

## 12th Yahya Cohen Memorial Lecture – The Cellular and Molecular Basis of Radiation-induced Sensori-neural Hearing Loss

Wong-Kein Low,<sup>1</sup>*FRCSEd, PhD, FAMS (ORL)*, Michelle GK Tan,<sup>2</sup>*PhD*, Alvin WC Chua,<sup>3</sup>*MSc*, Li Sun,<sup>2</sup>*MD*, De-Yun Wang,<sup>4</sup>*MD, PhD*

### Abstract

**Introduction:** Sensori-neural hearing loss (SNHL) is a frequent complication of conventional radiotherapy for head and neck tumours, especially nasopharyngeal carcinoma. To manage radiation-induced ototoxicity appropriately, an understanding of the cellular and molecular basis of this complication is necessary. **Materials and Methods:** A medline search of relevant literature was done, focusing on the radiation-induced cellular and molecular processes that lead to hair cell death in the cochlea. **Results:** Radiation-induced SNHL occurs in the cochlea, with the retro-cochlear pathways remaining functionally intact. By simulating radiotherapy regimes used clinically, radiation-induced cochlear cell degeneration in the absence of damage to the supporting structures and blood vessels has been demonstrated in animals. This could be due to apoptotic cochlear cell death, which has been shown to be associated with p53 upregulation and intra-cellular reactive oxygen species (ROS) generation. Oxidative stress may initiate the upstream processes that lead to apoptosis and other cell death mechanisms. **Conclusions:** A model of radiation-induced SNHL based on a dose and ROS-dependant cochlear cell apoptosis, is proposed. This model supports the feasibility of cochlear implantation, should one be clinically indicated. It can explain clinical observations such as radiation-induced SNHL being dose-dependent and affects the high frequencies more than the lower frequencies. It also opens up the possibility of preventive strategies targeted at different stages of the apoptotic process. Antioxidants look promising as effective agents to prevent radiation-induced ototoxicity; they target upstream processes leading to different cell death mechanisms that may co-exist in the population of damaged cells.

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### Introduction

Radiation-induced sensori-neural hearing loss (SNHL) has long been recognised as a complication of radiotherapy (RT) for head and neck tumours, if the auditory pathways had been included in the radiation fields. In Singapore, nasopharyngeal carcinoma (NPC) is common and the prevalence of SNHL after radiotherapy for NPC has been reported to be as high as 24%.<sup>1</sup> Radiation-induced ototoxicity is therefore an important clinical problem in Singapore. To be able to manage radiation-induced ototoxicity appropriately, a good understanding of the cellular and molecular basis of this complication is necessary.

### Site of Lesion

Kwong et al<sup>1</sup> reported sensori-neural hearing loss in NPC patients after RT, but did not differentiate between sensory or neural deafness. Although radiation-induced SNHL is generally believed to be a result of damage of the auditory sensory hair cells in the cochlea, it cannot be assumed that retro-cochlear neural deafness does not occur, either alone or in combination with cochlear deafness. The intactness of retro-cochlear auditory pathways is essential, if cochlear implantation were to be considered for restoration and rehabilitation of hearing loss in irradiated patients.

Being end-cells with no capacity for cell division, adult

<sup>1</sup> Department of Otolaryngology, Singapore General Hospital, Singapore

<sup>2</sup> Department of Clinical Research, Singapore General Hospital, Singapore

<sup>3</sup> Department of Plastic Reconstructive & Aesthetic Surgery, Singapore General Hospital, Singapore

<sup>4</sup> Department of Otolaryngology, National University of Singapore, Singapore

Address for Correspondence: Dr Wong-Kein Low, Director, Centre for Hearing and Ear Implants, Singapore General Hospital, Singapore 169608.

Email: low.wong.kein@sgh.com.sg

auditory neurons are said to have great resistance to direct killing by radiation.<sup>2</sup> Nevertheless, they are still susceptible to damage by radiation in high doses. It was demonstrated in chinchillas that cochlear nerve fiber degeneration occurred after exposure to high radiation doses; in ears exposed to 40 to 50Gy and 60 to 90Gy of radiation, the incidence rates were 31% and 62% respectively.<sup>3</sup>

In a study on NPC patients who have been treated by conventional RT, significant doses of radiation were delivered to the cochlea (mean 24.1 to 62.2 Gy) and region of the auditory nerve (mean 14.4 to 43.4 Gy).<sup>4</sup> Although some of the patients studied experienced SNHL after RT, evoked response audiometry showed no statistically significant difference in the pre- and post-RT inter-wave 1-5 latencies. This suggested that in the said radiation doses of exposure, damage had occurred in the cochlea but the retro-cochlear pathways remained functionally intact.

### Apoptotic Cochlear Hair Cell Damage

In a recent study on the cochlear cell line OC-k3 by Low et al,<sup>5</sup> flow cytometry and TUNEL assays were used to document gamma radiation-induced apoptosis. Apoptosis was found to occur predominantly at 72 hours post-irradiation and was dose-dependant, with more apoptotic cells seen at 20 Gy than at 5 Gy. A limitation of this study was that the findings were based on an ex-vivo experimental model using an immortalised cochlear cell line of the mouse. In an *in vivo* situation in mammals, the cochlea consists of a finite number of post-mitotic non-regenerating hair cells, which are influenced by the body's extra-cellular environment. This being the case, is it justifiable to extrapolate these experimental findings to an *in vivo* situation?

It is believed that etiologies of SNHL such as ageing and drug toxicity, share similar cell death mechanisms leading to a final common apoptotic pathway.<sup>6</sup> Radiation-induced apoptosis has been well demonstrated in non-cochlear cell systems and is generally accepted as an important mechanism of radiation-induced cell death *in vivo*.<sup>7,8</sup> Therefore, by relating our findings to what is already known, it is not unreasonable to expect radiation-induced apoptosis occurring in cochlear hair-cells *in vivo*.

Radiation-induced SNHL has been described to have either early or late-onset. Early-onset SNHL occur within hours or days after completion of RT, whereas late-onset radiation-induced SNHL may manifest months or years after exposure. It is conceivable that early-onset SNHL is the result of acute radiation-induced cochlear cell apoptosis. In late-onset SNHL, can a similar mechanism apply?

Late-onset radiation-induced SNHL had generally been attributed to progressive vascular compromise from radiation-induced vasculitis obliterans. It is interesting that

radiation-induced SNHL does not to have any correlation with vestibular dysfunction.<sup>9</sup> In a minute structure like the inner ear, it would be reasonable to expect both the hearing and vestibular functions to be affected by significant radiation-induced vascular changes. Although the late sequelae of radiation have often been said to be due to vascular injury, this has not been supported by hard evidence.<sup>10</sup> Theoretically, vascular injury can result in reduced vitality of the irradiated tissues which could potentially compound the injury without being the cause.

In animal studies, post-irradiation microscopic changes in the vascular and supporting structures of the cochlea had been observed but these could be attributed to experimental conditions such as the use of large radiation doses and radiation fields that were not uniform. Indeed, animal experiments using radiation regimes similar to those applied clinically had demonstrated cochlear hair cell degeneration in the absence of damage to vascular and supporting structures even up to 2 years post-irradiation.<sup>3</sup> Late biological effects of radiation are well-known and the hair cell degeneration observed may well represent a late biological response to the initial cellular damage.<sup>11</sup>

It is well accepted that radiation-induced SNHL is progressive in nature. The integrity of normal tissues or organs depends on the maintenance of a certain number of normally functioning mature cells. When the depletion of functioning cells reaches a critical level, a clinically detectable effect becomes apparent.<sup>2</sup> In the case of radiation-induced SNHL, the cochlea consists of a finite number of post-mitotic non-regenerating hair cells. A patient may experience hearing loss when a critical mass of hair cells is lost and it may take several months or years after radiation exposure before this stage is reached. Hence, "late"-onset radiation-induced SNHL may possibly represent the later stages of the progression in hair cell degeneration initiated by direct cellular injury during irradiation. Alternatively, the initial radiation-induced injury could have rendered the cells more susceptible to apoptosis following subsequent exposure to insults such as noise and ototoxic medications.

### The Role of p53

In the OC-k3 inner ear cell line, Low et al<sup>5</sup> found up-regulation of p53 related genes from micro-array studies. Western blotting confirmed up-regulation of p53 at 72 hours and phosphorylation at 3, 24, 48 and 72 hours after irradiation. It is now known that p53 can induce apoptosis through 2 mechanisms: one that depends on transactivation of apoptotic-related genes and another that involves translocation of p53 to the mitochondria where it can physically interact with and inactivates prosurvival Bcl-2 proteins. The 2 mechanisms most likely do not work independently of each other as transcriptional blockage

induced p53 accumulation in the mitochondria.<sup>12</sup> PUMA, a key BH3-only antagonist of Bcl-2 proteins, was thought to couple the nuclear and cytoplasmic functions of p53.<sup>13</sup>

### The Role of Reactive Oxygen Species

There has been compelling evidence in animal models, implicating reactive oxygen species (ROS) in the damage associated with non-radiation causes such as cochlear ischemia, noise trauma, presbycusis, meningitis-associated hearing loss and aminoglycoside and cisplatin ototoxicity.<sup>14</sup> Although the exact relationship between ROS and other cell death events is still not fully understood,<sup>15</sup> ROS is believed to play a key role in the promotion of apoptosis by affecting mitochondrial permeability, release of cytochrome c and activation of p53 and caspases.<sup>16</sup> In radiation-induced apoptosis in the OC-k3 inner ear cell line, Low et al<sup>5</sup> demonstrated dose-dependant intracellular generation of ROS at 1 hour post-irradiation and was believed to be an important triggering factor in the apoptotic process. ROS could explain the observation that high frequency hearing is preferentially damaged by radiation.<sup>17</sup> In an animal study on aminoglycoside ototoxicity, outer hair-cell death in the Organ of Corti were observed to follow a base-to-apex gradient, which was eliminated by the addition of antioxidants.<sup>18</sup> This was attributed to the outer hair cells in the basal coil (respond to higher frequency sounds) having much lower levels of glutathione than those in the apical region (respond to lower frequency sounds) and therefore, a lower antioxidant capacity.<sup>17</sup>

Based on a ROS-dependant apoptotic model of radiation-induced cochlear damage, specific intervention strategies can be considered. A prerequisite for the successful use of anti-apoptotic treatment is that it must be safe and must not interfere with the efficacy of the treatment. Treatment of the inner ear is especially feasible because antioxidants and other anti-apoptotic medications can be applied topically in the middle ear. In NPC, it may be argued that medication in the middle ear may spill into the post-nasal space via the Eustachian tube, which may then affect the effectiveness of radiotherapy. This may be overcome by delivering the medication specifically to the round window by the use of osmotic pumps or gelfoam application through myringotomies or ventilation tubes.

### Multiple Cell Death Mechanisms

Although the p53-dependent apoptosis is likely to play a key role in radiation-induced cochlear cell death, it is not necessarily the only mechanism involved. A number of different mechanisms leading to cell deaths have been described and may well be also involved in radiation-induced ototoxicity.

Firstly, necrotic cell death is expected to occur, especially in high radiation doses. Although this is a passive form of

cell death which does not encompass activation of any specific cellular programme, ROS can contribute to the death process by affecting lipid peroxidation of the cell membrane.<sup>19</sup>

Secondly, p53-independent mechanisms had been described. It had been noted that apoptosis induced by high LET radiation was not affected by cellular p53 gene status.<sup>20</sup> Ionising radiation has been found to induce caspase-dependent but p53-independent cell death in *Drosophila*.<sup>21</sup> This may possibly be due to ceramide formation as ceramide has been shown to trigger caspase activation during gamma-induced apoptosis of human glioma cells lacking functional p53.<sup>22</sup>

Thirdly, caspase-independent programmed cell death mechanisms had also been described in recent years. Non-caspase proteases such as cathepsins, calpains and granzymes are involved, resulting in intra-cellular signalling processes that lead to apoptotic- or necrotic-like morphological forms of programmed cell death.<sup>23</sup> Indeed, leupeptin, a calpain inhibitor, had been shown to protect inner ear cells from aminoglycoside ototoxicity.<sup>24</sup>

It can therefore, be argued that radiation-induced SNHL based on one stereotypical form of active cell death is likely to be an over-simplification. The coexistence of multiple cell death mechanisms in the same tissue is thought to be common and may even occur in the same cell population.<sup>25</sup> Multiple cellular organelles may trigger several pathways that may act independently or in concert.<sup>23</sup> This has implications for attempts to attenuate or prevent drug-induced hearing loss through manipulation of apoptotic pathways, in that downstream inhibition of a single pathway may not be sufficient to block all cell deaths. Nevertheless, intervention in the early stages of ototoxicity when the different modes of cell deaths may share common initial signalling processes, remains promising. Such upstream intervention approaches include strategies directed at the generation of reactive oxygen species.<sup>25</sup> In a recent study on humans, the principle of antioxidant protection against ototoxicity was proven.<sup>26</sup> In this double-blinded placebo-controlled study on patients receiving gentamicin treatment for acute infections, significantly more patients developed hearing loss in the placebo group compared to the group protected by aspirin, an antioxidant. Hence, antioxidants look promising in protecting against radiation-induced SNHL in the clinical setting.

### Conclusion

Radiation-induced SNHL is recognised as an important side-effect of radiotherapy if the auditory pathways had been included in the radiation fields. This is especially relevant in Singapore where NPC is common and radiotherapy is the main modality of treatment. A model of

radiation-induced damage of the sensor-neural auditory system is proposed, which is based on dose-dependent, ROS-related radiation-induced cochlear cell apoptosis without significant damage to the retro-cochlear pathway. This model supports the feasibility of cochlear implantation, should one be clinically indicated in such patients. It can explain clinical observations such as radiation-induced SNHL being dose-dependent and affects the high frequencies more than the lower frequencies. It also opens up the possibility of preventive strategies targeted at different stages of the apoptotic process. Antioxidants look promising as effective agents to prevent radiation-induced ototoxicity; they target upstream processes leading to different cell death mechanisms that may co-exist in the population of damaged cells.

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