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ASSESSMENT OF THE EFFECTS OF IBUPROFEN ASSOCIATION WITH NIFEDIPINE ON IBUPROFEN INDUCED HEPATOTOXICITY

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ABSTRACT. Ibuprofen is a nonsteroidal anti-inflammatory drug with large clinical applications, which implies its co-administration with different other medicines, to patients with a complex morbidity. A major unwanted consequences following ibuprofen administration in patients is its liver toxicity. We've studied the consequences of the co-administration of ibuprofen with nifedipine, a calcium channel blocker, aiming the finding of novel medicines associations for optimizing the ibuprofen therapy and reducing its liver toxicity.
Following these investigations we found out the association of nifedipine to ibuprofen therapy offers no significant protections against the hepatic injuries induced by ibuprofen. The experimental results we've obtained suggests that the excess of calcium ions plays no role in the ibuprofen induced hepatotoxicity, so that other causes might be involved in producing these lesions.

Keywords: ibuprofen, nifedipine, hepatotoxicity, hepato-protecting effect

INTRODUCTION

Ibuprofen is a derivative of the (±) 2-(p-isobutylfenil) propionic acid with analgesic, anti-inflammatory and antipyretic properties through inhibition of the prostaglandin biosynthesis (Vane J, 1983, Wissman G, 1987) following cyclooxigenase suppression. It is accounted that an isoenzyme belonging to the P_{450} cytochrome 2C (CYP_{450} TB 2C) subfamily is a major determinant of the Ibuprofen actions.

Ibuprofen is prescribed in the treatment of rheumatoid arthritis and osteo-arthritis (both in adults and children), in severe or prolonged inflammatory states, in dentistry and respectively in the respiratory pathology (Abramson SB, 2005). In gynecology ibuprofen is used in the treatment of dysmenorrhea and premature labor, because it inhibits the uterine motility – most probable following the decreasing of the synthesis of the stimulant prostaglandins (Owen PR, 1984).

In the same time ibuprofen is an analgesic and anti-inflammatory useful in the treatment of headache attacks and gouts exacerbations, its efficacy being the consequence of its ability to hinder the production of proinflammatory prostaglandins and, respectively, to diminish the macrophagic fagocitosis of the urates crystals (Insel PA, 2000). Another clinical indication of the ibuprofen is for the treatment of the fever syndromes in children aged 6 month or elders.

Because of its extended use in therapeutics, ibuprofen is often administered concomitant with different others medicines, in the case of same patients with a complex pathology.

Among the unwanted consequences following the ibuprofen administration is its liver toxicity.

We've studied the consequences of the association of ibuprofen with nifedipine, a calcium channel blocker, aiming for finding novel drug associations useful for diminishing of the ibuprofen hepatotoxicity risk.

MATERIALS AND METHODS

Materials

In order to evaluate the way the ibuprofen association with nifedipine influences the hepatotoxic effect of ibuprofen we've used an experimental model of induced toxic hepatitis using overdoses of this NSAID drug on the laboratory animals.

Experiments were undertaken on white Wistar male rats weighting 180 ± 3 g. The rats were maintained in standard laboratory conditions (at a light cycle of 12 hours and a room temperature of 20 ºC ± 2) and were feed with a balanced standard menu, 18 hours before the experiments receiving only drinking water *ad libitum*.

The experiments took place accordingly with the ethic norms regarding the experiments on laboratory animals.

The laboratory animals were randomly divided in 3 groups of 20 rats each, as follow:

- group 1 – in which rats were treated with 0.2 ml/100g ip of normal saline solution. This group has been considered the control group and was used for determining the normal values of the biochemical, hematological, pharmacodynamic and morphological parameters.
- group 2 – was the reference group for ibuprofen. The rats belonging to this group were treated with a single dose of ibuprofen (170 mg/kg bw ip)
- group 3 – included rats which were pretreated with nifedipine (15 mg/kg bw ip) and then, after an hour, were treated with ibuprofen (170 mg/kg bw ip)

24 hours following the administration of ibuprofen, between 8-10 a.m., heparinized blood was drawn from the retroorbital plex, the rats being then sacrificed under general anesthesia conditions.

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The liver was then removed, weighted and macroscopically examined. Samples of liver tissues were than collected and preserved in formaldehyde solution in order to be anatomo-pathological examined.

Drug used in the experiments

The following medicines were used in the experiments:

- Ibuprofen (170 mg/kg bw/ip) – obtained by dissolving Ibuprofen® pills, manufactured by Antibiotice SA
- Nifedipine (15 mg/kg bw/ip) – obtained by dissolving Nifedipine® pills, manufactured by Terapia SA
- Natrium Chloride 0.9% solution (0.2 ml/100g) ip, from the Ser fiziologic® vials, manufactured by Sicomed SA

All the solutions used in our experiments were prepared right before they were administered.

Methods

The biochemical parameters that were investigated included: enzymatic assessments (serum aminotransferases, alkaline phosphatase), determination of the plasmatic lipids levels (of seric triglycerides and cholesterol), glycemia values, disproteinemy tests. As for hematological parameters, the Quick protrombine time has been assessed.

The aminotransferases assessment was done using a colorimetric measure with 2,4-dinitro-fenil-hydrazine as reactant, with the help of a Specol photometer (Cucuianu M, 1998; Deutsch G, 1980).

The alkaline phosphatase was assessed using the Bodanski (Cucuianu M, 1998; Deutsch G, 1980) method, while glycemia was determined with the help of the orthotoluidine method (Cucuianu M, 1998; Deutsch G, 1980).

Seric triglycerides were determined using the Tixier method, while the total seric cholesterol values were assessed using the Watson method (Cucuianu M, 1998).

The Quick prothrombine time has been determined with the use of the Soulier method.

As for pharmacodinamic parameters the lengths of sleep induced by hexobarbital (1% solution 100 mg/kg bw ip) was used.

Also the liver weight was assessed as grams of liver weight / 100 grams of body weight.

For the microscopic examination, the liver samples were fixed in a 10% formaldehyde solution, being afterward included in paraffin. Histological sections were made and were subsequently colored with hematoxylin and eosin stain and then were examined using optical microscopy.

Statistics

The results obtained are presented as media ± standard deviation (SD).

In order to evaluate de relevance and the statistical significance of the results obtained, the data drawn from the experiments were processed statistically using the t-Student test. $P < 0.05$ was considered the minimal statistical significance level. All data were processed using with the help of statistical analysis software (Statistic for Windows).

RESULTS AND DISCUSSIONS

The median values ± SD of the investigated parameters of groups 1, 2 and 3 representing the control group, the ibuprofen reference group and respectively the group in which rats were initially treated with nifedipine and than treated with an overdose of ibuprofen are presented in tables 1 and 2.

It comes out that the administration of a 170 mg/kg bw ip overdose of ibuprofen induced the followings findings (table 1, 2):

- a significant rise of the aminotransferases (ASAT, ALAT) plasma levels relatively to the control group values
- a significant rise of the alkaline phosphatase activity as compared with the values recorded on the control group
- a significant lengthening of hexobarbital induced sleep as compared with the control group
- a significant rise of plasma cholesterol levels as compared with the control group values
- no significant changes in the values of the other parameters investigated were observed
- the lethality percent was of 15%

Administration of a 15 mg/kg bw ip of nifedipine followed by the administration of a 170 mg/kg bw ip of ibuprofen resulted in (table 1, 2):

- insignificant decrease of the ASAT, ALAT and alkaline phosphatase plasma levels comparatively to the reference group
- insignificant decrease of the prothrombine time and of the hexobarbital induced sleep, comparatively with the reference group
- insignificant decreasing of the liver weight relatively to the reference group
- insignificant lowering of plasmatic cholesterol levels as compared with the reference group
- insignificant changes in the mortality rate of the laboratory animals, a decreased from 15% to 10%

As from the morphopathological point of view:

- to the animals allotted to the ibuprofen reference group following the ibuprofen administration the followings changes were observed: hepatocytes' granular dystrophies, mostly centrilobular, as well as oedema of the Diesse's spaces (fig. 1)
- the same foundings as for the reference group were documented for the nifedipine pretreated group (fig. 2)

As it is well known the liver injuries induced by medicines represents a major clinical issue, as they represent as much as 5% of the total hepato-billiary pathology (Farrel, 1997).

Ibuprofen can induce liver changes to an average 15% of the patients that are using it (Abramson SB, 2005; Bareille MP 1992). These changes vary from

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light and transitory rises of hepatic enzymes to severe and fulminant hepatitis, usually in a mixt form (cytolytic and cholestatic) (Manoukian AV, 1996).

Because ibuprofen is prescribed on a large scale it is often possible to be co-administered with other medicines, including the calcium channels blockers – which are cytochrome P_{450} inhibitors ($CYP_{450}TB2C$) which is involved ibuprofen's liver biotransformation.

Table 1

Biochemical parameters (median val. ± DS) recorded at the groups treated with Ibuprofen (Ibuprofen reference group) and respectively pretreated with Nifedipine and then treated with Ibuprofen

	Control group (median val. ± DS)	Ibuprofen reference group (170 mg/bw ip)	Group pretreated with nifedipine (15 mg/bw) and then treated with ibuprofen (170 mg/bw ip)
Nr. of animals / group	20	20	20
Aminotransferases			
ASAT (UI/l)	17.14 ± 1.76	97.20 ± 8.69	92.75 ± 6.17
ALAT (UI/l)	12.45 ± 2.06	114.30 ± 9.36	109 ± 9.40
Alkaline phosphatase (UB)	31.70 ± 2.15	53.15 ± 4.05	50.65 ± 4.13
Plasmatic lipids			
Triglycerides (mg %)	90 ± 2.75	99.86 ± 8.75	88.44 ± 21.93
Cholesterol (mg %)	102 ± 18.69	141.44 ± 20	129.7 ± 7.8
Glycemia (mg %)	98.94 ± 2.00	87.71 ± 2.2	96.42 ± 3.2
The Tymol test (UML)	0.95 ± 0.66	2.87 ± 1.2	2.66 ± 0.84

Table 2

The hematological, pharmacodynamical and morphological parameters (median val. ± DS) and the mortality (%) at the groups treated with ibuprofen (ibuprofen reference group) and respectively pretreated with nifedipine and than treated with ibuprofen

	Control group (median val. ± DS)	Ibuprofen reference group (170 mg/bw ip)	Group pretreated with Nifedipine (15 mg/bw) and then treated with Ibuprofen (170 mg/bw ip)
Nr. of animals / group	20	20	20
Hematological parameter			
Protrombine time	11.70 ± 1.07	22.60 ± 3.50	20.90 ± 1.52
Pharmacodinamical parameter			
Lengths of the Hexobarbital induced sleep (min.)	28.65 ± 1.73	73.75 ± 4.97	71.10 ± 3.31
Morphological parameters			
Liver weight (g) / 100 g body weight	4.07 ± 0.23	5.58 ± 0.44	5.31 ± 0.40
Histopathological changes of the hepatic samples	Normal histological aspect	Granular dystrophy of some of the hepatocytes, Diesse's spaces oedema	Persistence of the hepatic changes
Mortality (%)	0	15%	10%

In our study the most frequently hepatic changes induced by ibuprofen were represented by the rise of ASAT and ALAT activity (which certifies the existence of hepatic lesion at the vascular pole of hepatocytes) and by a rise in alkaline phosphatase activity (a mark of billiary ducts injuries, respectively colangio-destructive cholestasis). Also lengthening of the protrombine time and of the hexobarbital induced sleep were documented. Lengthening of the sleep induced by hexobarbital is probably the result of decreased liver function with a consecutive incapacity of hexobarbital metabolism and a plasmatic rise of the free hexobarbital faction due to its displacement from the hepatic proteins by the ibuprofen action.

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The histopathological examination of the liver samples highlighted hepatocytes granular dystrophy and Diesse's spaces oedema.

The other test we have employed did not shown any major changes.

The reference data are rare and especially related with the clinical manifestations of hepatic or cholestatic reactions (Manoukian AV, 1996). According to Bjorkman D., the hepatic injuries induced by ibuprofen, as well as by other AINS, could be due to an idiosyncrasy reaction resulted from an immunological response or from a modified metabolic pathway (Bjorkman D., 1998).

No visible improvement of the hepatic lesions induced by ibuprofen has been documented at the laboratory animals pretreated with nifedipine, probably due to the fact that nifedipine (a well known cytochrome P_{450}TB inhibitor) has determined the diminishing of excretion and respectively of the biotransformation of ibuprofen into inactive metabolites.

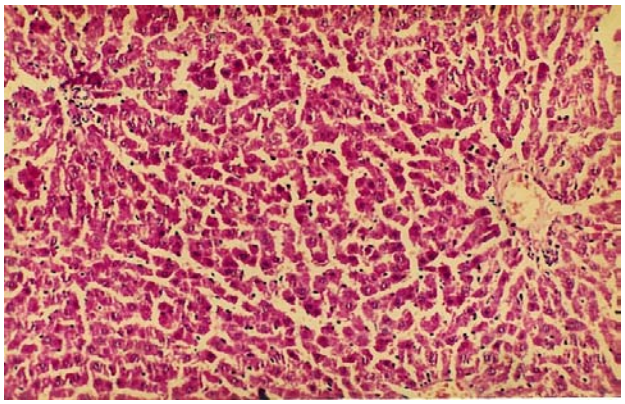

Fig. 1 The microscopical aspect of the hepatic lesions at the group 2 (HE stain)

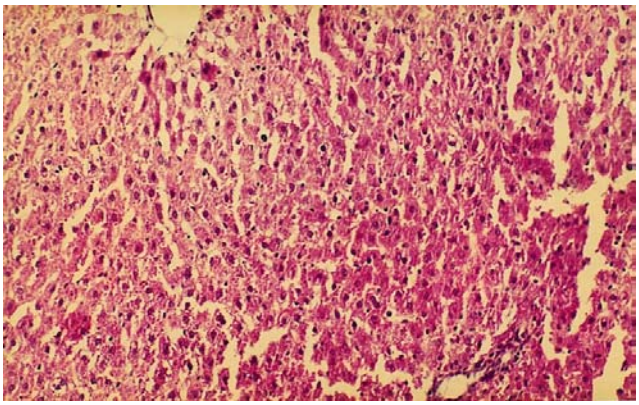

Fig. 2 The microscopical aspect of the hepatic lesions group 3 (HE stain)

CONCLUSIONS

Our research aimed to evaluate the consequences of ibuprofen association with the calcium channels blockers and nifedipine respectively

The most frequently hepatic changes induced by Ibuprofen were the rise in serum ASAT and ALAT and serum alkaline phosphatase, the lengthening of the protrombine time and the lengthening of the hexobarbital induced sleep.

Co-administering of ibuprofen with nifedipine did not offer any significant protection against the hepatic changes induced by ibuprofen.

The experimental results obtained suggest that the excess calcium ions are not playing a role in the ibuprofen induced hepatotoxicity, other causes being involved.

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