xenobiotic-induced retinal toxicities, making such injuries difficult to predict, avoid, and/or manage (6). Insight into one or more of the possible mechanisms by which drugs produce retinal damage may facilitate the development of future pharmaceuticals with reduced potential for precipitating injury.