Introduction

Evaluation of the auditory function is part of the functional assessment of the Central Nervous System in the follow-up studies mentioned in ICH S7A guideline for Safety Pharmacology Studies for Human Pharmaceuticals. The Auditory Brainstem Response globally tests functional integrity of the inner ear and auditory nerve, and their ability to properly transmit a sound signal along the whole pathway to the brainstem. The purpose of the present study was to validate the Auditory Brainstem Response (ABR) as a general evaluation of auditory loss in rats. Cisplatin was used to induce hearing function impairment as it is a well documented ototoxic compound which induces early outer hair cell death through free radical generations beginning at the basal turn of the cochlea.

Materials and Methods

Two groups of eight 8-week old male Sprague Dawley rats were given a single slow intravenous administration of cisplatin at 16 mg/kg. An additional group of 6 animals was used as a non treated control group. One of the cisplatin treated groups received a daily intraperitoneal injection of D-methionine (300mg/kg) for 4 days, starting on the day of cisplatin administration (figure 1).

Figure 1: Study design

Auditory evoked potentials and measurements of ABR thresholds were evaluated after auditory stimuli with pure tones of various frequencies (8, 16 and 32 kHz) delivered by 10 or 5 dB intensity decreasing sequences, in all animals under light isoflurane anaesthesia before treatment and on day 4.

- Tone bursts at set frequencies were generated by an arbitrary wave-form generator (model 75 wavetek). These audio stimuli were amplified and delivered to high frequency tweeter speakers tightly inserted into the external ear.

- Auditory brainstem responses were recorded by subcutaneously inserted stainless steel electrodes (Medtronic) located at the vertex and ipsilateral mastoid, with the lower back used as ground (figure 2). Signals were processed by an evoked potential measuring system (Neuropack Mu - Nihon Koden) (figure 3).

- The ABR threshold was defined as the stimulus level needed to produce a visual detection of at least one of the ABR waves. When no ABR wave was observed (deaf or almost deaf animals), the ABR thresholds were compared between groups before and after treatment by a two-way (time - treatment) analysis of variance (ANOVA) for repeated measurements using SAS PROC MIXED.

Results and discussion

No death was observed during the study, but clinical signs including piloerection and signs of diarrhoea were noted in cisplatin-treated animals. There was no difference in ABR threshold between groups at any stimulus frequency before treatment (Table 1).

Cisplatin induced a severe impairment of the auditory function 4 days after treatment, with ABR thresholds doubled when compared to control animals.

Table 1: Auditory Brainstem Response Threshold (dB) measured in rats before and 4 days after treatment

<table>
<thead>
<tr>
<th>Frequency (kHz)</th>
<th>Control Before treatment</th>
<th>Cisplatin Before treatment</th>
<th>Cisplatin + D-methionine Before treatment</th>
<th>Control 4 days after treatment</th>
<th>Cisplatin 4 days after treatment</th>
<th>Cisplatin + D-methionine 4 days after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>48.33 ± 5.16</td>
<td>39.29 ± 9.32</td>
<td>43.57 ± 6.90</td>
<td>91.43 ± 19.84</td>
<td>65.71 ± 17.66</td>
<td>77.74 ± 18.22</td>
</tr>
<tr>
<td>16</td>
<td>45.83 ± 6.65</td>
<td>46.43 ± 6.90</td>
<td>44.29 ± 5.35</td>
<td>100.79 ± 25.97</td>
<td>77.74 ± 18.22</td>
<td>87.91 ± 16.69</td>
</tr>
<tr>
<td>32</td>
<td>39.17 ± 13.57</td>
<td>41.43 ± 2.74</td>
<td>47.74 ± 5.67</td>
<td>107.86 ± 11.50</td>
<td>85.71 ± 16.69</td>
<td>93.56 ± 10.57</td>
</tr>
</tbody>
</table>

Although the ABR thresholds were higher in animals treated with cisplatin and D-methionine, they were reduced when compared to those measured in animals treated with cisplatin alone (Table 1, Figure 5).

Table: *p<0.05 in comparison to control; **p<0.01 and ***p<0.005 in comparison to cisplatin treated group.

Conclusion and discussion

These results demonstrate that cisplatin induced severe impairment of the auditory function in the rat as expected. Treatment with D-methionine was sufficient to protect the cochlea and to significantly reduce the toxicity level of cisplatin.

Therefore, Auditory Brainstem response in the rat can be used as a screening tool for the safety assessment of global auditory function. Cisplatin may be used as a positive ototoxic reference compound and combined with protective D-methionine for assessing the sensitivity of the test.