

# Use of a Nanoengineered Drug Carrier for Intracochlear Delivery of Otoprotective Substances in a Cochlear Implant Animal Model

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## Background

As cochlear implantation (CI) is being offered to people with greater residual hearing, preservation of inner ear function is becoming much more important. Likewise, very young children are now implanted and as technology improves, preservation of residual structure and function is important to maximise their future hearing. Whilst changes in surgical technique and refinements of electrode design have improved rates of hearing preservation, there is great scope for 'protection' of inner ear function with pharmacological intervention.

Direct trauma from the electrode may lead to necrosis and apoptosis, not only at the site of trauma, but also at more remote sites over time. Later, fibrosis around the electrode may contribute to delayed hearing loss. Pharmacologic intervention presents several challenges. To offer otoprotection, a drug must reach the appropriate part of the cochlea (generally the apex) in therapeutic concentration to protect hair cells, supporting cells, and neurons.

Adenosine acts as a cytoprotective substance released from tissues in response to stress. It has anti-oxidative, anti-inflammatory and anti-apoptotic effects in many tissues. Adenosine A<sub>1</sub> receptors are present in the cochlea and its agonists have been shown to ameliorate hearing loss following noise exposure and cisplatin treatment. In order to ensure sustained delivery to the cochlea, a number of slow release methods have been developed. Nanoengineered drug delivery carriers enable sustained release of substances over time, which is likely to be required to obtain therapeutic concentrations within the cochlea.

## Objectives

To investigate elution kinetics of nanoparticles (NP) *in vitro* for 3 adenosine receptor agonists (adenosine, ADAC (adenosine amine congener) and Regadenoson) using LCMS/MS techniques.

To develop approaches and then evaluate the use of a nanoengineered drug carrier to deliver ADAC, an Adenosine A<sub>1</sub> receptor agonist, that will protect against the development of cochlear injury during implantation in a guinea-pig model.

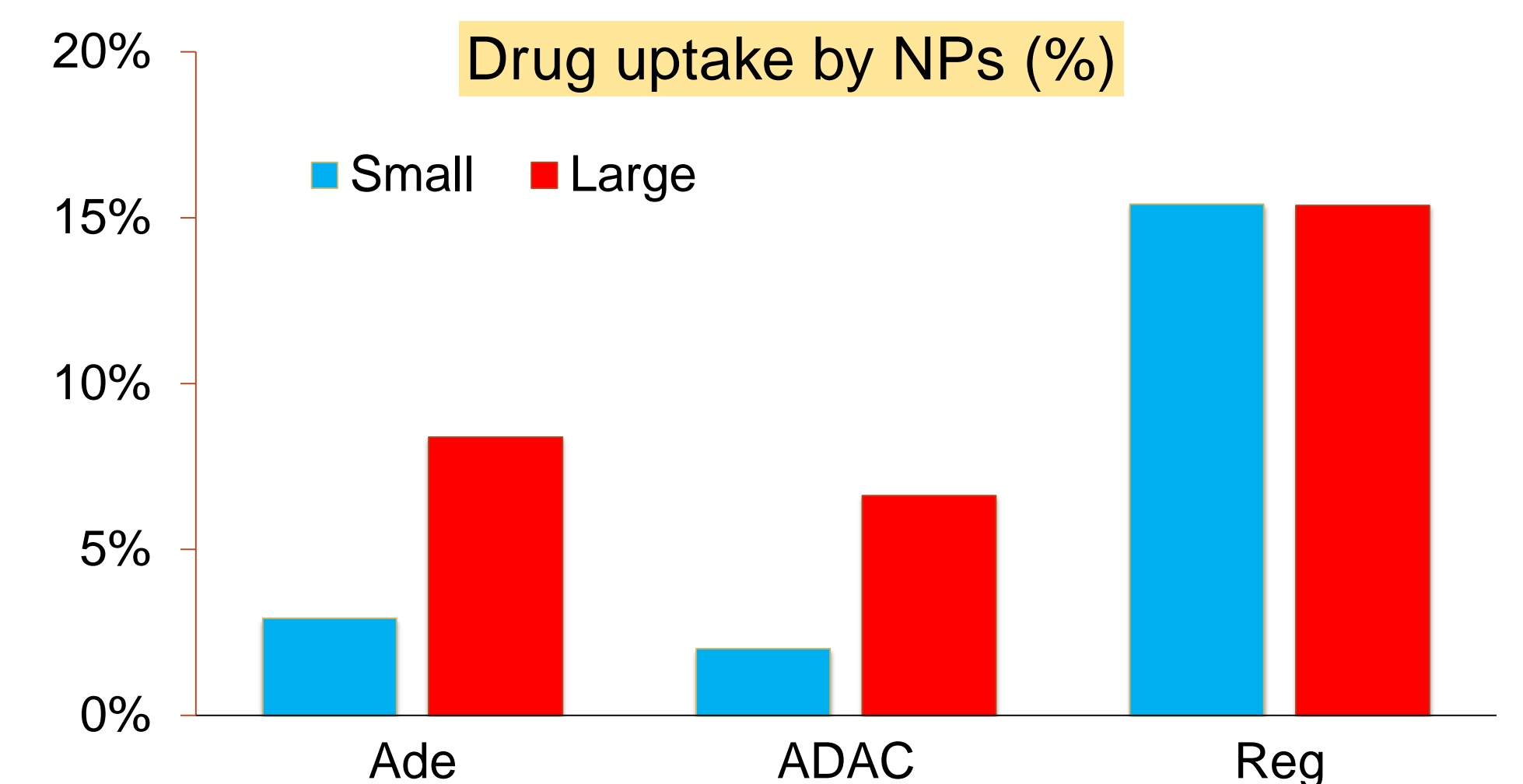
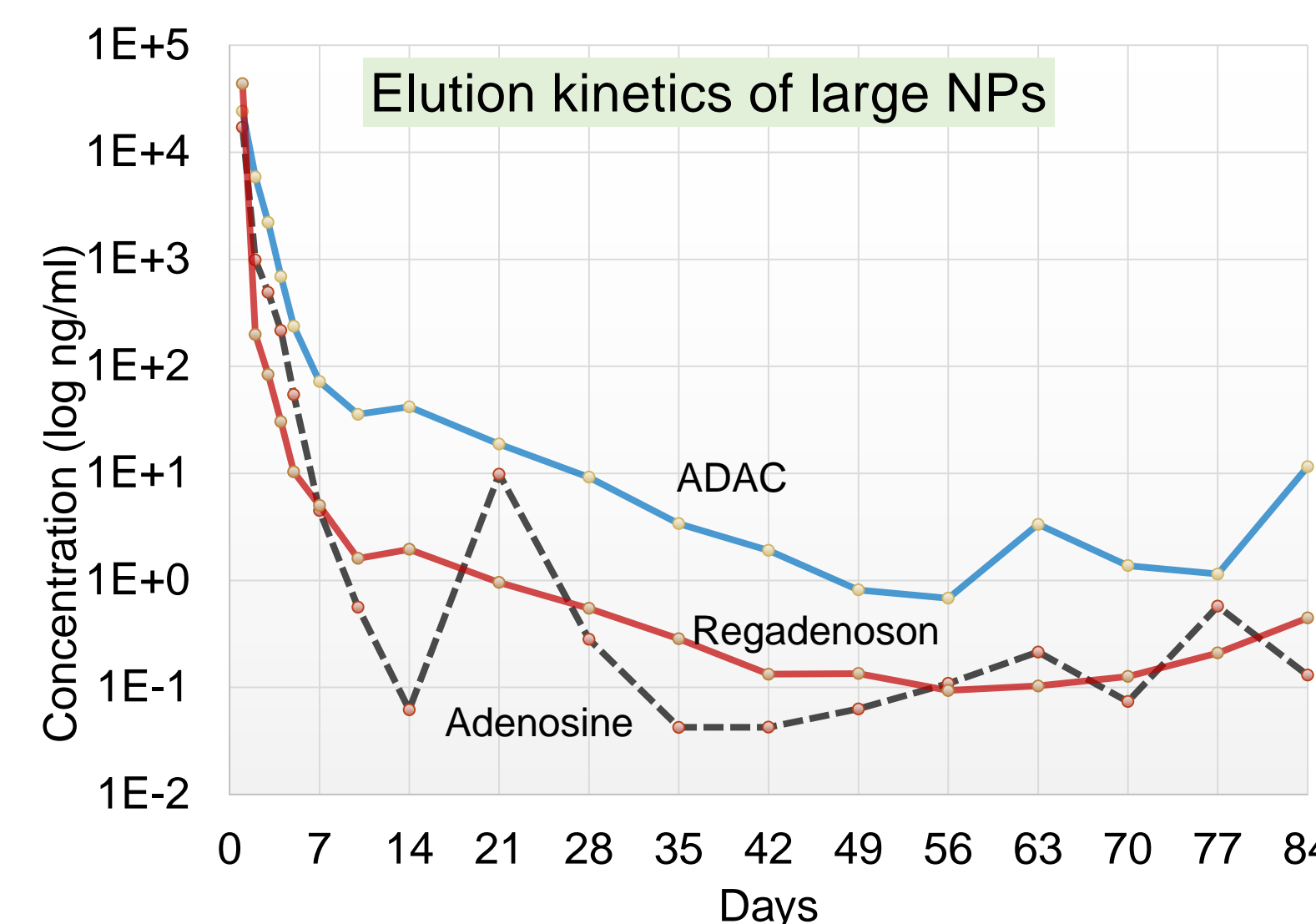
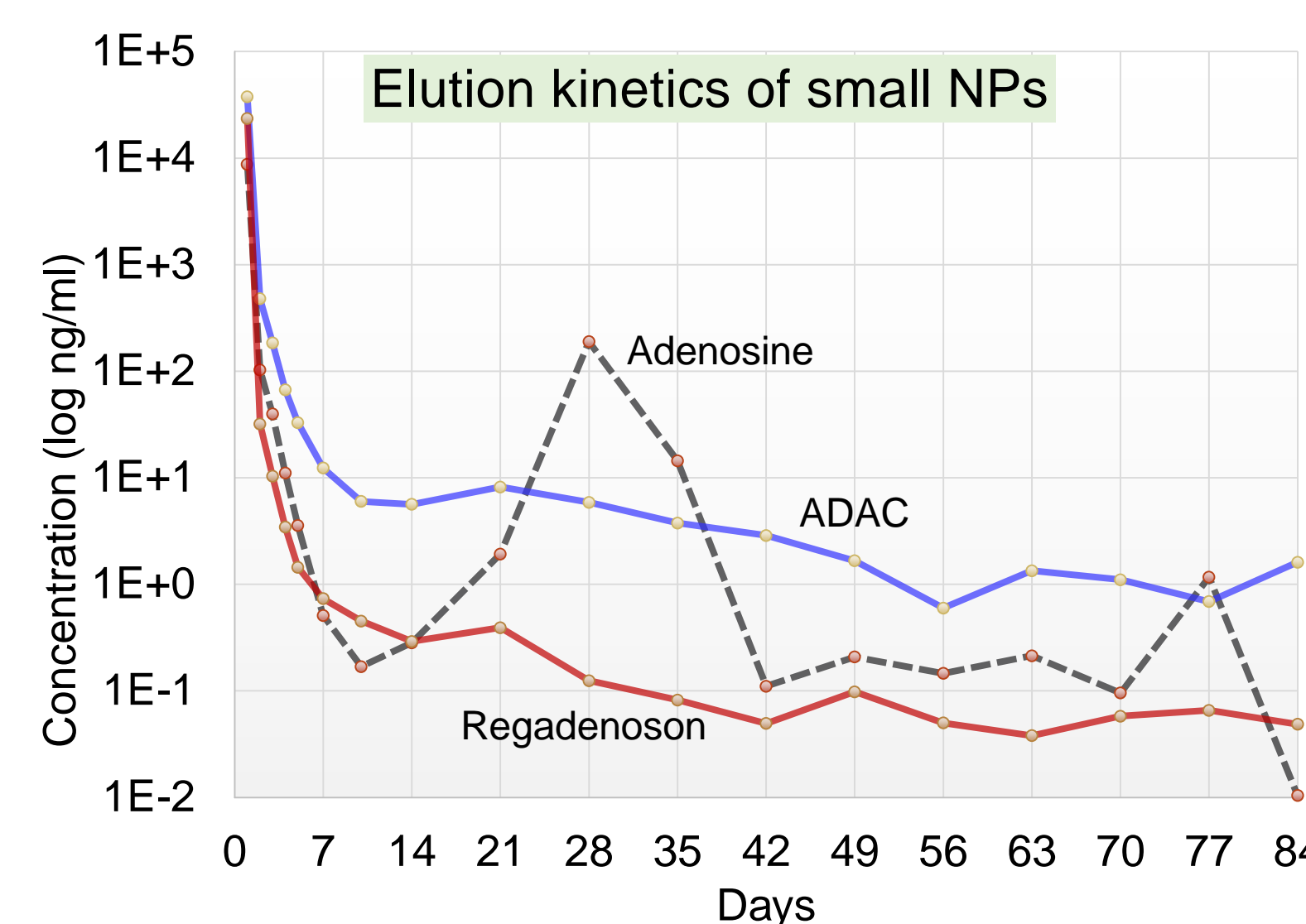
## Method

To study drug release kinetics, nanoparticle (NP) carriers were loaded with 100μM adenosine, ADAC and Regadenoson, and incubated in 100 μL artificial perilymph solution (APS) for up to 12 weeks. Samples were aliquoted at weekly intervals and concentration measured using liquid chromatography - mass spectrometry (LCMS/MS).

To eventually assess the impact of release of these compounds on cochlear injury we have developed an animal model of CI surgery. Guinea-pigs were exposed to noise (16kHz, 120dB SPL, 30min) to produce a lesion in the basal cochlear turn and permanent high frequency (>8kHz) threshold shift of 90-100dB assessed using Auditory Brainstem Responses (ABR). A non-functional CI (an electrode array with 4 platinum rings on a silicone carrier) was inserted via a cochleostomy to induce low frequency sensorineural hearing loss.

## In vitro elution kinetics observations

- Of the three compounds tested, ADAC delivered consistently higher concentration levels throughout. The difference narrows down over time. Even at 12<sup>th</sup> week, the concentration of ADAC is more than 3 times the minimum effective concentration (MEC).
- The unexpected spike in concentration of adenosine around 21-28 days in both large and small NPs could be due to inadvertent sampling of NPs into the LCMS.
- The MEC of ADAC for treating NIHL in rats is reported to be 0.85nM or 0.49ng/ml conc in blood. This would mean a further lower concentration required in perilymph of cochlea. With our data, it seems the concentrations attained at 12 weeks of elution with NPs are sufficient enough to provide the desired therapeutic benefits.



## Drug uptake by NPs

- Uptake of drug by the NPs – the larger NPs took up significantly higher conc of adenosine and ADAC, but with Regadenoson both size NP accumulated a similar amount.
- Regadenoson uptake by NPs was the highest, but it was also rapidly eluted in its first 10 days.
- Total uptake of ADAC is higher than adenosine but significantly lower than Regadenoson. However ADAC seems to elute from the NPs in a slow and steady fashion.

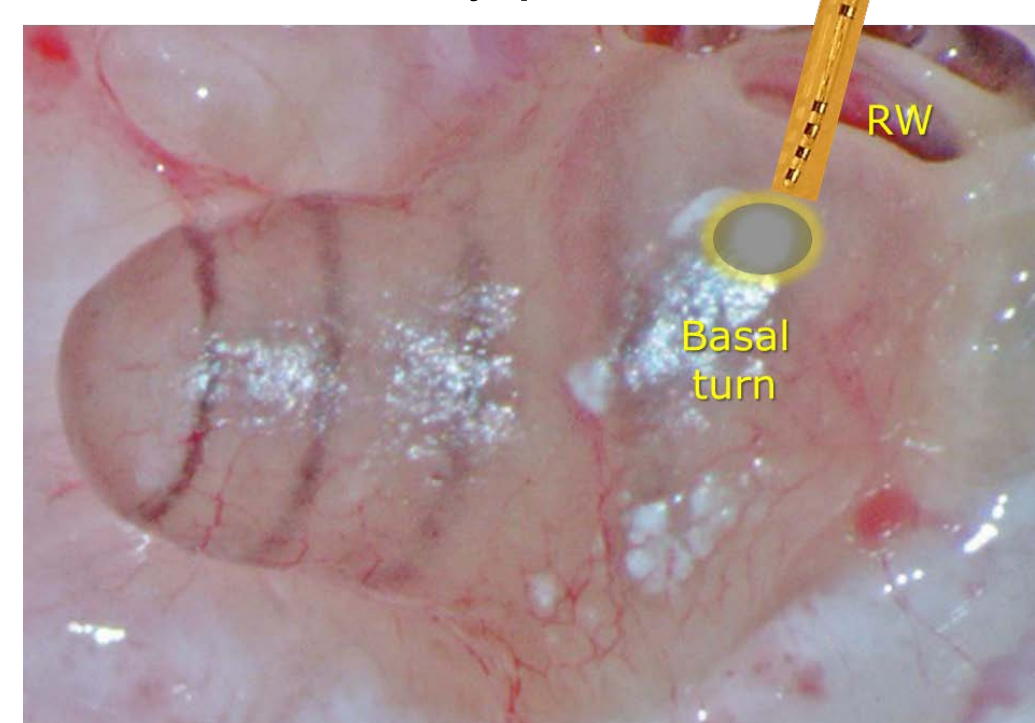
## Implant model: Noise induced hearing loss, cochlear implants and ABR's

- Exposure to noise (16kHz, 120dB, 30min) produced high frequency hearing loss in guinea pigs (16-24kHz), sparing low frequencies (2-8kHz).
- Dummy cochlear implants were inserted through a cochleostomy (1.6mm from round window) with an insertion depth of up to 3.2mm. This corresponds to frequency range of 12-28 kHz.
- Cochlear implant surgery affected the lower frequency region from 2-8kHz and raised the ABR thresholds by 20-30dB.

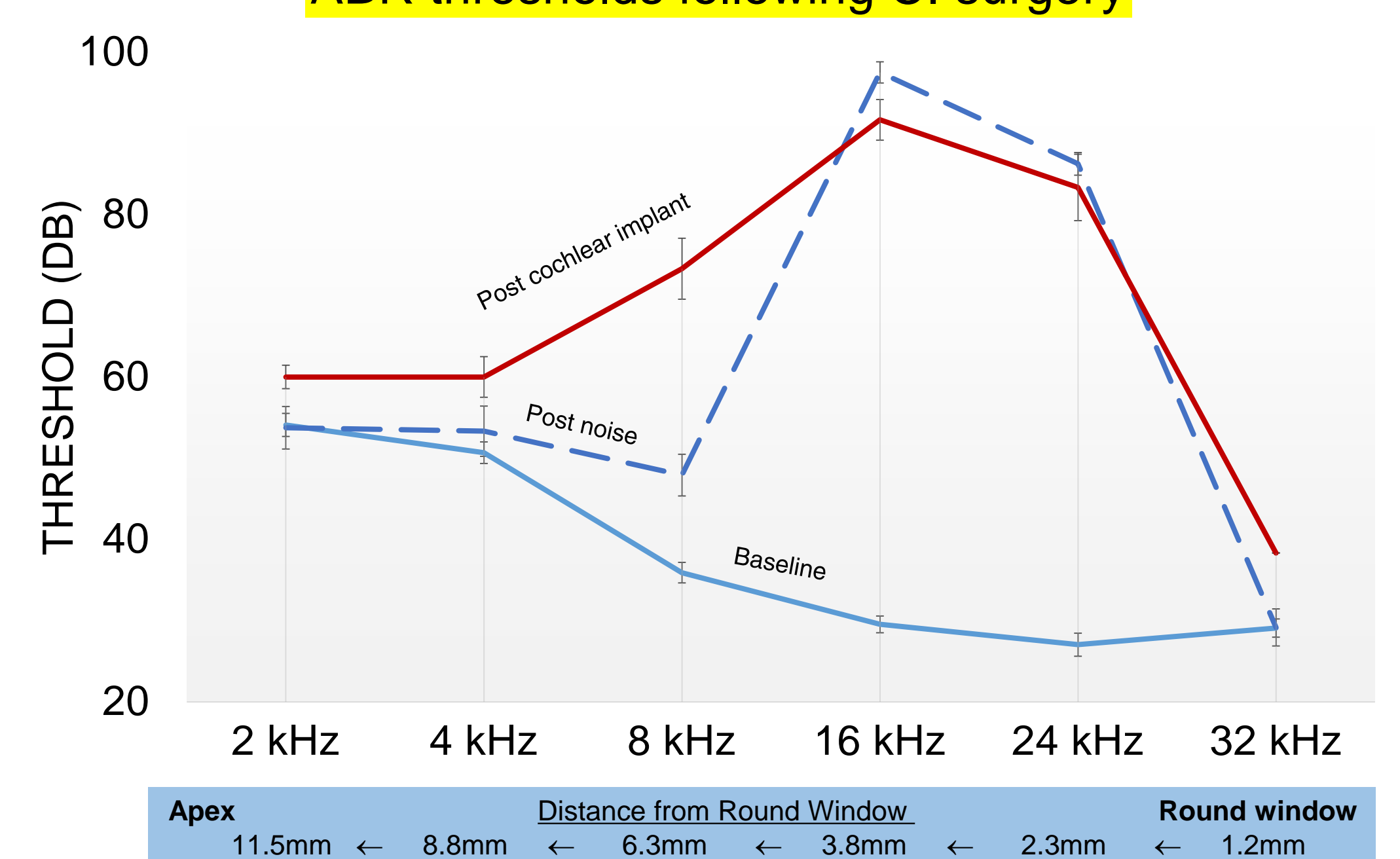


Dummy Cochlear implant.

Cochleostomy position.



## ABR thresholds following CI surgery



## Summary and Conclusions

ADAC elution kinetics were consistently higher than the other compounds, followed by Regadenoson and adenosine. The guinea-pig CI model has successfully achieved high frequency hearing loss from noise and low frequency loss from surgical trauma.

In-vitro studies have confirmed excellent release of Adenosine A<sub>1</sub> receptor agonists from the nanoengineered drug delivery carrier system. A reliable guinea-pig model has been developed for later use in both in-vivo pharmacokinetic studies and to investigate the otoprotective effects of Adenosine A<sub>1</sub> receptor agonists.