

# Hepatic and renal effects of azidothymidine and acyclovir on pregnant rats

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## Summary

The antiviral effect of azidothymidine (AZT) can be potentiated by acyclovir (ACV), and this drug association has been used in the management of HIV-infected patients. In the present study we examined the effects of this association on the livers and kidneys of both pregnant rats and their concepts. Previous data from this laboratory suggested that the deleterious effects of ACV on rat pregnancy are due to its extraplacental actions and these are, at least in part, counteracted by concomitant treatment with AZT. Kidneys and livers of pregnant rats were noticed to be much more sensitive to the toxic action of the drugs than those of their concepts, ACV eliciting much more evident morphological alterations than did AZT. Contrary to what was expected, in the group of rats treated with both drugs AZT was not able to diminish the severity of the alterations evoked by ACV. The proposed "protective" action of AZT against the abortive effect of ACV on rat pregnancy does not seem to be exerted through a renal or hepatic pathway.

*Key Words:* Rat pregnancy; Azidothymidine; Acyclovir; Kidney; Liver

## Introduction

The AIDS-pregnancy complex is today a subject of great concern, due not only to the increasing number of infected pregnant women but also to the potential embryopathic effects [1-3].

Zidovudine (azidothymidine, AZT) is currently the antiviral drug most used in HIV-infected patients, and is recommended for the prevention of vertical transmission of HIV-1 from mother to child. It was used for the first time by Horwitz and co-workers in the course of research for antineoplastic drugs, and reappeared later in 1974 when Ostertag *et al.* demonstrated its efficacy on the Friend virus-induced leukemia [4, 5].

It has been reported that the activity of AZT is potentiated *in vitro* by another antiviral drug, acyclovir [6, 7]. Thus, the beneficial effects of a combination of both drugs in the treatment of human acquired immunodeficiency syndrome (AIDS) were shown [8, 9].

Animal studies indicated that AZT does not cause teratogenic changes or chromosomal alterations when administered to rats from the 6th up to the 15th day of pregnancy in a daily dosage range of 125-500 mg [10]. Accordingly, Greene and co-workers and Klug and co-workers reported no deleterious effects on maternal weight gain or on offspring growth after administration of 200-300 mg/kg of AZT to 10 day pregnant rats [11, 12]. Notwithstanding, Stahlmann *et al.* reported a high mortality ratio in rats after oral or subcutaneous administration of 100 mg/kg of AZT three times a day on the 10th day of gestation [13].

With regard to acyclovir (ACV), the literature data are somewhat controversial. Stahlmann and co-workers and Chahoud *et al.* administered ACV to rats (50-300 mg/kg) from the 9th up to the 15th day of pregnancy and reported low maternal body weight gain and abnormalities of the fetal skull and vertebral column, followed by increased mortality rate among neonates [14-16]. Notwithstanding, Moore Jr and co-workers and Kolomiets *et al.* did not report embryo toxicity or deleterious effects on the offsprings of rats after treatment with ACV (15-20 mg/kg) from the 6th up to the 15th or from the 15th up to the 21st day of pregnancy [17, 18].

In a previous paper, we administered AZT (60 mg/kg b.w.) and/or ACV (60 mg/kg b.w.) to pregnant rats once a day from the 1st up to the 20th day of gestation [19]. It was shown that maternal body weight gain was severely affected by ACV; this effect was attenuated in rats treated with AZT+ACV and was virtually absent with AZT alone. We noticed that the abortive action of ACV was markedly diminished in the group treated with the association AZT+ACV. In addition, those data suggested that the deleterious effects of ACV on rat pregnancy were due to its extraplacental actions and these were, at least in part, counteracted by concomitant treatment with AZT.

Nephrotoxicity due to ACV is a well-documented hazard and includes various degrees of glomerular filtration impairment and tubulo-obstructive effects especially in the elderly and in patients with renal insufficiency [20-22].

In the present paper we investigated, by optical and electron transmission microscopy, the possibility that the effects of ACV previously seen on rat pregnancy would be related, at least in part, to some renal toxicity. Also, since AZT is importantly metabolized by the liver, we

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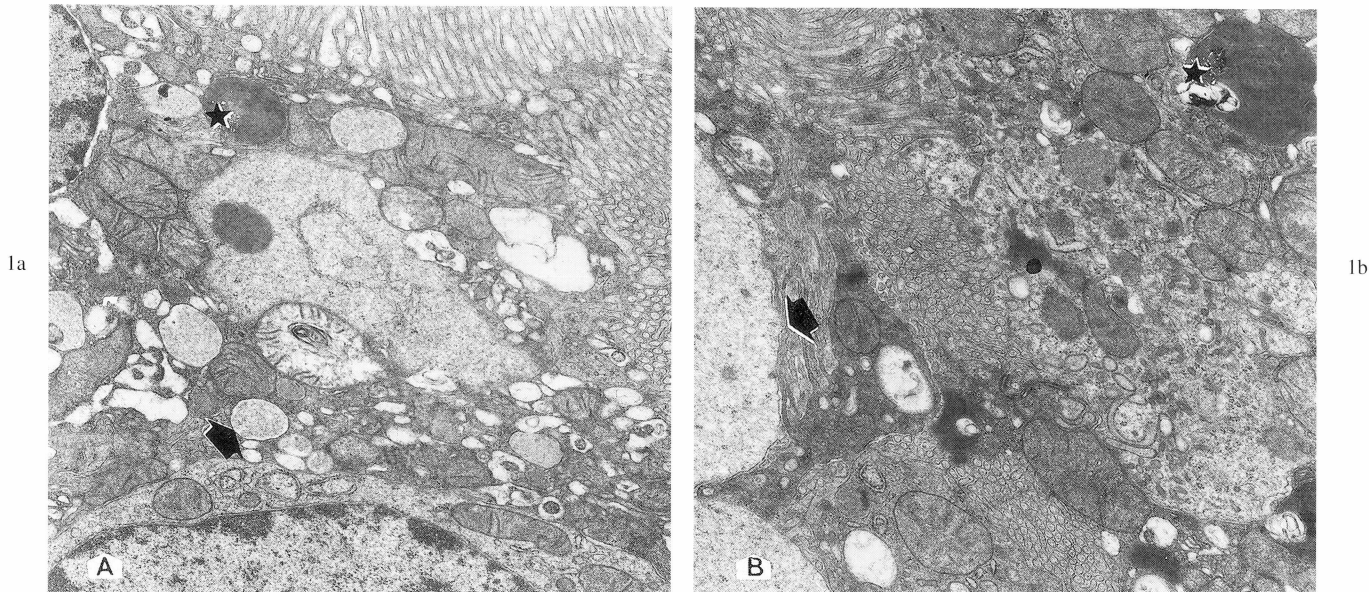


Figure 1. — Electronmicrographic aspects of proximal convoluted tubules from pregnant rats treated with acyclovir (A) or acyclovir plus azidothymidine (B), showing electron-dense bodies (marked with \*), vesicles containing an amorphous material (arrows) and mitochondria with few cristae (A, B = 21,000 X).

examined this organ after drug exposure. Finally, a presumable “protective” effect of AZT against the ACV toxicity could be anticipated on the basis of our previous results [19]. Being so, a group of animals treated with both drugs were also studied.

## Materials and Methods

### Animals and treatments

Female adult virgin, EPM-1 Wistar rats weighing 150-200 g under routine laboratory care were mated in the proportion of two females for every male for 2 hr. Pregnancy was determined according to Hamilton and Wolfe [23]. Sixty pregnant rats were then randomly divided into five groups, as follows. I, intact animals (no drug or drug vehicle); C (control), treated with 1 ml of glucose syrup (AZT vehicle) by gavage and 0.5 ml of a 0.1 N dilution of NaOH in physiological saline solution (ACV vehicle), subcutaneously; AZT+ACV, animals treated concomitantly with 60 mg/kg b.w. of zidovudine (Retrovir Syrup®, Welcome Lab.) by gavage and with 60 mg/kg b.w. of acyclovir (Zovirax®, Welcome Lab.) subcutaneously; AZT, animals treated with zidovudine by gavage as above and with 0.5 ml of a 0.1 N dilution of NaOH in physiological saline solution, subcutaneously; ACV, animals treated with acyclovir subcutaneously as above and with 1 ml of glucose syrup by gavage. Drugs and/or drug vehicles were given once a day. Treatments started on day 0 of pregnancy and extended until the 20th day of gestation.

At term (20th day) the animals were sacrificed by deep ether anesthesia. The abdominal cavity was opened and samples of both maternal and foetal kidneys were taken; some of them were immersed in Bouin's solution and others were immersed in a 2% glutaraldehyde solution. The same was done with maternal and foetal liver samples. These materials were then

routinely processed respectively for optical and electron transmission microscopy examination. An EM-900 model Zeiss electron microscope at 80 kV was used [24-29].

## Results and Discussion

Several papers have reported on the relative safety of the antiviral agent azidothymidine (AZT) in pregnancy. On the other hand, a widely used anti-herpes virus agent, acyclovir (ACV), may improve the therapeutic effect of AZT and could be a valuable combination drug in the treatment of AIDS.

Previous data from this laboratory pointed out that the untoward effects of ACV on rat pregnancy (low mother's body weight gain, low foetal weight at term, increased abortion rate) were somewhat counteracted by concomitant treatment with AZT, and were presumably due to extraplacental actions of the drug [19].

Microscopic observations of the kidneys revealed that rat foetal kidneys were not affected by any treatment (AZT, ACV or AZT+ACV). This finding confirms that of Chahoud and co-workers who administered 3 doses of ACV (100 mg/kg b.w.) on the 10th day of pregnancy [16]. Also, Kolomiets *et al.* administered 30-50 mg/kg b.w. daily from the 15th up to the 21st day of pregnancy and found no foetal kidney alterations [18]. On the other hand, however, it should be noticed that Stahlmann and co-workers reported the formation of crystals in the collecting tubules of rat foetal kidneys after the treatment of pregnant rats with ACV (100 mg/kg b.w.) on day 11.5 of gestation [14]. Such result could be explained on the basis of the differences in total drug dose, specific

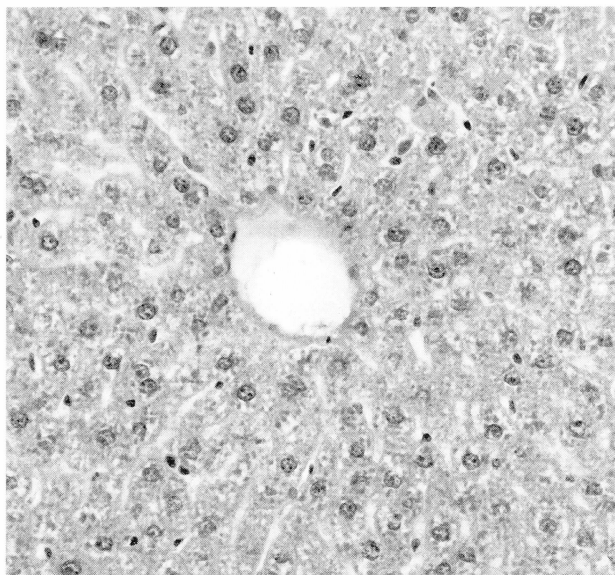


Figure 2. — Light microscopy view of the central portion of a liver lobule from a rat treated with acyclovir (300 X).

moment of the pregnancy and animal lineage used by these authors.

As expected from the observations of other authors and our own, maternal rat kidneys were much less affected by AZT than by ACV. In the AZT group, light microscopy revealed minor structural changes, including nuclear heterochromatism in some of the observed glomeruli. This finding is probably devoid of pharmacological significance, since it can be related to the functional status of the glomeruli at the moment of sacrifice. Ultrastructural images in this group were essentially normal.

In the groups ACV and ACV+AZT several alterations were detected under both light and electron microscopy examination. Optical images of the medullary region revealed dilated collecting tubules and some eosinophilic material in their lumina. Ultrastructural examinations showed organelle disorganization, increased concentration of electrondense bodies, reduction of mitochondrial cristae and electrontranslucent vesicles (Figure 1A). Especially in the ACV+AZT group, the proximal convoluted tubules had an enhanced population of lysosomes, mitochondrial cristolysis and large vesicles containing a sort of amorphous material (Figure 1B). These alterations could be related to the presence of ACV in the tubular fluid, since this drug is known to be eliminated mostly in an unaltered form in urine [17].

Similarly to what was observed for the kidneys, foetal livers showed no microscopical alterations after AZT, ACV or AZT+ACV treatments of pregnant rats during the entire period of pregnancy. This result is in agreement with observations by Moore Jr. and co-workers, Chahoud *et al.*, Kolomiets *et al.* and Greene and co-workers [11, 16-18]. On the other hand, several effects were noticed in maternal livers.

On light microscopy examination, the most evident alterations were seen in livers from ACV-treated pregnant rats, namely irregular and hyperchromatic nuclei and light areas within the cytoplasm (Figure 2). Electron microscopy revealed cellular effects in all drug-treated groups studied. In the AZT group we noticed mitochondria with few cristae, cytoplasmic derangement and translucent areas (Figure 2A). Livers from ACV-treated animals showed translucent areas, and those from AZT+ACV-treated animals were rich in translucent areas and lipidic bodies (Figure 2B).

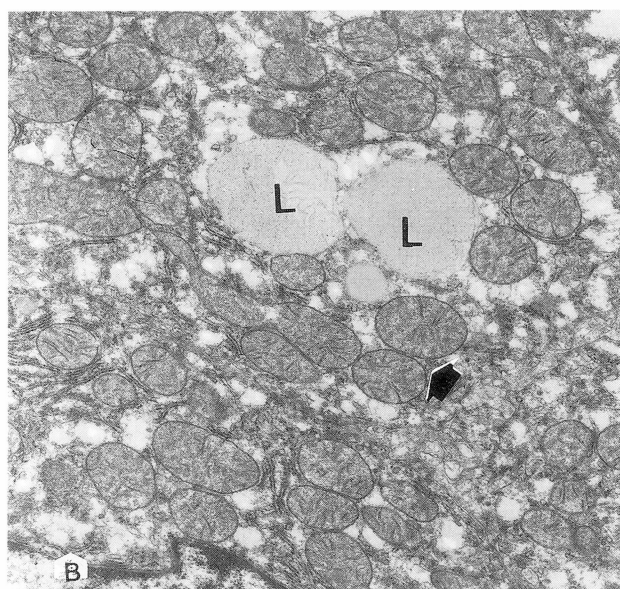
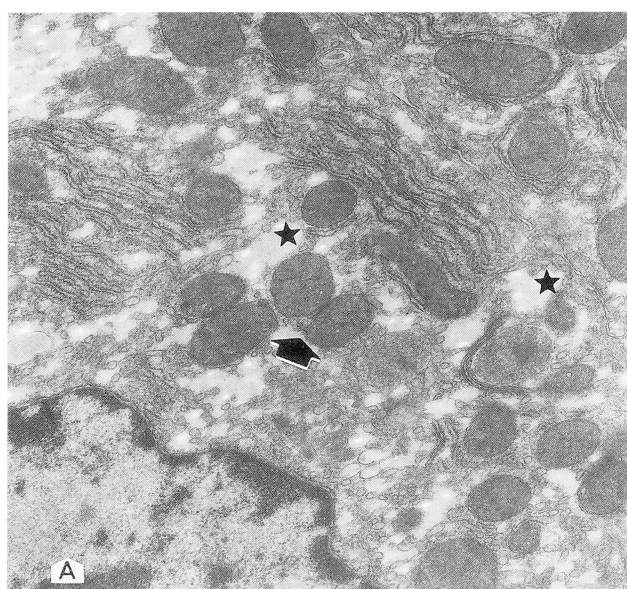


Figure 3. — Electronmicrographs showing aspects of hepatocytes from rats treated with acyclovir (A) or acyclovir plus azidothymidine (B). There are translucent areas within the cytoplasm (\*), mitochondria with few cristae (arrow) and lipid bodies (marked with L) (A = 20,500 X; B = 21,000 X).

The structural alterations seen after the AZT treatment of pregnant rats may be consequent to the biotransforming activity of this organ. In fact, AZT is conjugated in the liver with glucuronic acid and partly excreted in the bile, and most of the electrontranslucent areas (Figure 4) may represent dilatations of the agranular endoplasmic reticulum where drugs are metabolized [30-31]. In addition, 3'-amino,3'-deoxythymidine, a metabolite of AZT produced in the liver by a NADPH-dependent enzymatic system could be responsible for the cytotoxic effects on hepatocytes [32].

In conclusion, our previous data indicating some sort of beneficial drug interaction between AZT and ACV during rat pregnancy can not be explained on the basis of the present results. In other words, our results are indicative that the proposed "protective" action of AZT against the abortive effect of ACV on rat pregnancy is not exerted through a renal or hepatic pathway.

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