Amikacin-induced liver toxicity: correlations between biochemical indexes and ultrastructural features in an experimental model

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SUMMARY

In previous studies, aminoglycosides (AG) as gentamicin (G), dibekacin (D), tobramycin (T), netilmicin (N) and Sysomicin (S) were proved to induce ultrastructural alterations in the liver of experimental animals.

The aim of this studies is to investigate the effect of amikacin (AK) on rabbit liver which is commonly used in infections resistent to other AG; this was done studying both the common blood parameters and ultrastructural changes.

The study was accomplished in 24 New-Zealand rabbits, twelve received 20 mg/kg AK every 12 hours for 2 weeks.

Thereafter the animals were anesthesized ane liver slices were obtained for transmission electron microscopy.

As results obvious signs of primary and secondary microcholestasis associated to mitochondral cristae detachment and phospholipid aggregations were noted; this last finding was less marked when compared to previous studies employing other AG.

In the AK treated group, blood tests showed a significant increased in only Blood Urea Nitrogen (BUN) and an insignificant rise in AST levels.

Our findings are consistent with an AK induced liver toxicity albeit less evident with respect to the other AG.

Introduction

Amikacin is an aminoglycoside antibiotic proven to be effective in the treatment of Gram-negative infections which are resistent to therapy gentamicin.

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Its pharmacokinetics are similar to those of other aminoglycosides: since, in fact, it is a highly polar cation, poorly adsorbed at gastrointestinal level and for these reason must be administered in a parenteral way; furthermore, owing to its physio-chemical characteristics it is not metabolized by the liver and is thus completely excreted by the kidney in a unchanged from (almost exclusively by glomerular filtration), as shown by the discovery in the urine of 96 % of the dose administered during the 24 h period [1].

A small aliquota is, however, actively secreted by the hepatocytes in the bile: Rubinstein et al. [2] found 5,5 %, 15 %, and 9 % of the plasmatic concentration of amikacin in the bile, respectively 2, 3 and 4 h after it had been administered.

Ten to twelve h after administration the biliary concentrations of the molecule were higher than the plasmatic ones, on the contrary to other aminoglycosides [3].

Regarding aminoglycoside toxicity in general and that of amikacin in particular, experimental studies and clinical trials have shown that these drugs can be nephrotoxic and ototoxic [4, 5].

The toxicity of these antibiotics is related to the affinity to different body tissues: the stronger the bond higher concentrations in a determined organ, the maximum level is reached in the kidney especially the renal cortex.

The drug also accumulates in other body tissues, however at much lower levels among which the highest-concentration is found in the liver (5 ug/g of tissue) [6].

Not only due to the fact that amikacin accumulates in the liver but the fact it is also actively secreted by hepatocytes into the bile lead as to believe that not only is it nephrotoxic but it also hepatotoxic.

These hypotesis is supported by an increase in liver enzymes seen in some patients after aminoglycoside administration [7-9].

The fact that aminoglycosides cause liver damage has been shown by experimental models.

In particular, drug-induced ultrastructural alterations were observed in animals given low doses of gentamicin [10], sisomicin [11], dibekacin [12], tobramicin [13] and netilmicin [14] (consisting of phospholipidosis and microcholestasis), accompanied by a significant rise in the AST (gentamicin, tobramicin, netilmicin), ALT (dibekacin) and LDH (tobramicin) indicating functional a alteration in the metabolic areas of the cell.

This study was conducted in order to determine if amikacin, in a similar experimental model, is able to induce biochemical and ultrastructural hepatic lesions, also in view of the fact that amikacin was shown to be less nephrotoxic than the other aminoglycosides [5, 15-19].

We used a similar experimental model to determine if it is also less hepatotoxic.

Materials and methods

The research was carried out using 24 "New Zealand" rabbits aged 3 months, weighing and average 2,430 kg (ranging from 2,02 to 2,8 kg) and fed "ad libitum" with a commercial formula (MIL).

After a 7 day period of adaption to the new environment, fasting-blood samples were obtained via an intracardiac cannula and were centrifuged to determine serum urea, creatine, total bilirubin, transaminase, alkaline phosphatase, glucose, uric acid, cholesterol and triglyceride concentrations.

Than we randomly assigned the rabbits to 2 sub-groups of 12 were given

amikacin i.m. 40 mg/kg every 12 hours until the end of the experiment, the other 12 were given placebo and were used as the controls.

The daily amount of AK was determined by multiplying the dose commonly used in man by 5 (i.e. ratio between the metabolic rate of rabbit and man).

Blood samples were again taken 7 and 14 days after the beginning of experiment.

At the end of the study, the animals were anesthesized and liver slices were obtained.

The material was prefixed for 2 hours in a bath of 2,5 % gluteraldehyde solution buffered to a pH of 7,3-7,4 and was afterwards fixed in 1 % buffered Osmium tetraoxide solution for 1 hour; finally, it was dehydrated in an acetone solution and embedded in Epon.

Ultra-thin sections were cut with an LKB microtome, contrasted with uranyl acetate and lead citrate according to Reynolds and were then examined under an EM 300 Philips Electron Microscope.

Serum samples were immediately analysed by the SMAC II Technicon System. The data were statistically analysed by a two-way variance analysis (SP-23 design). The criterion of variance homogeneity tested by the Hartley method [20] was always fulfilled.

Results

Ultrastructurally we observed the following alterations in treated rabbit liver when compared to control:

a) a thickening of the pericanalicular web (see fig. 3), a slight enlargement of some biliary canalicula and intracanalicular biliary microtrombosis (see fig. 3-4).

The microvilli on the canalicula were normal both in amount and form.

b) an increased in the number and size of secondary lysosomes (multivesicular bodies, osmiophilic bodies and so forth) in the pericanalicular area (see fig. 3) some of which contained electron-dense lamellar material (phospholipidosis).

c) marked smooth endoplasmic reticulum hyperplasia (see fig. 1-4).

d) small intracytoplasmatic lipid droplets (see fig. 2).

e) alteration of the mitochondrial "cristae" which appear detached (see fig. 1).

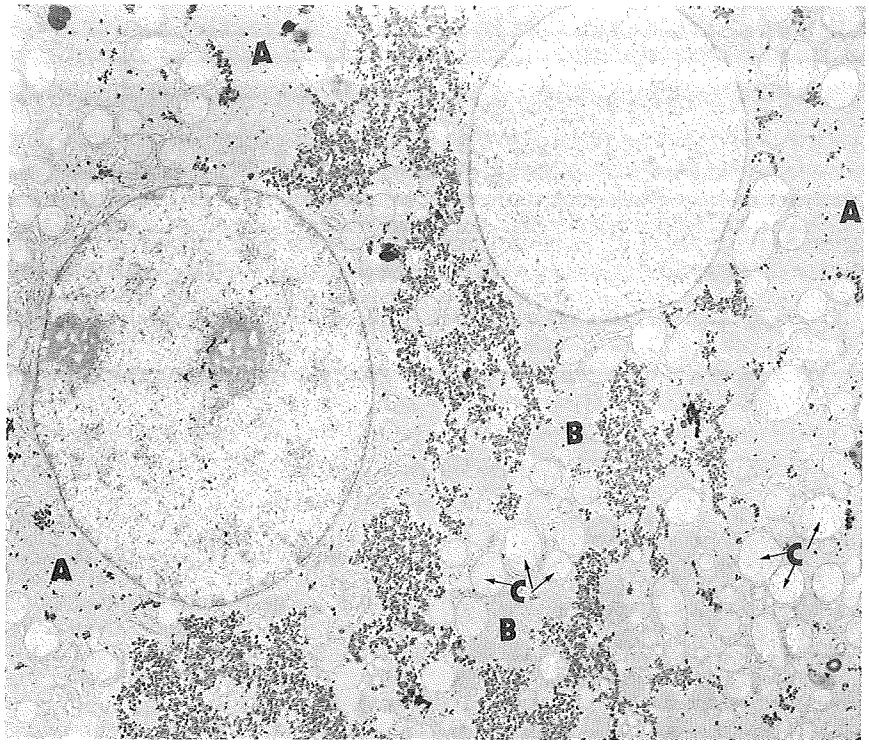

Fig. 1.—*Rabbit liver treated with amikacin (X 3.400). Binucleus hepatocite showing a notable smooth endoplasmic reticulum hyperplasia (A), with increase lysosomes number (B), and detachment mitocondrial cristae (C).*

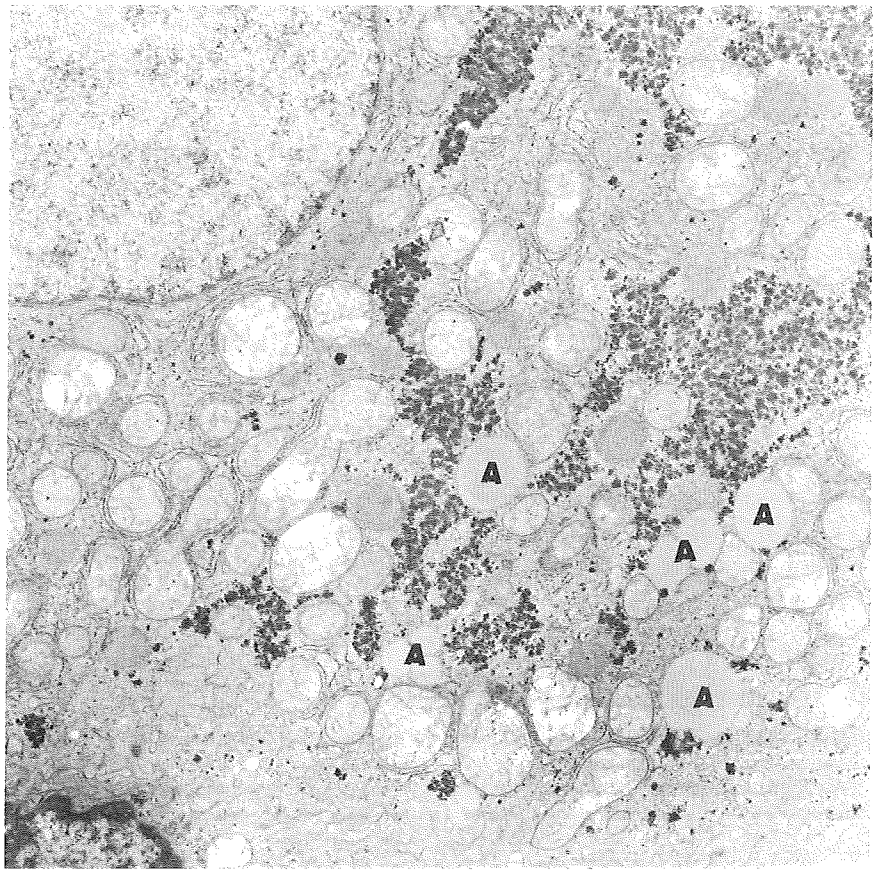

Fig. 2.—*Rabbit liver treated with amikacin (X 5.700). In this cytoplasmic area, several lipidic droplets are present (A).*

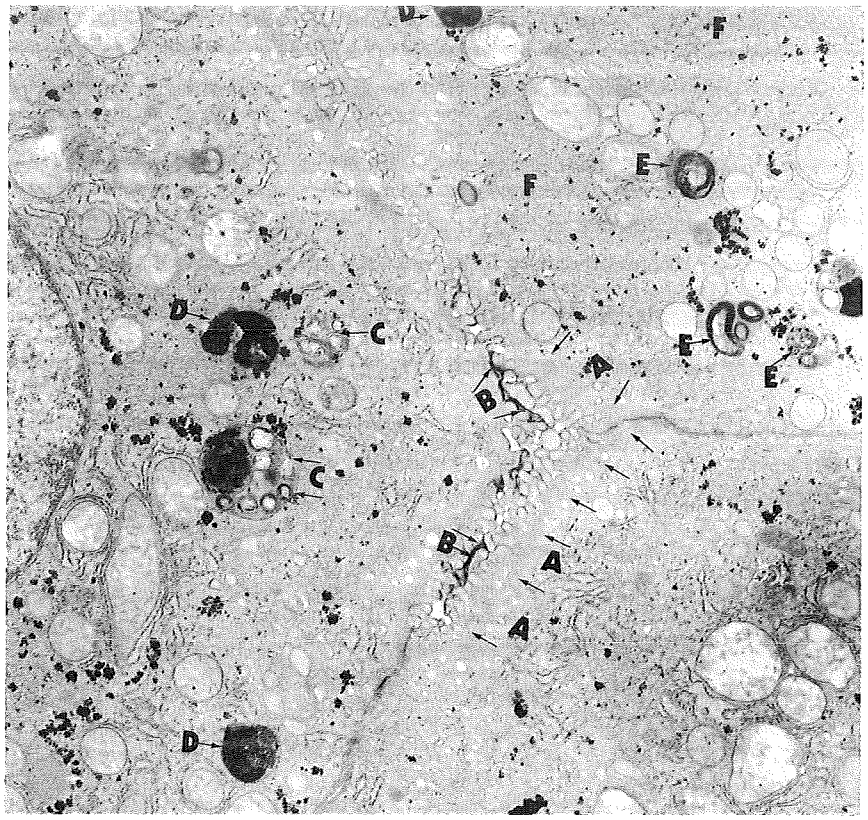

Fig. 3.—*Rabbit liver treated with amikacin (X 4.100). At the biliary pole level, are evident the next aspects: slight thickening of pericanalicular web (A); intracanalicular microtrombi (B); multi vescicular bodies (C); pericanalicular residual bodies (D); electron-dense lamellar material consisting of phospholipids (mild phospholipidosis) (E); smooth endoplasmic reticulum hyperplasia (F).*

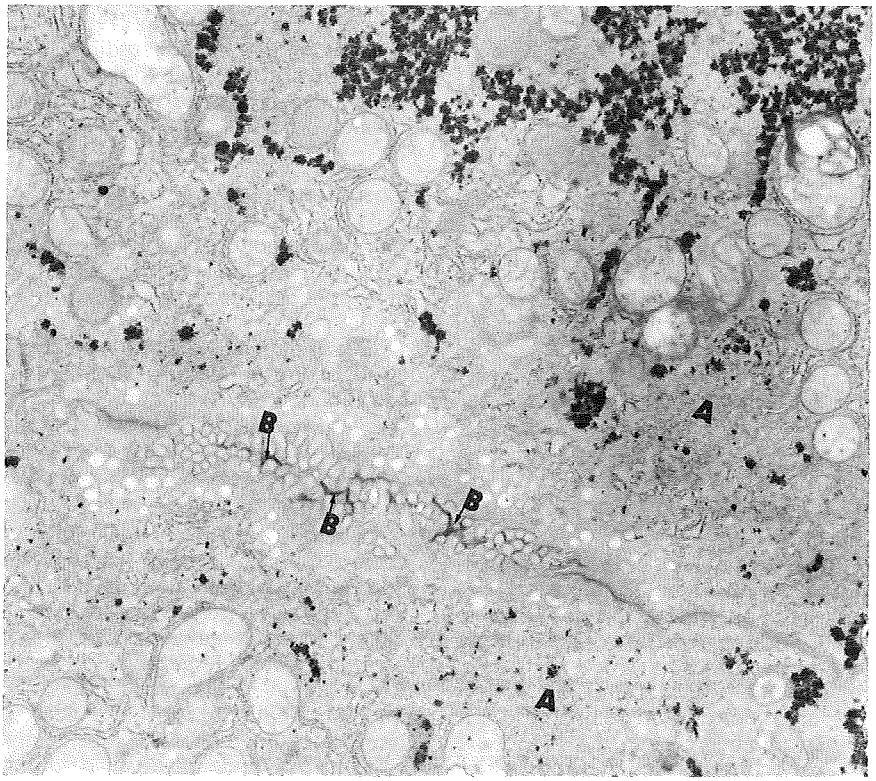

Fig. 4.—*Rabbit liver treated with amikacin (X 9.100). At the biliary pole level, we have another example of intracanalicular microtrombi (B), and smooth endoplasmic reticulum hyperplasia (A).*

Blood parameters

In order to statiscally eliminate the effect of spontaneous oscillation concerning the investigatigated parameters, the significance of the inferences between the trends of the treated and untreated animals were taken into consideration.

According to these criteria, the following differences were observed:

— significant increases in BUN ($p = 0{,}0019$ vs control group) ($p = 0{,}0005$) evident from the 1 st week of treatment (Fig. 5).

— a smaller increase in AST ($p = 0{,}10$), close to the level considered statistically significant (Fig. 6).

The other parameters studied showed no alterations neither in treated group nor in untreated animals.

Discussion

In this study, the administration of 40 mg/kg/day of amikacin induced cellular damage which was characterized by primary (see points a an b of the results) and secondary (see points c, d, e) [21] cholaestasis and a slight phospholipidosis.

These aspects are similar to those observed under similar conditions after administration of gentamicin [10], sisomicin [11], libekacin [12], tobramicin [13] and netilmicin [14].

This indicates a common mechanism of cellular damage in aminoglycosides. The damage seems to relate to the ability of these drugs to bind to both plasma membranes [22] and to organular membranes, for example, lysosomes [23] and mithocondria membranes [24].

Aminoglycosides are polications which permit them to bind to negatively loaded acidic phospholipids and in particular, to the phosphatidilinositol of the membranes [25].

Thus the formed aminoglycoside-phosphainositol compounds induce an alteration of phosphainositide metabolis which has repercussions on membrane function.

In particular, these aminoglycosides appear to have a greater affinity for lysosomes.

After active transport into the intracellular compartement aminoglycoside infact do link to the lysosomial phosphoinositol and thereby cause an inhibition of phospholipase A1 [23] and sphingomyelinases [23].

This leads to a reduction in the intralysosomial phospholipidic catabolism which in turn leads to an accumulation of phosphatidilcholine and sphingomyeline and thus phospholipidosis.

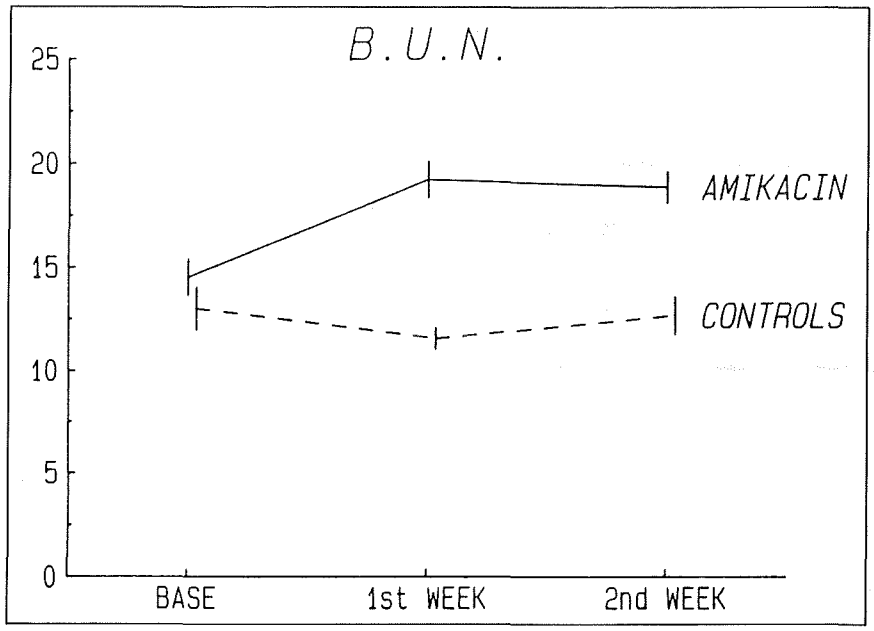

Fig. 5.—*Course of B.U.N. levels in treated (solid lines) and untreated (dotted lines) rabbits during the experiment. Values are indicated as Mean +/− E.S.M.*

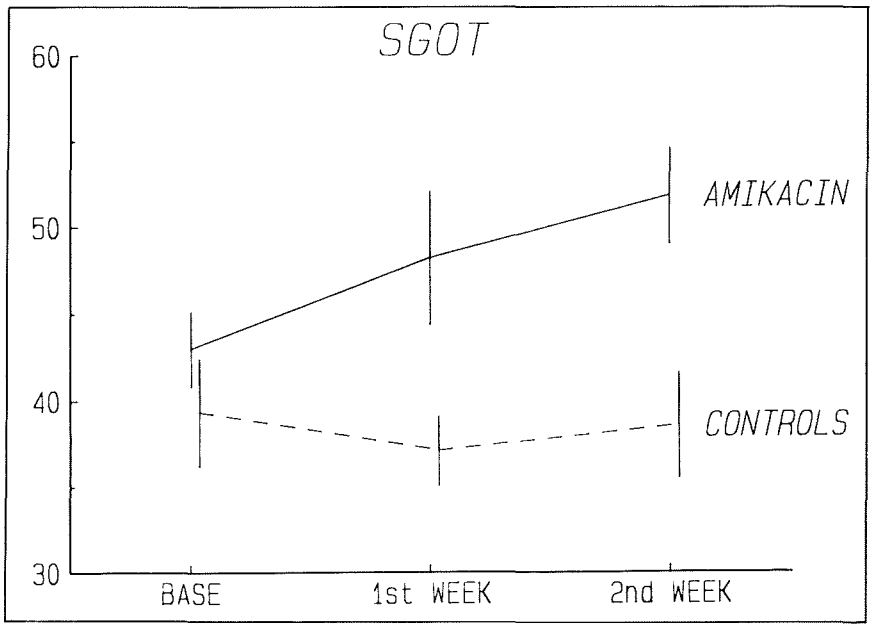

Fig. 6. —*Course of SGOT levels in treated (solid lines) and untreated (dotted lines) rabbits during the experiment. Values are indicated as Mean +/− E.S.M.*

An examination of the structure of amikacin explains why it causes a less widespread phospholipidosis than that seen with the other aminoglycosides [26].

Since the energy of interaction between amikacin and the lipidic bilayer is less than that seen with other aminoglycosides, this leads to less penetration of amikacin into the membrane, and consequently less phospholipase enzyme inhibition [26].

These properties are due to the number of aminogroups which interact with the phosphoric groups of phosphatidilinositol.

Only two in fact, of the four aminogroups of amikacin, form a bond with phospholipids; this is in comparison to 3- to 5 aminogroup possessed by other aminoglycosides [25].

This might explain amikacin's lower nephrotoxicity when compared to the other aminoglycosides [16].

Another important alteration which needs discussion is the presence of smooth endoplasmic reticulum hyperplasia (SER is almost invisible under normal conditions in rabbit liver).

Either an enzymatic induction or an induced microcholestasis is probably responsable for the SER hyperplasia seen with amikacin.

A functional alteration in the plasma membrane, due to the ability of phosphatidilinositol to bind to plasma membrane, might be the cause of the microcholestasis. Aminoglycosides cause an alteration of phosphainositide metabolism [22].

This determines a membrane disfunction, which in turn alters Ca^{++} transport and or intracellular Ca^{++} homeostasis, and therefore, alters the hepatocyte response to various stimuli.

Furthermore, aminoglycoside form compounds with phosphoinositol which causes a reduced Na-K ATP ase membrane activity [27].

It is a well-Known fact that a decreased Na-K ATP ase membrane activity leads to decreases in biliary secretion.

Finally, by altering mithocondrial metabolism, aminoglyside decrease the oxidative phosphorilation [28], and the result is less energy is available for biliary secretion [24].

Of the blood parameters, only AST increase but not significally.

Since AST resides in the inner mithocondrial membrane [30], we attribute the rise in AST to mithocondrial damage.

Unlike the other aminoglycosides, amikacin did not alter the level of the other blood parameters studied.

This is probably due to the specific biochemical properties of amikacin discussed earlier.

Conclusions

Amikacin induces a damaging effect not only in the kidney where it reaches high concentration, but also in liver tissue.

This is shown by a microcholestasis an slight phospholipidosis after amikacin administration in animals.

Even though it is difficult to draw clinical conclusion from an experimental, study, we would like emphasize the following points:

— before administering aminoglycosides in general and amikacin in particular, the clinician shoud take into account that their spectrum of adverse effects is much more vast than was commonly considered;

— caution in also required before the therapeutic use of amikacin in patients with liver diseases because it can both further damage already diseased cells and it increases the risk of nephrotoxicity [31];

— amikacin appears to be less toxic for both the kidney and the liver than the other aminoglycosides currently available.

However, further comparative studies on aminoglycoside toxicity are needed to confirm our results.

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TOXICIDAD HEPATICA INDUCIDA: CORRELACIONES ENTRE LOS PARAMETROS BIOQUIMICOS Y LAS ALTERACIONES ULTRAESTRUCTURALES EN UN MODELO EXPERIMENTAL

Resumen

Se ha demostrado en estudios previos que los aminoglucósidos como la gentamicina, dibekacina, tobramicina, netilmicina y sisomicina producen alteraciones ultraestructurales hepáticas en el animal de experimentación.

El objetivo de este estudio es investigar el efecto de la amikacina a dosis habitualmente utilizadas en infecciones resistentes a otros aminoglucósidos sobre el hígado de conejo, estudiando tanto los parámetros bioquímicos normales como las alteraciones ultraestructurales.

El estudio se realizó en 24 conejos New-Zealand, de los cuales 12 recibieron 20 mg/kg de amikacina cada 12 horas durante 2 semanas.

Los cortes de hígado se estudiaron por microscopía electrónica de transmisión.

Se observaron signos evidentes de microcolestasis primaria y secundaria junto con separación de las crestas mitocondriales y agregados fosfolipídicos. Este último signo era menos marcado en comparación con otros estudios previos con el resto de aminoglucósidos. En el grupo de pacientes tratados con amikacina, los estudios sanguíneos mostraron un aumento significativo de la urea sanguínea y un aumento no significativo de los niveles de AST.

Nuestros hallazgos confirman la existencia de toxicidad hepática inducida por amikacina, aunque menos evidente que la que producen otros aminoglucósidos.

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