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Upregulation of the Major Histocompatibility Complex (MHC-I) in Auditory Hair Cells Exposed To Interferon Alpha

Research Article

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Abstract

Previous studies have reported hearing loss in patients undergoing interferon-alpha (IFN-α) therapy. The mechanisms by which IFN-α causes hearing loss remain poorly understood. This study was designed to investigate if the immune reaction is one of these mechanisms. Real time-PCR was used to determine the expression of the major histocompatibility class-I (MHC-I) in HEI-OC1 auditory hair cell line. This gene is a marker for an immune response and it is known as H2K1 gene. The cochlear cells were treated by IFN-α (0, 200 & 2000U/ml) for 6, 12, 24 & 48 Hrs. Differential gene expression patterns encoding IFN-α-1, IFN-γ and H2K1 (MHC-I) were assessed by real-time PCR. The results revealed significant expression of the MHC-I gene in a dose- and time-dependent manner. This outcome indicates that IFN-α led to initiation of an immune reaction in the cochlear cell line. Therefore, the current in-vitro study indicates that the immune reaction might be the underlying mechanism of the hearing impairment observed in patients undergoing IFN-α therapy. These results imply that pre-treatment hearing evaluation and close monitoring of hearing function in patients undergoing long-term high-dose of IFN-α therapy are necessary to avoid or to minimize its adverse effect on hearing.

Keywords: Immune Response; Cochlear Cell Line; Major Histocompatibility; Interferon-Alpha.

Abbreviations: IFNs: interferons; IFN-α: interferon-alpha; IFN-β: interferon-beta; IFN-γ: interferon-gama; rt-PCR: real time polymerase chain reaction;HEI-OC: House Ear Institute- organ of Corti; STATs: signal transducers and activators of transcription; JAK: Janus Kinases; R1: receptor1;R2: receptor2; ISGF-3: interferon-stimulated gene factor-3; ISRE: interferon stimulated response element; GAS: gamma activating sequence; NK: natural killer cells; DCs: dendritic cells; CD: clusters of differerntiation; KLH: keyhole limpet hemocyanin ; ES:endolymphatic sac; FACS: fluoresce-activated cell sorter; SNHL: sensorineural hearing loss; FBS: fetal bovine serum.

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Introduction

Interferons (IFNs) are a family of natural proteins secreted in small amounts mainly by the cells of the immune system in response to viral infections, bacterial infections and tumors [47]. Because of its antiviral action, IFN-α is the cornerstone therapy for chronic viral hepatitis, including hepatitis C and hepatitis B. Also, because of its effect on the immune response, IFN-β is currently the most widely used therapy for multiple sclerosis [41]. As an antiproliferative, IFNs is used in treatment of a number of tumors including hairy cell leukemia [3], chronic myelogenous leukemia, non-Hodgkin lymphoma [27] Kaposi's Sarcoma [73], renal cell carcinoma [31], melanoma [8] and breast carcinoma [45].

Although IFNs have been successful as potent antivirals, immunomodulators and anticancer agents, treatment with IFN carries significant risks [38]. Many side effects have been reported with IFN therapy, including flu-like symptoms, depression, autoimmune reactions [10], and some blood disorders [66]. In addition, there are a considerable number of studies that have reported

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an association between hearing impairment and IFN therapy, in particular with IFN-α [20, 26, 29, 43, 47, 60, 65, 69, 70, 84]. The incidence of hearing impairment due to IFN is reported more frequently in patients with hepatitis C than in patients receiving IFN for other diseases. The reported cumulative dose that was found to be associated with hearing loss due to IFN-α was around 100 million units [20, 33, 46].

Different mechanisms have been suggested to explain IFN-induced SNHL, including direct toxic effect on the cochlear cells through apoptosis, inflammatory response, immune response and/or vascular effect that might lead to hemorrhage, ischemia and/or vasculitis of the stria vascularis [20, 26, 29].

The contributions of IFN-α to the immune response involve the activation of the immune system cells as well as the increased secretion of other cytokines that can affect the immune response. For instance, IFN-α induces activation of natural killer cells (NK cells), which are the key cells in the innate immune response [16]. IFN-α also activates T-lymphocytes, in particular, CD4+ and CD8+ T-cells and enhances their cytotoxicity, and thus promotes the adaptive immune response [88]. Other immune cells stimulated by IFN-α are the dendritic cells (DCs), which are considered professional antigen presenting cells (APCs) [21].

Also, IFN-α is a potent inducer of the major histocompatibility complexes (MHC class I for a greater extent and class II for less extent), the function of which is to hand off processed antigens to phagocytic cells for phagocytosis. Therefore, it is suggested that, IFN-α enhances antigen presentation to T cells [13]. Hence, an increase of the expression of MHC might indicate the immune-modulating effect of IFNs.

In addition, as an immune modulator, IFN-α stimulates the expression of other cytokines, such as IFN-γ, which ultimately affects the function and activity of many immune cells. IFN-γ is also essential for macrophage activation as well as T cell stimulation [14]. In addition, IFN-γ itself was found to be expressed in the inner ear as a result of antigin challenge in the endolymphatic sac [63, 64]. Moreover, Gloddek, et al. (2002) [32] found that repeated doses of IFN-γ led to significant increase in MHC-II molecules in the cochlear explants of the lateral wall, organ of Corti, modiolus, and spiral ganglia versus the untreated explants. Gloddek et al. (2002) [32] in light of their findings attributed the hearing loss associated with IFNs administration to their robust immunological effects on many cochlear structures.

Furthermore, studies on both animal and human have indicated that IFN-γ and IFN-α may upregulate MHC-I molecules on various types of normal cells [42, 49, 52] and tumor cells [1, 5, 7]. Therefore, through the activation of the cells of the immune system and increasing the secretion of IFN-γ, IFN-α is capable of initiating a global immune response and function as a key modulator of both innate and adaptive immunity. This means that IFNs can contribute to stimulation of an immune and/or autoimmune response, a sequence that might occur in the inner ear. Therefore, it can be speculated that IFN-α can provoke an immune reaction in the cochlea either directly through activation of cells of the immune system or through production of IFN-γ.

In summary, the occurrence of hearing loss in patients receiving interferon-alpha (IFN-α) therapy was reported by many studies; however, the underlying mechanism (s) for this hearing loss has not been delineated. Hence IFN-α contributes largely to the immune system and the immune response contributes to the etiology of a growing number of inner ear disorders; the question that remains is: is the hearing loss, noticed in patients receiving IFN-α, due to an immune reaction of the cochlear cells to IFN-α? Understanding the mechanisms by which IFN-α can cause hearing loss might open an avenue for therapeutic intervention that can protect the inner ear from the ototoxic effect of some medications in general and IFN-α in particular. Hence, the present study was designed to investigate the immune response of the cochlear cells to IFN-α treatment by examining the expression of the MHC-I gene in the HEI-OC1 cell line, using the real time polymerase chain reaction (rt-PCR). In particular, the following research questions were addressed:

1. Can IFN-α gene be expressed in the cochlear cells?
2. Will IFN-α act directly on the cochlear cells or through expression of IFN-γ?
3. Will IFN-α induce an immune response in the cochlear cells through increased expression of the MHC-I encoded by H2K1gene?

To answer the addressed questions, a cochlear hair cell line called HEI-OC (House Ear Institute, Los Angeles, CA) [44, 24, 68, 12] was used as an *in-vitro* system to examine the molecular events associated with treatment of the organ of Corti cells with IFN-α. In particular, the expression of IFN-α gene was examined to answer the first question. The expression of IFN-γ also was examined to find out if IFN-α acts directly on the cochlear cells or by enhancing the expression of IFN-γ. To detect if IFN-α can induce an immune response in the cochlear cells, the gene expression for the MHC-I was also examined.

Materials and Methods

Cell culture

The HEI-OC1 cell line was cultured on uncoated plastic culture flask, using Dulbecco's modified Eagle medium (DMEM) in the presence of 10% CO_2, 50 U/ml of recombinant IFN-γ, and 10% fetal bovine serum under two conditions, permissive conditions and non-permissive conditions. The permissive conditions were in the form of 33°C, 10 % CO_2, high-glucose DMEM media, 10% fetal bovine serum (FBS), and 50μ IFN-γ; whereas, the non-permissive conditions included 39°C, 5% CO_2, high-glucose DMEM, and 10%, FBS. When the cells in the culture flask reached around 85% confluence, they were seeded in the density of $5x10^4$ per well containing 2ml of complete medium, using 4 flat-bottomed 9-well plates, with a total of 36 wells. The 36 wells were divided into four groups according to the number of hours. They were labeled as: 6hrs, 12hrs, 24hrs, and 48hrs. Each of the four groups was divided into three subgroups—3 wells each (triplicate) according to the dose of IFN-α. The first subgroup was labeled as IFN0 acted as a control group, the second subgroup was labeled IFN200, and the third subgroup was labeled IFN2000. Table 1 summerizes the experimental deisgn used in the current study.

Treatment of the cell culture with IFN-α/PBS

When the cells in the culture plates reached around 85% confluence, they were treated by IFN-α or PBS corresponding to each subgroup. The IFN0 subgroup was treated with 2000U/ml of

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Table 1. Summary of experimental design.

Hours / Dose	6 hours	12 hours	24 hours	48 hours	Total # of wells
IFN0	IFN0/6	IFN0/12	IFN0/24	IFN0/48	12 wells
# of wells	3	3	3	3	
IFN200	IFN2000/6	IFN200/12	IFN40/24	IFN200/48	12 wells
# of wells	3	3	3	3	
IFN2000	IFN2000/6	IFN2000/12	IFN2000/24	IFN2000/48	12 wells
# of wells	3	3	3	3	
Total	9	9	9	9	36 wells

PBS (control/sham group), the IFN200 subgroup was treated by 200U/ml of IFN-α, and the IFN2000 subgroup was treated with 2000U/ml of IFN-α. All the plates were incubated in 39°C, 5% CO_2 for the corresponding period of time, namely 6hrs, 12hrs, 24hrs, and 48hrs. After each particular time, the cells were collected from each well and were subjected to cell viability assay.

Cell viability assay

Cell viability assay was performed to monitor the growth of the culture and to determine the effect of IFN-α on cell growth and cell replications. Cell viability was measured by Trypan blue exclusion. Briefly, 50μL of cell suspension was mixed gently with an equal volume of 0.4% Trypan blue in a test tube for five minutes at room temperature. Then 10μl of the mixture was placed in a hemocytometer to count the number of viable (unstained) and dead (stained) cells. The average number of unstained cells were calculated in each quadrant of the hemocytometer, and multiplied by 2×10^5 to count the cells/ml. The cell count was averaged across the three wells (triplicate) for each subgroup. Afterward, the cells were stored in -80C for RNA extraction.

RNA extraction

Cells were homogenized using a TissueLyzer (Qiagen, Valencia, CA). Total RNA was extracted with RNeasy Mini Kit (Qiagen) following manufacturer's instructions. RNA quality and quantity were evaluated by gel electrophoresis on a 1.5% agarose/Tris-Borate-EDTA (TBE) gel using ethidium bromide staining and 260/280 absorbance (ABS) ratio in a Beckman DU 600 Spectrophotometer (Beckman, Fullerton, CA, USA).

Reverse transcription

Reverse transcription (RT) of RNA was performed to convert the RNA resulting from the previous procedure into recombinant DNA (cDNA), using TaqMan Reverse transcription reagents. The reaction included 10.0μl of 10X PCR TaqMan Gold buffer II (Applied Biosystems), 30μl of 25 mM $MgCl_2$, 4 μl of 25 mM of each dNTP, 5μl of 100 μM of random primers (Gibco BRL), 2μl of RNasin (40 units; Applied Biosystems), 1.25μl of Super-Script II (250 units; Applied Biosystems) and 5μl (250 ng) of DNA-free total RNA in a final volume of 100μl. The reaction was incubated at 25°C for 10 min, 50°C for 50 min and 85°C for 5 min in a 2700 Thermocycler (Applied Biosystems Inc., Foster City, CA). The cDNA from this procedure was subjected to rt-PCR to reveal time and dose-dependent changes of the target genes in the hair cell culture challenged by IFN-α. In particular rt-PCR was used to measure the magnitude of expression, if any, of some genes that might be stimulated by IFN-α, namely IFN-α1, IFN-γ, and H2K1gene for MHC-I.

Real time Polymerase chain reaction (rt-PCR)

Gene-specific primers (Table 2) for the target genes (IFN-α1, IFN-γ, and H2K1gene for MHC-I) were designed using OLIGO Primer Analysis Software (v6.3, Molecular Biology Insights, Inc., Cascade, CO). The reverse transcription reaction was set-up with 250 ng of DNA-free total RNA, random primers and SuperScript III (Invitrogen). The reaction was incubated at 25°C for 10 min, 50°C for 50 min and 85°C for 5 min in a 2700 thermocycler (Applied Biosystems Inc., Foster City, CA). The real-time PCR reaction was set-up with SYBR Green PCR reagents (Applied Biosystems), including 5 μl of 10X SYBR PCR Buffer, 6μl of 25 mM $MgCl_2$, 4μl of each dNTPs (blended with 2.5 mM dATP, dGTP and dCTP, and 5 mM dUTP), 2.5μl of each gene-specific primer (5μM), 0.5μl of AmpErase UNG (0.5 U), 0.25μl of AmpliTaq Gold (1.25 U) and 5μl of cDNA in a final volume of 50μl. The conditions for the real-time PCR were 50°C for 2 min, 95°C for 12 min, and 40 cycles at 95°C for 15 sec, and 60°C for 1 min in an ABI PRISM 7900 Sequence Detection system (Applied Biosystems).

7300 Sequence Detection software (Applied Biosystems) was used for instrument control, automated data collection and data analysis. Relative quantification (fold difference) of the expression lev-

Table 2. Primers of the target genes.

Primer	Forward	Reverse
IFN-α1	5'-CTGATGGTCCTGGCGGTGCT-3'	5'-CCTTCTCCTGCGGGAATCCAA-3'
IFN-γ	5'-AGTCTCTTCTTGGATATCTG-GAGG-3'	5'-GTGTGATTCAATGACGCTTAT-GTT-3'
MHC-I	5'-CAGAGAGACTCAGGGCCTACC -3'	5'-GCGTCGCGTTCCCGTTCTT -3'

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els of each transcript was calculated using the $2^{-\Delta\Delta Ct}$ method [71] with modifications [51]. The ΔCt represents the Ct of the target gene normalized to 18S rRNA ($\Delta Ct = Ct_{Target} - Ct_{18S\ rRNA}$). Relative quantification (fold change) of the expression level of the target gene was calculated using a modified $2(\Delta\Delta Ct)$ method, where $\Delta\Delta Ct = (Ct_{Target} - Ct_{18S\ rRNA})_{Target} - (Ct_{Target} - Ct_{18S\ rRNA})$ Control.

Therefore, the data that collected in the current experiment included the fold change and the ΔCt. More than two fold-increase in the gene expression is considered a reliable indication of change in the expression of the gene relative to the control group (sham), which was normalized to the 18S rRNA gene [67].

On the other hand, the ΔCt was collected to quantitatively determine these genes that were expressed in the cochlear cells as a result of IFN-α treatment with respect to dose and time of IFN-α treatment. The ΔCt for each gene was compared across different doses and across different time points. Therefore, there were two independent variables (dose and time) with three and four levels respectively and one dependent variable (ΔCt), which created a 3X4 factorial design. (Figure 1).

The first level of the statistical analysis was achieved by calculating the fold-increase for each gene; the gene was considered reliably expressed if there was more than two-fold increase in the PCR products relative to the control group, which was normalized to the 18S rRNA. The second level of the analysis was to statistically test for the presence of significant effect of both the dose and the time (two independent variables) on the ΔCt of each gene. Therefore, two-way ANOVA was performed (four levels of time X three levels of the treatment). In particular, 3 separate two-way ANOVA for each gene was performed. Differences were considered significant if P-value < 0.05 (i.e. alpha=0.05). ANOVA testing was applied only for those genes that showed more than two-fold increase relative to the control. Afterward, a post hoc analysis, using the least difference test (LSD) was done to examine for significant differences across the three levels of the dose (IFN0, IFN200, and IFN2000); likewise, for significant differences among the four time points (6hrs, 12hrs, 24hrs, and 48hrs). LSD does not correct for alpha, which is beneficial for the current study as the least difference would be sufficient to justify significance for these kinds of data.

Results

Expression of IFN-α1gene

Figure 2 shows that there was about 2-4 folds increase of IFN-α1 gene in the case of 200 U/ml and about 3.5-6 folds increase in the case of 2000U/ml. The increase was more during the later hours of treatment, reaching its maximum level at 48 hours in both cases. Table 3 shows the output of the two-way ANOVA, which revealed a significant effect of the dose on the expression of the IFN-α1 (P<.05, P=.000). However, the effect of time in hours was not significant (P=0.342>0.05). The interaction between the dose and the hours was not significant (P=0.916), which means that the effect of dose was independent of the effect of the time of treatment. Post-hoc analysis reflected significant differences between IFN0 & IFN200, IFN0 & IFN2000, and IFN200 & IFN2000 (P=0.000, 0.000, & 0.004 respectively).

Expression of IFN-γ

The level of the expression IFN-γ gene was less than two folds (Figure 3), which indicates that IFN-γ gene was not expressed by IFN-α treatment even in small amount at the highest Ct cycle number used for PCR. Therefore, there was no need to run the two-way ANOVA in this case.

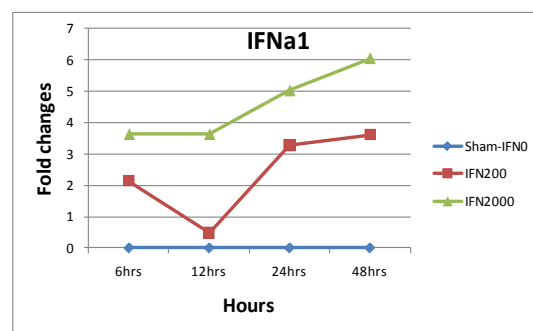**Figure 1. Relative fold changes in IFN-α1 gene expression.**

Figure 2: Relative fold changes in IFN-γ gene expression
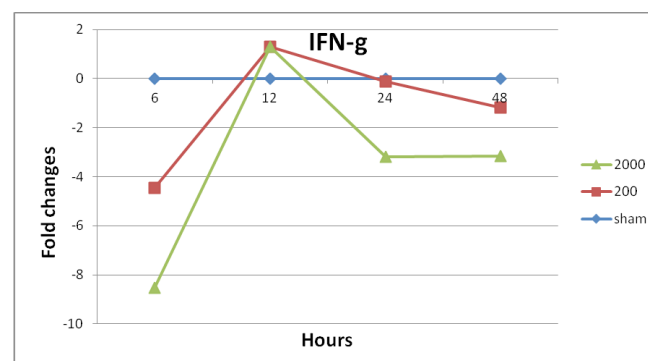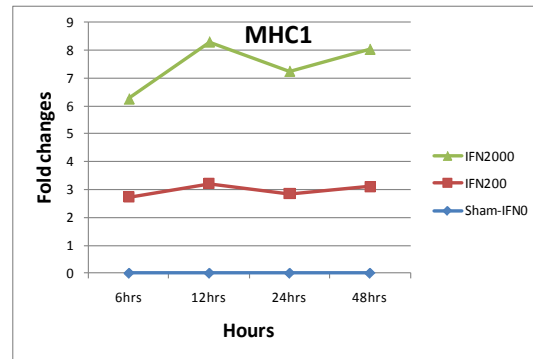

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Table 3. Output of two-way ANOVA for ΔCt of IFN-α1 gene.

Variable	df	Mean Square	F-value	Significance
Dose*	2	50.085	69.373	0.000
Time	3	0.845	1.170	0.342
Dose X time	6	0.237	0.328	0.916

*: The mean difference is significant at the 0.05 level

Figure 3. Relative fold changes of MHC-I gene expression.

Expression of the MHC-I gene

The result showed an increase in the MHC1 gene expression by about 3 folds in case of 200U of IFN-α versus 6-8 fold-increases in case of 2000U of IFN-α. It is noticed that the pattern of the fold change was similar for both cases (Table 4).

In addition, the two-way ANOVA indicated significant effects for both dose and time on the ΔCt of MHC-I gene, with P= 0.000 and 0.001 respectively (Table 5). The results of LSD test indicated significant differences between IFN0 & IFN200, IFN0 & IFN2000, and IFN200 & IFN2000 (P=0.000, 0.000, and 0.000 respectively) (Table 6). On the other hand, Table 7 shows the output of LSD test that indicated significant differences between 6 & 24 hrs, 12 & 24 hrs, 24 & 48 hrs time points (P=0.001, 0.029, and 0.000 respectively).

Table 4. Post-hoc analysis for the effect of dose on ΔCt for IFN-α1 gene.

Levels of dose	Std Error	Significance
IFN0-IFN200*	0.34688	0.000
IFN0-IFN2000*	0.34688	0.000
IFN200-IFN2000*	0.34688	0.004

*: The mean difference is significant at the 0.05 level

Table 5. Output of two-way ANOVA for ΔCt of MHC-I gene.

Variable	df	Mean Square	F-value	Significance
Dose*	2	15.618	246.631	0.000
Time*	3	0.464	7.320	0.001
Dose X hours	6	0.102	1.604	0.189

*: The mean difference is significant at the 0.05 level

Table 6. Post-hoc analysis for the effect of dose on ΔCt of MHC-II gene.

Levels of dose	Std Error	Significance
FN0-IFN200*	0.10273	0.000
IFN0-IFN2000*	0.10273	0.000
IFN200-IFN2000*	0.10273	0.000

*: The mean difference is significant at the 0.05 level

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Table 7. Post-hoc analysis for the effect of time on ΔCt of MHC-I gene.

Levels of time	Std Error	Significance
6-12 hrs	0.11863	0.116
6-24 hrs*	0.11863	0.001
6-48 hrs	0.11863	0.868
12-24 hrs*	0.11863	0.029
12-48 hrs	0.11863	0.085
24-48 hrs*	0.11863	0.000

Discussion

The first research question asked if IFN-α can be expressed in the cochlear cells. One subtype of IFN-α was tested to answer this question, i.e. the IFN-α1 gene. The results showed more than two fold-increases in the expression of the IFN-α1 gene (Figure 2). This increase was more apparent in case of the IFN2000 group than in the IFN200 group. These findings suggest that the dose of IFN-α affects the level of expression of IFN-α1. Therefore, it can be concluded that IFN-α gene can be expressed in the cochlear cells with subsequent expression of other IFN-α-induced genes, such as MHC-I as an immune response gene.

Although IFN-α is well known to induce IFN-γ [16, 61] and IFN-γ was found to be expressed in the inner ear [32, 33] the results of the current study revealed less than two fold-increases in the IFN-γ gene expression (Figure 3). This means that the growing cells from the cochlear cell line used in the current experiment did not produce IFN-γ. Many studies have shown that IFN-γ can be produced in the inner ear as a result of antigen challenge [32, 63] and in association with autoimmune SNH human subjects [53, 72]. Moreover, the expression of IFN-γ in the inner ear in these studies was associated with the expression of inflammatory markers called the intercellular adhesion molecules-1 (ICAM1) [63, 75] and immune response marker (MHC-II) [32]. This means that IFN-γ was associated with induction of inflammatory and immune responses in the inner ear. These findings provide evidence for the production of IFN-γ in the inner ear in association with inner ear injury or pathology, which is not the case in the current study. The events in the current study were not based on ongoing antigen challenges or stimulation of the immune system cells (the cochlear cell line used in the current study was not exposed to any kind of antigen or cell injury), rather they reflected molecular or genetic signaling events and this may explain the difference in findings.

At the molecular level, the results of the current study might be in agreement with the study of Nguyen, et al. (2000) [58] who found that IFN-α and IFN-β did not enhance the expression of IFN-γ. They attributed this to a negative regulation between type I-IFN (IFN-α & IFN-β) and type II-IFN to STAT1, which is involved in the signaling pathway of both types of IFN [25, 57]. When these two cytokines had an alternative signaling pathway that did not involve STAT1, they promoted the expression of IFN-γ [58]. Therefore, it can be argued that IFN-α used the Jak-STAT signaling pathway with activation of STAT1 in the cochlear cell culture, and hence, it was not able to promote the expression of IFN-γ. This pathway needs to be tested in order to elucidate this issue. Therefore, it can be concluded that treatment of cochlear cell line with IFN-α did not induce expression of the IFN-γ gene, which suggests that IFN-γ did not mediate the action of IFN-α; instead,

IFN-α might have acted directly on the cochlear cells.

On the other hand, the results showed more than two-fold increases in the expression of the MHC-I gene in the cochlear cells treated by IFN-α. The expression was relatively high in both doses of IFN-α and over all time points, which might suggest that MHC-I was expressed constantly in the treated cochlear cell culture. The function of the MHC-I molecules is to present processed antigen to cytotoxic T cells and bind to CD4 (co-stimulatory molecules) on the surface of T-cells. It is well-known that IFN-α induces activation of CD4+ T-cells and thus promotes their cytotoxicity, with subsequent expression of the MHC-I molecules [88]. In addition, IFN-α stimulates the DCs, which are considered professional APCs [21]. Therefore, IFN-α can enhance antigen presentation to T-cells with a subsequent expression of MHC-I [13]. Further procedures are needed to examine the existence of these cells in the growing HEI-OC1.

At the molecular level, the promoter (i.e., a part of the gene with which a stimulating molecule will attach to induce expression of that gene) of the MHC-I genes contains an interferon response sequence (IRS), which leads to expression of MHC-I once IFN-α is introduced [78]. Therefore, treatment of the cochlear cell culture with IFN-α most probably led to expression of MHC-I gene.

The results of the current study may be in agreement with what has been documented by many studies that MHC-I is a "hallmark" of type I-IFN, including IFN-α [17]. In addition, IFN-α treatment is important for colonel expansion and survival of the CD4+ and CD8+ T-cell populations (MHC-I bind to the surface of these cells) in an antigen-independent manner, a mechanism called "bystander effect" [62, 81]. Although this bystander effect is not as powerful as a response during viral infection, it still represents a unique mechanism of controlling an adaptive immune response [81]. Moreover, IFN-α causes an alteration of the MHC with increased expression of class I molecules on the tumor cells [59]. All these findings support the notion that IFN-α is a potent inducer of MHC-I even in the absence of antigen challenge.

Notably, lymphocytes (which are the cells of the adaptive immune response) are not normally present in the cochlea; however, the cochlear innate immunity is suggested to be responsible for initiating the adaptive immune response with subsequent recruitment of lymphocytes. Therefore treatment with IFN-α, which is well known by its priming action (initiates the innate immune response) and its bystander effect (needs no antigen) will promote the development of adaptive immune response with subsequent lymphocytic activation, leading to MHC-I production in the cochlear cell culture regardless of the absence of antigen exposure. This conclusion might explain the constant expression of MHC-I over all the time points and in both doses (200 & 2000U/ml).

Many studies have shown the involvement of lymphocytes, including CD4+ & CD8+ T-cells (cells of adaptive immunity) as well as NK cells (cells of innate immunity) with the production of IFN-γ and ICAM1 in the pathogenesis of immune-mediated inner ear disorders in human subjects as well as in experimental animals [6, 32, 53, 56, 87]. This association between hearing loss and the immune response suggests that IFN-α can cause hearing impairment by promoting the innate as well as the adaptive immune responses in the cochlea. This speculation is supported by the studies of Cadoni et al. (2001; 2003) [18, 19]. These investigators found anti-endothelial cell antibodies in 15 out of 32 (47%) patients who had sudden hearing loss due to IFN-α versus two out of 14 of the controls (14%). This suggested an association between sudden hearing loss and immune-mediated microvascular damage. These anti-endothelial cell antibodies react against surface antigens on the endothelial cells of the inner ear blood vessels, leading to their damage via a complement-mediated or antibody-dependent cellular cytotoxic mechanism. The same effect has been detected in retinal vascular lesions during interferon therapy [34]. Interferon therapy can provoke an immune response in the inner ear, resulting in hearing impairment.

Overall, the effects of IFN-α on the expression of MHC-I gene were dose and time dependent. Also, the pattern of changes in the gene expression across hours look to be identical for both doses (200 & 2000U/ml). These findings may be consistent with the dose-dependent response typically seen with cytokines treatment. For instance, low-dose treatment has a stimulating effect, whereas, high-dose produces tolerating or suppressant energies [15, 37, 77]. This observation did not point to an absolute stimulation or an absolute depression; rather it meant that low and high doses have distinctive outcomes. For example, Yoshino (1996) [89] found that a low dose of IFN-α caused downregulation of delayed hypersensitivity and cellular infiltration in lymph nodes. On the other hand, high doses were found to be clinically effective against neoplasm and against viral infection, but they are poorly tolerated by the patients [82]. Systemic administration of low doses of IFN-α during anti-neoplastic or antiviral therapy will act as a priming cytokines for the host immune system, resulting in protection from viral challenge and increase of the tolerance of the patient to the effect of IFN-α [9, 28]. Theoretically, low-doses of IFN-α mimic the early endogenous priming effect by innate immunity versus high-dose treatment that resembles the systemic adaptive immune response, which is usually vigorous and more generalized [80]. The innate and the adaptive immunity have distinctive cytokines production and distinctive cellular activation, which might make the outcome of low-dose treatment distinctive from the high-dose treatment.

This assumption would provide an explanation for some clinical findings associated with IFN-α treatment. For example, Kanemaru et al. (1997) [48] used IFN-α to treat patients with severe idiopathic SNHL due to viral infection. A significant number of those patients showed complete recovery after IFN-α therapy of a total cumulative dose of 30 million units. The recovery of the hearing loss in their study was most probably due, first, to enhancement of the antiviral effect that in turn overcame the viral assault in the inner ear. This antiviral effect was indicated by the concomitant increase in 2, 5 oligoadenylate synthetase (2,5 A-S) activity. Second, the recovery of the hearing loss might be attributed to a different pathway of IFN-α in the inner ear when it is used in low doses through the priming effect. This different pathway might have led to induction of other genes such as anti-inflammatory or anti-apoptotic genes with subsequent recovery of the hearing loss.

Furthermore, the results of the current study may be consistent with a considerable number of studies that found an association between SNHL and high doses of IFN-α therapy, especially in patients with hepatitis C [26, 65, 84, 29, 60, 20, 47]. The authors of these studies attributed this hearing loss to the inflammatory response or the immune response of IFN-α on the hair cells that might have led to apoptosis. The reported cumulative dose that was found to be associated with the occurrence of SNHL was more than 100 million units (i.e., about three times the cumulative dose used in the treatment of SNHL in the study of Kanemaru et al. (1997) [48]. Therefore, it can be concluded that IFN-α, when used in small doses, can produce different effects on the cochlear cells from when it was used in large doses. This suggests that IFN-α has dual actions; protective in small doses and harmful in large doses.

Conclusion

IFN-α1 gene can be expressed in the cochlear cells as a result of IFN-α treatment with subsequent expression of MHC-I gene, which is a an immune response gene. This immune response was mediated by IFN-α, not through IFN-γ. The administration schedule of IFN-α treatment may play an important role on its biological and therapeutic effects by shaping the innate and the adaptive immune responses. In particular, low-dose of IFN-α can be used to protect against viral infection or immune-mediated disorders. On the other hand careful observation and follow ups might be necessary in case of long-term, high-dose therapy of IFN-α; two measures that can be applied to protect against hearing impairment.

Directions for Future Research

- A wide range of doses that extended from 10 to 100,000U/ml can be used. This will help in determining different biological characteristics of IFN-α that are largely dependent on dose. Also, repeated doses might be helpful to mimic the actual therapeutic course of IFN-α and to help in magnifying an immune response.
- Other markers for immune responses such as markers for CD4+, CD8+ and NK cells might be helpful in understanding the mechanisms involved in IFN-α-induced hearing loss.
- Immunohistochemical studies might be helpful in determining the functional effect of these genes that are affected by IFN-α treatment by detecting their corresponding proteins.
- Animal models can be used to mimic the biological events that can occur in vivo as a result of IFN-α treatment.
- Prospective studies that include subjects receiving IFN-α therapy for different diseases (such as hepatitis C, hepatitis B, malignancies.etc) need to be conducted. These patients should have their hearing tested before starting the therapy and every three or six months during the therapy. The hearing evaluation should include basic audiological testing as well as otoacoustic emissions to detect early changes that might occur in the cochlea.

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