Toluene-Induced Hearing Loss in the Guinea Pig

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Toluene is a high-production industrial solvent, which can disrupt the auditory system in rats. However, toluene-induced hearing loss is species dependent. For instance, despite long-lasting exposures to high concentrations of aromatic solvent, no study has yet succeeded in causing convincing hearing loss in the guinea pig. This latter species can be characterized by two metabolic particularities: a high amount of hepatic cytochrome P-450s (P-450s) and a high concentration of glutathione in the cochlea. It is therefore likely that the efficiency of both the hepatic and cochlear metabolisms plays a key role in the innocuousness of the hearing of guinea pigs to exposure to solvent. The present study was carried out to test the auditory resistance to toluene in glutathione-depleted guinea pigs whose the P-450 activity was partly inhibited. To this end, animals on a low-protein diet received a general P-450 inhibitor, namely SKF525-A. Meanwhile, they were exposed to 1750 ppm toluene for 4 weeks, 5 days/week, 6 h/day. Auditory function was tested by electrocochleography and completed by histological analyses. For the first time, a significant toluene-induced hearing loss was provoked in the P-450–inhibited guinea pigs. However, the ototoxic process caused by the solvent exposure was different from that observed in the rat. Only the stria vascularis and the spiral fibers were disrupted in the apical coil of the cochlea. The protective mechanisms developed by guinea pigs are discussed in the present publication.

Key Words: ototoxicity; toluene; glutathione; cytochrome; diet; SKF525-A; hearing loss.

Toluene-induced hearing loss was assessed in the rat by Pryor et al. (1991). Then, the research projects on toluene-induced hearing loss were numerous and almost always carried out in the rat due to its vulnerability to aromatic solvents (Campo et al., 1997; Crofton et al., 1994; Johnson and Canlon, 1994). On the other hand, the auditory receptor of the guinea pig is known to be solvent resistant (Campo et al., 1993; Fechter, 1993; Lataye et al., 2003; McWilliams et al., 2000), but the reasons why the organ of Corti of guinea pigs can resist solvent are still questionable.

Several toxicokinetic studies allowed a better understanding of the difference in uptake and clearance of the solvent between guinea pig and rat (Campo et al., 2006). All these results pointed in the same direction: When both species are exposed in the same experimental conditions, the half-life of toluene is longer in the rat than in the guinea pig. This might be one possible reason to explain the difference in cochlear sensitivity to toluene between rats and guinea pigs but likely not the only one.

As far as we know, there are two major mechanisms which might explain toluene-induced hearing loss: (1) a poisoning of Deiters and Hensen’s cells, which are both important to maintain the K+ homeostasis in the vicinity of outer hair cells (Wangemann, 2002) and (2) an oxidative cell injury, such as lipid peroxidation (Campo et al., 2001). Today, no investigation has yet been performed to evaluate the hepatic metabolism and the cochlear glutathione (GSH) impacts on cochlear resistance to solvent in the guinea pig, though any advance in our understanding could be of real interest, even for humans.

The hepatic metabolism of xenobiotics like toluene depends on the activity of the different forms of cytochrome P-450s (P-450s). Indeed, the P-450 superfamily is a hemoprotein that plays a pivotal role in the hepatic metabolism of a wide variety of chemical compounds (Lewis, 2003). Several gene families exist in all mammals. It has become evident that many of these P-450 enzymes exhibit partially overlapping substrate specificities. For instance, the oxidation pathway of toluene mainly involves P-450 2E1, which results in hippuric acid (HA), whereas P-450 1A2 mainly leads to cresols (Campo et al., 1998).

The amount of GSH (L-γ-glutamyl-L-cysteinyl-glycine) contained in the cochlea appears to be an additional element...
A total of 22 male guinea pigs were used in the pilot study and 43 in the main subchronic study. The albino guinea pigs were provided by Charles Rivers Laboratories (L’Arbresle, France) and weighed between 320 and 380 g on arrival at the animal facility. They were housed in individual cages (350 × 180 × 184 mm) for 2 weeks before the start of the experiments. Food and tap water were available ad libitum except during exposure. The animals were on a normal day/night cycle, that is, lighting was on 12 h/day. The temperature in the animal quarters was 22°C ± 2°C and the relative humidity was 55 ± 10%.

The guinea pigs were kept on two different diets, as described in Table 1. Whichever the experiment considered, pharmacokinetic or long-lasting experiment, the diet was initiated 15 days before the beginning of the toluene exposure. The animals with a standard diet were housed with irradiated corncob bedding, whereas those on a low-protein diet were housed with certified cellulose bedding in order to avoid an uncontrolled intake of protein (Dietex Ltd., Saint Gratien, France). While conducting the research described in this article, the investigators adhered to the Guide for Care and Use of Laboratory Animals mandated by the French Conseil d’Etat in Decree n° 87-848 published in the French Journal Officiel on 20 October 1987.

Experimental Protocols

**Efficiency of the SKF treatment.** Fifty milligrams per kilogram body weight was the highest dose that the guinea pigs could endure while retaining proper physiological functions during the duration of the experiment. The SKF powder (from Sigma, Saint Quentin Fallavier, France) was dissolved in a solution of 0.9% NaCl. A single dose of 50 mg/kg was sc administrated to reduce the P-450 2E1 activity. Four SKF-treated animals and four controls were used for this particular pilot study. One hour after the treatment, the livers were isolated and a microsomal suspension was prepared before being stored at −20°C.

P-450 activity was evaluated by quantitative analysis, the principle of which was based on the oxidation of 4-nitrophenol (4-NP) in 4-nitrocatechol (4-NC) by microsomal P-450 2E1 (Reinke and Moyer, 1985). The rate of 4-NC formation was measured at 518 nm (Shimadzu UV-1240) and expressed per protein quantity: pmole/min/mg protein. The linearity of the 4-NC formation was first checked as a function of both incubation time and protein concentration. The microsomes of liver (1 mg/ml) were mixed with cofactors (MgCl2, 3mM, G-6-P 5mM, G-6-P dehydrogenase 1 U/ml, NADP+ 1mM). After a 30-min preincubation period at 37°C, the reaction was triggered by adding 100µM of 4-NP diluted in a 0.1M phosphate buffer (pH 7.4). The incubation lasted 4 h in a shaking water bath. The reaction was stopped with 100 µl of HClO4 (11 N), and the samples were frozen at −20°C for 15 min to precipitate the proteins. The samples were then centrifuged at 20,000 × g for 5 min. Finally, 200 µl of NaOH (10 N) was added to 1 ml of supernatant to reveal the colorimetric reaction.

**Consequences of the SKF treatment on the time course of toluene in blood.** Fourteen anesthetized guinea pigs were used in this investigation of toxicokinetics: five were on a low-protein diet, four were on a standard diet, and five others were on a diet and SKF treated. The SKF treatment consisted in an injection of 50 mg/kg body weight, 1 h before inducing the anesthesia.

One polypropylene catheter (0 = 0.58 mm) was fitted into the jugular as an iv route and another one into the carotid artery to collect arterial blood samples. An initial 250-µl sample of arterial blood was collected 15 min before the toluene injection to obtain a reference value.

**Table 1** Diets

<table>
<thead>
<tr>
<th>Diet</th>
<th>Protein (%)</th>
<th>Assimilated glucides (%)</th>
<th>Fibers (%)</th>
<th>Mineral (%)</th>
<th>Lipid (%)</th>
<th>Humidity (%)</th>
<th>Methionine (%)</th>
<th>Cysteine (%)</th>
<th>Ascorbic acid (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>16.0</td>
<td>50.5</td>
<td>13.3</td>
<td>6.0</td>
<td>3.5</td>
<td>10.0</td>
<td>0.21</td>
<td>0.25</td>
<td>800</td>
</tr>
<tr>
<td>Low protein</td>
<td>7.0</td>
<td>50.0</td>
<td>26.0</td>
<td>4.0</td>
<td>6.0</td>
<td>7.0</td>
<td>0.09</td>
<td>0.10</td>
<td>800</td>
</tr>
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</table>

**Materials and Methods**

**Animals and Diets**

The aim of the present study was to determine the relationship between the hepatic metabolism (P-450 and GSH) of toluene and its ototoxic potency in the guinea pig. Assuming a crucial role for toluene (mother molecule) in the relationship between the hepatic metabolism (P-450 and GSH) of toluene and its ototoxic potency in the guinea pig. The microsomal suspension was prepared before being stored at −20°C.

**Pilot Experiments**

**Corrections of the SKF treatment on the time course of toluene in blood.** Fourteen anesthetized guinea pigs were used in this investigation of toxicokinetics: five were on a low-protein diet, four were on a standard diet, and five others were on a diet and SKF treated. The SKF treatment consisted in an injection of 50 mg/kg body weight, 1 h before inducing the anesthesia.

One polypropylene catheter (0 = 0.58 mm) was fitted into the jugular as an iv route and another one into the carotid artery to collect arterial blood samples. An initial 250-µl sample of arterial blood was collected 15 min before the toluene injection to obtain a reference value.
The animals received a 300-μl bolus of intralipid (fat emulsion) containing 28 μl of toluene (43 mg/kg). We chose such a bolus (300 μl of intralipid + 28 μl of toluene) in order to compare the present results with previous toxicokinetic data obtained in both rats and guinea pigs (Campos et al., 2006). The bolus was iv injected over a period of 1.5 min; 250-μl samples of arterial blood were collected and dispensed into 1.5-ml glass containers at 1, 2, 5, 10, 30, 60, 120, and 180 min following the end of the injection. After each blood collection, a 250-μl bolus of saline solution was injected to prevent a pressure shock. The blood samples were gently shaken and then frozen (−30°C) with no impact on the subsequent measurements.

The method used to measure the toluene concentrations has already been detailed in Campos et al. (1999). Briefly, toluene was extracted from the blood sample with a 1-ml aliquot of CS2, containing m-xylene as an internal standard in order to establish phase separation. After shaking, the samples were centrifuged at 1500 × g for 15 min at −4°C. The lower CS2 layer was pipetted above the olfactory bulbs. The measuring and reference electrodes were insulated platinum wire was inserted through a guide hole under visual control. A retroauricular incision was made and the otic capsule exposed by cutting through the connective tissue and the neck musculature. The tip of an insulated wire was inserted through a guide hole under visual control. When the electrode tip was on the edge of the round window, the electrode was anchored onto the otic capsule with dental cement. A reference electrode was placed above the olfactory bulbs. The measuring and reference electrodes were fastened to a transistor socket and fixed with dental cement to the skull. This electrophysiological technique allowed the auditory-evoked potentials to be recorded. Hearing testing was performed in an audiometric room, 4 weeks after the end of the toluene exposure. The anesthetized guinea pigs were placed in a restraining device that ensured a constant distance (15 cm) and orientation from the left pinna to the speaker (B&L 2405). The generation of the sound stimuli and the signal treatment were performed with a Tucker-Davis technologies apparatus equipped with Biosig software. The acoustic stimuli, 2 cycles for the rise/fall ramp and 4 cycles for the plateau, were filtered clicks and gated sinusoidal stimuli at 2, 4, 8, 16, and 32 kHz.

The acoustic stimuli were emitted at a rate of 20/s. The analysis window lasted 10 ms. After amplification (× 2.103) and filtering between 30 and 3000 Hz, the electrocochleographic potentials were fed into a signal averager (n = 260) to determine thresholds. A trough-to-peak amplitude of 15 μV was considered as the threshold value in our experimental conditions.

Urine collection and HA analysis. The urinary levels of HA, the end product of the oxidative pathway, were used to monitor the hepatic and renal metabolisms shift due to either the SKF treatment or the diet for the 4-week exposure to 1750 ppm toluene. The animals were placed in metabolic-type cages with free access to food and water from 1500 h on Wednesday to 0830 h on Thursday. During this 17-h and 30-min period, refrigerated urine was collected, then frozen, and stored at −20°C.
HA was determined with a cleanup procedure using solid-phase extraction (SPE) cartridges, which was first applied to urinary or standard samples. Each urinary sample was centrifuged (1750 \( g \) for 10 min at 10°C, and a 60-\( \mu l \) aliquot of urine was added to an activated SAX column (BondElut cartridge; Varian, Les Ulis, France). An 800-\( \mu l \) aliquot of 3-methyl hippuric acid (MHA; the internal standard; Aldrich, Saint Quentin Fallavier, France, 98%; 1 g/l) was then added. Similarly, standard samples were prepared using 60 \( \mu l \) of an HA (Aldrich, 99%; 10 g/l) aqueous standard solution and 800 \( \mu l \) of the aqueous MHA solution. After rinsing with water, the HA and the internal standard were eluted from the SPE columns using 3 ml of a methanol/acetic acid (MeOH/AcOH: 9/1; vol/vol) mixture. The elution layers were dried at 70°C for 40 min and the residues reconstituted in an aliquot of the high-performance liquid chromatography eluant (4 ml; water/MeOH/AcOH: 60/40/1; vol/vol/vol). The chromatographic separation was performed at a flow rate of 1 ml/min in a Lichrospher C8 column (length 250, i.d. 4.6 mm, 5 \( \mu m \)). The Shimadzu SPD 10 AVp UV detector was set at 254 nm. Variability in duplicate samples was less than 2% with a logarithm of the odds 4 mg/l in water.

HA was expressed per gram of creatinine measured by Jaffe’s method. An RX Daytona (CR3814) machine and a Randox kit were used to measure urinary creatinine.

**Histology.** The animals were sacrificed immediately after the audiogram. They were injected with a heavy dose of ketamine (75 mg/kg) and then fixed by intracardiac perfusion of 300 ml of a mixture of 4% paraformaldehyde and 3% glutaraldehyde in a trihydrate solution of sodium cacodylate (0.1M, pH 7.4). The cochleae were then removed and soaked in the fixation solution. After primary 24-h fixation, the cochleae were postfixed with OsO4 1% in 0.1M cacodylate buffer (pH 7.4) for 1 h and finally washed in a trihydrate solution of sodium cacodylate. For the surface preparation (cochleogram), the left or right cochleae were chosen randomly from each animal. They were drilled in 70% ethanol to obtain a thin layer of bone. The bony shell was then picked away from the apex to the base to isolate the entire organ of Corti. The trimmed organ of Corti (including the hook) was mounted in glycerine as a surface preparation. Hair cells were counted as present if the stereocilia, the cuticular plate, or the cell nucleus could be visualized. No attempt was made to assess the degree of possible cellular damage to surviving cells. Cochleograms were constructed from the surface preparation. The frequency-place map established by Greenwood (1990) was used to superimpose the frequency coordinates on the length coordinates of the organ of Corti. A different protocol was used for semithin sections. Three cochleae per group were immersed at room temperature in 0.7M EDTA for 5 h. The cochleae were dehydrated in ascending concentrations of ethanol up to 100% and embedded with resin.
FIG. 6. Average cochleograms ($n = 4$) obtained from control guinea pigs (A), from toluene-exposed guinea pigs (B), from toluene-exposed guinea pigs kept on diet (C), from toluene-exposed guinea pigs treated with 50 mg/kg of SKF (D), from animals exposed to toluene, treated with SKF, and kept on a diet (E). Toluene exposure: 1750 ppm toluene, 6 h/day, 5 days/week, for 4 weeks. Abscissa: length of the entire spiral course of the organ of Corti as a function of frequency, according to Greenwood (1990). Ordinate: hair cell loss in percent. OHC1, first row of outer hair cells; OHC2, second row; OHC3, third row; IHC, inner hair cells.
(Epon/Araldite). Semithin (2 μm) sections were stained with cresyl violet and examined by light microscopy.

Statistics

Statgraphics (Centurion XV version) software was used to run all the statistical analyses. An ANOVA was run to test the auditory thresholds and the blood toluene concentration obtained with the different groups. A non-parametric test, Kruskal-Wallis (K), was used to determine the statistical significance of the HA concentration, the P-450 2E1 activity, and the GSH concentration. Alpha levels of 0.05 (significant at 95%) were used for the significance of the tests.

RESULTS

Pilot Experiments

Efficiency of the SKF Treatment

Figure 2 shows that SKF treatment at 50 mg/kg sc significantly (K = 5.33, p = 0.021) reduced the activity of P-450 2E1 from 112 to 44 pmole/min/mg protein, which corresponds to an inhibition of 60.7%. This activity was evaluated 1 h after the SKF injection.

Toluene Concentration in Blood

Figure 3 illustrates the blood-borne toluene time course obtained after an iv injection of a 300-μl bolus of intralipid containing 28 μl of toluene. Three hours after the bolus injection, the blood burden of the animals had almost disappeared. From a general point of view, the treatment did not succeed in increasing the toluene concentration in the blood, even when the animals were on a low-protein diet.

Subchronic Experiment

Audiometry

Figure 4 illustrates the mean threshold values of cochlear-evoked potentials obtained from unexposed groups, including the control group, and for the four groups of toluene-exposed guinea pigs as a function of audiometric frequency. It shows that the greatest auditory sensitivity (~10 dB) was obtained at 8 kHz, even though the threshold measured at 16 kHz was low (~15 dB). The thresholds of the cochlear-evoked potentials increased at higher (32 kHz) and lower frequencies (2–4 kHz). The four toluene-exposed groups were (1) exposed to toluene alone, (2) exposed to toluene and on a diet, (3) exposed to toluene and treated with SKF, and (4) on a diet, exposed to toluene and treated with SKF. Guinea pigs from the TOL SKF DIET and TOL SKF groups had cochlear potential thresholds which were significantly higher, $F_{\text{group}}(6,257) = 11.49; p < 0.001$, than those obtained with the other groups. This 20-dB increase in auditory threshold was quite homogenous from 2 to 32 kHz. The cochlear potential thresholds measured with the DIET ($n = 5$) group were similar to those obtained with SKF ($n = 5$) and SKF DIET ($n = 7$) groups. For the sake of clarity, they have not been depicted.

The HA

The effects of group treatment were consistent across the 4 weeks of treatment, so only the urinary concentrations of HA obtained the fourth week are presented in Figure 5. The variations in HA measured as a function of the different parameters, namely diet, toluene exposure, and SKF treatment,
were all highly significant ($K = 48.5; p < 0.001$) except for the SKF group, which showed no significant variation in HA excretion ($5.6 \pm 0.9 \text{ g/g of creatinine}$) compared to that of the CTRL group ($4.4 \pm 0.4 \text{ g/g of creatinine}$).

The guinea pigs coming from the low-protein group excreted about 66% less HA than the CTRL group. As expected, toluene exposure significantly increased in HA excretion to $14.6 \pm 2.7 \text{ g/g of creatinine}$. The HA concentration obtained with the TOL group was threefold higher than that obtained with the CTRL group but not different from TOL SKF and TOL SKF DIET. Only the urinary HA excreted by the TOL DIET group was higher ($24.6 \pm 5.6 \text{ g/g of creatinine}$) than that of the TOL group.

**Histology**

**Cytocochleograms.** By and large, the average cytocochleograms obtained from the four toluene-exposed groups were all rather flat in the frequency range tested by cochlear-evoked potentials and not very different from the control group (Fig. 6a). Whatever the group considered, the hair cell losses observed at the apex of the cochleae ($> 0.2 \text{ kHz}$) were commonly observed in the controls. The absence of cells in the extreme apical part of the cochlea does not correspond to lesions but illustrates the natural organization of the hair cells for this species.

The average cytocochleograms of the TOL (Fig. 6b) and TOL DIET (Fig. 6c) groups were quite similar to cytocochleogram of the controls (Fig. 6a). The average cytocochleograms of the TOL SKF (Fig. 6d) and TOL SKF DIET (Fig. 6e) groups showed greater hair cell losses than those obtained in TOL or TOL DIET groups (Figs. 6b and 6c). But such differences do not explain the increase in the audiometric thresholds in frequencies ranging from 2 to 32 kHz.

**Semithin sections.** All the semithin sections (2 µm) showed throughout this article were representative of a specific group of treated or nontreated guinea pigs. Three sections in a row were observed for each cochlea analyzed. As expected from the auditory results, the morphological results obtained with the CTRL group were not different from those obtained from the SKF or the Diet groups. In the same manner, the morphological data obtained with TOL + SKF group were quite similar to those obtained from the TOL DIET SKF group.

**Apical Organ of Corti and Melanin Granules.** Melanin pigments occur as dark brown granules (Fig. 7) with
a decreasing distribution from the apex to the base of the
guinea pig’s cochlea. In the toluene-exposed guinea pigs,
several vacuoles can be seen within the granules. These may be
due to oxidative stress. Compared to the control organ of Corti,
the melanin granules found at the apex of the cochlea appear to
be less consistent and subdivided.

Afferent Fibers. The upper panel of Figure 8 shows the three
rows of outer hair cells, the inner hair cells, and the myelinated
fibers. Although the inner hair cells are still present in the
bottom panel of Figure 8, most of the spiral fibers have
disappeared in the cochleae of animals belonging to the TOL
SKF DIET group. The loss of fibers is not as obvious in the
TOL group as in the TOL SKF DIET group. The SKF
treatment and the diet considerably exacerbated the loss of
spiral fibers in the apical part of the cochlea when the guinea
pigs were exposed to toluene.

Stria Vascularis. The upper panel of Figure 9 shows that the
stria vascularis (SV) of albino guinea pigs consists of three
main layers of cells: (1) marginal cells, which form a layer
called the luminal surface; (2) basal cells, which form a thicker
layer adjacent to the spiral ligament; and (3) intermediate cells,
which are distributed between the marginal and the basal cells,
often in close proximity to the capillary blood supply. In all the
panels of Figure 9, it is also noticeable that intermediate cells have amelanotic melanocytes.

The guinea pigs on a low-protein diet, exposed to toluene,
and treated with SKF had an abnormal conformation of the
apical SV; the intermediate cell volume is considerably reduced
in the TOL SKF DIET group. Only one blood vessel is still
distinguishable in the bottom panel of Figure 9.

DISCUSSION

To the best of our knowledge, the current investigation is the
first and only experiment, which has succeeded in provoking
a toluene-induced hearing loss by inhalation in the guinea pig.
To increase the auditory thresholds of guinea pigs, the
experimental conditions were particularly harsh: the toluene-
exposed guinea pigs were treated with a general P-450
inhibitor, namely SKF, and some of them were kept on
a low-protein diet to decrease P-450 2E1 activity. Such
experimental conditions were chosen only to better understand
the protective mechanisms developed by the guinea pig’s
hearing to resist toluene exposure. Despite a 50% decrease in
P-450 2E1 activity for the first hour and the choice of a diet
containing very low quantities of methionine and cysteine, we
did not succeed in increasing the toluene burden in blood. In
spite of the demanding experimental conditions used in this
investigation, it is possible that the SKF-inhibited livers of the
guinea pigs were still capable of metabolizing the quantity of
inhaled toluene. In our opinion, it is unlikely that guinea pigs
can eliminate blood toluene via urine and breath. Indeed, in
a previous study (Campo et al., 2006), the authors showed
a large difference in the toluene toxicokinetics parameters
(uptake, clearance) between rat and guinea pigs even after an iv
administration of the solvent. During these experiments, the
animals did not urinate (they did not receive food and water the
day before the experiment) and the modification of the breath
rhythm was more easily observed in the rats.

Another big difference between these two species is that the
histopathological observations of toluene-exposed cochleae are
completely different from those already reported in the rat
(Campo et al., 2001). For instance, a high outer hair cell
resistance was observed, except maybe in the extreme apex of
the cochlea. More surprising was the obvious loss of spiral
fibers, which are myelinated dendrites with their cellular body
located in the spiral ganglia. It is worthy of note that their
losses match an abnormal aspect of the SV at the apical coil of
the cochlea, which plays a fundamental physiologic role in
hearing. On the other hand, all the histopathological observa-
tions described above do not explain the increase in the
auditory thresholds in frequencies ranging from 2 to 32 kHz.
Indeed, the histopathological data are observable beyond the frequency range tested in the present experiment.

The major function of the SV is to keep a store of electrochemical energy, which is required in acoustic transduction (Salt and Konishi, 1986). The SV consists of three main layers of cells that extend along the entire length of the cochlea. A single layer of marginal cells forms the luminal surface, while the basal cells form a layer several cells thick adjacent to the spiral ligament. Intermediate cells are distributed between the marginal and the basal cells, often in close proximity to the capillary blood supply. In the SKF-inhibited guinea pigs on a diet, the intermediate cells were absent at the apical coil of the cochlea. These intermediate cells are kind of melanocytes (Hilding and Ginzburg, 1977), even though they are unable to synthesize melanin pigment in albino guinea pigs (Conlee et al., 1994). Nevertheless, this pigment can be found in Hensen’s cells and could play an unexpected protective role. Indeed, melanin is a complex polymeric molecule with a multilayer structure. A non-negligible part, approximately 20%, of the melanin mass consists of lipids (Zecca et al., 2003). The physicochemical properties of the melanin polymer that allows binding of lipophilic substances could modulate the toxic effects of solvents and their related metabolites. We already know that melanin can bind a variety of metals and act as a sequesterer of reactive Fe ions (Zecca et al., 2003); more generally, it could constitute a high-capacity storage trapping system and, as such, might prevent neuronal damage.

As far as we know, the major histopathological observations such as spiral fibers and intermediate cells losses in the apical coil of the cochlea have never been reported in the literature, whatever the model of animal used. This means that even in toluene-exposed rats, no such cochlear disruptions have ever been mentioned. Therefore, both spiral fibers and intermediate cells could constitute characteristics of toluene-induced hearing loss in the guinea pig, the apical cochlea being more sensitive to toluene than the basal cochlea. A differential vulnerability between basal and apical cochlea has already been evoked in the guinea pig exposed to different xenobiotics. For instance, the destruction of outer hair cells by aminosides or by cisplatin progresses in a base-to-apex gradient (Forge and Schacht, 2000), meaning that the apex-to-base gradient of sensitivity to toluene observed in the present study was completely different from that described with other ototoxic agents (Sha et al., 2001). As a result, the toxic mechanism provoking spiral fiber and intermediate cell losses are totally different from that described for the rat in the literature. How can we explain this?

Recently, a number of authors have proposed that the differential cellular distribution of GSH may explain the differential susceptibility between base and apex (Lautermann et al., 1997; Usami et al., 1996). Since the GSH concentration in the sensory epithelium of the guinea pig is eightfold higher than that of the rat (Sha et al., 2001), the GSH distribution might explain the difference in susceptibility to toluene between rat and guinea pig (Lautermann et al., 1997). But the GSH distribution throughout the cochlea is higher in the apical than in the basal coil of the cochlea, and this is not in favor of an apex-to-base gradient of spiral fibers and intermediate cells losses due to toluene. Consequently, we must admit that the direct link between toluene ototoxicity and inner ear glutathione is still not established by the present experiment.

So, how can we explain the histopathological data observed in this study? In fact, we did not succeed in increasing the blood toluene burden in spite of the use of a P-450 inhibitor and the fact that the guinea pigs were kept on a diet. Although the liver is the organ in which the metabolism of xenobiotics is the most important (De Bethizy and Hayes, 1989), extrahaepatic organs can also be the targets for xenobiotics requiring bioactivation reactions. Besides, the high blood flow received by the central nervous system makes the brain a major target for many lipophilic xenobiotics, like aromatic solvents. Today, nobody denies either a depressive effect (Evans and Balster, 1991; Maguin et al., 2009) or a neurotoxic effect of toluene on the central nervous system (Greenberg, 1997). On this basis, reduced P-450 activity and/or the presence of a weaker pool of GSH in the central nervous system could make it more vulnerable to the deleterious effects of prolonged exposure to toluene and, thus, cause hearing dysfunction. In other words, the subjects on the low-protein diet and with a reduced P-450 activity in the central auditory pathways (including glutamatergic afferent fibers) could suffer from metabolic insults such as reactive oxygen species (Monks et al., 1999). By extrapolation from studies on the central nervous system, one would assume that inhibited P-450 and a low pool of GSH might lessen the toxic effects of different compounds including glutamate (Miyamoto et al., 1989). So, it is possible that the central auditory pathways of SKF-inhibited guinea pigs on a diet were more insulated than the auditory receptors. The fact that the inner hair cells survived despite the loss of the spiral fibers speaks in favor of a nervous toxic effect. Indeed, we cannot say that the loss of the apical spiral fibers was due to a deafferentation of the inner hair cells but rather to a degenerative process like Wallerian degeneration.

In summary, despite the use of a general P-450 inhibitor, the guinea pigs on a diet were capable of metabolizing the inhaled toluene like the nontreated controls. This was not the case for rats (data not shown), so the guinea pig can metabolize toluene more efficiently than the rat, probably because of the higher level of hepatic P-450 in the former than the latter (Shimada et al., 1997). Nevertheless, we provoked a toluene-induced hearing loss characterized by a disruption of the SV and by the loss of spiral fibers at the apical coil of the cochlea. The resistance of the outer hair cells throughout the organ of Corti could be due to (1) the presence of melanin granules within the Hensen’s cells and (2) the high concentration of GSH in the sensory epithelium, specifically in the apex. In the other hand, the spiral fiber loss is not linked to the GSH distribution in the cochlea but might be due to a central effect of toluene that we plan to study in the near future.
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