Ciguatera: A public health perspective

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Abstract

Ciguatera fish poisoning is a seafood-borne illness caused by consumption of fish that have accumulated lipid-soluble ciguatoxins. In the United States, ciguatera is responsible for the highest reported incidence of food-borne illness outbreaks attributed to finfish, and it is reported to hold this distinction globally. Ciguatoxins traverse the marine food web from primary producers, Gambierdiscus spp., to commonly consumed fish in tropical and subtropical regions of the world. Ciguatoxins comprise 12 known congeners among Caribbean and tropical Atlantic fish and 29 reported congeners among Pacific fish. Expanding trade in fisheries from ciguatera-endemic regions contributes to wider distribution and increasing frequency of disease among seafood consumers in non-endemic regions. Ciguatoxins produce a complex array of gastrointestinal, neurological and cardiovascular symptoms. Treatment options are very limited and supportive in nature. Information derived from the study of ciguatera outbreaks has improved clinical recognition, confirmation, and timely treatment. Such studies are equally important for the differentiation of ciguatoxin profiles in fish from one region to the next, the determination of toxicity thresholds in humans, and the formulation of safety limits. Analytical information from case and outbreak investigations was used to derive Pacific and Caribbean ciguatoxin threshold contamination rates for adverse effects in seafood consumers. To these threshold estimates 10-fold safety factors were applied to address individual human risk factors; uncertainty in the amount of fish consumed; and analytical accuracy. The studies may serve as the basis for industry and consumer advisory levels of 0.10 ppb C-CTX-1 equivalent toxicity in fish from the tropical Atlantic, Gulf of Mexico, Caribbean, and 0.01 ppb P-CTX-1 equivalent toxicity in fish from Pacific regions.

1. Introduction

Ciguatera fish poisoning is a food-borne disease endemic to tropical and subtropical coral reef regions of the world. It is contracted by consumption of finfish that have accumulated lipid-soluble toxins produced by microalgae (dinoflagellates) of the genus Gambierdiscus. The vectors and symptoms of this disease in the Caribbean and South Pacific have been described in the literature since the 18th century, with mentions of illness consistent with ciguatera dating back to the 16th century (e.g. Halstead, 1967). Current estimates of ciguatera prevalence in endemic regions range from less than 0.1% of populations of continental land masses (e.g., Queensland, Australia; Florida, USA) to greater than 50% of populations of small islands of the South Pacific and Caribbean (see reviews by Lewis, 1986a; Lange, 1994; Fleming et al., 1998, 2001). Ciguatera has over time become a hazard to consumers in non-endemic regions because of expanding international trade in seafood from tropical fisheries. In 2007, the European Union and the United States imported greater than 80% of their fishery products to meet consumer demand. In the U.S., approximately 2.4% of fishery imports originated from the islands of Oceania (excluding Australia and New

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Zealand) and 2.0% of fishery imports originated from tropical Atlantic and Caribbean sources (NMFS, 2008). Oceania and the tropical Atlantic and Caribbean encompass ciguatera-endemic regions and, consequently, the incidence of ciguatera disease in the continental U.S. has been linked to these sources. The recognition of ciguatera in non-endemic regions led public health institutions worldwide to rank ciguatera as the most common food-borne disease related to the consumption of finfish (De Fouw et al., 1999; Lehane, 2000).

On a global level, the collection of ciguatera epidemiological data has been inefficient. The public health impact of the disease is significantly underestimated because of a generalized reticence to report illness; this reflects the lack of conviction that anything can be done to cure the disease or ameliorate symptoms. In non-endemic regions, underreporting appears to result from the lack of diagnostic recognition of ciguatera poisoning by consumers and medical practitioners (McKee et al., 2001). It is estimated that less than 20% of ciguatera illnesses are reported. Lewis and Sellin (1992) estimated that over 25,000 people worldwide are affected annually by ciguatera. Fleming et al. (1998) ventured a broader estimate of 50,000–500,000 poisonings per year worldwide, while Tosteson (1995) suggested that 20,000–40,000 illnesses per year occur in Puerto Rico and the American Virgin Islands alone. The incidence and worldwide distribution of ciguatera is suggested to be on the increase (DeHaro et al., 2003; Levine, 1995; Lehane and Lewis, 2000; Poon-King et al., 2004), paralleling a worldwide increase in harmful algae bloom (HAB) events (Anderson, 1989; Hallegraeff, 1992, 1993; Lechuga-Deveze and Sierra-Beltran, 1995). Recent observations consistent with geographic expansion of ciguatera include the first reports of consumer illness and detection of ciguatoxic fish from the Canary Islands of the eastern Atlantic (Perez-Arellano et al., 2005), the western Gulf of Mexico (Villareal et al., 2007), and the eastern Mediterranean (Bentur and Spanier, 2007). Implicated meal remnants of the fish caught within the respective regions were confirmed to contain ciguatoxins and species of Gambierdiscus were identified from the respective coastal waters. Additional evidence consistent with expansion of ciguatera into new regions of the globe include observations of Gambierdiscus species at Crete Island (Aligizaki and Nikolaidis, 2008); Rio de Janeiro, Brazil (Nascimento et al., 2008); Cau Island, Viet Nam (The et al., 2008); and Hong Kong (Lu and Hodgkiss, 2004). It was suggested that HAB intensification and expansion are linked to anthropogenic (Ruff, 1989) and naturally occurring environmental changes, including global warming and increased nutrient loading (Smyda, 1989). Increases in sea surface temperature (SST) were associated with increased dinoflagellate abundance and fish toxicity in Puerto Rico (Tosteson et al., 1998; Tosteson, 2004). Chateau-Degat et al. (2005) reported a positive correlation between SST and Gambierdiscus abundance in Tahiti and used those data and human case incidence to develop a predictive model for disease. Several explanations were suggested for this relationship between elevated temperatures and increased ciguatera incidence, including the enhancement of denuded coraline substrates for Gambierdiscus through coral bleaching. Physical disturbances of coral reefs (e.g., dredging, harbor construction) were also associated with increased Gambierdiscus abundance (Lewis, 1986a; Bruslé, 1997) and outbreaks of ciguatera (Ruff, 1989; De Sylva, 1999). More recently, petroleum production platforms and state-sponsored artificial reef programs were shown to provide substrates that support coral and other components of the tropical benthos, including Gambierdiscus (Villareal et al., 2007). The introduced structures created new habitat in the upper euphotic zone and served as fish aggregation points, thereby accelerating toxin transfer from Gambierdiscus to prized food fish. The authors suggested that the structures could have unintended consequences for human health, particularly if rising SST over the next century alter benthic biota distributions and fish migration patterns. These concerns also extend to proposals for off-shore aquaculture operations or off-shore wind farms which would also add new substrate for benthic flora.

Although recognition of disease incidence in non-endemic regions has raised general concern, the public health and economic impacts of ciguatera have long existed and are particularly high in remote island nations and territories of the Pacific (Lewis, 1986b, 1992a, b) and Caribbean. Many of these affected communities are at marginal socioeconomic levels and fish represent an important source of protein and income. High disease incidence rates in some of the smaller islands of the South Pacific (Lewis, 1986a) and discouragement of commercial fisheries development due to the threat of ciguatera in export markets (e.g. Sadovy, 1999; Wong et al., 2005) significantly hinder human welfare in many small island communities. The risk of fish poisoning is broadly recognized by resident populations in endemic regions. In most cases, smaller reef fish are preferred because smaller fish are considered less likely to be poisonous. The odds are better for smaller fish to be less toxic or nontoxic, but smaller species and smaller specimens of apex predators can be as toxic as larger species of known repute. Island residents waste very little of the fish they catch when preparing meals (e.g., fish stew with head and viscera), a practice that negates the margin of safety sought by selection of the smaller species because of higher toxin concentrations in organ tissues. Many residents of the ciguatera-endemic Caribbean and Pacific consume subthreshold levels of toxin on a regular basis (e.g. Goodman et al., 2003; Glaziou and Martin, 1993). The toxins accumulate in their systems until that point where toxicity threshold is reached and symptoms appear. The afflicted then stop eating fish for a time, are treated using traditional remedies (e.g., Pink Pepper, Schinus terebenthifolius, in New Caledonia and Vanuatu; Bourdy et al., 1992; Garrec et al., 2005) and, when symptoms subside, they resume fish consumption.

While the distribution of ciguatoxic fish is often described as global between latitudes 35° north and 35° south, the actual locations of toxic fish within this broad geographic range are discrete and heterogeneous. There are many areas that are relatively free of ciguatera, that are often found in close proximity to areas of high risk for ciguatera. Lewis (2006) describes, for example, the southern reef of Tarawa and the western reef of Maraki in...
The Republic of Kiribati as high risk areas for ciguatera, while the remaining reefs of these atolls are low-risk areas. Indeed, the toxicity of tropical fish depends on where the fish are caught, and because toxin is accumulated through trophic transfer from the primary production level, the length of time fish reside and feed in a toxic area is an important variable. The heterogeneous distribution of toxic reefs is consistent with the territorial nature of most tropical fish species. The same surmise can be made for the semi-pelagic species of fish known for ciguatera (e.g., Scomberomorus cavalla and Caranx latus). For the fisherman or consumer, there are no methods to discern how long a fish has lived in a particular area. Experience and historical knowledge of toxic reefs within any particular region appear to be the only meaningful mitigating factors for the avoidance of toxic fish.

2. The source of ciguatera toxins

Building on the pioneering works of Randall (1958), Helfrich and Banner (1963), Helfrich et al. (1968) and Banner et al. (1960), Banner and Helfrich (1964), Yasumoto et al. (1971, 1976) selected Ctenochaetus striatus (Surgeonfish) with known reputation for causing ciguatera for detailed study. They correlated disc-shaped dinoflagellates in Surgeonfish stomach contents with toxicity of viscera extracts. Extracts of the dinoflagellate collected from populations epiphytizing calcareous algae and encrusting dead coral in the Gambier Islands yielded fractions toxicologically and chemically consistent with those extracted from ciguatoxic fish. Examination of field-collected and laboratory cultured samples of this dinoflagellate confirmed the benthic link to ciguatera toxins (Yasumoto et al., 1977, 1979, 1980; Bagnis et al., 1980). The dinoflagellate was designated a new genus and species, Gambierdiscus toxicus (Adachi and Fukuyo, 1979). Since the initial discovery of G. toxicus, several new species have been added to the genus including G. belizeanus (Faust, 1995), G. yasumotoi (Holmes, 1998), G. pacificus, G. australis, and G. polynesiensis (Chinain et al., 1999a). However, recent work by Richlen et al. (2008) and Litaker et al. (2008) using detailed morphological and rDNA sequence data question the original assumption of a single cosmopolitan G. toxicus species and the more recent designation of new species based upon morphological characteristics alone. Genetic clustering suggests that a wide-ranging complex of multiple cryptic species exists.

Gambierdiscus spp. are single-cell algae (division Prorphyta) that occupy benthic and epiphytic niches in ciguatera-endemic regions. These dinoflagellates differ from better known open-water “red-tide” species in that they do not form conspicuous surface blooms. Gambierdiscus spp. are frequently found in sparse populations associated with macroalgae or encrusting denuded coraline surfaces, and they can form dense submerged populations in protected locations. Significant variation in toxin production occurs within the genus. This variation is reflected in regional differences in ciguatera toxin profiles associated with fish and the symptoms reported in outbreaks in the three major ciguatera-endemic regions, (Caribbean Sea and the Pacific and Indian Oceans). The distribution of ciguatoxic finfish, often characterized as spatially irregular, is consistent with the distribution of benthic dinoflagellate communities in low energy systems; e.g., mangrove systems in the Caribbean and leeward sides of coral reefs and atolls (personal observations; Tindall and Morton, 1998; Stewart, 1988) where physical structures provide some degree of protection from energetic water movement. The protected environments in which Gambierdiscus spp. are found in abundance are, in some cases, nursery grounds for fishes and invertebrates which ultimately populate higher energy coral reef areas. Dietary intake of G. toxicus was shown to disturb the equilibrium of fish; as a result predation escape abilities might be diminished (Davin et al., 1986, 1988; Kohler et al., 1989; Lewis, 1992a, b; Gonzalez et al., 1994). The toxins also impair embryonic and larval stages of development in fish, decrease larval survivability, and may expedite toxin transfer from Gambierdiscus spp. to higher trophic levels (Ungerer and Thomas, 1996; Edmunds et al., 1999; Colman et al., 2004). The aforementioned observations of adverse effects on intermediate trophic levels are consistent with a rapid 3 month transit of ciguatera toxins from peak densities of Gambierdiscus spp. to peak number of cases of ciguatera reported in French Polynesia (Chateau-Degat et al., 2005).

Laboratory studies show considerable variation in toxicity within the genus Gambierdiscus (Sperr and Doucette, 1996). Although extracts from field collections of G. toxicus from French Polynesia yielded toxins which were chemically consistent with ciguatoxins isolated from fish (Bagnis et al., 1980; Yasumoto et al., 1971), very few clonal cultures from those collections reproduced the same results. Holmes et al. (1991) reported that the production of toxins was limited to certain strains of G. toxicus and that wild cells produced 100-fold greater quantities of toxins than cultured cells. Variations in toxin yield were also observed in field populations in the Caribbean (McMillan et al., 1986) and Pacific (Chinain et al., 1999b) where G. toxicus biomass did not correlate with toxicity. This led to the supposition that ciguatera outbreaks occur when environmental conditions favor the growth of highly toxic genetic strains within a population (Holmes et al., 1994; Holmes and Lewis, 1994; Chinain et al., 1999b). Laboratory studies suggest that individual G. toxicus strains were adapted to particular environmental regimes. bomber et al. (1988) reported reduced growth rates at high irradiance and increased toxicity at higher temperatures (>28°C) for a number of strains. Morton et al. (1993) showed a 200-fold difference in G. toxicus toxicity depending on the light, salinity, and temperature conditions used during culture. More recently, studies in genetic speciation suggest that G. toxicus is not a single cosmopolitan species but a wide-ranging complex of multiple cryptic species. This could partly explain the seemingly random patterns of toxicity (Richlen et al., 2008). Few G. toxicus strains have been the focus of toxicity studies and limited data has been gathered for the newer species designates. The toxicity of G. belizeanus is not known, and the description of G. yasumotoi included a brief mention of maito toxin-like activity. Chinain et al. (1999b) reported ciguatoxin-like activity from G. pacificus, G. australis, and G. polynesiensis, with low levels of
activity in *G. pacificus* and *G. australis*, and “exceptionally high” activity in two *G. polyneisensis* strains. All of the known species except *yasumoti* were reported to occur in Belize (Faust, 1995; Choinin et al., 1999a). Because the different Gambierdiscus spp. co-occur and appear to vary in toxicity, changes in the proportion of Gambierdiscus spp. in a population appear to contribute to variable toxicity in natural populations.

The first ciguatoxin structure elucidated from *G. toxicus* was designated CTX-4B (M_+H^+_, m/z 1061.6) and was consistent with a deoxy precursor to CTX-1 from moray eel (Murata et al., 1989, 1990). Subsequent studies identified, from *G. toxicus* and fish, a C-52 epimer of CTX-4B designated CTX4A; 52-epi-54-deoxy congener of CTX-1; and a series of congeners containing oxocine E-rings including a decylated-deoxy congener CTX-3C; 51-hydroxy-CTX-3C; and 2,3-dihydroxy-CTX-3C (Satake et al., 1993, 1997, 1998). All appear to be precursors to oxidized CTX-1 congeners identified from toxic fish (Murata et al., 1990; Lewis and Holmes, 1993). The less polar CTX-1 precursors identified from *G. toxicus* exhibit median lethal potencies in the mouse model approximately 10-fold less potent than CTX-1 from fish. In addition to the lipid-soluble ciguatoxins, *G. toxicus* produces the water-soluble toxins, maftotoxin, gambierol and gambieric acids (see review by Wright and Cembella, 1998). These compounds are not thought to be significant contributors to ciguatera fish poisoning due to their water-soluble nature and low oral potency.

### 3. Assimilation and metabolism of toxins in fish

Ciguatera toxins in finfish comprise larger assemblages of ciguatoxin congeners. The Pacific and Caribbean ciguatoxins differ slightly in structure and toxicity. The more abundant and potent Pacific ciguatoxins are piscine metabolites of Gambierdiscus toxins and are assimilated and metabolized through multiple trophic levels of the marine food web (Lewis and Holmes, 1993). Similar evidence for the Caribbean ciguatoxins is lacking in the absence of structure elucidation of Caribbean Gambierdiscus toxins but most surmise the same must be true. The principal and most toxic Pacific ciguatoxin was first isolated from moray eels in 1967 (Scheuer et al., 1967; Tachibana et al., 1987). Structure elucidation of the principal Pacific ciguatoxin and its precursor-toxin CTX-4B from *G. toxicus* was completed in 1989 (Murata et al., 1989, 1990). The ciguatoxins are lipid-soluble polyether compounds with skeletal structures of 13–14 transfused ether rings. They are odorless, colorless, devoid of heteroatoms other than oxygen, and bear few conjugated bonds. These physical features made them very difficult to detect by conventional means (e.g., UV-absorption) at the time of elucidation. CTX-1, has a mass of 1110.6 daltons and a molecular formula of C_{60}H_{92}O_{19} (Murata et al., 1989, 1990). It is a potent sodium channel agonist and exhibits extreme lethality in mouse models with a median lethal dose of 0.25 μg/kg. Investigations of ciguatoxic fish from the central and western Pacific, led by Ysumoto (Japan), Lewis (Australia) and Legrand (France), determined that multiple CTX-1 congeners were present in toxic fish. The body of work also suggested that a select few were truly abundant, and, in most cases, CTX-1 dominated toxin profiles. The contribution of CTX-1 to composite toxicity in mice, and, by extension, to humans, was estimated at 90% (Lewis et al., 1991a, b). Although numerous precursors, isomers, and congeners were detected, insufficient amounts of the less abundant toxins were recovered for structural elucidation by NMR. However, using fragmentation patterns of the structures of CTX-1 and CTX-4B as templates many congeners were identified by a combination of FAB/MS/MS and synthetic conversions to known structures (Yasumoto et al., 2000). Approximately 29 congeners were identified from fish and comprise products of acylation, epimerization, hydroxylation, and oxidation of CTX-4B and CTX-3C.

Similar studies were performed in the Caribbean (Crouch et al., 1995; Vernoux and Lewis, 1997) and, in 2002, the isolation and characterization of Indian Ocean CTX was reported by Hamilton et al. (2002a, b). Caribbean ciguatoxin (C-CTX-1) was first described by Lewis et al. (1998) with a mass of 1140.6 Da and a molecular formula of C_{62}H_{92}O_{19}. Like Pacific CTX, it is a potent sodium channel agonist and exhibits a median lethal dose in the mouse model of 3.7 μg/kg; it is approximately 10-fold less potent than P-CTX-1. The case for dominance of a single Caribbean ciguatoxin appears less convincing than is the case in the Pacific. A single, and apparently dominant, Caribbean ciguatoxin (presumptive MH^+_, m/z 1123.6) was isolated and partially characterized from *Sphyraena barracuda* and *Caranx latus* (Crouch et al., 1995). This dominant ciguatoxin and at least two minor toxins were also observed in *Scomberomorus cavalla* (greater amberjack) meal remnants from a ciguatera outbreak among U.S. soldiers in Haiti in 1995 (Poli et al., 1997). In the latter two studies, the toxins were not obtained in sufficient quantities for structural elucidation by NMR, and there was some question as to the mass assignment of the principal toxin (i.e., m/z 1123.6). In 1997, five ciguatoxins were isolated from *C. latus* (Vernoux and Lewis, 1997) and the structures of two, (C-CTX-1: MH^+_, m/z 1141.6 and its C-56 epimer C-CTX-2) were elucidated by NMR and MS (Lewis et al., 1998). It was estimated that C-CTX-1 and C-CTX-2 contribute 40–60% to total fish toxicity in the waters of St. Barts in the French West Indies (Pottier et al., 2002). Seven additional C-CTX congeners and three additional isomers of C-CTX-1 or C-CTX-2 were identified (Pottier et al., 2002). The isomers and congeners identified comprised products of epimerization, hydroxylation, and oxidation of C-CTX-1 or C-CTX-2. A system for annotation of ciguatoxins from the Caribbean and Pacific was proposed by Vernoux and Lewis (1997) to distinguish CTX source and structural variants in which the acronym CTX is used with a letter prefix to indicate region of origin (i.e., P for Pacific; C, for Caribbean; and I, for Indian) and a postscript numbering system to indicate the chronological order of reporting.

### 4. Symptoms, diagnosis, and treatment of ciguatera

Variation in symptom patterns in the Pacific Ocean, Indian Ocean, and Caribbean Sea regions have been attributed to the different suites of ciguatoxins identified from those regions (Lewis, 2000). In the Caribbean, gastrointestinal symptoms and signs are characteristic in
the acute phase (Lawrence et al., 1980), and are followed closely by neurologic, especially peripheral, neurologic symptoms. In the Pacific and Indian Ocean regions, the neurological symptoms and signs are more pronounced in the acute phase with occasional reports of more severe neurologic effects, including coma (Bagnis et al., 1979). Onset of symptoms and signs typically begins within 0.5–12 h of eating a toxic fish, and the acute phase often abates within 24 h (Hokama, 1988). Cardiovascular problems (generally a combination of bradycardia with hypotension) may be present during this acute period. In the Pacific and Indian Oceans, there are reports of rapid progression to respiratory distress, coma, and occasionally death (DeFusco et al., 1993; Lange, 1987; Habermehl et al., 1994). More extensive and subjective neurological signs may emerge in a few hours to two weeks after exposure. Paresthesias, numbness and tingling of perioral region and extremities and temperature related dysesthesias (i.e., hot and cold temperature sensation reversals) are considered characteristic symptoms of ciguatera fish poisoning (Pearn, 2001). Studies suggested that the unusual sensations represent tingling or “electric shock” pain rather than a true reversal of hot and cold perception (Cameron and Capra, 1993). Cameron and Capra (1993) concluded that the ciguatoxins induced abnormal discharges in the peripheral A-delta and peripheral C-modal nociceptor fibers involved in temperature sensation and may also be involved in the intense itching that patients report. Nerve conduction studies in a limited number of patients with acute illness, demonstrated a generalized disturbance in sensory and motor conduction with significant prolongation of the refractory periods and the supernormal period of excitability (Cameron et al., 1991).

Other subjective neurological symptoms include metallic taste, pruritis, arthralgia, and myalgias (muscle aches, especially in the legs) and sensations of loose teeth (Poon-King et al., 2004). Cerebellar signs and tremors may present up to 10 days after initial exposure (Chungue et al., 1993). Headache, which appears non-localized, intense, and prolonged may be a presenting sign (Pearn, 2001). General weakness, hyporeflexia, and dysphagia may also be found. While cranial and peripheral nerves appear to dominate the clinical picture, Karlis et al. (2000) reported that 11% of the neurological symptoms observed were indicative of central nervous system involvement (paralysis, ataxia, stupor, confusion) which is usually indicative of the most severe cases (Cameron and Capra, 1993). A wide range of neurobehavioral symptoms were reported from the Pacific and Indian Ocean regions following ciguatera poisoning, including fatigue, anxiety, depression, hysteria, “neurosis,” memory disturbance and mental inefficiencies (Bagnis et al., 1979; Gillespie et al., 1986; Lipkin, 1989; DeFusco et al., 1993; Quod and Turquet, 1996; Karlis et al., 2000; Arena et al., 2004; Friedman et al., 2007; Williams et al., 2008). Depression in many patients accompanies slow regression of the paresthesias, weakness, fatigue, and complaints of general malaise, suggesting a secondary reaction to chronic illness. In contrast, neurocognitive complaints (memory, mental inefficiencies) may be the direct result of neurotoxic exposure, i.e., a general cognitive inefficiency as a result of diminished cerebral functioning or secondary to fatigue, depression, or general malaise.

Acute gastrointestinal problems typically resolve within 24–28 h and cardiovascular disorders reverse within 48–72 h (Butera et al., 2000; Hokama, 1988). Recovery from neurologic symptoms is longer and less predictable, ranging from 1 week to 6 months (Butera et al., 2000; Lange et al., 1992; Morris et al., 1982a, b; Poon-King et al., 2004). Chronic illness may occur in a subset of patients, and is characterized by a vague and poorly defined combination of recurring neurologic and neuropsychological symptoms. Pruritus, arthralgia and fatigue can persist for months or years (Gillespie et al., 1986). The fatigue can be so debilitating that it resembles a Chronic Fatigue Syndrome (Pearn, 1996, 1997) and evidence of causal linkage to chronic phase lipids was suggested (Hokama et al., 2003a, b, 2006, 2008). Chronicity may reflect lengthy persistence of ciguatoxins in the body (Chan and Kwok, 2001) or lowered neural thresholds responding to dietary or behavioral stimuli unrelated to ciguatoxins. The persistence of symptoms in some patients for several years is not unusual. In one study (Lange et al., 1992), 65% of the patients had symptoms for 6 months or longer with recurrence up to two years.

Based on observations that repeated exposures to ciguatera toxins might be associated with a more severe clinical illness, it was hypothesized that the fat-soluble ciguatoxins accumulate in humans and lower threshold tolerances (Bagnis et al., 1979; Glaziou and Martin, 1993; Hokama, 1988). Increasing age and weight, presumably associated with greater lifetime exposures and greater capacity for toxin storage, have also been linked to the duration and severity of symptoms (Katz et al., 1993). An alternate explanation is that the ciguatoxins cause irreversible sub-clinical damage. Physical or dietary behaviors (e.g., exercise, alcohol consumption, or excessive caffeine) or repeat exposure to sub-threshold levels of ciguatera toxins may induce symptom recurrence.

Diagnosis of ciguatera by medical professionals remains a matter of exclusionary assessment. There are currently no reliable biomarkers that can be used to confirm diagnosis of ciguatera in clinical settings, although studies using animals (Morey et al., 2008; Ryan et al., 2007; Dechaourou et al., 2005a, b, 2007) suggest that detection of ciguatoxin in clinical samples may be possible in the near future. Using a murine model, progress was made in the use of blood collection cards for sample collection and highly sensitive in vitro assays for detection of ciguatoxin activity were optimized for blood matrix (Dechaourou et al., 2005a). At present, however, diagnosis is based on presenting symptoms and time course, dietary history of reef fish consumption, and the exclusion of other diagnoses that could account for symptoms that are common to other etiologies (e.g., Perkins and Morgan, 2004). Ciguatera has some symptoms in common with illness caused by neurotoxic shellfish poisonings, scombroid poisoning, pathogenic bacteria and enteric viruses (Ting and Brown, 2001), organophosphate pesticide poisoning, eosinophilic meningitis, multiple sclerosis and other neurologic conditions (Friedman et al., 2008). However, variation occurs in the clinical syndrome and severity of disease. This appears to reflect individual risk factors among patients in different parts of the world, and the geographic differences in ciguatoxin profiles within and among species of ciguatoxic
fish. The current standard for diagnosis of ciguatera includes confirmation of ciguatoxins in the consumed fish by laboratory methods (e.g., see Detection in Fish, below). A outbreak investigation that reports multiple individuals consuming the same fish, and with symptoms and time course consistent with ciguatera, is strongly supportive of the diagnosis.

In the Pacific and Caribbean, a variety of traditional herbal medicines and remedies have been used to treat ciguatera. Some of the plants and preparations identified (Bourdy et al., 1992) were found to ameliorate ciguatoxin effects in animal models (Amade and Laurent, 1991). Extracts of Argusia argentea and Schinus terebenthifolius had beneficial effects on mice fed toxic moray eel liver. Davallia solida acted like mannitol on isolated frog axonal preparations exposed to ciguatoxin (Benoit et al., 2000). However, active agents in many plant preparations have not been identified and there are no controlled studies on their efficacy and safety for treating ciguatera. Various medical treatments for specific symptoms have been tried, but with variable success (reviewed by Friedman et al., 2008).

Fluoxetine for chronic fatigue (Berlin et al., 1992); amitriptyline for paresthesias, pruritis, and headaches (Lange et al., 1992: Davis and Villar, 1986); acetaminophen and nifedipine for headaches (Calvert et al., 1987; Berlin et al., 1992); and gabapentin was used to treat pain (Perez et al., 2001). In all cases, randomized trials to assess fitness for purpose are lacking. Obstacles to randomized clinical trials of prospective treatments for ciguatera include the spontaneous nature of disease occurrence, a general lack of resources for conducting clinical trials in those places where it occurs with greater frequency, the lack of biomarkers and clinical tests to confirm the diagnosis of ciguatera, typically small patient cohorts, variability among patients in time to treatment, and differences between Pacific Ocean, Indian Ocean, and Caribbean toxin profiles in fish.

Palafox et al. (1988) first described treatment of ciguatera by intravenous (IV) mannitol with complete resolution of symptoms in 17 of 24 patients within 48 h of infusion; these included two cases of coma and one case of shock that improved within minutes. Similar results (Pearn et al., 1989; Blythe et al., 1992) suggested that treatment with IV mannitol abbreviated morbidity and chronicity. IV mannitol infusion is the most studied therapy for ciguatera, and the only therapy assessed by randomized clinical trials (Bagnis et al., 1992; Schnorff et al., 2002). IV mannitol is administered at 0–1.0 g/kg body weight over a 30–45 min period within 48–72 h of ingestion of toxic fish (Palafox et al., 1988; Blythe et al., 2001). Beneficial effects were reported for up to several weeks after intoxication (Blythe et al., 1992, 1994; Schwarz et al., 2008). The effect of mannitol infusion was thought to be mediated by the osmotic reduction of neuronal edema (Pearn, 2001) and sodium channel dependent reduction in binding effects (Birinyi-Strachan et al., 2005a, b). In the latter study and that of Bagnis et al. (1992), mannitol effects were distinguished from control treatments including hyper- and isosmolar d-sorbitol, sucrose, glucose, vitamin B, calcium gluconate and free radical scavenging agents Trolox® and L-ascorbic acid. Contradictory findings were reported, however, by Schnorff et al. (2002) in a double-blind and randomized study in which saline was found to be as effective as mannitol in ameliorating the symptoms of ciguatera. Nicholson and Lewis (2006) noted that clinical use of mannitol may induce excessive loss of fluids in patients suffering from acute diarrhea and vomiting, and that patients experiencing bradycardia and hypotension are at higher risk of cardiac failure if infused with high doses of mannitol. Lewis and King (1996) suggested that mannitol should not be administered until the patient is adequately rehydrated. Mannitol therapy has been recommended for two primary goals: reduction of acute neurologic symptoms and prevention of chronic neurologic symptoms.

5. Pharmacology of ciguatoxins

Symptoms of ciguatera were long recognized as indicative of central and peripheral nervous system injury. Early studies identified sodium dependent, and tetrodotoxin sensitive, excitatory cell depolarization in a variety of isolated nerve and muscle tissue preparations (e.g., Rayner, 1970; Ohshika, 1971; Miyahara et al., 1979). Voltage-clamp studies suggested that ciguatoxins cause spontaneous, and enhance evoked, action potentials by lowering activation thresholds and delayed repolarization of voltage-gated sodium channels (Benoit et al., 1986; Bider et al., 1984; Molgo et al., 1990). A continuous and large body of work further elucidated cellular effects and molecular mechanisms of ciguatoxin actions including selective binding and competition with another polyether toxin, brevetoxin, for “site 5” on voltage-gated sodium channels (Bider et al., 1984; Poli et al., 1986, 1997; Sharkey et al., 1987; Lombet et al., 1987; Lewis et al., 1991a, b; Gawley et al., 1992; Trainer et al., 1994; Dechraoui et al., 1999); elevation of intracellular calcium levels (Lewis and Endean, 1986; Molgo et al., 1993); stimulation of spontaneous and evoked neurotransmitter release from synaptosomes and motor nerve terminals (Molgo et al., 1990; Hamblin et al., 1995; Brock et al., 1995); axonal and schwannn cell edema (Allsop et al., 1986; Benoit et al., 1996; Mattei et al., 1999); induction of tetrodotoxin-sensitive leakage current in dorsal root ganglion neurons (Strachan et al., 1999); and blockade of voltage-gated potassium channels (Hidalgo et al., 2002; Birinyi-Strachan et al., 2005a, b). While both ciguatoxins and brevetoxin elicit membrane depolarization, ciguatoxin induces unique spontaneous single channel events in sensory neurons (Hogg et al., 1998). Birinyi-Strachan et al. (2005a, b) observed ciguatoxins to induce a blockade of voltage-gated potassium channels contributing to neuronal excitability in rat parasympathetic neurons. Other reports suggested that ciguatoxins prolong action potential duration after hyperpolarization via blockade of voltage-gated potassium channels (Birinyi-Strachan et al., 2005a, b; Benoit and Legrand, 1992) and induce leakage current (Strachan et al., 1999). Hogg et al. (2002) also observed that ciguatoxins induce unique oscillations in membrane potential and action potential firing in rat cultured dorsal root ganglion neurons. Dechraoui et al. (2006) noted the differential expression of voltage-gated sodium channel isoforms of nervous, skeletal and cardiac tissues and assessed the relative affinity of brevetoxins and ciguatoxin in the rat and the fish Centropristis striata models by
radioligand (tritiated brevetoxin-3) competitive binding. Results indicated a species related resistance of heart voltage-gated sodium channels in the rat and comparable sensitivity between the species for brain and skeletal muscle.

Although the neurological symptoms observed in clinical cases of ciguatera poisoning are believed to be consistent with the direct interaction of ciguatoxins with voltage-gated sodium channels (see review by Lehane and Lewis, 2000), Kumar-Roine et al. (2008) argued that the unique effects of ciguatoxins on voltage-gated sodium channels did not fully explain the spectrum of symptoms elicited by ciguatoxins. They cited a number of studies where sodium channel activator affects, and ciguatoxin activation of L-type calcium channels, were mediated by nitric oxide (NO) via the neuronal nitric oxide synthase (nNOS) pathway, and that nNOS and inducible NOS (iNOS) were also implicated in nociceptive and inflammatory responses to other types of envenomation. They observed that Pacific ciguatoxin-1B induced prolonged dose- and time-dependent NO production in murine macrophages through modulation of iNOS expression. Based on these observations and studies showing elevated levels of NOS-induced inflammatory cytokines, l-citrulline and neopterin in the urine and serum of ciguatera patients, the authors hypothesized a cascade of excessive N-methyl-d-aspartate (NMDA) receptor activity due to prolonged voltage-gated sodium channel activity. This might result in calcium influx, with activation of constitutive NOS (cNOS) and production of NO; the latter reacts with superoxide radicals to produce peroxynitrite anion creating an oxidative stress that activates the transcription factor NF-KB, and stimulating iNOS gene transcription leading to further NO production. While direct evidence is lacking for ciguatoxin activation of NMDA receptors, it has been shown for brevetoxins (Dravid et al., 2005; Berman and Murray, 1999; Massensini et al., 2003). The hypothesis appears to be consistent with free radical scavenging activity reported for mannitol and active agents of traditional herbal remedies (e.g. mangiferin in D. solida).

Depressed body temperature in mice following intraperitoneal administration of toxic fish extracts was noted in early studies (Doorenbos et al., 1976; Hoffman et al., 1983; Sawyer et al., 1984; Lewis et al., 1993). Peng et al. (1995) presented evidence for CNS effects of ciguatoxin and brevetoxin on thermoregulatory centers of the brain based upon localization of Fos in the medial preoptic (MPO) nucleus in mice and perhaps reflecting changes in core body temperature. Ciguatoxin activity on sodium channels of excitable membranes and the thermal dysthesia in patients was reported to result from alteration of C-polymodal nociceptors (Cameron and Capra, 1993). More recently Gatti et al. (2008) in a retrospective study of ciguatera cases in French Polynesia reported body temperature depression under 36.5 °C in 48 of 80 documented patient files. The latter observation appears to be a first report and is consistent with CNS activity reported in prior studies (see review by Gordon and Randell, 2005).

6. Detection of ciguatera toxins

The ideal method for the detection and quantification of ciguatoxins in fish, a method that is simple, rapid and accurately measures toxicity relative to human susceptibility is yet to be developed. Over the written history of ciguatera a wide variety of methods have been devised to detect and reject toxic fish. Many traditional methods such as discoloration of silver coins or copper wire or the repulsion of flies and ants were readily discredited (Banner and Helfrich, 1964). Other in vivo methods including the mongoose assay (Banner et al., 1960) and cat assay (Lewis, 1987; Bagnis et al., 1985a, b) were simple but cumbersome, non-quantitative and ethically objectionable. The brine shrimp (Granade et al., 1976; Hungerford, 1993), mosquito (Bagnis et al., 1985a, b, 1987) and diptera larva (Labrousse and Matile, 1996) assays also were fairly simple but ambiguous and unconvincing. Isolated tissue assays including the guinea pig ileum assay (Dickey et al., 1982), the guinea pig atrium assay (Lewis, 1988; Lewis and Endean, 1986), the isolated frog nerve assay (Benoit et al., 1986), and hemolytic assays using human and mouse blood cells (Escolana De Mota et al., 1986) showed greater sensitivity but in most cases technical difficulty or ethical objections precluded them from wider application.

The mouse bioassay, based on the method described by Banner et al. (1960), refined and described in detail by Yasumoto et al. (1984), is presently the most widely used assay for the detection of ciguatoxins in fish. The method specifies intraperitoneal injection of serially diluted semi-purified fish extracts into mice and 24 h observation for signs of toxicity. Characteristic signs of toxicity qualify ciguatoxins in extracts and the relationship between dose and time to death is used to quantify toxicity. Total lethality is expressed in mouse units (MU). Calculation of fish toxicity (Lewis and Sellin, 1992; Lewis et al., 1991a, b) is approximated by log MU = 2.3 log (1 + T – 1), where MU is the number of mouse units of ciguatoxin injected and T time to death in hours. One MU is the LD50 dose for a 20 g mouse which is equivalent to 5, 48 and 18 ng of PacificCTX-1, CTX-2 and CTX-3, respectively. Additional purification of herbivorous fish extracts can be performed to separate the polar maitotoxins from lipid-soluble ciguatoxins (Yokoyama et al., 1988; Holmes et al., 1991; Holmes and Lewis, 1994; Legrand et al., 1992a, b) since the maitotoxins exhibit low oral potency, and do not appear to accumulate in piscivorous fish causing ciguatera in humans. The mouse assay has been used extensively in research settings and to a lesser extent in monitoring market fish. It shares some of the disadvantages noted for other in vivo assays, including maintenance of animal colonies, cumbersome execution and ethically compromised in addition to questions of acceptable levels of sensitivity and specificity to be preferred in modern methods of public health importance.

Alternatives to in vivo testing were developed to detect ciguatoxins using sodium channel specific cytotoxicity (Manger et al., 1993, 1995) and sodium channel receptor binding in rat brain synaptosomal preparations (Lombet et al., 1987; Lewis et al., 1991a, b; Poli et al., 1997). The assays can detect subpicogram levels of ciguatoxins in fish extracts and provide qualitative and quantitative estimates of toxicity. Both assays can be formatted for high sample throughput (e.g. Van Dolah et al., 1994). The neuro-2a mouse neuroblast cell assay (Manger et al., 1993, 1995) is...
a ouabain-veratridine-dependent in vitro screening method for sodium channel specific algal toxins. Assay specificity is achieved by pretreatment of neuroblasts with veratridine to initiate sodium channel gating and ouabain to block the sodium-potassium pump which would otherwise permit cells to compensate for sodium ion flux. Neuroblasts that are not treated with veratridine and ouabain are not affected by sodium channel specific toxins. Both treated and untreated neuroblasts, however, are susceptible to toxins acting through other vital metabolic mechanisms. Through appropriate experimental design the cell assay can discriminate between toxins that activate (e.g., brevetoxins, ciguatoxins) or block (e.g., tetrodotoxins, saxitoxins) voltage-gated sodium channels, and also distinguish between sodium channel specific toxins and those acting through other modes of action. The assay provides a composite response to all sodium channel specific toxins in an extract and it cannot discriminate molecular species acting in the same manner or determine what other modes of action may be where toxicity is not sodium channel specific. The neuro-2a mouse neuroblast cell assay is used routinely for research and outbreak applications in some laboratories (e.g., Dickey et al., 1999; Dickey, 2008; Dechraoui et al., 2005a).

The receptor binding assay is based on binding competition between ciguatoxin standard or sample with tritiated brevetoxin for the sodium channel receptor. The bound and unbound toxins are separated by centrifugation or filtration, and the amount of bound radiolabeled brevetoxin is measured by liquid scintillation counting. Samples are quantitated by comparison with a standard competition curve, generated by addition of increasing concentrations of unlabeled ciguatoxin or brevetoxin to a mixture of receptor and tritiated brevetoxin. Binding competition can be carried out in traditional test-tube format or adapted to a microplate format and quantified using a microplate scintillation counter to permit high sample throughput (Poli et al., 1997; Van Dolah et al., 1994; Dechraoui et al., 2005a; Darius et al., 2007). Receptor assays provide a quantitative estimate of composite toxicity much like the cytotoxicity assay where all toxins in an extract bind to the sodium channel receptor. Relative binding affinities correlate with their relative toxic potencies. Ciguatoxin detection limits are at the pmol range. A disadvantage to the application of receptor assays for ciguatoxins, and other algal toxins, is that they are technically complex, and require the use of radiolabeled materials with attendant licensing requirements. Modifications of receptor assays to colorimetric or fluorometric end points have generally not been successful because colorimetric or fluorometric tags tend to decrease binding affinity of the toxin. However, a membrane potential-based assay for brevetoxins was developed using synaptoneurosomes isolated from mice. The assay detected the depolarizing effect of brevetoxins as enhancements of the veratridine-dependent increase in fluorescence of the voltage-sensitive fluorescent probe rhodamine 6 G. The assay was relatively rapid and detected brevetoxin activity in the nanomolar range. The synaptoneurosomal technique was shown to compare favorably with the cytotoxicity assay, the receptor binding assay and HPLC/MS (David et al., 2003).

The development of immunoassays for ciguatoxins in fish tissues appear to have the best potential for meeting the utilitarian requirements of speed, simplicity and accuracy in the measurement of toxicity relative to human susceptibility. Initial development if immunoassays for ciguatera began with a radio immunoassay format using polyclonal antibody to a partially purified ciguatoxin preparation (Hokama et al., 1977). In succeeding iterations of polyclonal and monoclonal antibody based assay developments (Hokama et al., 1984, 1998; Hokama 1983, 1990; Park 1994), specificity, i.e. cross-reactivity with other toxins including okadaic acid, maitotoxin and brevetoxin, and unacceptable rates of false positives and false negatives (Dickey et al., 1994; Wong et al., 2005) remained obstacles to success (see review by Van Dolah and Ramsdell, 2001). The later monoclonal antibody assays developed for ciguatera were also reported to react with abnormal lipids in sera of chronic fatigue syndrome, chronic ciguatera fish poisoning, hepatitis B and cancer patients suggesting a causal link (Hokama et al., 2003a, b, 2006, 2008). More recently reported developments of mouse and chicken antibodies specific to synthetic fragments of ciguatoxin were reported to produce reliable and accurate results in screening fish populations for ciguatoxins (Campora et al., 2008a, b). The alternative strategy to produce anti-ciguatoxin antibodies using protein-conjugated synthetic fragments of ciguatoxins (Pauillac et al., 2000, 1998) appeared to be successful and sufficiently sensitive for identifying ciguatoxins in fish tissue but the antiserum was reportedly lost before thorough evaluation could be performed. Tsumuraya et al. (2006) noted the extremely low content, and difficulty in recovery, of ciguatoxins in contaminated fish necessary for anti-ciguatoxin antibody development. They used synthetic haptons in producing anti-ciguatoxin monoclonal antibodies against both ends of ciguatoxin CTX-3C (Oguri et al., 2003). The paired antibodies were used to develop a sandwich enzyme-linked immunosorbent assay (ELISA) that detected CTX-3C at the ppb level without cross-reactivity to other related marine toxins, including brevetoxin A, brevetoxin B, okadaic acid (OA), and maitotoxin. This report was followed closely by the generation of monoclonal antibodies against the right wing of ciguatoxin CTX-1B and 51-hydroxy-CTX-3C (Tsumuraya et al., 2006), also without cross-reactivity to the other marine toxins tested for the anti-CTX-3C preparation.

The application of mass spectrometry (MS) played a critical role in the structure elucidation of most of the ciguatoxin congeners recovered in trace quantities from toxic fish and Gambierdiscus. Using as templates the fragmentation patterns of those ciguatoxins obtained in sufficient quantity for NMR structure elucidation, the structures of the many congeners were deduced by a combination of FAB/MS/MS and synthetic conversions to known structures (Yasumoto et al., 2000). More recent advancements in MS technology have dramatically improved the analytical capabilities, reduced expense, size and technical complexity of instrumentation, to afford wider access to the analytical power of the technology. Prior to these developments MS was utilized in relatively few laboratories and required extensive purification and concentration of ciguatera toxins recovered from toxic fish (e.g. Lewis and...
risk level of extracts from the Caribbean Sea suggested an estimated using P-CTX-1 as an internal standard, the analysis of fish ppb P-CTX-1 and 0.10 ppb C-CTX-1 were detectable, and MS/MS) of P-CTX-1 and C-CTX-1 in spiked and naturally reversed-phase HPLC/tandem mass spectrometry (HPLC-

Lewis et al. (1999). They described a method for gradient determination of toxicity thresholds in humans, and the application of immunoassays where inference of toxicity through epitopic (i.e. structural) recognition of toxin by antibody is made less definitive where affinities for multiple toxins or congeners are known, or likely to be, variable; where toxin or congener profiles in fish are known to be variable; where relative toxic potencies are poorly known; and where toxin or congener standards are not widely available. Accordingly, it was found that in the assessment of toxic fish for consumer protection a two-part protocol comprising in vitro mouse neuroblastoma cell assay to measure toxic potency and LC-MS/MS to confirm the molecular presence of ciguatoxins provided the most appropriate information for decisions of public health and economic importance.

7. Ciguatoxin adverse affect levels

The availability and analysis of meal remnants directly linked to ciguatera disease is infrequent. Most often, implicated meal remnants are discarded and the only samples available for analysis are from implicated “lots” of fish. In commercial practice the term “lot” can mean fish caught at the same time from a particular location; fish sold as a single grouping and perhaps co-mingled with others from different capture locations; or fish sold by a particular vendor with little or no record of origins. Therefore, traceback to a specific lot may not be helpful in identifying the source of toxic fish. While the availability of fish from implicated lots is potentially useful for circumstantial inference, the documented variability of ciguatoxin burden in fish from any particular region limits the usefulness of such analyzes for fish poisoning confirmation, much less the assessment of exposure thresholds. Information derived from the study of ciguatera disease outbreaks has improved clinical recognition and timely treatment of this disease. Where implicated meal remnants are available such studies are equally important for the differentiation of ciguatoxin profiles in fish from one region to the next, the determination of toxicity thresholds in humans, and the development of regulatory policy to protect consumers.

In the Pacific Lehane and Lewis (2000) noted that mild outbreaks of ciguatera occur after exposures of 1 ng P-CTX-1/kg body weight. They assumed consumption of 500 g fish/meal to derive a threshold ciguatoxin contamination rate in fish of 0.1 ppb P-CTX-1 equivalent toxicity using the mouse bioassay. Applying a 10X safety factor to address individual human risk factors, uncertainty in the amount of fish consumed, and uncertainty in assay accuracy, the authors suggested that a “safe” fish would contain no more that 0.01 ppb P-CTX-1 equivalent toxicity (see also Lehane, 2000). Similarly, in the Caribbean Vernoux and Lewis (1997) estimated a threshold contamination rate of 1.0 ppb C-CTX-1 equivalent toxicity using the mouse bioassay, and no more than 0.1 ppb C-CTX-1 for safe fish after a 10X safety factor is applied. A review of exposure levels in 109 outbreaks of ciguatera in the United States from 1998–2008 was consistent with the suggested adverse effects thresholds and “safe” contamination rates for C-CTX-1 and P-CTX-1 equivalent toxicity in fish (Dickey et al., 2008; Dickey, 2008). These estimates were derived from analyses of implicated “meal remnant” fish tissues using a tiered protocol of in vitro neuroblastoma cell assay to measure toxicity and LC-MS/MS to confirm the molecular presence of ciguatoxins in fish tissues. The aforementioned studies may serve as the basis for industry and consumer advisory levels of 0.10 ppb C-CTX-1 equivalent toxicity in fish from the tropical Atlantic, Gulf of Mexico, Caribbean, and 0.01 ppb P-CTX-1 equivalent toxicity in fish from Pacific regions.

8. Summary

Establishment of industry and consumer guidance for controlling the ciguatera hazard and prevent illness has been constrained by many factors. Much work remains before risk assessments and effective management plans can be undertaken. Interdisciplinary science has made progress in developing the critical information required for ciguatera risk analysis. The principal ciguatera toxins found in fish have been identified and are traceable through the food web to a single genus of dinoflagellate, Gambierdiscus spp.; the toxins produced by Gambierdiscus spp. are assimilated and metabolized through trophic transfer in many species of food fish; and the burden of toxins accumulated by fish that induce ciguatera fish poisoning among consumers can be measured. Significant progress in ciguatera research has been made in recent years but many questions pertaining to environmental and public health risk analysis remain to be addressed.

Conflict of interest

The authors declare that there are no conflicts of interest.

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