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Cortisol Levels in the Human Perilymph after Intravenous Administration of Prednisolone

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Key Words
Perilymph · Cortisol · Prednisolone · Otosclerosis

Abstract
Cortisone is used in various inner ear disorders such as sudden hearing loss. However, it is not known if the doses of prednisolone employed in therapy increase the cortisol level in the inner ear. To evaluate the level of cortisol within the perilymph after intravenous administration of 125 and 250 mg of prednisolone, serum and perilymph samples of 29 consecutive patients with clinical otosclerosis subjected to stapedectomy were collected. Cortisol levels were determined by RIA. The perilymphatic cortisol level was significantly increased in the group with 250 mg of prednisolone while the perilymphatic cortisol levels were not significantly different between the control group and the patients treated with 125 mg. Although the therapeutic dose of prednisolone is not known, we conclude that the application of 250 mg has a greater impact on the inner ear than 125 mg.

Introduction

The effectiveness of corticosteroid therapy in inner ear disorders such as sudden hearing loss, blast injury, tinnitus, Menière's disease and auto-immune diseases of the inner ear has been discussed for many years [Moskowitz et al., 1984; McCabe, 1979; Henry, 1992; Kitajiri et al., 2002; Lee and Marcus, 2002]. It has been demonstrated in animals exposed to noise trauma that recovery of hearing function is better in the corticosteroid-treated group [Lamm and Arnold, 1999]. In addition, pretreatment with corticosteroids effectively reduces noise-induced cochlear damage and hearing loss [Henry,1992; Michel et al., 1993; Wang and Liberman 2002].

Corticosteroids exert part of their salutary effects through a potent suppression of inflammatory responses. Glucocorticoid receptors have been shown to be widely expressed throughout the human body. The presence of these receptors within the inner ear of mammalians has been shown by immunohistochemistry, in situ hybridization and PCR [Erichsen et al., 1996; Furuta et al., 1994]. It has recently been shown in functional animal studies that prednisolone (in the range of therapeutic plasma concentrations) increases the K^+ secretion of the stria vascularis [Lee and Marcus, 2002].

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Clinical experiences and retrospective studies have given contrasting results about the influence of prednisolone in idiopathic sensorineural hearing loss (SNHL) [Wilkins et al., 1987; Kitajiri et al., 2002]. However, in the treatment of idiopathic SNHL the administration of cortisone is the mainstay. The optimal drug dose and route of administration are currently unknown. Because of the known side-effects, systemic application should be avoided in several cases (e.g. diabetes, heart failure), and the local pharmacotherapy of the inner ear could be a useful alternative. Various authors report in clinical studies the intratympanic application of prednisolone for the treatment of Menière's disease [Shea and Ge, 1996; Sennaroglu et al., 1999; Hirvonen et al., 2000]. However, there are currently no published data about human perilymphatic cortisol levels after application of therapeutically employed doses. Because of the well-known anatomical differences between the human and e.g. the inner ear of guinea pigs, data about human inner ear drug concentrations after corticosteroid administration are needed.

The aim of this study was to find out if cortisol can be measured within the human perilymph after application of doses actually used in the therapy of acute inner ear disorders such as idiopathic SNHL and to analyse the correlation between the drug dose applied and drug levels reached in serum and perilymph.

Materials and Methods

After having obtained their informed consent, 29 consecutive patients suffering from clinical otosclerosis undergoing stapedectomy in local anaesthesia were included in this prospective study. The patients' ages ranged from 24 to 58 years with an average of 38 years. The distribution men:women was 1:1.5, and the body weight ranged from 54 to 86 kg with an average of 72 kg. On the day of surgery, a 10-ml serum sample was taken at 7 a.m. and stored at –20°C. The patients were randomly divided into 3 groups. Group I (n = 9) received 125 mg prednisolone (prednisolone-21-hydrogen-succinate, Solu Decortin H, Merck, Darmstadt, Germany) intravenously and group II received (n = 10) 250 mg prednisolone intravenously. The control group (n = 10), received Ringer's saline solution but no prednisolone. The injections in all 3 groups were given approximately 35 (± 11) min before opening the oval window. Perilymph (1–2.5 µl) from all patients was sampled after removal of the stapes footplate during stapedectomy using capillary forces of sterilized glass capillaries (Clark Electromedical Instruments, Pangbourne, UK). These capillaries were prepared by pulling them out over a gas flame resulting in glass capillaries with an inner diameter of about 100 µm. Sampling was done by touching the surface of the perilymph at the level of the oval window. Capillaries were never introduced into the vestibulum. The perilymph samples were stored after gentle centrifugation at –20°C. At the time of perilymph sampling, a second serum sample was taken and stored at –20°C. Cortisol in serum and perilymph was measured using a non-extraction ^{125}I-RIA kit from DRG Instruments GmbH (Marburg, Germany). In brief, cortisol as analyte and a ^{125}I-labelled cortisol tracer are competing for a fixed number of rabbit anti-cortisol-antibody-binding sites. The separation of free from bound antigen is achieved by polyethylene glycol as a precipitating aid. The amount of ^{125}I-labelled cortisol bound to the specific antibody is inversely proportional to the endogenous analyte concentration.

For the measurement of the perilymph specimen, all standards and samples were applied in a volume of 10 µl instead of 25 µl, whereas antiserum and tracer solutions were added as prescribed in the original assay protocol. For this, 1 µl of vigorously centrifuged perilymph was prediluted with assay diluent to a final volume of 10 µl.

Albumin in serum and perilymph was measured on a Hitachi 747 analyser (Roche Diagnostics, Mannheim, Germany) as colorimetric assay with bromocresol green, using the original reagent kit and following the manufacturer's instructions to evaluate the amount of blood contamination. The perilymph samples were analysed in a 1:10 dilution as described above.

Statistical analysis was performed employing distribution-free tests (Kruskal-Wallis test, Mann-Whitney test, Spearman correlation coefficient). The method of Marcus et al. [1976] for post hoc comparison was used where necessary. Statistical significance was set to 5%. All tests were two-sided. The statistical significance was calculated using the program SPSS, version 10.0.5.

This research has been approved by the ethic committee of the Technical University of Munich (12/20/99).

Results

In all samples of serum and perilymph, cortisol levels could be determined by the use of RIA. In the evaluation of cortisol levels, eventual blood contamination of the perilymph was calculated considering the albumin perilymph-serum gradient of 1:35 [Thalmann et al., 1992].

The concentration of cortisol within the perilymph showed a statistically significant difference between group II (86.5 ± 62.8 µg/dl) and the control group (28.5 ± 45.9 µg/dl; p = 0.021) on one side, and between groups II and I (19.1 ± 21.1 µg/dl; p = 0.007) on the other side. No significant difference could be found between the perilymph cortisol levels of group I and the control group (table 1, fig. 1).

Serum cortisol levels before surgery showed no statistically significant differences between the 3 groups (control group 16.7 ± 5.8 µg/dl; group I, 18.5 ± 10.5 µg/dl; group II, 16.2 ± 5.8 µg/dl) (fig. 2).

The concentration of cortisol in the serum sample taken at the time of perilymph sampling showed significant differences between the control group (17.2 ± 10.1 µg/dl) and the 2 groups with prednisolone administration (group I, 282.1 ± 328.4 µg/dl; group II, 347.5 ± 184.1 µg/dl), as expected. In contrast, there was no significant difference between groups I and II (fig. 3).

Table 1. Cortisol concentrations (μg/dl) in perilymph and serum

Group	Perilymph	Serum I	Serum II
Control	2.5	10.2	14.0
Control	18.0	26.0	19.0
Control	14.0	19.0	24.0
Control	156.0	17.0	13.2
Control	18.0	24.4	17.0
Control	5.0	12.1	14.0
Control	17.0	8.0	12.0
Control	2.5	14.0	16.0
Control	15.0	19.0	2.0
Control	37.0	18.0	41.0
125 SDH	21.2	26.0	100.0
125 SDH	4.0	10.3	740.0
125 SDH	68.0	41.0	164.0
125 SDH	14.0	24.2	84.0
125 SDH	0.2	17.0	820.0
125 SDH	5.0	12.2	23.0
125 SDH	31.0	11.2	9.4
125 SDH	24.0	7.2	39.0
125 SDH	5.0	18.0	560.0
250 SDH	170.0	26.0	207.0
250 SDH	152.0	19.0	660.0
250 SDH	75.0	18.0	315.0
250 SDH	41.0	22.0	377.0
250 SDH	48.0	14.0	322.0
250 SDH	7.0	12.0	300.0
250 SDH	120.0	13.0	620.0
250 SDH	90.0		315.0
250 SDH	0.2	6.0	19.9
250 SDH	162.0	16.1	340.0

All patients (control: without prednisolone; 125 SDH: group I, with 125 mg prednisolone; 250 SDH: group II, with 250 mg prednisolone) with the relative values of cortisol (μg/dl). Perilymph: sample value after calculation and subtraction of the blood contamination; serum I: sample taken before surgery; serum II: taken at the time of perilymph sampling.

The average ratio perilymph:serum in the control group was 1:0.4, 1:1.8 in group I and 1:3.9 in group II. Because of large interindividual differences there was no significant correlation between serum levels and perilymph levels of cortisol in both the control and the cortisol-treated groups. The correlation values in the control group ($r = 0.15$, $p = 0.68$), in group I (with 125 mg of prednisolone; $r = 0.59$, $p = 0.09$) and in group II (with 250 mg of prednisolone; $r = 0.34$, $p = 0.34$) were not significantly different (fig. 4).

High levels of cortisol within the serum and low levels of cortisol within the perilymph were detected in 3 patients of group I.

Discussion

Since there is increasing evidence for an inflammatory pathomechanism of inner ear disorders such as idiopathic SNHL and Menière's disease, the use of anti-inflammatory drugs has been proposed as a first-line treatment [Moskowitz et al., 1984; Wilkins et al., 1987; Shea and Ge, 1996; Sennaroglu et al., 1999; Hirvonen et al., 2000]. However, contrasting results about the effectiveness, undesirable side-effects after systemic administration of glucocorticoids and the need of high doses for reaching a therapeutic effect have impeded a standardized, universally accepted therapy concept. There are only a few basic experimental data available concerning inner ear drug concentrations after systemic or local administration. In the present study, we analysed – to our knowledge for the first time in humans – levels of cortisol within the perilymph after endovenous administration of corticosteroids. All patients had been informed about the study and their treatment and had given their consent. The administration of prednisolone is supposed to protect the inner ear against noise-induced cochlear damage and hearing loss, which may occur during stapedectomy [Henry, 1992; Michel et al., 1993; Wang and Liberman, 2002]. The doses of prednisolone used by us are commonly given in the therapy of sudden hearing loss. In this study we show that intravenous administration of 250 mg of prednisolone (an equivalent to 3.4 mg/kg; group II) significantly increases cortisol levels within the perilymph. The ratio perilymph:serum was about 1:4 in group II. These results are in good agreement with the animal study data of Parnes' standard dose group [Parnes et al., 1999]. They analysed in guinea pigs the drug concentration in serum, perilymph and cerebrospinal fluid after intravenous, oral and local/intratympanic administration of glucocorticoids by high-performance liquid chromatography. For intravenous administration, 2 different concentrations were used: a standard and a high dose (0.2 mg/kg dexamethasone and 4 mg/kg for hydrocortisone and methylprednisolone or 8 mg/kg for dexamethasone and 20 mg/kg for hydrocortisone and methylprednisolone, respectively). In their high-dose group, the ratio perilymph:serum was about 1:10 (1:3 for hydrocortisone, 1:7 for methylprednisolone).

In our group I (125 mg of prednisolone, an equivalent to 1.7 mg/kg) in comparison with the control group we observed increased levels of cortisol within the perilymph and serum. The ratio perilymph:serum was 1:1.8. However, these results were statistically not significant because of large interindividual differences. Parnes et al.

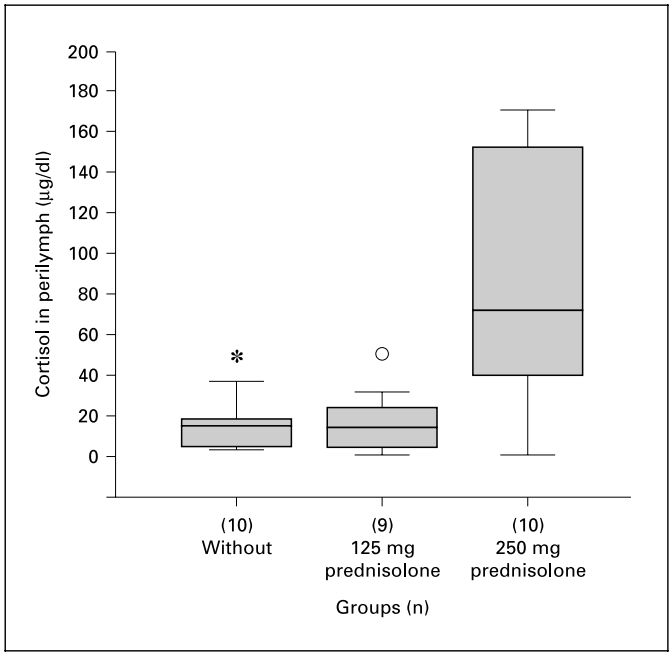

Fig. 1. The boxplots show the median, minimum and maximum, interquartile range, outliers (○) and extreme cases (*) of individual variables of the cortisol concentration in the perilymph.

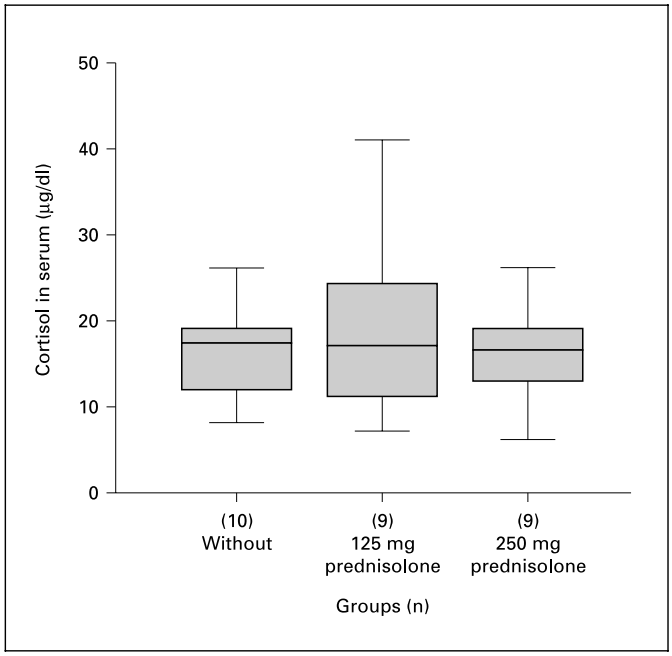

Fig. 2. The boxplots show the median, minimum and maximum, and interquartile range of individual variables of the cortisol concentration in the serum before surgery.

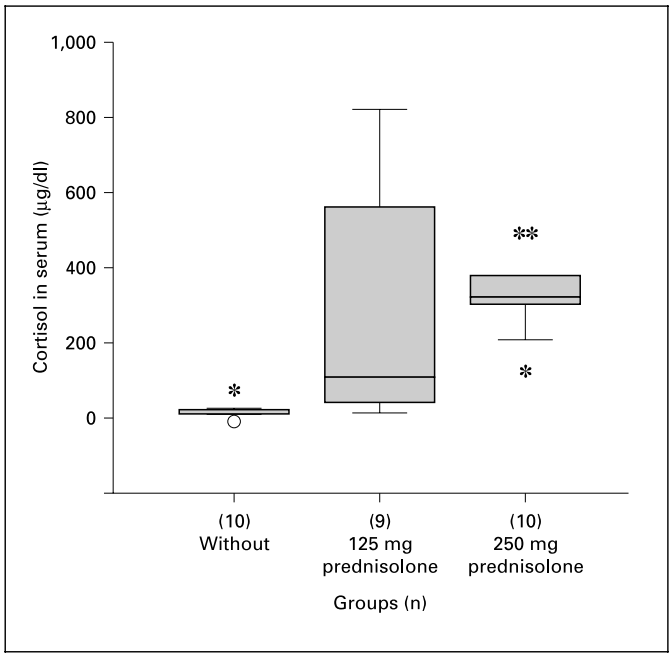

Fig. 3. Concentration of cortisol within the serum in the 3 groups after administration of prednisolone. The boxplots show the median, minimum and maximum, interquartile range, outliers (○) and extreme cases (*) of individual variables.

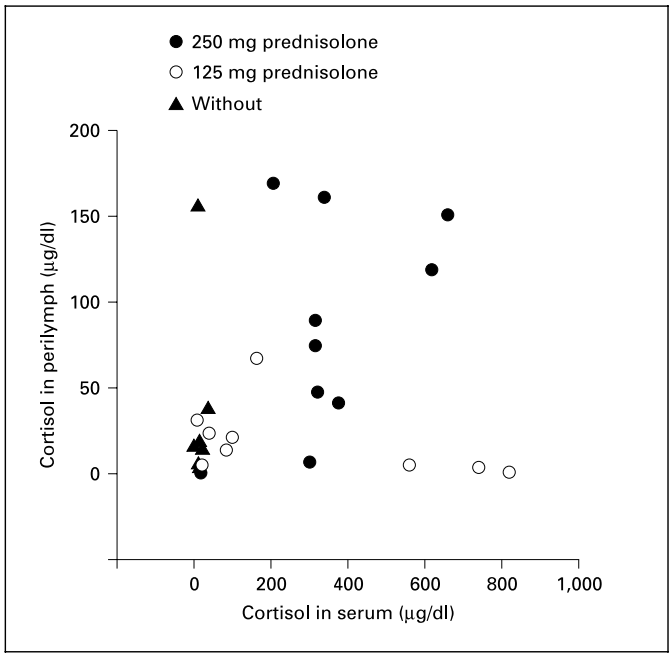

Fig. 4. No correlation between the concentrations of cortisol within serum and perilymph in the control group and the groups with 125 and 250 mg of prednisolone could be found.

[1999] found in their standard dose group (0.2 mg/kg dexamethasone and 4 mg/kg for hydrocortisone and methylprednisolone) no measurable levels in serum and perilymph for dexamethasone while hydrocortisone and methylprednisolone led to measurable perilymph and serum levels. In their standard dose trial, no significant differences between the levels in the serum and in the perilymph were seen [Parnes et al., 1999].

In our control group, 3 patients showed higher cortisol levels in the perilymph than in the serum. On one side, measuring errors cannot be excluded. On the other side, differences in the metabolism of corticosteroids in the inner ear including the binding capacity must be considered. Tobita et al. [2002] analysed the uptake and elimination pattern of prednisolone in cochlear tissue, brain, hepatic tissue and in serum. They observed a slower elimination of prednisolone from the cochlear tissue compared to brain, liver and serum. Increased levels of drugs within the perilymph in comparison with the serum levels have been measured by Parnes et al. [1999] in their standard dose group, too.

In our study, we found large interindividual differences of the cortisol levels in serum and perilymph in all groups. One reason for the differences in the perilymph values might be blood contamination. Therefore, the risk of blood contamination was reduced by extremely careful handling of the glass capillaries at the time of perilymph sampling. Before opening the inner ear, suprarenin (1:1,000, Aventis, Frankfurt/Main, Germany) was applied to the middle ear mucous membrane. Furthermore, macroscopically contaminated perilymph samples were discarded. Microscopic blood contamination, which can never be excluded completely, was estimated by measuring the albumin concentration in serum and perilymph. Since the gradient of albumin between perilymph and serum has been determined as 1:35 [Thalmann et al., 1992], the amount of blood contamination could be calculated from the sample volume and the albumin concentration. Thereby the amount of cortisol from blood contamination could be subtracted from the total cortisol level measured in the perilymph. Due to the minute amounts of perilymph some calculation errors cannot be excluded. However, our results are in good agreement with those of the study of Parnes et al. [1999].

In 1 control case, we observed an unexpectedly high perilymph cortisol concentration while the serum cortisol of this patient was at the average level. Erroneous single or multiple application of prednisolone on the morning of surgery was excluded. A possible explanation for the high perilymph and normal serum level could be an excitatory status of the patient since prednisolone clears the serum much faster than the perilymph [Parnes et al., 1999; Tobita et al., 2002]. We analysed the measuring and calculation error of our detection system. The critical difference for the dilution of the perilymph and the 'measurement noise' of the test was calculated at 0.31. The overall error can be assumed to be around 30% if the determination errors of cortisol and albumin measurement in perilymph and serum are considered. Both explanations above may not account for the extremely high value, leaving this result unexplained. This extreme value did not have any influence on the significance of the results. The statistical tests used are not based on normal distribution, and they consider this extreme value as the highest but do not account for the value itself.

Even the excellent controlled animal study of Parnes et al. [1999] with large amounts of perilymph (10 μl) had marked standard deviations. In their standard dose group, the standard deviation was 100% in the serum and in the perilymph [Parnes et al., 1999, table XI].

Large interindividual differences are due to variations of endogenous cortisol production and pharmacokinetics in serum and perilymph. Therefore no consistent correlation between cortisol level in serum and perilymph was found in our study.

To explain the large interindividual differences, we analysed other parameters such as the interval between drug administration and probe sampling, but we did not find any correlation [unpubl. observations]. Furthermore, the amount of blood contamination showed no correlation with the interindividual cortisol level differences of the perilymph.

The time point of perilymph sampling may influence the amounts of cortisol measured. We have taken the samples at about 35 min (± 11 min). Obviously, it cannot be excluded that a higher cortisol level can be found at an earlier or a later time point. Prednisolone concentration in homogenized cochlear tissue of guinea pigs taken at 0.5, 1, 2, 4 and 8 h after intravenous injection of prednisolone (100 mg/kg) peaked at 1 h and at 0.5 h in serum [Tobita et al., 2002]. In the time period between 0.5 and 1 h, neither tissue nor serum were analysed and the whole cochlea prednisolone concentration was examined and not perilymph in particular. We assume that the peak value in the perilymph is reached before the peak value in the cochlear tissue. Of note is the high dose of prednisolone used by Tobita et al. [2002] (100 mg/kg) and by the high-dose group of Parnes et al. [1999] (20 mg/kg) which exceeded the human dose by far. The pharmacokinetic of prednisolone is difficult to determine in the human as firstly a high

number of patients is needed to overcome the large interindividual differences and secondly only a minute amount of sample in the microlitre range is available.

Our sampling volume of the perilymph was limited to small quantities. The patients in our study suffering from otosclerosis had a very good sensorineural hearing with an air-bone gap of 20 up to 40 dB (SPL). To avoid any damage to the inner ear, the perilymph sampling was done very carefully using only the capillary forces, and only 1–2.5 µl of perilymphatic fluid could be obtained. As described above, the tip of the glass capillary was never introduced within the vestibulum. Up to now we have used perilymph sampling in more than 100 patients subjected to stapedectomy, and with a very careful sampling procedure and taking only small volumes by capillary force, we have never had a postsurgery SNHL. In the standard dose group of the study of Parnes et al. [1999], despite a perilymph sampling volume of 10 µl, large standard deviations of the drug concentrations within the perilymph were observed. To our knowledge, other studies investigating perilymph levels of cortisol after glucocorticoid administration are not yet available.

Recently, Becvarovski et al. [2002] have published their observation about the gentamicin levels after intravenous administration in the human labyrinth in patients subjected to translabyrinthine acoustic neuroma surgery. They found a rapid increase in the intralabyrinthine gentamicin level and interindividual variations of gentamicin levels. In particular, there were in some cases higher intralabyrinthine gentamicin levels than the levels in the serum. The phenomenon of having higher concentrations within the perilymph than in the serum was also observed in our study and could be due to local metabolism including different binding capacities within the inner ear and the blood-perilymph barrier as suggested by Tobita et al. [2002].

We conclude that the intravenous administration of 250 mg of prednisolone (corresponding to 3.5 mg/kg body weight), as used in the clinical practice for inner ear disorders such as sudden hearing loss or Menière's disease, are needed to reach a significant increase in cortisol concentrations in the perilymphatic fluid. By contrast, the administration of 125 mg of prednisolone is not sufficient to increase the level of cortisol significantly within the perilymph. Concerning the therapeutic effectiveness of doses used in therapy and in our investigation, further studies are needed.

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