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The role of antioxidant vitamins in Ciprofloxacin induced oxidative stress in pancreas

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ABSTRACT: The present study was undertaken to evaluate the effect potential of Ciprofloxacin (CPFX) to induce pancreatitis in rats with reference to its doses of treatment along with restoration of anti-oxidant vitamin supplementations. Adult male wistar strain weighing about 150 – 250g were subdivided into seven groups. I – Control group received normal saline. II Low dose group: 250mg of ciprofloxacin /kg. body weight /day. III High dose group: 400mg of ciprofloxacin /kg. body weight/day. IV High dose + Vitamin A: 400mg. of ciprofloxacin followed by 7.5mg.of vitamin A /Kg. body weight/day. V High dose + Vitamin C: 400mg. of ciprofloxacin followed by 500mg. of vitamin C /Kg. body weight/day. VI High dose + Vitamin E: 400mg. of ciprofloxacin followed by 600mg. of vitamin E / kg. body weight/day. VII High dose withdrawal: 400mg. of ciprofloxacin as an oral dose and were allowed a withdrawal period of the drug for further seven days. Body weight, organ weight, insulin levels and histopathology of pancreas were studied. The drug did not cause any significant alteration in the body weight. Whereas, an increase in the organ weight of the pancreas and rise in insulin levels were noted after the drug treatment. In low dose treatment group, the compactness of the exocrine pancreatic cells is considerably reduced and endocrine islet cells showed extensive damage to α and β cells. In high dose treatment group the α and β cells had disappeared and endocrine pancreas.

KEYWORDS: Ciprofloxacin, oxidative stress, pancreas, vitamin A, Vitamin C, Vitamin E

INTRODUCTION

Quinolones are potent antimicrobials with broad spectrum activity and are effective against a wide range of infections caused by Gram – positive and gram – negative bacteria including *Bacillus anthracis*.[1] They exert their bactericidal effect by inhibiting the bacterial DNA gyrase, a type II topoisomerase.[2] They have the advantage of possessing relatively well tolerated.[1] However, clinical experience has indicated that they have some undesirable side effects including cutaneous reactions like phototoxicity, juvenile cartilage toxicity, and although the incidence is very low, adverse central nervous system reactions including epileptogeni convolutions.[3,4] The mechanism underlying these adverse effects still not well known. Interactions of quinolones with dopamine and opiate receptors were also postulated.[5] Fluoroquinolone antibiotic ciprofloxacin (CPFX) induces oxiadative stress in cerebral and hepatic tissues of rats as indicated by significant level of lipid peroxidation and alterations in glutathione (GSH) redox status.[6] Vitamins are well established to act as antioxidants in many systems.

Since this drug is used for prolonged period, it will be worthwhile to study its effect on endocrine organs especially pancreas, in order to assess its toxic effects, if any. In the recent years, toxicity by any agent working through lipid peroxidation, reactive oxygen species (ROS) generation is known to be counteracted by many anti-oxidants, one among them being the vitamins supplementations. In this study also, the role of vitamins A, C and E have been investigated for their probable rescue effects from the ciprofloxacin-induced toxicity in pancreas, if any.

MATERIALS AND METHODS

Animals

Wistar strain albino rats weighing about 180-200g, readily amenable to ease of handling and restraint during various dosing parameters, have been selected for this study. The rats were procured from Tamil Nadu Agricultural University, Coimbatore, and acclimatized to our animal house conditions for 2 weeks. The animals were housed in a well ventilated, temperature and humidity

controlled animal house with constant 12 ± 1 hours light and dark schedule. They were provided with standard diet and clean water *ad libitum.* They were then carefully monitored until the end of the experiment. The experiments were approved by the Animals Care Committee of the Institute (722/02/a/CPCSEA).

Experimental protocol

The animals were divided into seven groups as follows. The animals were treated with Ciprofloxacin and vitamins supplementation at 12 hours interval for seven consecutive days (Short duration).

1. **Control group**: Received 0.9% saline orally
1. **Low dose**: Received 250mg of ciprofloxacin /60kg. body weight /day
2. **High dose**: Received 400mg of ciprofloxacin /60kg. body weight/day
3. **High dose: + Vitamin A supplementation**: Received 400mg. of ciprofloxacin followed by 400 mg.of vitamin A /60Kg. body weight/day.
4. **High dose + Vitamin C supplementation**: Received 400mg. of ciprofloxacin followed by 400mg. of vitamin C /60 Kg. body weight/day.
5. **High dose + Vitamin E supplementation**: Received 400mg. Of ciprofloxacin followed by 400mg. of vitamin E / 60 kg. body weight/day.
6. **High dose withdrawal**: Received 400mg. of ciprofloxacin as an oral dose and were allowed a withdrawal period of the drug for further seven days

Sample collection and histological study

The animals were weighed before and after treatment. Twenty-four hour after the last treatment schedule the animals were sacrificed by decapitation method. The pancreas was dissected out and weighed. The pancreas was then fixed for histological studies in freshly prepared Bouin's fluid, dehydrated in a graded ethanol series, cleared in benzene and embedded in paraffin wax. Tissues were sectioned at 6 um and the sections were stained with periodic acid – schiff (PAS) and counter stained with haematoxylin.

RESULTS AND DISCUSSION

The exocrine pancreas presented marked inflammation of the acinar cells after CPFX treatment in the present study suggestive of acute pancreatitis. Inflammation of the pancreas, almost always associated with acinar cell injury, is termed pancreatitis.[7] Acute pancreatitis is caused by the distructive effects of pancreatic enzymes which run much, within the pancreatic parenchyma.[8,9] Drugs have been implicated in the aetiology of acute pancreatitis.[10,11,12] In control group (Fig 1), the pancreas shows normalcy. It consists of α cells, β cells, δ cells acinar cells, blood vessels and connecting tubules. In low dose treated group (Fig 2), the compactness of the cells was reduced. Endocrine islet cells became damaged with reduced number of light and dark cells. In high dose (Fig 3), islet cells are highly damaged with disappearance light and dark cells and clumping of all the cells lead to fibrocystis.

In the endocrine pancreas, presence of antagonic substance leads to a constant overproduction of insulin and consequently to hypertrophy of the islets and then to hydropic degeneration and hyalinization. The exocrine pancreas presented marked inflammation of the acinar cells after ciprofloxacin treatment in the present study. Inflammation of the pancreas almost always associated with acinar celll injury is termed as pancreatitis. Acute pancreatitis includes a mild, self limited form termed interstitial or edematous pancreatitis and a move serious, severe type. The initial stage of pancreatitis is characterized by swelling and edema and this may be the condition observed. This stage may lead to necrosis of pancreatic tissue. Acute pancreatitis is caused by the destructive effects of pancreatic enzymes which run among within the pancreatic parenchyma.

In the present study also we observed that, in low dose treated animals there was a acinar cell injury i.e. pancreatitis. Whereas in high dose treated groups necrosis of pancreatic tissue was observed. Drug have been implicated in the aetiology of acute pancreatitis. Anectodal case reports are found associated with pancreatitis with the NSAIDs namely sulindac,[14] indomethacin[15] keto profen,[16] naproxen,[17] aspirin,[18] piroxicam,[19] mefenamic acid[20] and ibuprofen.[21] In the present study ciprofloxacin also caused acute pancreatitis.

In low dose treated animals, all the pancreatic cells are minimally damaged whereas in high dose treatment, all the cells are damaged and reduced in their number particularly B cells. Vitamin 'A' supplementation (Fig 6) greatly recovers the pancreatic toxicity induced by ciprofloxacin.

Vitamin E, as a scavenger of peroxyl radicals, is probably the most important, inhibitor of the free-radical chain reaction of lipid peroxidation in animals. Initiation of lipid peroxidation can be prevented by enzymes that savenger ROS / RNS and proteins that sequester transition metal ions. Sequestration of metal ions also prevents them from decomposing perioxides into chain-propagating peroxyl and alkoxyl radicals. The dietary content of vitamin E is one factor that affects the sensitivity of laboratory animals to certain toxins or to tissue insults such as ischaemia reperfusion.[22] Vitamin 'C' (Fig 5) and 'E' (fig 4) supplementation had also showed similar restorative effect. Whereas, withdrawal group gives partial recovery of pancreatic toxicity (Fig 7).

The pivotal roles of nutritional antioxidants are clearly understood, as evidenced by the antioxidative function of vitamin E, which is frequently used as a food additive as well as supplement to protect against oxidation. Vitamin A and C also have antioxidative functions. These compounds react directly with ROS and decrease their toxicity.

Vitamins are well established to act as anti-oxidants in many systems. In this study also their supplementation proved to be effective in restoring the balance of the tissue and lowering the ciprofloxacin toxicity. Drug withdrawal was not very effective like vitamin supplementations in the restorative activity suggesting a need for an extended period of drug withdrawal needed by the pancreas.

Fig – 1 Control Group

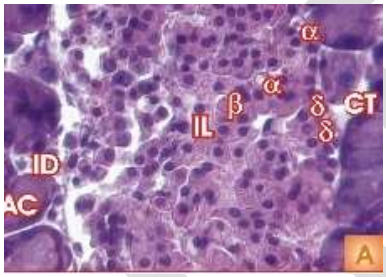


Fig – 2 Low Dose Ciprofloxacin

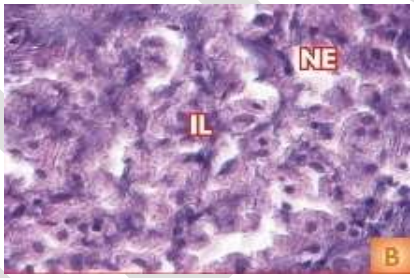


Fig - 3 High Dose Ciprofloxacin

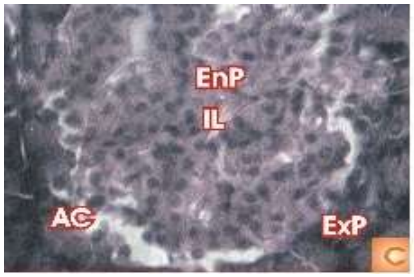


Fig – 4 High Dose + Vit ' E' Group

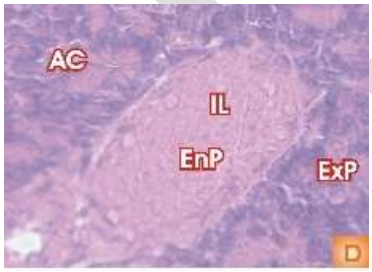


Fig – 5 High Dose + Vit 'C' Group

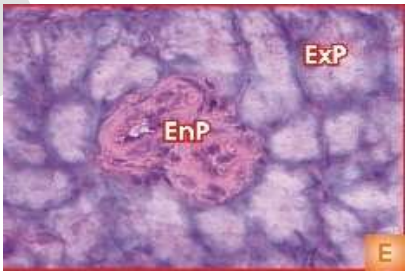


Fig – 6 High Dose + Vit 'A' Group

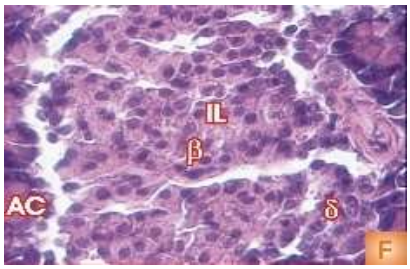
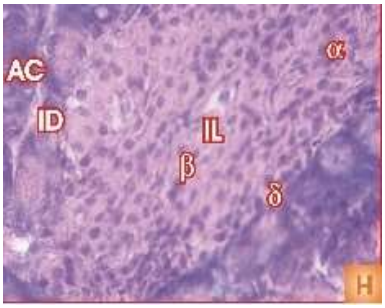


Fig –7 High Dose + Withdrawal Group



L E G E N D

AC	-	Acinar cell
ID	-	Intralobular duct
IL	-	Islets of Langerhans
B	-	B or β cells
A	-	A or α cells
D	-	D or δ cells
CV	-	Connective tubules
BV	-	Blood vessels
EnP	-	Endocrine Pancreas
ExP	-	Exocrine Pancreas
NE	-	Necrosis

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CONCLUSION

In the present study we found the ciprofloxacin induced pancreatic toxicity and Vitamin 'A' as an very good rescue agent. Drug withdrawal shows partial recovery. So we should avoid to taking too much of antibiotics.

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