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MiniReview

Concentration–Response Relationship of Hearing Impairment Caused by Quinine and Salicylate: Pharmacological Similarities but Different Molecular Mechanisms

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Abstract: This review has the purpose to summarize concentration–effect studies made with quinine and to compare the effects on hearing between quinine and salicylate. Quinine and salicylate have roles in experimental hearing research and may induce pronounced and reversible hearing impairment when administered in sizeable doses. The quinine-induced increase in hearing threshold and its recovery can be analysed according to 'the psychophysical power function'. The power function is a special case of the Hill equation when the stimulus (e.g. a drug concentration) is exceedingly small compared with the concentration that would elicit a half-maximum response. Quinine and salicylate induce sensorineural hearing impairment and tinnitus when given in higher dose ranges in man. The drugs influence the presence, magnitude, and quality of audiological responses, such as spontaneous and evoked otoacoustic emissions. Quinine reversibly reduces frequency selectivity and hearing sensitivity, whereas the self-attained most comfortable speech level and the acoustic stapedius reflex are not affected, that is the dynamic range of hearing is reversibly reduced. This observation supports the view that quinine acts on the outer hair cell of the cochlea. Both drugs share a protective effect against the permanent hearing damages caused by gentamicin. This action is interpreted as a request for functioning mechanoelectric transducer (MET) channels to elicit the ill effect of aminoglycosides. Both drugs may interfere with the cochlear amplifier through blocking MET channels and the motor protein prestin. This review finds considerable overlap between type and extent of pharmacological actions of quinine and salicylate, supposedly caused by partly shared mechanisms of action but performed with different molecular mechanisms.

The aim of this MiniReview was to explore key findings from the few concentration–effect (pharmacokinetic–pharmacodynamic, PK-PD) studies that have been performed with quinine, to understand the extent and mechanisms of reversibly impaired hearing. With respect to salicylate, concentration–effect studies are scarce. Considering the overlap of sensorineural effects of quinine and aspirin, we have compared studies on their respective mechanisms to interfere with hearing. Quinine has been used as a drug since the 17th century, in the beginning as bark from the cinchona tree. The most significant medical indication is severe malaria where it still has a role. It has been argued that the availability of quinine was a key factor in making Africa safe for non-immune Europeans as malaria was a deadly threat [1]. Quinine has been used to induce abortion and has killed many women because of its general toxicity. Both quinine and its isomer quinidine affect ion channels and cause life-threatening arrhythmia taken in overdose. Salicylate was known to the ancients and manufactured synthetically in the 19th century. Its neuropharmacological effects on hearing both peripherally and centrally have recently been updated [2].

The hearing organ serves as a transducer performing the first step in the chain of receiving a physical stimulus and treating it in a complex way to be ultimately relayed to the brain. A normal dynamic hearing range from 0 to 120 dB corresponds to a change in sound pressure of 10^{12}. If viewed as energy flow, this value shall be squared. This gigantic range has been commented on by the British mathematician Ian Stewart [3]: 'Evolution pretty much had to come up with something like a logarithmic scale, because the external world presents our senses with stimuli over a huge range of sizes'. At even higher sound intensities, the hearing organ will break down irreversibly.

Literature Search

PubMed (National Library of Medicine, USA) was consulted for literature at several occasions starting in March 2014. A search on 'quinine and hearing' gave 92 hits in February 2016

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while an updated search on 'aspirin and hearing loss' in February 2016 gave 137 hits. 'Salicylate and hearing loss' differed by one post (138). More searches were done to explore certain related entities, for example the production of some authors found in the primary searches and search terms, for example psychophysical power expression/function, 'Borg scale' and MET channels. The term Borg scale returns about 1600 hits.

Quinine-induced Hearing Impairment

As part of an institutional hearing research project carried out at the Departments of Physiology, Audiology and Clinical Pharmacology at the Karolinska Institutet [1,4], the characteristics of quinine effects on hearing were studied in guinea pigs [5] and subsequently in healthy volunteers [6–8] and in patients [9].

Studies in Guinea Pigs

The effects of quinine on the cellular mechanics in an isolated cochlear preparation were explored [10] before turning to the measurement of hearing in the live animal [5]. Hearing impairment related to quinine concentration was studied in anaesthetized guinea pigs whose temporal bone was opened to uncover the cochlea. A microelectrode was placed on the round window for electrical recording and analysis of neural activity from the cochlea. A sound stimulus tone burst at 8 kHz was generated by a small loudspeaker and conveyed through a plastic tube glued to the external ear canal.

The recorded signal is generated by the first generation of neurons in the cochlea that relay primary, amplified and processed auditory stimulations to the cerebral cortex for further processing. The nerve activity was detected for 10 msec. after each sound pulse, and each sampling was based on 300 sound pulses. An algorithm was used to classify whether the anaesthetized animal had 'heard' the sound. Quinine was administered intravenously as one or more doses varying from 5 to 50 mg/kg. Blood samples of 0.1 ml were drawn and analysed for quinine using a modified HPLC method originally developed for clinical analysis of the antiarrhythmic compound quinidine.

Representative results from three of the 14 guinea pigs are shown in figs 1 and 2. The observed changes in hearing thresholds (dB) and quinine concentrations in blood are shown to vary systematically with time. Animal a: had one dose, and animals b and c: had two doses at separate times. Figure 2 shows the same data but displayed as hearing threshold shift *versus* quinine blood concentration. The lines between the dots represent a curve fit according to the models presented by Stevens [11] and later by Borg using the properties of rating scales to quantitate the perception of strain imposed by physical work.

The model that has been applied in perception research is $R = cS^n$ or $R = c(S-b)^n$, where R is a response, c is a proportionality constant, S is a stimulus and b is a fitted parameter that represents a stimulus >0 and which may move the curve along the x-axis. Thus, b equals a quinine concentration that would not at all influence hearing. Using an exponential expression allows more flexibility than to select a fixed loga-

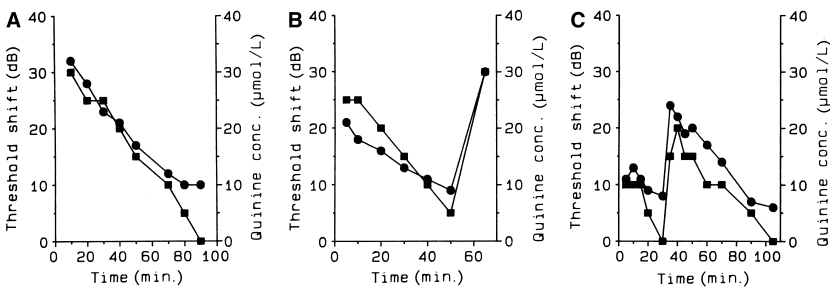

Fig. 1. Hearing threshold shifts (■) and blood concentration of quinine (●) after iv. administration of quinine in guinea pigs. (A) 50 mg/kg given at 0 time, (B) 30 mg/kg at 0 time and 40 mg/kg at 60 min, (C) 30 mg/kg at 0 time and 40 mg/kg at 30 min (with permission from [5], fig. 1).

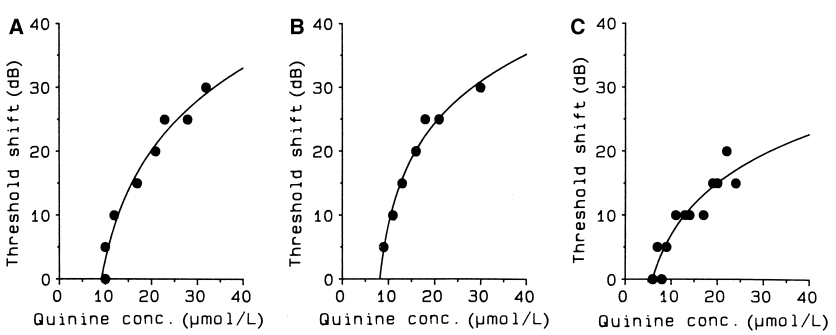

Fig. 2. Hearing threshold shifts (L) *versus* quinine concentrations. The same data as presented differently in fig. 1. The curves represent the fit to the model $L = 10(\log k + a(\log(S-b)$ (with permission from [5], fig. 2).

rithmic scale as suggested by Fechner in the 19th century [11]. In different sensory experiments, the exponent has varied between 0.3 and 3 [11].

The hearing threshold is expressed as the logarithm of a ratio between the intensity of a sound that is used for testing and the intensity of a sound at a certain reference level, which is usually the normal hearing threshold. Thus, the right-hand part of $R = c(S-b)^n$ was also log-transformed when finding estimates for the three model parameters c, b and n by a non-linear fitting procedure. Figure 2 supports the application of the suggested 'psychophysical power function' to describe reversible hearing impairment caused by quinine in the guinea pig. It was possible to get convergence on parameter estimates of the effect–concentration model in 13 of the 14 animals [5]. The proportionality parameter c was estimated to 5.3 ± 15 S.D., the parameter b was 5.1 ± 2.9 S.D., and the exponent n was estimated to 2.5 ± 1.0 S.D.

Studies in Man

To test whether this PK-PD model of decreased hearing in guinea pigs was valid in human beings, three oral doses were given in random order and single blind to six healthy volunteers [6]. The condition in human beings added one more complexity, namely a noticeable and consistent delay between the progression of the concentration and the resulting effect, known as hysteresis. One way to allow for counter-clockwise hysteresis is to add a virtual effect compartment that requires time to equilibrate with the central blood compartment in the pharmacokinetic model. The effect compartment is assumed to be the site of the effect. The same calculation approach to estimate the parameters of the reduced Hill equation as used for the guinea pigs showing no hysteresis (figs 1 and 2) was then applied. A plausible explanation for the species difference in relation to the presence of hysteresis could be that the guinea pig is a much smaller animal and therefore having greater relative metabolic and circulatory activity. This greater activity would lead to a more rapid equilibration for drug access between blood and endolymph of the cochlea with virtually no delay in the development of the effect compared with the course of the blood concentration. A discussion of 10 major factors causing hysteresis loops was recently published, mentioning, for example, distribution delay, tolerance and feedback regulation [12].

Figure 3A shows the measured threshold shifts after three doses in one individual. Hearing impairment was evaluated with a sweep technique between 0.5 and 8 kHz. Figure 3B displays quinine plasma concentrations, the delayed concentration curve in the virtual effect compartment and the observed hearing threshold shifts together with the fitted curve according to the model, all *versus* time. Finally, fig. 3C depicts threshold shifts *versus* calculated quinine concentrations in the effect compartment. The results of this study [6] were followed up in an extended volunteer study with two oral doses of the same size and one iv. infusion over 6 hr administered

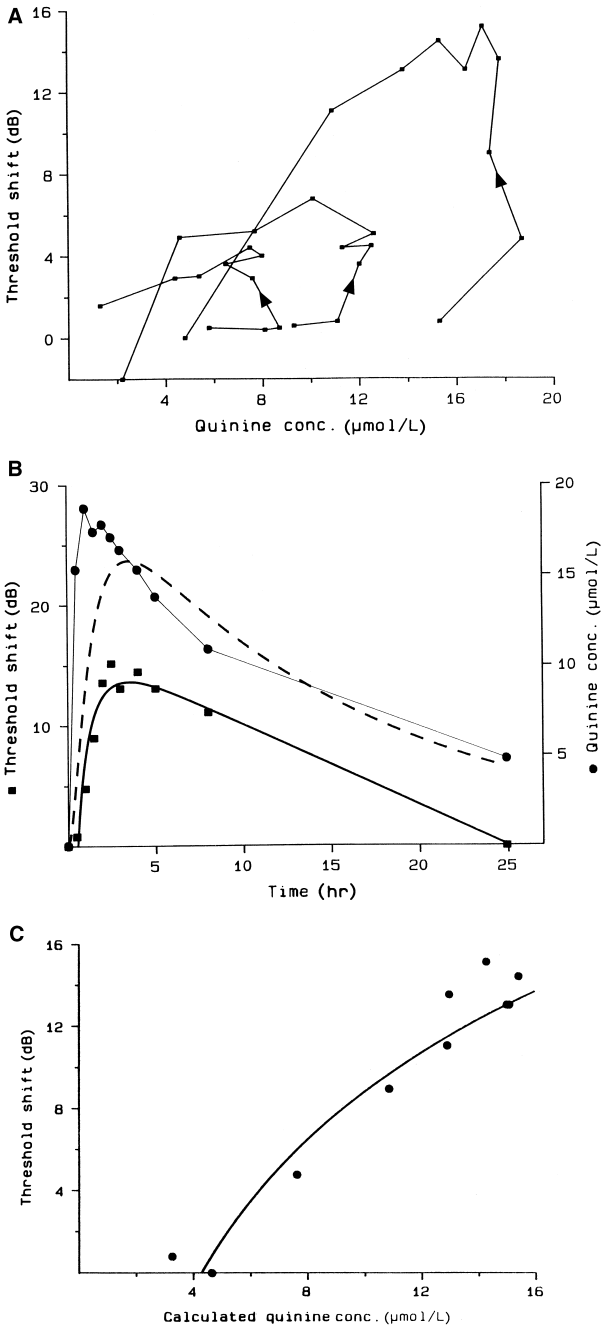

Fig. 3. (A) Curves showing the change in the effect–concentration relationship after oral intake of 5, 10 and 15 mg/kg of quinine in individual LJ. Arrows on curves indicate direction of time. (with permission from [6], fig. 3). (B) Plasma concentrations of quinine (●) in individual LJ., calculated effect compartment concentration (— —), observed hearing impairment as threshold shift (■) and fitted line (——) according to estimated parameters (a = 2.41, k = 0.03) (with permission from [6], fig. 4). (C) Relationship between observed hearing impairment (L) and the calculated effect compartment quinine concentration (C_e, ●) in individual LJ. after the intake of 1.5 mg/kg of quinine. The solid line was generated with the estimated effect model parameters as $L = 10(\log 0.03 + 2.41 \log C_e)$. (with permission from [6], fig. 5).

to six healthy volunteers [7]. The results were consistent and the design allowed for more estimates of the obtained kinetic and dynamic model parameters, although with considerable variability, probably dependent on properties of the models, the range of available data and measurement error. Hysteresis was present both after two oral doses and after one iv. infusion administered over 6 hr. The estimates of the rate constant for transport into the virtual effect compartment were approximately 0.8/hr, which indicates an equilibration half-time of about 1 hr for the effect compartment.

A subsequent study had entirely the focus on detailed audiological measurements as quinine was infused by a computer-assisted pump to attain three pseudostable plasma concentration states in one volunteer [8]. Hearing thresholds were measured at three frequencies (1000, 2000 and 4000 Hz). This experimental design abolished any visible hysteresis. The same pattern as seen in the guinea pigs relating blood concentration to hearing impairment was observed with plasma-derived concentrations. Unbound quinine was also determined and the characteristic pattern is visible in both (fig. 4). According to current theory, it is the unbound concentration that equilibrates with the site of action. The maximum and completely reversible hearing impairment amounted to 46 dB in this volunteer.

The possibility to use the patient's hearing loss as a test for adequate compliance to quinine therapy in the treatment of malaria has been documented [9]. All patients had pronounced subjective and objective hearing loss down to 45 dB during quinine treatment. The hearing loss was equally distributed over the frequencies 0.5–8 kHz. The patients reported normal hearing after treatment, and this was supported by normal audiograms. The authors recommend that audiometry could be used to monitor treatment when drug analysis cannot be performed. In another study, 10 patients with malaria were treated with quinine infusions [13]. Nine of them had audiograms that could be evaluated and showed transitory hearing impairment ranging from 15 to >45 dB (median 35 dB). All audiograms at follow-up were within normal limits.

PK-PD Modelling Using two Methods with Agreeing Results

In our studies on the hearing impairment caused by quinine, we consistently used the 'psychophysical power expression' to evaluate the data as a pharmacodynamic model related to receptor theory (Hill equation) was chosen. The curve fitting procedure was successful and a striking consistency can be seen between data points and model-generated curves in figs 2, 3C and 4.

There is a close and remarkable relationship between the 'psychophysical power function' and the Hill equation, which is a classical expression of the pharmacological effect. Its familiar form is: $E = E_{max} \cdot C^s/(C^s_{50\%} + C^s)$, where E is the actual effect, E_{max} the maximal drug-induced effect, C the actual concentration, $C_{50\%}$ the concentration that would produce half E_{max} and s an exponent that is usually called the slope factor and that greatly influences the steepness of the effect–concentration curve. The Hill equation can be derived by a reasoning framework that starts with the law of mass action taking into consideration the association between a drug molecule and a receptor. Because the number of receptors is assumed to be limited, one should expect a maximum drug-induced effect (E_{max}) when all available receptors are

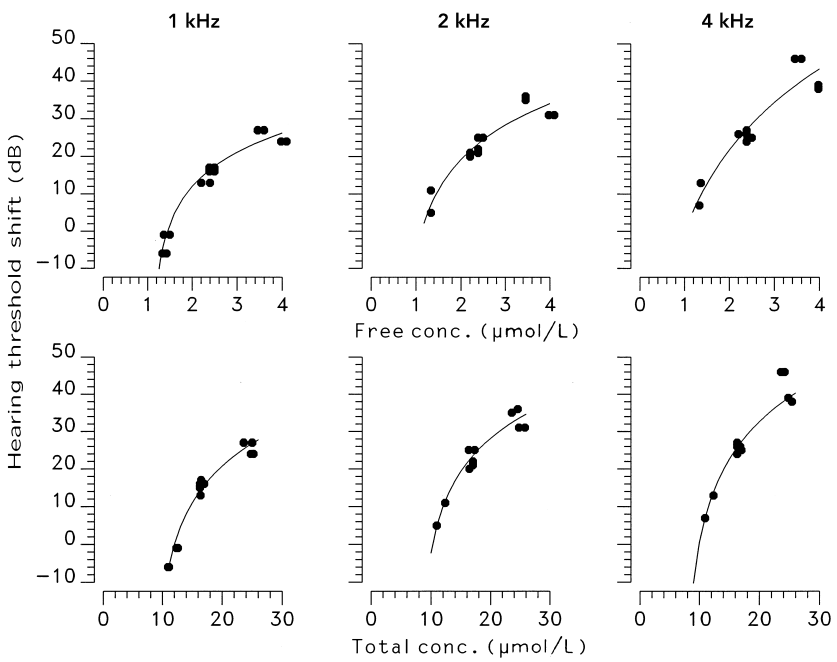

Fig. 4. Observed hearing threshold shift (dB) at 1, 2 and 4 kHz *versus* measured free (upper part) and total (lower part) plasma quinine concentrations obtained in a volunteer infused to three steady state levels of quinine. The solid lines were generated with the estimated model parameters. (with permission from [8], fig. 6).

occupied. It follows that there is a concentration ($C_{50\%}$) that will produce half E_{max}.

Assuming that the drug concentration that gives a certain effect is far smaller than ($C_{50\%}$), the Hill equation shown above can be simplified to $E = k \cdot C^s$, which is identical to the 'psychophysical power function' $R = c(S)^n$. Thus, the two models that have separate historical origins take the same shape when the drug concentration is exceedingly small compared with $C_{50\%}$. The decibel scale used for hearing is the $\log_{10}$ of the ratio between a sound intensity that is audible and a reference for normal hearing threshold. We thus fitted the model parameters to the $\log_{10}$ of the 'psychophysical power function'.

A standard programme was applied for the parameter estimates, NONLIN from SAS Institute (Cary, NC, USA) in studies [5–7]. An alternate approach called semiparametric pharmacodynamics modelling was also applied [7]. The original table is shown as Table 1. No major or systematic differences in the parameter estimates between the two different methods can be seen. Although convergence of the fitting procedures to give parameter estimates always occurred, the '95% asymptotic confidence intervals' were considerable and coefficients of variation may be calculated, for example, for the global means row of Table 1, showing the result of PK-PD modelling being 27, 37, 67, 158, 27 and 30%, respectively. We interpret this wide variability as a result of the computational conditions related to the limited availability of data over a wide range of concentrations which are inaccessible partly because of the toxicity of quinine. The largest variability is in the parameter k which is highly influenced by the preceding calculation of k_{eo}, the parameter of the transport to the virtual effect compartment and the inaccessible approach of the curve to the *y*-axis.

One may conclude that the estimates of the exponent in the reduced Hill equation were consistently above unity 2.5 ± 1.0 S.D. [5]. The same parameter in human beings had a median of 1.54 (range 0.22–4.18) [6] and a mean of 2.13 ± 0.57 at 1 kHz and 3.44 ± 1.04 at 2 kHz, respectively [7].

For the quinine hearing impairment effect, it is fair to consider the concentration ranges that we have studied to be low in relation to presumed concentration where kinetic saturation (diminishing returns) of the effect could start. The general toxicity of the drug precludes such experiments and no sign of diminishing returns was seen. Hudspeth [14] emphasizes that the compressive nonlinear amplification handling by the hair cells shows 'power law behaviour'. The basilar membrane that vibrates in relation to the level of stimulation has been shown to follow a power law relation in some species.

Other Audiological Responses: Comparison between Quinine and Aspirin and their Effects on Cochlear Function

Quinine.

A concordant view in the literature is that ototoxicity is reversible and dose and concentration dependent. We have not found any evidence-based reports of permanent hearing loss as seen, for example, for aminoglycosides and cisplatin for which histopathological damages of the cochlea are observed in addition to well-demonstrated persistent hearing damage.

There are changes in the cellular mechanics of the isolated cochlea preparation [10] after the application of 0.5–4 mM quinine. Isolated outer hair cells (OHCs) increase their length after the application of 1.5 mM quinine [15], which can now be interpreted as a sign of hyperpolarization. OHC motility is reduced and thus the active force, together with the elongation of the cells, when exposed to 5 mM quinine solution [16]. The infusion of quinine into the scala tympani in concentrations 0.05–5 mM showed dose-dependent and reversible attenuation of electrically evoked otoacoustic emissions [17]. In a study on isolated OHC that were exposed to 0.05–1.5 mM quinine initial hyperpolarization and subsequent depolarization of the cell membrane were noticed during a 15–20-min.

Table 1.

Estimates of pharmacodynamic parameters after quinine were administered in two oral doses (15 mg/kg) on two occasions and as a constant rate iv. infusion over 6 hr (15 mg/kg) in six healthy volunteers. (With permission from [7], table 1).

	K_{eo} (/hr)		k		γ	
Parameters	1000 Hz	2000 Hz	1000 Hz	2000 Hz	1000 Hz	2000 Hz
Pharmacokinetic–pharmacodynamic modelling						
First oral dose	0.88 ± 0.25	1.14 ± 0.49	0.04 ± 0.05	0.08 ± 0.13	2.70 ± 082	3.60 ± 1.22
Second oral dose	0.79 ± 0.37	1.16 ± 0.65	0.30 ± 0.28	0.25 ± 0.52	1.70 ± 0.56	3.40 ± 1.49
Infusion	0.48 ± 0.24	0.68 ± 0.34	0.11 ± 0.17	0.03 ± 0.08	1.97 ± 0.89	3.30 ± 1.12
Global mean	0.71 ± 0.19	0.99 ± 0.37	0.15 ± 0.10	0.12 ± 0.19	2.13 ± 0.57[1]	3.44 ± 1.04
Semiparametric pharmacodynamic modelling						
First oral dose	0.89 ± 0.28	1.10 ± 0.53	0.05 ± 0.04	0.06 ± 0.06	2.30 ± 0.43	2.90 ± 1.32
Second oral dose	0.73 ± 0.33	0.99 ± 0.26[2]	0.27 ± 0.30	0.30 ± 0.60[2]	1.80 ± 0.63	2.90 ± 1.23[2]
Infusion	0.50 ± 0.17	0.59 ± 0.24[2]	0.20 ± 0.28	0.04 ± 0.06[2]	2.20 ± 0.96	3.40 ± 1.60[2]
Global mean	0.70 ± 0.14	0.90 ± 0.27	0.17 ± 0.12	0.13 ± 0.17	2.12 ± 0.46	3.02 ± 1.11

Data are mean values ± S.D. Global mean was calculated by first averaging the results of the three dosings for each subject and then calculating the mean of the six values.

[1] $p < 0.05$ when compared with 2000 Hz.

[2] n = 5.

observation [18]. Quinine solutions from 0.05 to 5 mM have been applied in these animal studies. Plasma concentrations in the 10–30 μM range are associated with marked hearing impairment in anaesthetized guinea pigs [5], and approximately, the same range is reported for plasma concentrations in man [6–9,19–21]. Considerably higher quinine concentrations were used in these *ex vivo* investigations compared with the lower measured concentrations in *in vivo* experiments. Thus, examining the structure, micromechanics and membrane physiology of OHC support the hypothesis that OHC reversibly interacts with quinine in relation to quinine concentrations in blood or plasma to cause transitory hearing impairment.

The cochlea produces faint sounds (otoacoustic emissions, OAEs) that can be measured in the outer ear canal. These emissions may be spontaneous, a response to a triggering click sound (click evoked) or a distortion product caused by continuous two-tone stimulation. They can also be evoked by electrical stimulation [17]. The click-evoked OAEs are now used to screen hearing ability in the newborn [22,23]. The spontaneous OAEs, which are known to be the most vulnerable form of emissions, are completely abolished by quinine administration [21,24]. The spontaneous OAEs may have their origin as a consequence of the OHC amplifier properties that may operate according to a mathematical and engineering concept called Hopf bifurcation [14]. When at a critically low level of stimulation, the system becomes unstable, generating self-oscillations that reflect very high gain. However, the click-evoked OAEs are decreased in relation to increasing quinine concentrations and decreasing hearing ability expressed as a pure-tone threshold shift (fig. 5) [8,19,20,25]. Moreover, quinine attenuates the distortion product OAEs [19–21], which is a reflection of a change in the compressive nonlinearity of the cochlear amplifier. The distortion product OAEs have been shown to be dependent on the function of horizontal links between adjacent OHC stereocilia [26]. It is generally agreed that the OHC is the source of these various forms of OAEs.

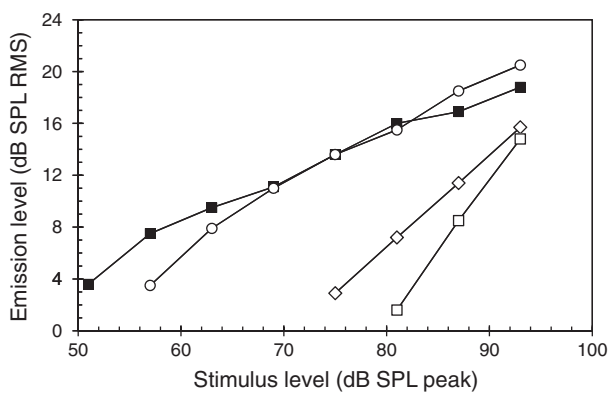

Fig. 5. The size of click-evoked otoacoustic emissions in relation to stimulus level. From top to bottom, before quinine administration (■) and at stable iv. plasma concentrations of 12 (○), 16 (◇) and 24 (□) μM. Corresponding hearing threshold shifts (1000–4000 Hz) at the three plasma concentrations were 3.4, 21.0 and 34.1 dB (With permission from [8], fig. 4).

In contrast, the protective reflex of the hearing organ, known as the stapedius reflex, was unchanged and reacted to the same strength of stimulus when hearing was maximally decreased by 46 dB pure-tone threshold shift in the human volunteer compared to before quinine was given [8]. This observation is consistent with other reports [20–25]. The stapedius reflex is triggered at relatively strong sounds, about 85 dB hearing level [20]. The OHCs have their operational range at lower sound intensities, as experiments in mice show that extinction of OHCs increased the hearing threshold by about 50 dB, a level clearly below where the stapedius reflex becomes activated [27]. This, together with unchanged brainstem responses at high stimulus levels [8] and unchanged self-attained most comfortable speech level [20], points at the OHC function as the target for quinine. The change in these indices when quinine was administered can be summarized as a reduction in the dynamic range of the human auditory system [20], which is understood as the range of sound stimuli that can be handled and amplified in an optimal way, not adding discomfort or other disturbances.

Quinine is most likely distributed to both inner hair cells (IHCs) and OHCs which are influenced through their mechanotransducer (mechanoelectrical or MET) channels. The concept of MET (or MT) channels is still more functional than alluding to fully characterized channels. They have been cautiously named the MET 'channel complex' [27] and addressed as 'elusive' [14,28]. It was recently stated that the 'molecular identity of this ion channel is still unclear' [29]. Some very limited suggestions are at hand for part of their structure [28,30,31]. Mechanotransduction exists to convert vibrations into a nerve signal to be further handled by the brain. The inverse concept, electromechanical transduction, is used to name the process of producing motile activity (electromotility) participating in the cochlear amplification and being the origin of otoacoustic emissions. The OHCs are assessed to have both mechanoelectric and electromechanic capacities [32]. Quinine was suggested to affect electromechanical transduction in the OHC thereby impairing hearing reversibly [1,17]. The terms in the area of mechanotransduction and the abbreviations used have not been fully harmonized.

The OHCs contain a protein called prestin [27,33] that is needed for the electromechanical transduction being part of the cochlear amplification process. The amplifier participates in the primary treatment of the sound waves that reach the cochlea, that is the energy brought to the hearing organ is treated in a nonlinear and frequency-dependent fashion. Prestin, which is described as 'the motor protein' of the OHCs [33], is part of a gene family of *anion* exchangers (specifically SLC26A5) [34]. Experiments in gene-deficient mice have corroborated the crucial importance of prestin for electromotor activity in cochlear amplification [34].

It has been shown that quinine in the concentration range encountered in human studies blocks the MET current (fig. 6). Farris *et al.* [28] found a total block of hemi-gap-junctional channels in an auditory hair cell preparation from turtle and discussed the propensity of partially charged amines to enter the pore of the channel electrochemically. At physiological pH

of blood, approximately 40% of quinine is positively charged, which fits well into the concept of a negative charge close to the mouth of the channel. The more bulky part of the molecule is thought to block other entries as long as it stays in place [28]. In contrast to the focus on anions regarding the properties of prestin, *cations* are critically involved in the mechanisms at the core of mechanoelectrical transduction in hair cells. The sequence of events is thought to start with K^+ inflow and depolarization as a response to movements of the stereocilia. This process happens within microseconds. Next, follows the inflow of Ca^{2+} through voltage-gated channels. Intracellular Ca^{2+} activates channel passage of K^+ to exit the cell [14]. The resulting potential of the receptor cell will release the neurotransmitter glutamate and thus propagate the signal to the brain [14].

It seems plausible that quinine blocks channels opening to K^+ in the OHC, which interferes with their physiological action. Both mechanoelectrical and electromechanical transduction are exerted by the OHC [32]. K^+ and Na^+ were inhibited by realistic quinine concentrations in cultured spiral ganglion cells [35]. Quinine is considered a standard K^+-channel blocker [36]. A recent example of quinine inhibition of a K^+ channel important for the motility of spermatozoa has been reported [37].

Aspirin and salicylate.

The likewise old remedy aspirin is known to cause tinnitus and impaired hearing when taken in overdose. A systematic review involving 185,000 individuals extracted from 37 publications has recently been published with the main aim to evaluate the effect on audiometric measurements in different dose ranges [38]. The authors found sensorineural hearing loss documented for daily doses from 4 to 10 g per day with a reported threshold shift of 15–112 dB. The authors concluded, 'the effect was dose-dependent and reversible in the short-term'. Aspirin, the third most used drug in the world, is employed as an analgesic, antipyretic, anti-inflammatory agent and is also recommended in low dose to decrease the risk of myocardial infarction in men [38].

Aspirin effects overlap to a considerable extent those of quinine [2]. An early observation was that the evoked emission response was tapered after the intake of aspirin [39]. Further, spontaneous otoacoustic emissions were abolished after the intake of 16 doses of 325 mg aspirin every 6 hr [40]. Aspirin reversibly reduces overall otoacoustic emission amplitudes [41]. The evoked distortion products were reduced in amplitude [42] and changed in fine structure [41,43]. Similar effects have been observed for quinine as discussed above. However, reports on graded effects of aspirin on click-evoked emission level or stapedius reflex, as was the case for quinine, have not been found in the literature.

It has been shown (fig. 7) that salicylate, which is negatively charged at physiological pH, acts as a competitive antagonist at the anion binding site of prestin [44]. The inhibition of channel current occurs at concentrations of salicylate comparable with those found in human beings [45]. Whether quinine, which is not present as anion, could interact at the same binding site seems less likely and supports the hypothesis that quinine and salicylate mediate their effects through different binding sites and may be through different channels.

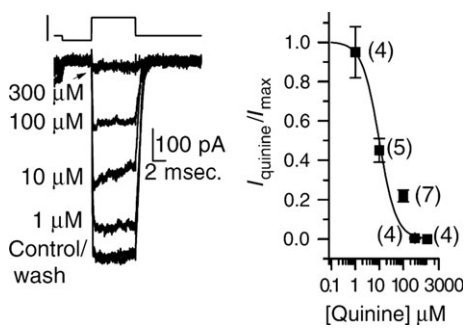

Fig. 6. Quinine inhibits the function of MET channels in a tissue preparation from the red-eared slider turtle. Hair cell bundles were maximally stimulated with a stiff glass probe attached to a piezo-electric element. Concentration-dependent decrease in channel current as response to stimulation (left). Analysis of the data according to the Hill equation (right). The half-maximum decrease in relative channel current was reached at 10 µM quinine. (with permission from [28], fig. 6A,B).

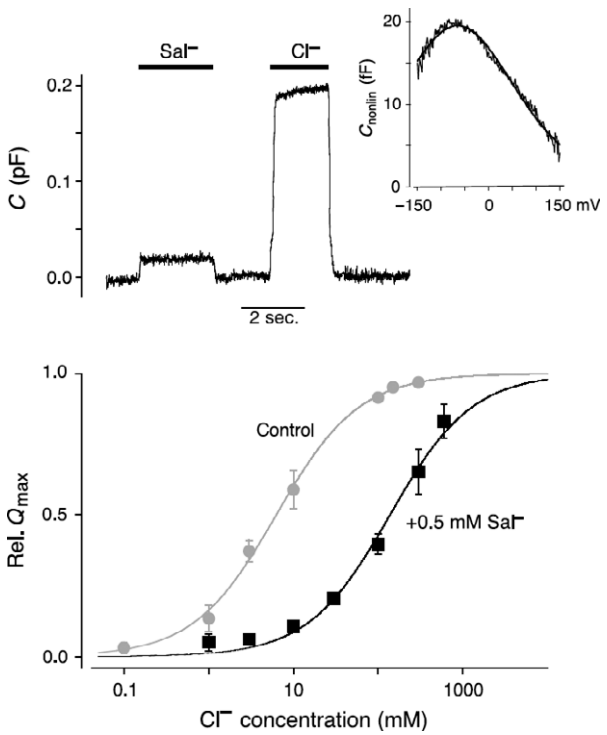

Fig. 7. Salicylate inhibits voltage-sensitive channels of prestin in inside-out patches from the rat OHC as shown by lack of channel conductance when exposed to salicylate compared with chloride (upper left). Voltage dependence was shallow when salicylate was present (upper right). The dependence of relative change in the maximum channel current on a salicylate concentration analysed according to the Hill equation showing a major decrease of the current associated with 0.5 mM salicylate, but almost identical shape or slope factor (lower part), (with permission from [44], fig. 5).

Aspirin and quinine have been evaluated in neurophysiological animal models, both being tinnitus-inducing agents [46]. Consistently, similar effects of both drugs on firing rates for spontaneous and stimulus-driven multi-unit activity in primary auditory cortex, anterior auditory field and secondary auditory cortex have been found [46]. Salicylate and quinine significantly increased spontaneous firing rates in secondary auditory cortex, whereas the rates were reduced in primary auditory cortex and the anterior auditory field. The authors suggest a central effect of quinine and salicylate in addition to their peripheral ototoxic effects. However, a study of the input–output relation of brain stem response suggested different effects caused by very high doses of salicylate, quinine and furosemide on IHC and OHC functions [47]. In a recent review, the possibility of extended toxic effects, including the formation of a superoxide radical on the spiral ganglion, is discussed [38]. One noteworthy remark is that clinically observed hearing loss in the range of 100 dB [45] exceeds what is expected if salicylate only interfered with OHC-mediated cochlear amplification. Dieler *et al.* [18] conclude in their study on isolated OHCs '. . .the underlying mechanisms of quinine ototoxicity are considerably different to that of salicylate although both substances clinically lead to identical symptoms'.

Concentration–response relationships for hearing loss *versus* total and unbound salicylate concentration in eight individuals have been reported [45]. A significant linear correlation was found between the hearing loss in dB and unbound salicylate concentration. Unbound fractions of salicylate are greatly dependent on total concentrations and may be several times higher in patients intoxicated with salicylate than at normal therapeutic concentrations. Apart from the studies reviewed here [6,8–10], PK-PD investigations have not been found for impaired hearing and for other audiological measurements.

Mechanotransduction as a Route for Ototoxicity

The use of the group of aminoglycoside antibiotics for treating severe infections and the anticancer drug cisplatin is associated with a notorious disadvantage of causing permanent hearing loss, especially when doses are pushed upwards. Functionally intact mechanotransduction seems to be needed to elicit aminoglycoside and cisplatin toxicity [30,31]. Blocking the MET channels with quinine and curare prevents hair cell loss after exposure to gentamicin in rat cochlear explants [30]. The lateral line in fish is affected by quinine, probably making the cilia stiffer [1]. Quinine-treated zebrafish are protected from cisplatin-induced hair cell death in their lateral-line sensory organ [30]. That was also the case in two zebrafish mutants known to lack functional mechanotransduction [31]. The same principal author [31] and collaborators have reported the results of the screen of a library of 10,000 small molecules in the zebrafish lateral-line high-throughput test of inhibition of cisplatin-induced hair cell death. They found two candidates for further development. However, the structure of these molecules was not disclosed.

Salicylate has been shown in guinea pigs to counteract the ototoxic effect of gentamicin as it decreased the hearing shift from 60 dB in controls to 20 dB when salicylate was given concomitantly [48]. In a placebo-controlled study, 195 patients scheduled for infection treatment with gentamicin together with aspirin 3 g daily for 14 days were found to have a significantly lower incidence of hearing loss (3%) than control patients receiving placebo (13%) [49].

Future Therapeutic Lead

The finding of a protective action of quinine and salicylate, decreasing permanent hearing damage caused by certain therapeutically important drugs has gained interest. An account of results through screening the lateral-line sensory organ of the zebrafish is available [50]. Pharmaceuticals from a library of FDA-approved drugs were screened and among them, 10 molecules were found to protect against at least two of the ototoxic drugs neomycin, gentamicin, kanamycin and cisplatin. Drugs from many pharmacological groups were among the protective 10 compounds, suggesting that several molecular mechanisms may be involved. Some of the drugs blocked gentamicin uptake into the hair cells, indicating that MET channels or other ways of entry may operate. The development of this therapeutic lead should hopefully continue.

Conclusion

The effects of quinine and salicylate to reversibly impair hearing can be analysed by well-known pharmacological principles such as the Hill equation. At the ion channel level, both drugs cause effects on channel currents that can be conventionally analysed according to the typical S-shaped appearance of the Hill equation with a plateau signifying the maximum obtainable effect. Its reduced form known as the psychophysical power function is applied for the concentration–effect relationship of hearing impairment, since no sign of diminishing returns could be traced at the concentrations of quinine that can be tolerated in the guinea pig and in man.

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