The English Summary – Master Thesis

EFFECTS OF CERTAIN AGENTS ON IATROGENIC HEPATOTOXICITY IN EXPERIMENTAL ANIMALS

Thesis

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Summary & Conclusion

In the present study, the hepatotoxic effects of four drugs in current medical use, namely paracetamol, phenobarbitone, isoniazid, and rifampicin, were investigated in experimental rats. Three successful submaximal hepatotoxicity models were constructed, based on the doses reported in the literature as well as on pilot experimental trials. These are paracetamol, paracetamol-phenobarbitone, and isoniazid-rifampicin hepatotoxicity models.

For setting of the first model, paracetamol was administered in single intraperitoneal doses of 500-700 mg/kg to adult fasted and fed rats, and in single oral doses of 400-1000 mg/kg to adult fasted rats. Oral doses of 600 and 800 mg/kg, administered to fasted adult rats, were selected as submaximal hepatotoxic paracetamol doses.

In the second model, phenobarbitone was co-administered with paracetamol to adult rats. Phenobarbitone was administered via the intraperitoneal route, in a dose of

75 mg/kg/day for three consecutive days, followed by a single oral dose of paracetamol, 600 mg/kg, on fourth day.

For setting of the third model, isoniazid and rifampicin were individually administered to young rats via the intraperitoneal route, each in a dose of 50 mg/kg/day for 21 days, and co-administered in intraperitoneal doses of 50 and 100 mg/kg/day of each drug for 21 days. Isoniazid-rifampicin co-administration to young rats (each in a dose of 50 mg/kg/day, i.p., 21 days) was selected to represent submaximal hepatotoxicity.

Adult rats were fasted 18 hours before paracetamol administration and remained fasted for 24 hours after paracetamol administration then sacrificed. Young rats were deprived of food 12 hours after the last drug administration, remained fasted for 12 hours, then sacrificed. In all models, animals were sacrificed 24 hours after the last hepatotoxic dose administration.

Three drugs, thought to act through different protective mechanisms, were studied to assess their hepatoprotective potentials in the previously-mentioned hepatotoxicity models. These are N-acetylcysteine as an antioxidant and glutathione precursor, cimetidine as a liver microsomal enzyme inhibitor, and nifedipine as a calcium channel blocker.

To study the effect of N-acetylcysteine in normal adult rats, it was administered in a single oral dose of 1200 mg/kg. When it was studied for possible hepatoprotective effect against paracetamol-induced hepatotoxicity, it was administered in single oral doses of 500 and 1200 mg/kg, one hour before paracetamol.

To explore the effect of N-acetylcysteine on normal young rats, it was administered in a dose of 100 mg/kg/day, i.p., for 21 days. To study the possible hepatoprotective effect against isoniazid-rifampicin-induced hepatotoxicity, it was given in repetitive intraperitoneal doses of 50 and 100 mg/kg/day for 21 days, parallel to isoniazid and rifampicin.

To investigate the effect of cimetidine on normal adult rats, it was administered in two consecutive doses, each of 100 mg/kg, i.p., with 2-hour interval. When it was tested as a hepatoprotectant against paracetamol- and paracetamol-phenobarbitone-induced hepatotoxicities, it was administered in a single

intraperitoneal dose of 100 mg/kg one hour before paracetamol, or in two consecutive intraperitoneal doses, each of 100 mg/kg, given one hour before and one hour after paracetamol.

To study the effect of cimetidine on normal young rats, it was administered repetitively in a dose of 40 mg/kg/day, i.p., for 21 days. When tested against isoniazid-rifampicin-induced hepatotoxicity, it was administered in repetitive intraperitoneal doses of 20 and 40 mg/kg/day, 21 days, parallel to isoniazid and rifampicin.

When the effect of nifedipine was explored in normal adult rats, it was administered in two consecutive doses, each of 25 mg/kg, i.p., with 8-hour interval. When tested against paracetamol-induced hepatotoxicity, nifedipine was administered in a single intraperitoneal dose of 25 mg/kg one hour before paracetamol, or in two consecutive intraperitoneal doses, each of 25 mg/kg, given one hour before and seven hours after paracetamol.

To investigate the effect of nifedipine in normal young rats, it was administered repetitively in a dose of 10 mg/kg/day, i.p., for 21 days. When tested against isoniazid-rifampicin-induced hepatotoxicity, it was given in repetitive intraperitoneal doses of 5 and 10 mg/kg/day, 21 days, parallel to isoniazid and rifampicin.

Serum activities of glutamic-pyruvic transaminase (GPT), glutamic-oxaloacetic transaminase (GOT), and lactate dehydrogenase (LDH) enzymes were estimated as biomarkers of liver injury. Serum malondialdehyde (MDA) level, hepatic glutathione (GSH) content, and hepatic cytosolic glutathione-S-transferase (GST) activity were measured as oxidative stress biomarkers. Liver calcium content and liver weight/body weight ratio were estimated as parameters related to hepatotoxic calcium deregulation and inflammation, respectively. The biochemical estimations were confirmed by a histopathological study.

The main findings of the present investigation can be summarized as follows:

1. Acute oral N-acetylcysteine administration to normal adult rats (1200 mg/kg, p.o., single dose), or repetitive intraperitoneal administration to normal young rats (100 mg/kg/day, i.p., 21 days), did not significantly affect serum GPT

activity, liver GSH and calcium contents, liver cytosolic GST activity and liver weight/body weight ratio.

2. Acute intraperitoneal cimetidine administration to normal adult rats (100 mg/kg, i.p., twice with 2-hour interval), or repetitive administration to young rats (40 mg/kg/day, i.p., 21 days), resulted in significant elevations of serum GPT activity, but did not significantly affect liver GSH and calcium contents, liver cytosolic GST activity, and liver weight/body weight ratio.
3. Acute intraperitoneal nifedipine administration to adult rats (25 mg/kg, i.p., twice with 8-hour interval) resulted in a significant elevation of serum GPT activity, but did not significantly affect liver GSH and calcium contents, liver cytosolic GST activity, and liver weight/body weight ratio. Repetitive intraperitoneal nifedipine administration to young rats (10 mg/kg/day, i.p., 21 days) did not significantly affect serum GPT activity, liver GSH and calcium contents, liver cytosolic GST activity, and liver weight/body weight ratio.
4. Acute administration of paracetamol to adult fasted rats in a single intraperitoneal dose of 500 mg/kg resulted in significant elevations of serum GPT and GOT activities but did not significantly affect liver weight/body weight ratio. Acute administration of paracetamol to adult fasted rats in a single intraperitoneal dose of 700 mg/kg resulted in significant increases of serum GPT and GOT activities as well as liver weight/body weight ratio. Acute administration of paracetamol in single oral doses of 500 and 700 mg/kg to fed rats did not significantly affect serum GPT and GOT activities as well as liver weight/body weight ratio.
5. Oral and intraperitoneal routes of paracetamol administration to adult fasted rats in a single dose of 500 mg/kg did not significantly differ regarding serum GPT and GOT activities as well as liver weight/body weight ratio. Oral and intraperitoneal routes of paracetamol administration in a single dose of 700 mg/kg showed significant differences with respect to serum GPT and GOT activities but not liver weight/body weight ratio.
6. Acute administration of paracetamol to adult fasted rats in a single oral dose of 400 mg/kg did not significantly affect serum GPT and GOT activities, serum MDA level, hepatic GSH content, and liver weight/body weight ratio.

7. Acute administration of paracetamol to adult fasted rats in single oral doses of 500-1000 mg/kg significantly increased serum GPT, GOT and LDH activities, significantly increased serum MDA level and hepatic calcium content, significantly decreased hepatic GSH content and hepatic cytosolic GST activity, and resulted in the occurrence of histopathological lesions, but did not significantly affect liver weight/ body weight ratio in doses up to 800 mg/kg.
8. N-acetylcysteine administration (500, 1200 mg/kg, p.o.) one hour before paracetamol (600 mg/kg, p.o.) significantly lowered paracetamol-induced rises of serum GPT, GOT, and LDH activities, significantly lowered paracetamol-induced elevations of serum MDA level and hepatic calcium content, and significantly improved paracetamol-induced suppression of hepatic GSH content and hepatic cytosolic GST activity, but did not significantly affect liver weight/body weight ratio of paracetamol-treated rats. N-acetylcysteine administration (500, 1200 mg/kg, p.o.) improved liver histopathology of paracetamol-treated rats.
9. N-acetylcysteine administration (500 mg/kg, p.o.) one hour before paracetamol (800 mg/kg, p.o.) significantly lowered paracetamol-induced rises of serum GPT and GOT activities as well as paracetamol-induced elevation of serum MDA level, but did not significantly affect hepatic GSH content and liver weight/body weight ratio of paracetamol-treated rats. N-acetylcysteine administration (1200 mg/kg, p.o.) one hour before paracetamol (800 mg/kg, p.o.) significantly lowered paracetamol-induced rises of serum GPT and GOT activities and paracetamol-induced elevation of serum MDA level, and significantly improved paracetamol-induced suppression of hepatic GSH content, but did not significantly affect liver weight/body weight ratio of paracetamol-treated rats.
10. Cimetidine administration (100 mg/kg, i.p.) one hour before, or one hour before and one hour after, paracetamol (600 mg/kg, p.o.) did not significantly affect serum GPT, GOT, and LDH activities, serum MDA level, liver GSH and calcium contents, liver cytosolic GST activity, and liver weight/body weight ratio of paracetamol-treated rats.

11. Nifedipine administration (25 mg/kg, i.p.) one hour before paracetamol (600 mg/kg) did not significantly affect serum GPT and GOT activities, liver GSH content, and liver weight/body weight ratio of paracetamol-treated rats. Nifedipine administration (25 mg/kg, i.p.) one hour before and seven hours after paracetamol (600 mg/kg) significantly decreased paracetamol-induced rises of serum GPT, GOT, and LDH activities, as well as paracetamol-induced rises of serum MDA level and hepatic calcium content. Nifedipine administration (25 mg/kg, i.p.) one hour before and seven hours after paracetamol (600 mg/kg) did not significantly affect hepatic GSH content, hepatic cytosolic GST activity, and liver weight/body weight ratio of paracetamol-treated rats, but improved liver histopathology.
12. Nifedipine administration (25 mg/kg, i.p.) one hour before and seven hours after paracetamol (800 mg/kg) did not significantly affect serum GPT and GOT activities, serum MDA level, liver GSH content, and liver weight/body weight ratio of paracetamol-treated rats.
13. Repetitive short-term intraperitoneal administration of phenobarbitone to adult rats (75 mg/kg/day, 3 days) significantly increased liver cytosolic GST activity, slightly affected liver histopathology, and did not significantly affect serum GPT, GOT, and LDH activities, serum MDA level, hepatic GSH and calcium contents, and liver weight/body weight ratio.
14. Paracetamol (600 mg/kg, p.o.)-phenobarbitone (75 mg/kg, i.p., 3 days)-co-administration significantly increased serum GPT, GOT, and LDH activities, significantly increased serum MDA level, liver calcium content, and liver weight/body weight ratio, significantly decreased liver GSH content, and dramatically affected liver histopathology, but did not significantly affect liver cytosolic GST activity.
15. Phenobarbitone administration (75 mg/kg/day, i.p., 3 days) significantly potentiated paracetamol (600 mg/kg, p.o.)-induced rises of serum GPT and GOT activities, as well as paracetamol-induced elevation of serum MDA level.
16. Cimetidine administration (100 mg/kg, i.p.) to paracetamol (600 mg/kg, p.o.)-phenobarbitone (75 mg/kg/day, i.p., 3 days) co-treated rats, one hour before

paracetamol, significantly decreased paracetamol-phenobarbitone-induced elevation of serum GOT activity, but did not significantly affect serum GPT activity of paracetamol-phenpbarbitone co-treated rats.

17. Cimetidine administration (100 mg/kg, i.p.) to paracetamol (600 mg/kg, p.o.)-phenobarbitone (75 mg/kg/day, i.p., 3 days) co-treated rats, in two consecutive doses given one hour before and one hour after paracetamol, significantly decreased paracetamol-phenobarbitone-induced rises of serum GPT, GOT, and LDH activities, significantly reduced paracetamol-phenobarbitone-induced elevations of serum MDA level and hepatic calcium content, and significantly increased paracetamol-phenobarbitone-induced suppressions of hepatic GSH content and hepatic cytosolic GST activity. Cimetidine administration (100 mg/kg, i.p.) to paracetamol (600 mg/kg, p.o.)-phenobarbitone (75 mg/kg/day, i.p., 3 days) co-treated rats, in two consecutive doses given one hour before and one hour after paracetamol, improved liver histopathology but did not significantly affect liver weight/body weight ratio of paracetamol-phenobarbitone co-treated rats.

18. Repetitive long-term administration of isoniazid alone (50 mg/kg/day, i.p.) or rifampicin alone (50 mg/kg/day, i.p.) to young rats for 21 days did not significantly affect serum GPT and GOT activities, liver GSH content, and liver weight/body weight ratio.

19. Repetitive long-term co-administration of isoniazid (50 mg/kg/day, i.p.) and rifampicin (50 mg/kg/day, i.p.) to young rats for 21 days significantly increased serum GPT, GOT and LDH activities, significantly elevated serum MDA level, significantly lowered hepatic GSH content and hepatic cytosolic GST activity, and resulted in the occurrence of histopathological lesions, but did not significantly affect liver weight/body weight ratio or liver calcium content. Repetitive long-term co-administration of isoniazid (100 mg/kg/day, i.p.) and rifampicin (100 mg/kg/day, i.p.) to young rats for 21 days significantly raised serum GPT, GOT and LDH activities, significantly increased serum MDA level, significantly suppressed hepatic GSH content and hepatic cytosolic GST activity, significantly increased liver weight/body weight ratio, and resulted in the occurrence of histopathological lesions, but did not significantly affect liver calcium content.

20. N-acetylcysteine administration (50 mg/kg/day, i.p., 21 days) to isoniazid (50 mg/kg/day, i.p., 21 days)-rifampicin (50 mg/kg/day, i.p., 21 days) co-treated rats did not significantly affect serum GPT and GOT activities, liver GSH content, and liver weight/body weight ratio, and did not improve liver histopathology.
21. N-acetylcysteine administration (100 mg/kg/day, i.p., 21 days) to isoniazid (50 mg/kg/day, i.p., 21 days)-rifampicin (50 mg/kg/day, i.p., 21 days) co-treated rats significantly decreased isoniazid-rifampicin-induced elevations of serum GPT, GOT, and LDH activities, significantly lowered isoniazid-rifampicin-induced elevations of serum MDA level, significantly improved isoniazid-rifampicin-induced suppression of hepatic GSH content and hepatic cytosolic GST activity, and improved liver histopathology, but did not significantly affect liver weight/body weight ratio and liver calcium content.
22. Cimetidine administration (20 mg/kg/day, i.p., 21 days) to isoniazid (50 mg/kg/day, i.p., 21 days)-rifampicin (50 mg/kg/day, i.p., 21 days) co-treated rats significantly decreased isoniazid-rifampicin-induced elevations of serum GPT and GOT activities and improved liver histopathology, but did not significantly affect liver GSH content and liver weight/body weight ratio.
23. Cimetidine administration (40 mg/kg/day, i.p., 21 days) to isoniazid (50 mg/kg/day, i.p., 21 days)-rifampicin (50 mg/kg/day, i.p., 21 days) co-treated rats significantly decreased isoniazid-rifampicin-induced elevations of serum GPT, GOT, and LDH activities, significantly lowered isoniazid-rifampicin-induced elevations of serum MDA level, significantly improved isoniazid-rifampicin-induced suppression of liver GSH content, and improved liver histopathology, but did not significantly affect liver calcium content, liver cytosolic GST activity, and liver weight/body weight ratio.
24. Nifedipine administration (5 mg/kg/day, i.p., 21 days) to isoniazid (50 mg/kg/day, i.p., 21 days)-rifampicin (50 mg/kg/day, i.p., 21 days) co-treated rats did not significantly affect serum GPT and GOT activities, liver GSH content, and liver weight/body weight ratio.
25. Nifedipine administration (10 mg/kg/day, i.p., 21 days) to isoniazid (50 mg/kg/day, i.p., 21 days)-rifampicin (50 mg/kg/day, i.p., 21 days) co-treated

rats significantly increased serum GPT, GOT, and LDH activities and significantly suppressed liver GSH content, but did not significantly affect serum MDA level, liver calcium content, liver cytosolic GST activity, and liver weight/body weight ratio.

CONCLUSION:

According to the results of the present study, we can conclude the following:

1. Paracetamol and isoniazid are true hepatotoxic agents. A common feature of the two drugs is that they are both converted to reactive toxic electrophilic metabolites by the liver microsomal CYP450 enzyme system, and the metabolites bind to hepatic GSH and then to hepatocellular macromolecules, yielding their hepatotoxic effects. Another common feature is that the hepatotoxicities of both drugs are associated with oxidative stress and compromised cellular antioxidant defense mechanisms. The reactive metabolites initiate lipid peroxidation either directly or through reactive oxygen species. They differ in the sense that paracetamol hepatotoxicity, unlike isoniazid hepatotoxicity, is associated with dramatic increases of liver calcium content.
2. Phenobarbitone and rifampicin are not considered true hepatotoxins, and their hepatotoxicities are mainly attributed to their ability to stimulate liver microsomal enzymes and hence to potentiate the hepatotoxicities of the true hepatotoxins, paracetamol and isoniazid, respectively, via stimulating the production of their reactive toxic metabolites.
3. Paracetamol is hepatotoxic to adult fasted rats in a single oral or intraperitoneal dose administration. Fasting is essential for the induction of paracetamol hepatotoxicity in experimental rats. Oral and intraperitoneal routes of administration show comparable hepatotoxicities. A threshold hepatotoxic paracetamol dose exists, below which paracetamol shows no appreciable hepatotoxicity.
4. Paracetamol-phenobarbitone and isoniazid-rifampicin combinations are hepatotoxic to adult and young rats in acute and long-term dose regimens, respectively.

5. N-acetylcysteine exhibits a significant hepatoprotective effect against paracetamol-induced hepatotoxicity in adult rats as well as against isoniazid-rifampicin-induced hepatotoxicity in young rats. The hepatoprotective effect is most probably attributed to its antioxidant effect, either directly or through stimulation of hepatic GSH biosynthesis. That is, antioxidant therapy seems to be an effective tool against paracetamol- and isoniazid-induced hepatotoxicities.

6. Cimetidine is hepatoprotective against paracetamol-phenobarbitone-induced hepatotoxicity in adult rats, but not against paracetamol-induced hepatotoxicity in adult rats. It is also hepatoprotective against isoniazid-rifampicin-induced hepatotoxicity in young rats. In either case, the hepatoprotective effect is most probably attributed to the potent liver microsomal enzyme inhibitory effect of cimetidine. The effect is evident only when liver microsomal enzymes that are in the inhibitory spectrum of cimetidine, and that are responsible for the biotransformation of the hepatotoxins to their toxic metabolites, are involved. This is the case in paracetamol-phenobarbitone-induced and isoniazid-rifampicin-induced hepatotoxicities, where CYP3A4 is most probably the involved subfamily. That is, liver microsomal enzyme inhibition may be an effective hepatoprotective tool against drugs whose hepatotoxicities depend on their biotransformation to reactive toxic metabolites.

7. Nifedipine exhibits a significant hepatoprotective effect against paracetamol-induced hepatotoxicity in adult rats. On the other hand, nifedipine is not hepatoprotective against isoniazid-rifampicin-induced hepatotoxicity in young rats. The hepatoprotective effect against paracetamol-induced hepatotoxicity is most probably attributed to the control of calcium homeostasis and inhibition of cellular calcium influx. That is, calcium channel blockers may have significant hepatoprotective activities only against drugs whose hepatotoxicities are associated with calcium deregulation.

8. Nifedipine administration significantly potentiates isoniazid-rifampicin-induced hepatotoxicity in young rats. The effect is most probably attributed to the liver microsomal CYP3A4 enzyme induction together with the lack of a hepatoprotective effect. In case of paracetamol-induced hepatotoxicity,

however, the hepatoprotective effect of nifedipine, through inhibition of calcium influx, may overwhelm the hepatotoxic effect of the drug through CYP450 induction.

9. Compared to cimetidine and nifedipine, N-acetylcysteine is a safer hepatoprotective and has a wider hepatoprotective spectrum.

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