INFLUENCE OF SOME ANTI-INFLAMMATORY, ANTIPYRETIC AND ANALGESIC AGENTS ON URINARY ENZYME LEVEL IN RATS

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Abstract......The influence of single oral dose of anti-inflammatory, antipyretic and analgesic agents on urinary enzymes was investigated in rats as a indicator of nephrotoxic effect. Urinary LDH activity was significantly elevated by aspirin, ketophenylbutazone, aminopyrine, phenacetin and acetaminophen. These drugs increased also H/M ratio of LDH isoenzymes. Although other test drugs have no effect on LDH in urine phenylbutazone and indomethacin elevated GPT and AI-P, oxyphenbutazone did γ–GT and anthranilic acid derivatives did AI–P and γ–GT. Other drugs such as sodium salicylate, ibufenac, ibuprofen, bucolome, aminopropylone, sulfinpyrazone, benzydamine and mepiperizole did not significantly influence any enzyme activities measured in urine.

Key words: Urinary enzymes, Nephrotoxicity, Anti-inflammatory agents, Analgesics.

INTRODUCTION

The kidney is a target organ for the toxic action of various drugs and chemicals, because the drug is possible to make higher concentration in the kidney than in blood or other organs and to irritate the tubular cells. The renal damages have been generally tested by the methods as follows; the renal functional tests such as glomerular filtration rate or renal clearance of creatinine or urea, the concentration of these nonprotein nitrogenous substances in blood, the measurement of urinary abnormal substances such as protein, blood or tubular cells and so on. The currently used renal functional tests are complicated and not suited for the routine screening in great number of experimental animals. Several studies have indicated that significant histologically visible damage can occur before those common renal tests are affected (Sharratt and Frazer 1963, Rosenbaum et al 1967, Carbenedo et al 1974, and Obek et al 1974). However, histological examination of the kidney requires organ removal or biopsy, which eliminates this method as a general screen for renal damages. Therefore, a more sensitive and more simple functional test of nephropathy is needed. The renal enzyme excretion method has been proposed to be a sen-
sitive and nondestructive test of renal toxicity (Ellis et al 1973 and Patel et al 1975). Raab (1971) has reported that the studies of renal enzyme excretion must be considered as the most sensitive method for the detection of renal disturbances and slight changes in tubular permeability or increased desquamation of tubular cells are accompanied by a rise of certain enzymatic activities in urine. Nomiyama et al (1973) and Stroo and Hook (1977) have also suggested that the assay of urinary enzymes may be useful in the detection of early injury in renal tubules.

In modern clinical pharmacology, one of the major problems consists in the toxic or side effects caused by drugs. We have studied the pharmacology of nonsteroid anti-inflammatory agents which were developed recently. Therefore, the present work was attempted to determine the influence of anti-inflammatory, antipyretic and analgesic agents on the kidneys and the urinary enzyme levels were measured in rats.

MATERIALS AND METHODS

Treatment of animals

Wistar male rats weighing 140-160g were used. The experimental groups were formed by 10 animals. Animals were fasted 18 hr prior to drug administration and during subsequent urine collection. Water was supplied ad libitum at all times. The urine was collected in stainless steel metabolic cages after drug administration. After urine collection, a blood sample was obtained from aorta by syringe and serum was separated by centrifuge.

Chemicals

The drugs used were aspirin, sodium salicylate, diclofenac sodium, phenylbutazone, oxyphenbutazone, sulfanpyrazone, phenacetin, (Fujisawa), ibufenac, ibuprofen (Kakenyaku), mefenamic acid, flufenamic acid (Sanyo), indomethacin (Banyu), bucolome (Takeda), ketophenylbutazone (Kyowahakko), aminopyrine (Iwaki), aminopropylone (Nihonshinyaku), benzydamine (Yoshitomi), mepirizole (Daiichi) and acetaminophen (Yamanouchi). Drug was administered orally to animals as a volume of 3 ml/100g of body weight, suspended by addition of gum arabicum. From the preliminary test, animals were applied comparatively high dose which is nearly a daily dose in humans. Furthermore, mercuric chloride was also investigated by intramuscular injection as a control nephrotoxic agent.

Assays of enzymes and nonprotein nitrogenous substances

Urine for the enzyme assays was centrifuged for a short time at 3000 rpm and dialyzed against running tap water for 18 hr, in order to eliminate the obstacles of drugs and concentrate the enzymes. The enzyme activities were assessed in the dialyzed urine and serum by the methods as follows; lactic dehydrogenase (LDH ; EC 1.1.1.27) according to the method of Wroblewski and La Due (1955), glutamic oxalacetic transaminase (GOT ; EC 2.6.1.1) and glutamic pyruvic transaminase (GPT ; EC 2.6.1.2) by the method of Henry et al (1960), γ-glutamyl transpeptidase (γ-GT ; EC 2.3.2.2) by Szasz (1969), alkaline phosphatase (Al-P ; EC 3.1.3.1) by Bessey et al (1946). The values of enzyme activities are measured by centrifugal fast analyzer (Rotochem II, Amicon) and
Anti-inflammatory agents and urinary enzyme level

are expressed in mU/ml or as ratio on 100 of control. Creatinine and urea nitrogen were measured in non-dialyzed urine and serum according to the methods of alkaline picric acid (Fabiny and Ertingshausen, 1971) and urease indophenol (Fawcett and Scott, 1960) respectively. Creatinine clearance was also calculated from urine volume.

Analysis of urinary LDH isoenzymes

There are 5 isoenzymes in the urine LDH enzyme. Then, after the urine was dialyzed and concentrated to about 100 times by millipore filter of minicon B15 (Amicon), LDH isoenzymes were assessed by means of agar gel electrophoresis followed by the staining using nitroblue tetrazolium.

RESULTS

I Effect on enzyme activities and nonprotein nitrogenous substance levels in urine and serum

1. Mercuric chloride

Rats given single intramuscular injection of mercuric chloride at doses of 1.0, 2.5 or 5.0mg/kg were tested for urinary enzyme levels 24 and 48 hr after injection. The result is shown in table 1. In 24 hr urine after injection, the smallest dose (1.0mg/kg) of mercuric chloride enhanced significantly LDH and GPT activities and 2.5 or 5.0mg/kg showed a marked increase in all test enzyme activities. In 48 hr urine, the smallest dose of the drug showed no significant changes in all test enzymes, but 2.5mg/kg continued the increase in all enzyme activities. While by injection of 5.0mg/kg, rats were died after 24 hr. In serum which was collected 48 hr after injection of mercuric chloride, the smallest dose of the drug did not show the significant changes in all test enzymes, BUN, creatinine and creatinine clearance. The injection of 2.5mg/kg enhanced significantly LDH and GOT activities, BUN and creatinine in serum and resulted in a decrease of creatinine clearance. The changes of urinary enzyme activities by drugs, were found to appear an early stage after administration. Therefore, the effect of test drugs was investigated only in urine up to 16 hr after administration and the results are summerized in table 2.

2. Salicylates and phenylacetic acid derivatives

All test drugs did not affect significantly urine volumes. Aspirin given orally at 900mg/kg enhanced significantly LDH, γ-GT activities and creatinine level in 16 hr urine. In serum of 16 hr after aspirin administration, only Al-P activity was significantly higher than that of control. Sodium salicylate at 900mg/kg, ibufenac and ibuprofen at 300mg/kg did not significantly altered all test enzyme activities in both urine and serum.

3. Anthranilic acid derivatives

The significant increase in urien enzyme activities were found in Al-P activity by mefenamic acid at 300 mg/kg, Al-P and γ-GT by flufenamic acid at 300 mg/kg and γ-GT by diclofenac sodium at 25 mg/kg. In serum, flufenamic acid enhanced only LDH activity, but other two drugs did not influence on enzyme activities and nitrogenous substance
Table 1. Effect of mercuric chloride on enzyme activities, BUN, creatinine and its clearance in urine and serum of rats.

<table>
<thead>
<tr>
<th>Dose (mg/kg.m)</th>
<th>Urine volume(ml)</th>
<th>LDH (mU/ml)</th>
<th>GOT (mU/ml)</th>
<th>GPT (mU/ml)</th>
<th>Al-P (mU/ml)</th>
<th>γ-GT (mU/ml)</th>
<th>BUN (mg/dl)</th>
<th>Creat mg/dl</th>
<th>Creat. clea. % to control</th>
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<tbody>
<tr>
<td>0-24 hr</td>
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<td>Control</td>
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</table>

Blood was collected 48 hr after drug administration.

LDH = Lactic dehydrogenase, GOT = Glutamic oxaloacetictransaminase, GPT = Glutamic pyruvic transaminase, Al-P = Alkaline phosphatase,
γ-GT = γ-glutamyl transeptidase, BUN = Blood urea nitrogen, Creat = Creatinine, Creat. clea. = Creatinine clearance, *p<0.05, **p<0.01.
Abbreviation and significant level are the same for all tables.

Table 2. Effect of anti-inflammatory, antipyretic and analgesic drugs on enzyme activities, creatinine, its clearance and BUN in urine and serum of rats (as ratio on control).

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Dose (mg/kg.p.o.)</th>
<th>Urine Volume</th>
<th>LDH mU/ml</th>
<th>GOT mU/ml</th>
<th>GPT mU/ml</th>
<th>Al-P mU/ml</th>
<th>γ-GT mU/ml</th>
<th>Creat mg/dl</th>
<th>BUN mg/dl</th>
<th>Al-P mU/ml</th>
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<td>110</td>
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<tr>
<td>Phenacetin</td>
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</tbody>
</table>

Urine was collected from 0 to 16 hr and blood was collected 16 hr after drug oral administration.
Anti-inflammatory agents and urinary enzyme levels.

4. Indomethacin and bucolome

Indomethacin at 25 mg/kg elevated significantly GPT and AI-P activities in urine, but did not alter the enzyme activities in serum. Bucolome did not influence on the enzyme activities and nitrogenous substance levels in both urine and serum.

5. Pyrazolone derivatives

Phenylbutazone, oxphenbutazone, ketophenylbutazone, aminopyrine, sulfinpyrazone were tested at 300 mg/kg and aminopropylene at 400 mg/kg. The significant increase in urinary enzyme activities were found in GPT and Al-P by phenylbutazone, in γ-GT by oxphenbutazone, in LDH by ketophenylbutazone and aminopyrine. The increase in serum enzyme activities was found in LDH by aminopyrine, aminopropylene and sulfinpyrazone. No significant changes in nitrogenous substance levels were observed by all pyrazolone derivatives in both urine and serum.

6. Basic anti-inflammatory drugs

Benzydamine hydrochloride and mepirizole were tested at 300 mg/kg. In urine, no significant changes were observed in both drugs. In serum, both drugs enhanced markedly only LDH activity.

7. Anilin derivatives

Phenacetin and acetaminophen were tested at 600 mg/kg. Phenacetin enhanced significantly both LDH and Al-P activities in urine. On the other hand, acetaminophen enhanced significantly both LDH and GOT activities in urine. The two drugs did not influence on enzyme activities and nitrogenous substance levels in serum.

| Table 3. Effect of anti-inflammatory, antipyretic and analgesic drugs on proportion of LDH isoenzyme activity in rat urine. |
|---|---|---|---|---|
| Drugs | Dose mg/kg, p.o. | Proportion of LDH isoenzyme (%) |
| | | ISO | H | I | II | III | IV | V |
| Control |  |  |  |  |  |  |  |  |
| Aspirin |  |  |  |  |  |  |  |  |
| Na-salicylate |  |  |  |  |  |  |  |  |
| Ibuprofen |  |  |  |  |  |  |  |  |
| Indomethacin |  |  |  |  |  |  |  |  |
| Bucolome |  |  |  |  |  |  |  |  |
| Phenylbutazone |  |  |  |  |  |  |  |  |
| Oxyphenbutazone |  |  |  |  |  |  |  |  |
| Ketophenylbutazone |  |  |  |  |  |  |  |  |
| Aminopyrine |  |  |  |  |  |  |  |  |
| Phenacetin |  |  |  |  |  |  |  |  |
| Acetaminophen |  |  |  |  |  |  |  |  |

Urine was collected for 16 hr after drug administration.
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II Analysis of urinary LDH isoenzymes

The result on proportion of urinary LDH isoenzymes are shown in table 3. Salicylates and pyrazolone derivatives decreased V type of LDH isoenzymes and increased I and II types. On the contrary, phenylacetic acid derivatives increased V type and decreased I and II types. Other drugs, especially anilin derivatives which enhanced markedly urinary LDH enzyme activity, showed no significant effect on proportion of LDH isoenzymes.

DISCUSSION

The occurrence of renal impairment seems to become one of the major problems among the unwanted actions of drugs. Nephrotoxicity of nonsteroid anti-inflammatory drugs is still a subject for discussion (Gilman 1964 and Rosner 1974). The findings of kidney damages by analgesics containing phenacetin, also directed attention to anti-inflammatory agents which are used widely in clinic. The influence of anti-inflammatory, antipyretic and analgesic drugs on the kidneys was investigated by the determination of urinary enzyme activities, because the method has been reported to be more simple, sensitive and useful for detecting early renal damages than the currently used other methods.

At first, the effect of mercuric chloride known as a nephrotoxic agent, was tested. Mercuric chloride enhanced dose-dependently the activities of all test enzymes and the increase was more marked in 24 hr urine than in 48 hr. Urinary enzyme activities, especially LDH, were significantly enhanced at lower dose than at one which increased nitrogenous substances. Moreover, when the isoenzyme of LDH was investigated by electrophoresis, mercuric chloride showed the increase of I and II types of isoenzyme that suggests the damages of proximal tubules in the kidneys, accompanied by the decrease of IV and V types. The urinary enzyme activities did not change in parallel with the serum enzyme activities. Therefore, the increase of enzyme activities in urine is hardly due to the enzymes which are released from injured organs other than the kidneys. From the results of mercuric chloride, the assay of urinary enzymes was shown to be a useful method for the detection of drug-induced nephrotoxicity.

The results obtained by anti-inflammatory, antipyretic and analgesic agents showed that some drugs results in the increase level of urinary enzymes. Aspirin showed the significant increase of LDH and γ-GT activities in urine. This effect corresponds to the results reported by Raab (1968, 1969) and Trnavsky and Rosensky (1976) who observed an increase of urinary enzyme activities by aspirin in rats. In a experiment with volunteers, a higher excretion of tubular cells in urine was observed by treatment with aspirin (Prescott 1965, 1966 and Prescott et al 1963). This increased excretion of tubular epithelial cells was accompanied by an increase of some enzymes in urine (Dubach and Josch 1967). Therefore, it was suggested that aspirin affected the renal tubules, desquamate the tubular cells and released some enzymes from the cells. While the measurement of LDH isoenzyme pattern revealed that aspirin induced the damages of proximal tubules because of the increase of I and II types. Trnavsky and Rosensky have reported that
Anti-inflammatory agents and urinary enzyme level

sodium salicylate also enhanced significantly some enzymes in urine. On the contrary, it
was not found in the present study that sodium salicylate and phenylacetic acid derivatives
increase the enzyme level in urine. Urinary LDH activity was markedly elevated by
aniline derivatives and significantly by some of pyrazolone derivatives. These drugs
increased also H (Iand II types)/M (IV and V types) ratio of LDH isoenzyme. Therefore,
it is conceivable that aniline derivatives may have nephrotoxic effect on proximal tubules
and aspirin and pyrazolone derivatives could not be denied to induce the renal damages.
Anthrinal acid derivatives such as mefenamic acid, flufenamic acid and diclofenac sodium
elevated significantly Al-P and γ-GT activities though have no effect on LDH. Indomethacin
increased GPT and Al-P activities in urine. From these results, it is supposed that
fenamates and indomethacin may injure tubular cells though they are less nephrotoxic.
Other test drugs did not influence the urinary enzyme activities.

Many nonsteroid anti-inflammatory drugs have been reported to cause renal papillary
necrosis after prolonged administration to rats (Brown and Hardy 1968, Kincaid-Smith
1970, Kaump 1966 and Emmerson et al 1973). Although it is pointed out that anti-inflam-
atory drugs have a possibility induced renal damages, definitive evidence linking
nonsteroid anti-inflammatory drugs to renal damages is lacking. From our results, it is
not possible to draw any conclusion for clinical pharmacology of nonsteroid anti-inflam-
atory drugs. However, our findings point to possible toxic effects of these substances
on kidney tubules.

SUMMARY

The influence of the treatment with anti-inflammatory, antipyretic and analgesic
agents on urinary enzyme activities was investigated in rats. In the urine collected for
16 hr after single oral administration of drugs, LDH and its isoenzyme, GOT, GPT, Al-P,
γ-GT activities and nonprotein nitrogenous substances were measured as well as in serum.
From the preliminary test used mercuric chloride, the assay of urinary enzymes was found
to be a useful method for the detection of nephrotoxic effect induced by drugs. Urinary
LDH activity was significantly elevated by aspirin, ketophenylbutazone, aminopyrine, phe-
nacetin and acetaminophen. These drugs increased also H/M ratio of LDH isoenzyme
types. Phenylbutazone and oxyphenbutazone did not significantly increase LDH activity,
but the former elevated GPT and Al-P activities and the latter did γ-GT. While an-
thranilic acid derivatives elevated significantly Al-P and γ-GT activities though have no
effect on LDH in urine. Indomethacin increased GPT and Al-P activities. Other test
drugs such as sodium salicylate, ibufenac, ibuprofen, bucolome, aminopropylone, sulfim-
pyrazone, benzydamine and mepirizole did not influence the urinary enzyme activities.

REFERENCES

Brown, D.M. and Hardy, T.L. (1968). Short-term study of the effect of phenacetin, phenazone and


Trnávsky, K. and Rovensky, J. (1976). Influence of some antirheumatic drugs and cytostatics on
Anti-inflammatory agents and urinary enzyme level.

Urinary enzyme level. Pharmacology, 14, 378-384.