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Ototoxicity of paclitaxel in rat cochlear organotypic cultures

Yang Dong [a,b], Dalian Ding [b], Haiyan Jiang [b], Jian-rong Shi [a], Richard Salvi [b], Jerome A. Roth [c,*]

[a] Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China
[b] Center for Hearing and Deafness, University at Buffalo, NY 14214, USA
[c] Department of Pharmacology and Toxicology, University at Buffalo, NY 14214, USA

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ABSTRACT

Paclitaxel (taxol) is a widely used antineoplastic drug employed alone or in combination to treat many forms of cancer. Paclitaxel blocks microtubule depolymerization thereby stabilizing microtubules and suppressing cell proliferation and other cellular processes. Previous reports indicate that paclitaxel can cause mild to moderate sensorineural hearing loss and some histopathologic changes in the mouse cochlea; however, damage to the neurons and the underlying cell death mechanisms are poorly understood. To evaluate the ototoxicity of paclitaxel in more detail, cochlear organotypic cultures from postnatal day 3 rats were treated with paclitaxel for 24 or 48 h with doses ranging from 1 to 30 μM. No obvious histopathologies were observed after 24 h treatment with any of the paclitaxel doses employed, but with 48 h treatment, paclitaxel damaged cochlear hair cells in a dose-dependent manner and also damaged auditory nerve fibers and spiral ganglion neurons (SGN) near the base of the cochlea. TUNEL labeling was negative in the organ of Corti, but positive in SGN with karyorrhexis 48 h after 30 μM paclitaxel treatment. In addition, caspase-6, caspase-8 and caspase-9 labeling was present in SGN treated with 30 μM paclitaxel for 48 h. These results suggest that caspase-dependent apoptotic pathways are involved in paclitaxel-induced damage of SGN, but not hair cells in cochlea.

Introduction

Paclitaxel is an antineoplastic drug widely used for the treatment of ovarian, breast, lung and, cervical cancers as well as many head and neck neoplasms (Forastiere et al., 1993; Hunter et al., 2011; Jeansonne et al., 2011; Bicaku et al., 2012). As a tubulin stabilizer, paclitaxel inhibits proliferation of tumor cells by inducing tubulin polymerization, hindering the formation of the mitotic spindle causing the arrest of proliferating cells in the G2/M-phase of the cell cycle which ultimately leads to tumor cell death by either apoptosis or phagocytosis (Tishler et al., 1992; Foland et al., 2005). Critical side-effects of paclitaxel include neurotoxicity, hypersensitivity reactions, hematologic toxicity, cardiac disturbances and gastrointestinal tract symptoms (Arbuck et al., 1993; Laskin et al., 1993; Rowinsky et al., 1993) limiting its clinical use. Peripheral neuropathy is the most common side-effect of paclitaxel resulting in a variety of sensory symptoms such as numbness and paresthesia in a glove-and-stocking distribution (du Bois et al., 1999; Pace et al., 2007). In neurons, paclitaxel-induced tubulin polymerization interferes with axonal transport which presumably is responsible for the peripheral neuropathy. The neurotoxic effects of paclitaxel on the dorsal root ganglia (DRG) are dose and time dependent and often result in sensory disorders such as allodynia and hyperalgesia and a reduction of sensory nerve conduction velocity (Cavaletti et al., 2000). The damaging effects of paclitaxel on peripheral axons are thought to be due to it neurotoxic effects on satellite cells, peripheral glial cells that form the protective and supporting envelope of myelin surrounding peripheral axons (Kiya et al., 2011).

Since paclitaxel preferentially damages sensory neurons in the dorsal root ganglion, it may be predicted to similarly disrupt neurons within the auditory periphery. Although several clinical reports suggest that paclitaxel can cause mild to moderate sensorineural hearing loss (Gogas et al., 1996; Tibaldi et al., 1998), direct evidence elucidating the nature and locations of its toxic effects on sensory hair cells, cochlear support cells and neurons is lacking. One reason factor that clouds our understanding of the ototoxic of paclitaxel is that it is often co-administered with a variety of other antineoplastic agents, such as cisplatin (Ding et al., 2011a; Jamesdaniel et al., 2012), that are known to cause hearing loss (Bellmunt et al., 2012). Consequently, functional deficits to the auditory system occurring during or after antitumor treatment were almost always attributed to other ototoxic drugs (Cavaletti et al., 1997; Wandt et al., 1999). Another possible reason why paclitaxel by itself may not be considered ototoxic is that it may cause a "hidden hearing loss" by primarily damaging the auditory nerve fibers rather than the outer hair cells (OHC). Damage to auditory nerve fibers or inner hair cells (IHC) has little effect on hearing thresholds when assessed with the "gold standard" audiogram (Lobarinas et al., 2013).

Abbreviations: taxol, Paclitaxel; SGN, spiral ganglion neurons; OHC, outer hair cells; IHC, inner hair cells; ABR, auditory brainstem response; ANF, auditory nerve fibers.

* Corresponding author at: Department of Pharmacology and Toxicology, 11 Cary Hall, University at Buffalo, Buffalo, NY 14214, USA.
E-mail address: jaroth@buffalo.edu (J.A. Roth).

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However, if paclitaxel were to selectively damage auditory nerve fibers within the inner ear its toxic effects might only be detected by measuring the compound action potential (Ding et al., 1999; Salvi et al., 1999) or the auditory brainstem response (ABR), in particular wave I (Starr et al., 2001; Ye et al., 2012). This is consistent with previous animal studies in which auditory brainstem response (ABR) threshold elevations were observed in mice and guinea pigs with intact sensory hair cell suggesting that selective nerve damage, characteristic of peripheral neuropathy, is responsible for the normal cochlear potentials (Atas et al., 2006). Thus, the histopathological consequences of paclitaxel treatment may be interesting to investigate under well controlled conditions to determine if paclitaxel damages the sensory hair cells or if it preferentially damages the nerve fibers of the spiral ganglion neurons (SGN) similar to "auditory neuropathy," a condition in which the hair cells are functional normal, but the auditory nerve is damaged thereby preventing sound transmission to the central nervous system (Starr et al., 2003; Berlin et al., 2005; Zeng et al., 2005; Berlin et al., 2010). Accordingly, studies were performed to determine whether paclitaxel by itself is damaging to the sensory hair cells, supporting cells and neurons in the inner ear. To accomplish this, we applied various doses of paclitaxel to cochlear organotypic cultures from postnatal day three (P3) rats and identified the histopathological changes occurring in the hair cells, support cells, SGN and their nerve fibers.

Materials and methods

Cochlear organotypic cultures and paclitaxel treatment. Cochlear organotypic cultures were prepared from postnatal day 3 SASCO Sprague-Dawley rats following procedures similar to those described previously (Wei et al., 2010; Ding et al., 2011b). The cochlea was removed and the entire organ of Corti and SGN transferred on to rat tail type I collagen gel in basal Eagle medium containing 2% sodium carbonate. A 15-μl drop of the collagen solution was placed on the surface of a 35 mm culture dish and allowed to gel for approximately 30 min. Afterwards, 1.3 ml of culture medium (0.01 g/ml bovine serum albumin, 1% Serum-Free Supplement (Sigma I-1884), 2.4% of 20% glucose, 0.2% penicillin G, 1% BSA, 2 mM glutamine, 95.4% of 1 BME) was added to the dish. The cultures were placed in an incubator at 37 °C in 5% CO_2 overnight. On the following day, fresh medium was added alone or in the presence of various concentrations of paclitaxel. Paclitaxel stock solution was made at a concentration of 30 mM in 1 μl DMSO and diluted in culture medium to a final paclitaxel concentration of 0 μM, 1 μM, 10 μM or 30 μM. Cochlear explants were treated with culture medium containing 1 μM, 10 μM or 30 μM paclitaxel or control medium (0 μM paclitaxel) containing an equivalent concentration of DMSO (0.001 μl) and cultured in 5% CO_2 at 37 °C in a humidified atmosphere. These experiments were approved by the University at Buffalo's Animal Care and Use Committee.

Histological evaluation. At the end of each experiment, the cochlear explants were fixed with 10% formalin for 2 h and subsequently washed with 0.1 M phosphate buffered saline (PBS). Auditory nerve fibers and SGN were labeled and evaluated along the entire length of the cochlea (Ding et al., 2011b). Specimens were immunolabeled with primary monoclonal antibody against neuronal class III β-tubulin (Covance, TUJ1, # MMS-435P; 1:100 dilution) overnight at 4 °C. After washing with PBS three times, specimens were labeled with a secondary antibody conjugated with Cy3 (goat anti-mouse IgG, Jackson ImmunoResearch; #115-165-205, lot 80054; 1:200 dilution) for 60 min at room temperature. To visualize F-actin that is heavily expressed in the stereocilia bundles and cuticular plate of the hair cells specimens were labeled with phalloidin conjugated to Alexa Fluor 488 (Life Technologies, A12379; lot 1488579; 1:100 dilution); labeling was carried out after completing the secondary antibody labeling. To assess paclitaxel-induced pathological changes to the nucleus, specimens were stained with To-Pro-3 (Life Technologies; #53605; Lot 1351934; 1 mM stock solution diluted 1:1000) for 20 min following the manufacturer's protocol. After rinsing with PBS, specimens were mounted on glass slides in glycerin, coverslipped and examined using a confocal microscope (Zeiss LSM-510 meta, step size 0.5 mm per slice) using appropriate filters to detect the red fluorescence of Cy3 labeling in auditory nerve fibers and SGN (excitation 550 nm, emission 570 nm), green fluorescence of Alexa Fluor 488 in the stereocilia bundles and cuticular plate of hair cells (excitation 488 nm, emission 520 nm) and far red fluorescence of To-Pro-3 in the nucleus (excitation 642 nm, emission 661 nm). Confocal images were stored on disk and processed using Confocal Assistant and Adobe Photoshop 5.5 software.

Hair cells counts. To quantify hair cell loss produced by paclitaxel, cochlear cultures were examined under a fluorescence microscope (Zeiss Axioskop 400×) equipped with appropriate filters for detection of Alexa Fluor 488 fluorescence. The numbers of missing IHC and OHC were counted in consecutive 0.24 mm segments along the entire length of the cochlea (Ding et al., 2011b). Hair cells were classified as missing if the stereocilia and the cuticular plate were absent. Using hair cell count data from normal SASCO Sprague-Dawley rats as a reference (i.e., lab norms), a cochleogram was constructed showing the percent IHC and OHC loss as a function of the percent distance from the apex of the cochlea. Hair cell losses from individual cochleograms were averaged to generate a mean cochleogram for each condition using custom software.

TUNEL and caspase staining. Cochlear explants were treated with 30 μM paclitaxel in culture medium or control medium for 48 h to evaluate the extent of TUNEL labeling using the APO-BrdU TUNEL Assay Kit (Life Technologies, #A23210) according to the manufacturer's protocol. To determine if cell death was caspase-mediated, cochlear explants were also evaluated for the expression of activated caspases-6, -8 or -9 using fluorescently-labeled caspase-6 inhibitor (CaspaTag 6, FAM-VEID-FMK, # 654, 1:30 in solvent, Intergen), caspase-8 inhibitor (CaspaTag 8, # 8004, 1:30 in solvent, Intergen) or caspase-9 inhibitor (CaspaTag 9, # 8003, 1:30 in solvent, Intergen) following the manufacturer's instructions. The caspase inhibitors enter living cells and irreversibly bind to the specific activated caspase present in cells undergoing apoptosis (Amstad et al., 2001). Cochlear specimens were incubated for 1 h at 37 °C with a caspase-6, -8 or -9 probe ($n = 6$/caspase probe). Samples were subsequently fixed with 10% formalin in PBS for 20 min and then labeled with To-Pro-3 as described above. Cochlear cultures were mounted in glycerin on glass slides and examined with a confocal microscope using appropriate filters to detect the carboxyfluorescein activated caspases (excitation 488 nm, emission 529 nm) and with To-Pro-3 which labels the nuclei. Images were processed with Advanced Imaging Microscopy (version 4.0, Carl Zeiss) and Adobe Photoshop as described previously (Qi et al., 2008).

Results

Paclitaxel damaged hair cells, auditory nerve fibers and SGN

After 24 h treatment, no obvious histopathologies were observed in hair cells, auditory nerve fibers or SGN of organotypic cultures ($n = 5$/condition 1 μM, 10 μM and 30 μM) with any of the doses of paclitaxel employed (data not shown). However, after 48 h exposure, paclitaxel produced significant damage to cochlear hair cells which increased in a dose-dependent manner. Fig. 1A shows an example of a normal control cochlea maintained in culture for 48 h ($n = 5$). Staining with Alexa Fluor 488 revealed bright green staining of the stereocilia and to a lesser extent the cuticular plate of the three orderly rows of OHC and one row of IHC (Fig. 1A). Cell nuclei were labeled with To-Pro-3;

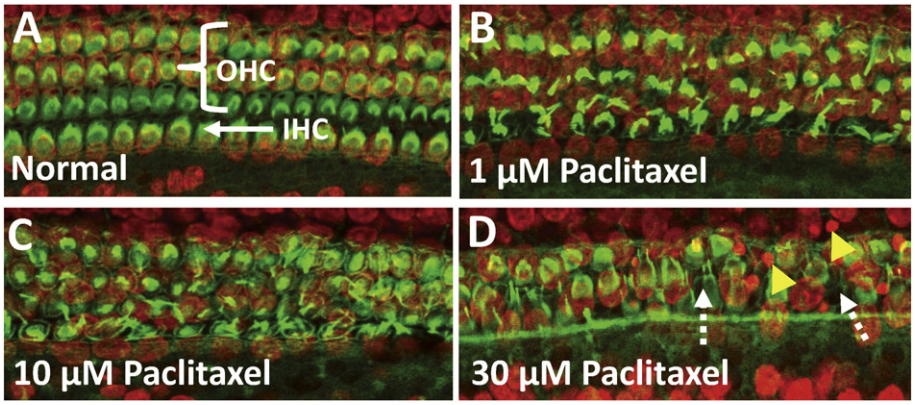

Fig. 1. Representative confocal images of cochlear surface preparations (upper basal turn, $n = 5$/condition) double labeled with Alexa-Fluor 488-phalloidin (green), which labels the stereocilia and cuticular plate of outer hair cells (OHC) and inner hair cells (IHC) and ToPro-3 (red) which labels nuclei. Cochleas were cultured for 48 h. (A) Normal control culture and cultures treated with paclitaxel concentrations of (B) 1 μM, (C) 10 μM or (D) 30 μM. (B and C) Stereocilia were disordered, but nuclei were present with 1 and 50 μM paclitaxel. (D) Most stereocilia missing, some nuclear condensation (yellow arrowhead) and a few missing hair cells (dashed white arrow).

to more easily visualize the blue fluorescent signal of To-Pro-3, signal was converted to red. Fig. 1(B–D) shows representative examples illustrating the staining patterns observed in the upper basal turn after 48 h treatment with paclitaxel (1 to 30 μM). Treatment for 48 h with 1 μM (Fig. 1B, $n = 5$) and 10 μM (Fig. 1C, $n = 5$) paclitaxel resulted in a dose-dependent increase in stereocilia disarray and damage. Increasing the concentration of paclitaxel to 30 μM ($n = 5$) resulted in almost total loss of stereocilia, the loss of some hair cells indicated by the absence of the cuticular plate and nuclear shrinkage and condensation of some hair cell nuclei (Fig. 1D).

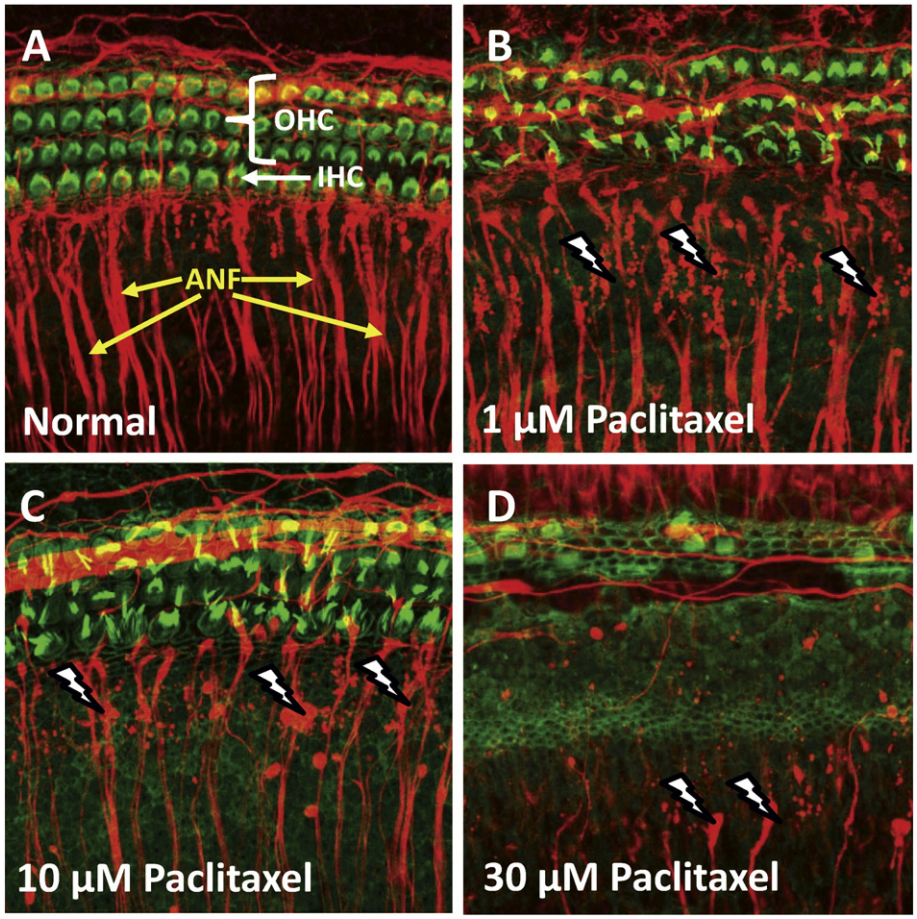

Fig. 2. Representative photomicrographs taken from the lower portion of the apical turn of cochlear explants maintained in culture for 48 h. Specimens ($n = 5$/condition) were double-stained with β-tubulin (red) and phalloidin (green). (A) Normal controls; note three orderly rows of OHC (bracket) and one row of IHC (white arrow) and thick fascicles of ANF (yellow arrows) projecting radially to the hair cells. Cultures treated with 1 μM (B), 5 μM (C), or 30 μM (D) of paclitaxel. As the dose of paclitaxel increased from 1 to 30 μM hair cell and ANF damage increased in a dose-dependent manner. Jagged, white arrows label degenerating ANF.

Fig. 2 provides representative examples of the auditory nerve fibers (ANF) ($n = 5$/condition; images taken from the lower portion of the apical turn) as they project out to the hair cells in normal and paclitaxel treated cochlear explants maintained in culture for 48 h. Specimens were double-stained with Alexa 488 conjugated phalloidin and an antibody against β-tubulin. In normal control cultures, thick fascicles of ANF extend out radially to innervate the OHC and IHC (Fig. 2A). In cultures treated with 1 μM or 10 μM paclitaxel, considerable stereocilia damage and disarray were evident on the hair cells. This was associated with ANF damage characterized by nerve fiber thinning, fragmentation and bleb formation on the ANF (Fig. 2B); the blebs were quite large in some cases (Fig. 2C). When the concentration of paclitaxel was increased to 30 μM, many hair cells were destroyed and most ANF were missing (Fig. 2D). Damage to ANF after 48 h treatment with paclitaxel was concentration dependent and occurred at concentrations as low as 1 μM paclitaxel.

The SGN, which give rise to the radial ANF, were assessed under the same control and experimental conditions as in Fig. 2 ($n = 5$/condition). Representative photomicrographs taken from the upper basal turn are shown in Fig. 3. In controls, the SGN somas were large, round and densely packed. ANF can be seen emerging from some SGN (Fig. 3A). The cytoplasm, but not the nucleolus, of the SGN was intensely labeled with tubulin (Fig. 3A). Paclitaxel treatment damaged the SGN in a concentration dependent manner. Treatment with 1 μM produced mild shrinkage of SGN (Fig. 3B) and caused a slight reduction in the number of SGN. In addition tubulin staining of the cytoplasm was less intense in paclitaxel treated samples than in controls and in a few cases the SGN were condensed. As the dose of paclitaxel increased from 10 to 30 μM, the number SGN decreased and there was greater shrinkage and condensation of the SGN soma (Figs. 3C-D).

Paclitaxel dose-dependent hair cell damage

To evaluate the location and degree of cochlear pathology induced by paclitaxel, mean cochleograms ($n = 7$) were measured to show the percent loss of OHC (OHC1, 2, 3) and IHC after 48 h treatment with paclitaxel. Few IHC and OHC were missing in control cultures (Fig. 4A). Treatment with 1 μM paclitaxel (Fig. 4B) induced only minor hair cell loss in the base of the cochlea (80–100%, Fig. 4B). Interestingly, 10 μM paclitaxel resulted in moderate IHC loss, but minor OHC loss in the base of the cochlea (Fig. 4C, 60–100%). When the dose was increased to 30 μM paclitaxel, there was considerable loss of IHC and OHC (Fig. 4D). Hair cell losses decreased from the base (100%) to apex (10%) and surprisingly losses were again greater for IHC than OHC unlike other ototoxic drugs that typically cause greater OHC than IHC damage (Ding et al., 2011a; Jamesdaniel et al., 2012).

Paclitaxel induced apoptosis of SGN

To determine if cell death was mediated by apoptosis, TUNEL staining of SGN was examined in the upper basal turn ($n = 4$/condition) following treatment with 0 μM (control) or 30 μM paclitaxel for 48 h. TUNEL labeling was absent in SGN in normal control explants

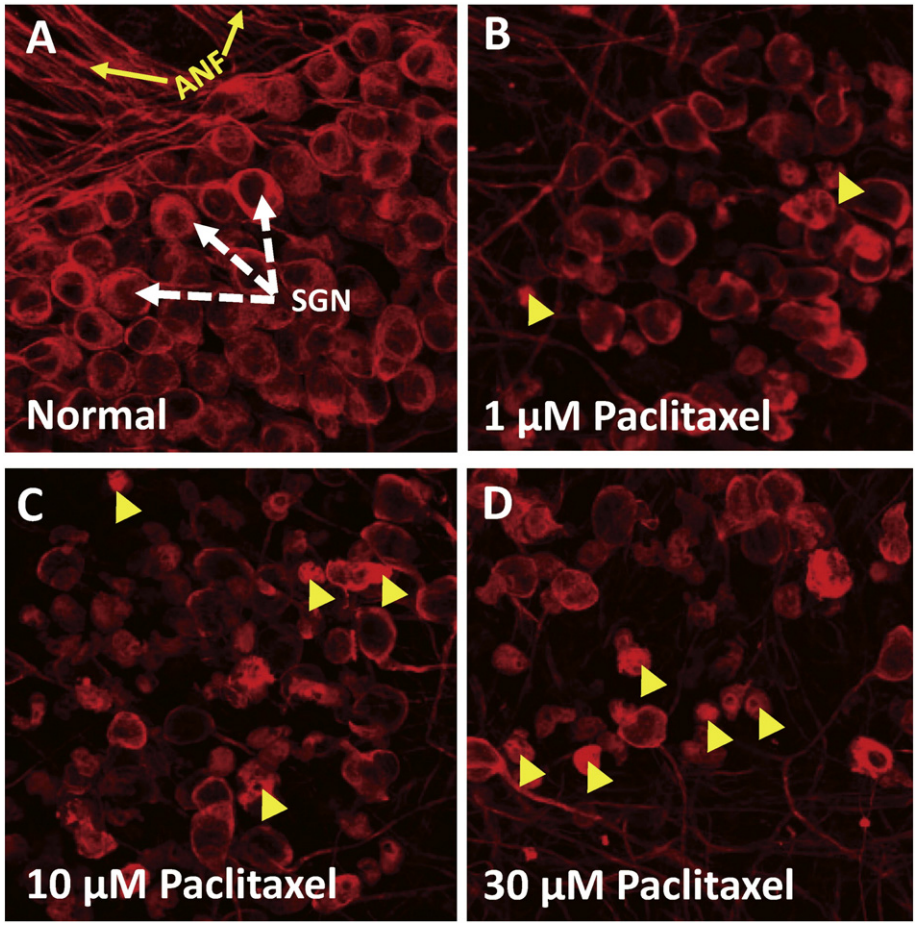

Fig. 3. Representative photomicrographs of SGN from upper basal turn ($n = 5$/condition) cultured for 48 h and afterwards immunolabeled with β-tubulin. (A) Normal control cultures with large, round, densely package SGN (dashed arrows) and ANF (yellow arrows) extending radially toward the hair cells. (B) SGN treated with 1 μM paclitaxel. Note mild soma shrinkage, decreased tubulin staining, and a few condensed cells (yellow arrowhead). (C and D) Treatment with 10 and 30 μM paclitaxel increased SGN condensation (yellow arrowheads) and decreased the number of SGN.

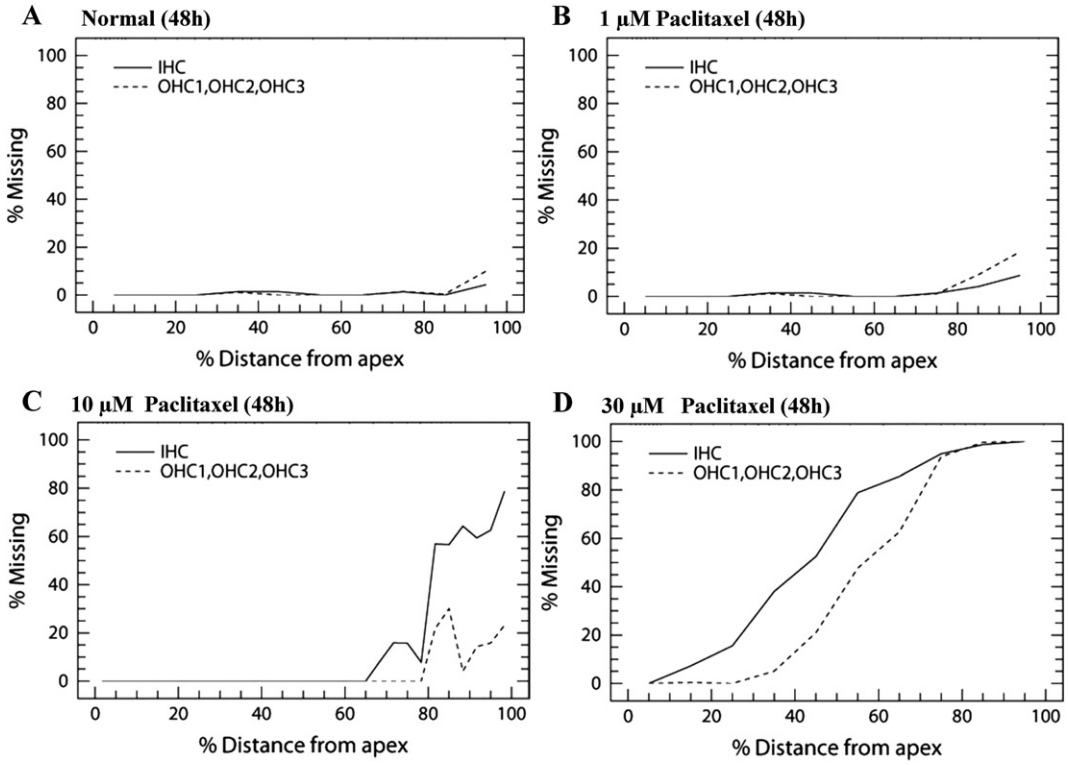

Fig. 4. Mean ($n = 7$/group) cochleograms obtained from cochleas cultured for 48 h. Cochleograms show the percentage of missing IHC and OHC (rows 1–3) as a function of percent distance from the apex of the cochlea. Data from (A) normal controls and cultures treated with (B) 1 μM, (C) 10 μM and 30 μM paclitaxel. IHC and OHC losses increased with paclitaxel dose. IHC losses were typically greater than OHC loss. Hair cell loss was greatest near the base of the cochlea (100% distance from apex) and decreased toward the apex.

(Fig. 5A). In contrast, paclitaxel treatment resulted in condensed and fragmented nuclei (karyorrhexis) in degenerating SGN (Fig. 5B) suggesting that cell death proceeds via apoptotic mechanisms.

Paclitaxel activates caspases in SGN

To determine whether paclitaxel-induced SGN cell death was caspase mediated, activated caspase-6, -8 and -9 staining was evaluated in control cochlear cultures ($n = 4$/caspase) and cultures ($n = 4$/caspase) treated for 48 h with 30 μM paclitaxel using specific fluorogenic cell-permeable, carboxyfluorescein probes. The activated forms of caspase-6, -8 and -9 were not detected in SGN in control cultures (Figs. 6A, C, and E, upper basal turn). However, after paclitaxel treatment, the activated species of caspase-8 (Fig. 6B), -9 (Fig. 6D) and -6 (Fig. 6F) were seen in some SGN (green/yellow/orange labeling, upper basal turn). In contrast, there was no evidence of caspase-8, -9 and -6 staining in the organ of Corti after 48 h treatment with 30 μM paclitaxel (Figs. 7A–C, upper

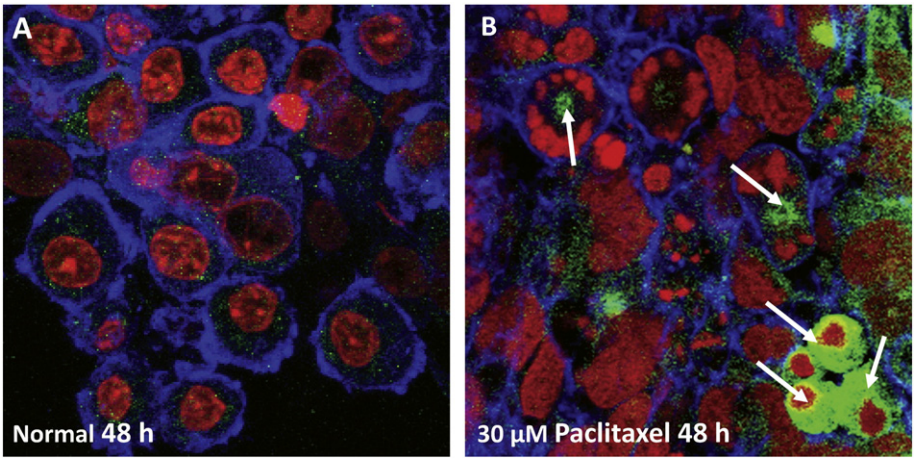

Fig. 5. Representative images (upper basal turn) of SGN obtained from normal control ($n = 4$) and paclitaxel ($n = 4$) treated cultures that were triple-stained with β-tubulin (blue), To-Pro 3 (red) and TUNEL (green). (A) TUNEL labeling was absent in SGN from normal controls after 48 h in culture. (B) After 48 h treatment with 30 μM paclitaxel TUNEL labeling (white arrows) was present in SGN with condensed and fragmented (karyorrhexis) nuclei.

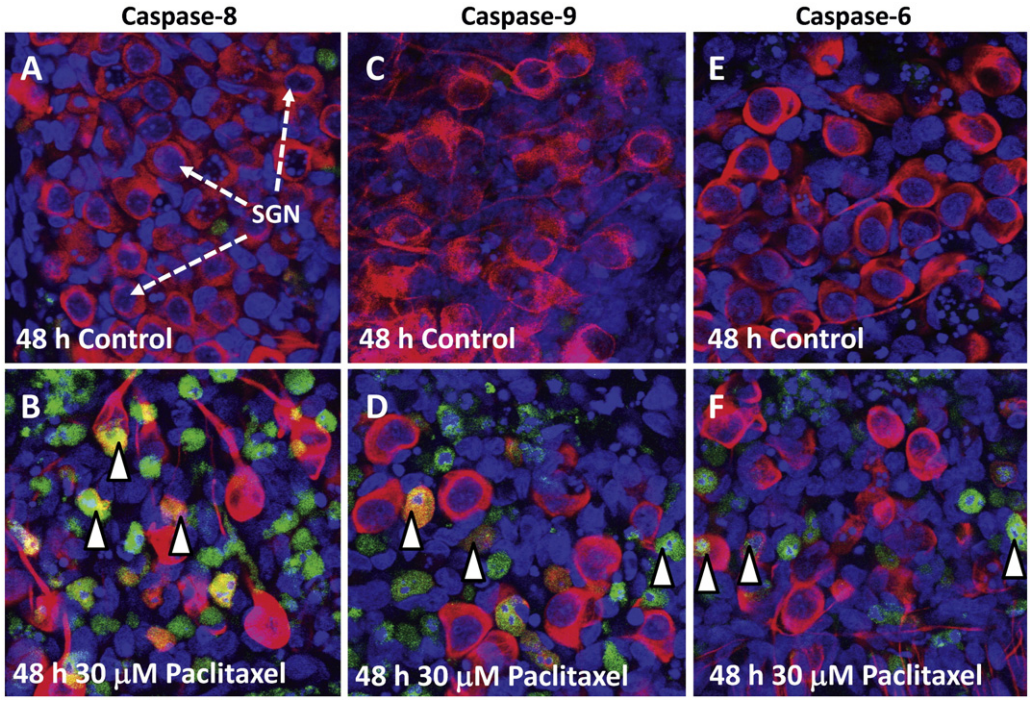

Fig. 6. Representative images (upper basal turn) of SGN obtained from control ($n = 4$) and paclitaxel ($n = 4$) treated (30 μM, 48 h) cochlear cultures. SGN labeled with β-tubulin (red) and one of three cell permeable, fluorogenic probes (green); each of the probes preferentially labeled one of the three caspases; specifically caspase-6, -8 or -9. All three caspases, caspase-8 (A), caspase-9 (C), and caspase-6 (E) were absent from control cultures. After paclitaxel treatment, caspase-8 (B), caspase-9 (D), and caspase-6 (F) were detected in some SGN (yellow/green, yellow dashed arrow) or small support cells (white arrowhead points to green, yellow caspase label) adjacent to SGN.

basal turn, $n = 4$/caspase). These results suggest that paclitaxel-mediated SGN cell death is caspase mediated whereas hair cell death in the organ of Corti is caspase-independent.

Discussion

Because of the cytotoxicity and tissue penetration of antineoplastic agents, many of these compounds have the potential to enter into the inner ear and cause hearing impairment (Cummings, 1968; Stadnicki et al., 1975; Lugassy and Shapira, 1990). The typical mechanisms by which anticancer drugs kill cancer cells may be quite different from the processes that induce cell death in non-proliferating hair cells and neurons of the inner ear. Hirose et al. (2011) screened a library of 88 anti-cancer drugs (National Cancer Institute Approved Oncology Drugs Set) for the ability to damage hair cells in the zebrafish lateral line. Agents found to promote hair cell degeneration included cisplatin as well as a number of other anticancer drugs. Moreover, combined treatment with several of these drugs synergistically enhanced hair cell loss. However, paclitaxel was not identified as toxic in this zebrafish drug screen. A number of case reports provide circumstantial evidence that other anti-cancer drugs besides those identified in the zebrafish drug screen promote hearing loss (Tibaldi et al., 1998; Moss et al., 1999; Attili et al., 2008). Unfortunately, the potential of these drugs to induce hearing loss is difficult to interpret based on clinical findings because patients are typically treated with multiple anti-cancer agents making it difficult to attribute ototoxicity to any single drug in the cocktail. Because of well-known species differences, drug screen testing in fish, in which hair cell proliferation can occur, may not accurately recapitulate toxicities in humans or other mammals. For example, paclitaxel was one of many drugs noted by Hirose et al. (2011) as not promoting hair cell degeneration. Failure of paclitaxel to induce zebrafish hair cell loss is not totally unexpected as the toxic actions may be cell and/or

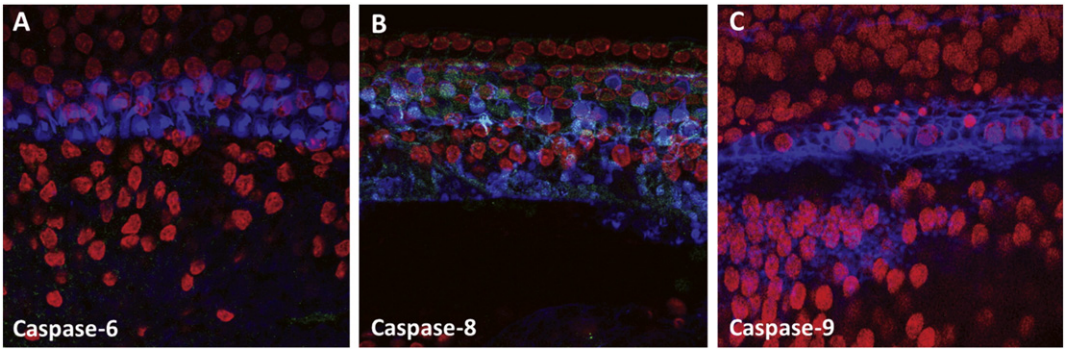

Fig. 7. Representative confocal images (upper basal turn, $n = 4$/caspase) obtained from the organ of Corti after 48 h treatment with 30 μM paclitaxel. Specimens were triple-stained with phalloidin (blue, actin labeling), To-Pro 3 (red, nuclear labeling) and caspase-6, caspase-8 or caspase-9 (green). Caspase-6 (A), caspase-8 (B), and caspase-9 were not detected in the organ of Corti under these conditions even though all three caspases were observed in some SGN and adjacent support cells (see Fig. 6).

species specific. Since paclitaxel induces tubulin polymerization, it may be less likely to affect hair cell viability. Induction of tubulin polymerization is thought to interfere with axonal transport in post-mitotic, non-dividing neurons, which is believed to be responsible for much of the toxic actions and peripheral neuropathies seen in patients taking paclitaxel. The lack of change in the hearing threshold previously reported for paclitaxel may be due to the fact that it is less toxic to hair cells as indicated by the studies of Hirose et al. (2011) and the findings reported in this paper where low dose (1 μM) paclitaxel, although capable of damaging hair cell stereocilia (Fig. 2B), had little effect on cuticular plate. In contrast, low dose paclitaxel causes considerable damage and loss of ANF. At higher doses, however, paclitaxel caused considerable damage to both hair cells and nerve fibers.

Given the accepted toxic mechanism of paclitaxel, it is not surprising that significant damage occurred to both the auditory nerve fibers and SGN since various isoforms of β-tubulin are expressed in both hair cell and neurons (Hallworth and Luduena, 2000; Jensen-Smith et al., 2003). Microtubules play an important role in axonal transport and since paclitaxel is a microtubule-stabilizing agent, paclitaxel is anticipated to severely disrupt axon transport leading to the degeneration of nerve fibers. Damage to SGN and auditory nerve fibers is concentration dependent and observed at all concentrations of paclitaxel employed. It should be noted that loss of SGN in the cochlea may not result in elevation of hearing thresholds as measured by the traditional audiogram, which is primarily caused by OHC loss. This probably accounts for the fact that paclitaxel has not been identified as a major ototoxic drug. It is also important to stress that the neurotoxic actions of paclitaxel observed at the lowest concentration employed (1 μM) in our study are comparable to blood concentrations achieved in cancer patients taking the drug (Kobayashi et al., 2007; Ando et al., 2012).

Identification of the pathways promoting cell injury which lead to cell death are key to understanding the mechanisms by which paclitaxel promotes degeneration of the cells within the inner ear. Paclitaxel-evoked peripheral neuropathy is associated with swollen and vacuolated mitochondria as well as opening of the mitochondrial permeability transition pore (PTP) in axons (Flatters and Bennett, 2006). Both these responses, upon treatment with paclitaxel, are also observed in non-neuronal tissue as well (Andre et al., 2000; Varbiro et al., 2001). Opening of the PTP by paclitaxel results in increased permeability of mitochondria to a variety of ions and induces release of calcium from the mitochondria (Kidd et al., 2002) thus leading to increased oxidative stress and apoptotic cell death. Paclitaxel was also reported to induce apoptosis in cortical neurons by regulating JNK activity and its downstream transcription and inhibition of the PI3K/AKT pathway (Figueroa-Masot et al., 2001). Similarly paclitaxel induced sustained phosphorylation of JNK/SAPK in human neuroblastoma SH-SY5Y cells suggesting cell death occurs via an apoptotic mechanism possibly independent on its effect on tubulin (Nicolini et al., 2003). In contrast, paclitaxel induced cell death in post-mitotic non-dividing DRG neurons occurs predominantly by necrosis as demonstrated by morphological and molecular analysis (Scuteri et al., 2006). Results presented herein suggest that paclitaxel-induced hair cell and SGN damage may similarly be initiated by different cytotoxic mechanisms. For example, paclitaxel treatment resulted in positive TUNEL staining in the SGN indicative of an apoptotic mechanism, whereas TUNEL staining was not observed in hair cells implying that cell death occurs via other degenerative process. Confirming this difference is the observation that 30 μM paclitaxel-treatment for 48 h induced activation of the apoptotic markers caspase-6, -8 and -9 in degenerating SGN but failed to induce caspase labeling in the organ of Corti. As to why the toxic mechanism of paclitaxel is different in these cells is not known but may suggest difference in the interaction of this drug possibly with the distribution or function of different forms of β-tubulin present in these cells (Hallworth and Luduena, 2000; Jensen-Smith et al., 2003). The subtypes and distribution of β-tubulin may also account for differences in the sensitivity of IHC and OHC to paclitaxel as noted herein (Hallworth and Luduena, 2000).

In conclusion, our results demonstrate for the first time that paclitaxel has the potential to cause hearing impairment at concentrations that can be achieved in vivo. Paclitaxel appears to be more toxic to auditory nerve fibers and SGN than hair cells. Paclitaxel-induced damage to SGN and ANF may go largely undetected by conventional audiometric testing which is mainly geared to detection of OHC damage. In addition, the mechanism of cellular toxicity was also different between nerve fibers and hair cells as neuronal tissue proceeds via an apoptotic process whereas hair cell loss most likely occurs via necrosis. Based on these findings, the potential ototoxic mechanism of paclitaxel will likely be additive with other antineoplastic agents known to induce hearing loss.

Conflict of interest

There is no conflict of interest which effects objectivity in regard to publishing this paper.

Acknowledgments

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