One sample antipyrine clearance after 90% partial hepatectomy in the rat

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ABSTRACT - Antipyrine clearance was estimated by a one-sample saliva technique before and after 90% partial hepatectomy in nine rats. For comparison, the hepatic contents of cytochrome P450 and serum alanine amino transaminase activity were determined in vitro. Antipyrine clearance and hepatic cytochrome P450 were reduced according to the reduction in liver weight following hepatectomy. During hepatic regeneration, antipyrine clearance and liver weight increased identically, whereas total cytochrome P450 recovered more slowly, being 71% of initial values at the time when antipyrine clearance and liver weight had recovered. Serum alanine amino transferase activity increased 10–20 times 24 h after hepatectomy, and normalized after 52 h. The hepatic glutathione content per gram liver weight was unchanged during the regeneration, suggesting intact detoxification during hepatic regeneration. This study demonstrates that, although the assessment requires some time, and that a value cannot be attached to a fixed time-point, the one-sample antipyrine saliva clearance is a quantitative in vivo estimate of 'functional hepatic mass'. The test can easily be applied in animal studies where such a measure is requested.

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Antipyrine is extensively used as a model substrate to assess hepatic mixed-function oxidase activity in vivo. It is exclusively metabolized by oxidative routes in the liver and, compared to the metabolism of other drugs, its rate of metabolism is slow. Owing to the low clearance, it is insensitive to changes in hepatic blood flow. The clearance of antipyrine has been used to estimate 'functional hepatic mass' in man (1) and in animals (2), and it is well documented that liver disease (1) and experimental hepatic necrosis (3) reduce antipyrine clearance.

Inherent variability in drug metabolism, including genetically determined variations between subjects (or individual animals), has in human studies led to the common use of cross-over experimental designs. For a number of practical reasons this approach has not been exploited extensively in investigations on small animals. However, recent developments have provided a simple method for estimation of antipyrine clearance (2, 4). By this method, antipyrine clearance is determined non-invasively, using oral administration of antipyrine and estimation of the concentration of antipyrine in saliva, making repeated investigations in the same animal possible.

In the present study this approach was used to study antipyrine clearance repeatedly in a small number of animals subjected to 90% partial hepatectomy.
Material and methods

Female Wistar rats weighing about 200 g were fed Ro-
stock® rat pellets and tap water ad libitum, and housed
in individual cages with constant temperature and hu-
midity. Between 9 and 11 a.m., either a 90% partial
hepatectomy (5) or a sham operation consisting of lapar-
otomy with manipulation of the liver was performed
under diethyl ether anaesthesia. After surgery, pellets
and tap water were withdrawn for 72 h and the animals
had access to only 20% glucose in water. After 72 h the
animals had free access again to tap water and pellets.

Nine animals received antipyrine 1 ml (10 mg/ml) by
gastric tube 1 day before 90% hepatectomy, immediately
after the surgical procedure, and 48, 144, 168, 216, 240
and 312 h later. Saliva, 50-100 μl, was sampled 3–5 min
after s.c. injection of pilocarpine 0.15 mg, which does
not interfere with either the volume of distribution or the
half-life of antipyrine (6). Saliva samples were obtained
immediately before the antipyrine administration, and
in case of residual antipyrine from the previous dose, the
dose given was corrected by subtraction (concentration
times the volume of distribution of antipyrine). This
correction was only necessary for the 48 h estimation;
at maximum the correction accounted for 19% of the
dose, a value unlikely to introduce considerable bias. The
sample for the calculation of the one-sample antipyrine
clearance (2) was taken according to the optimal time
(7) predicted from the assumptions that reduction in
clearance corresponds to the 90% reduction in liver
weight immediately after hepatectomy and that total
recovery of antipyrine clearance occurs after 360 h. In
control animals samples were taken after 5 h (2).

Antipyrine is known to enhance its own metabolism
by enzyme induction. The degree of self-induction was
investigated in five animals, given antipyrine and pilocar-
pine at the same time as described above. Saliva was
sampled only after the initial and the last antipyrine
dosage.

In another series, groups of four animals, treated
identically but not given antipyrine and pilocarpine,
were investigated immediately or 24, 36, 52, 72, 120,
168, 216 and 360 h after 90% hepatectomy. Animals
dying before investigation were replaced, to maintain a
group size of four. Not more than one animal had to be
replaced in any group.

The animals were anaesthetized with diethyl ether and
bled from a 19-gauge needle inserted into the aorta, and
serum alanine amino transferase activity was estimated
(Autoanlyser, ACA, Dupont Instruments). The liver
was removed, blotted on filter paper, weighed, and stored
in liquid nitrogen for 1–12 h. After thawing, the liver
was homogenized in a Potter-Elvehjem glass teflon
homogenizer with 10 ml of isotonic KC1 (153 mmol/l) for
determination of cytochrome P450 (8), protein (9) and
total glutathione concentrations (10) by the methods
indicated.

Results

Antipyrine clearance prior to and from zero to
312 h after 90% partial hepatectomy is shown in
Table 1. Antipyrine clearance was reduced from
0.73 ± 0.02 (mean ± SEM) to 0.14 ± 0.01 ml/min
48 h after hepatectomy, i.e. 19% of control value.
After 240 h, antipyrine clearance returned to ini-
tial values.

Self-induction by the antipyrine doses admin-
nistered in this study, i.e. 10 mg administered 8
times over 312 h, increased the antipyrine clear-
ance by 0.10 ± 0.03 ml/min (mean ± SEM, p < 0.01
paired t-test).

In Table 2 the total hepatic cytochrome P450
content, serum alanine amino transferase activity,
the total hepatic glutathione and the liver weight
at various times after 90% partial hepatectomy
are shown. The results are from estimates on
groups of four animals after the intervals given
as mean ± SEM. Hepatectomy reduced the total
hepatic cytochrome P450 from 377 ± 18 to 49 ± 2
nmol (mean ± SEM), i.e. to 13% of control value.
After 360 h the total hepatic cytochrome P450
content was 71% of control value (p < 0.01).

Liver weight was reduced from 8.66 ± 0.42 to
1.20 ± 0.05 g by the hepatectomy (mean ± SEM),
also by 86%, by the hepatectomy. After 360 h, liver
weight was 6.80 ± 0.70 g, which is not statistically
significant from the control value (p > 0.05, t-test).

Serum alanine amino transferase activity rose
from 23.8 ± 4.1 U/l to a maximum of 370 ± 45
after 24 h. After 52 h the activity was identical to
control values.

The hepatic glutathione concentrations were

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<td>Antipyrine clearance (ml/min) before and after (hours) 90% partial hepatectomy in nine rats</td>
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identical at all intervals after hepatectomy (Table 2, p > 0.20, one-way analysis of variance).

Discussion

We have demonstrated that partial hepatectomy leaving 14% of the liver weight reduces the total hepatic cytochrome P450 amount to 13% and antipyrine clearance to 19% of initial values. However, whereas the estimation of liver weight and total hepatic cytochrome P450 is confined to a well-defined time point, the clearance of antipyrine is estimated from the elimination of antipyrine over a period of about 5 h and in extreme cases up to 48 h after the hepatectomy. During this period, liver weight and cytochrome P450 increase about 1.3–1.6 times, and antipyrine clearance most probably also increases during this period. It is therefore more appropriate to assign a time point of about 24–36 h to this antipyrine clearance value. In that case antipyrine clearance can be considered to be reduced according to the reduction in hepatic mass, indicating that antipyrine clearance is a measure of 'functional hepatic mass'. The antipyrine one-sample saliva clearance method has several advantages: 1) it can be estimated several times in the same animal, thereby reducing the number of animals to be investigated; 2) the difference which the test is able to detect is smaller owing to less influence from inter-individual variation; 3) the test is non-invasive; 4) since only one sample has to be analysed, the test requires less resources. The test, however, also has some disadvantages: 1) it is not possible to assign the clearance to an exact time point, and in case of a severe reduction of hepatic function the test reflects a time interval of about 48 h, whereas an almost normal liver function reflects an interval of 3–5 h; 2) estimating the clearance repeatedly makes it impossible to obtain information from liver biopsies etc. This however can be overcome by estimating antipyrine clearance once only in each animal if the saliva (or plasma) sample is taken when the animal is killed.

During the regeneration of the liver, antipyrine clearance and liver weight were closely related and returned to initial values at the same time. Total hepatic cytochrome P450, however, recovered more slowly, being 71% of initial values at the
time when antipyrine clearance and liver weight had returned to initial values. The reason for this discrepancy is not clear: one explanation is that antipyrine is metabolized by a few cytochrome P450 isozymes (11), and the amount of these may not reflect the total amount of P450. The repeated antipyrine doses may also induce the isozymes metabolizing antipyrine. The degree of this self-induction was estimated to be about 0.1 ml/min, corresponding to about 14% of control values. With minor restrictions this study demonstrates that the one-sample antipyrine saliva clearance reflects the liver size and is a quantitative measure of 'functional hepatic mass', also during hepatic regeneration. The one-sample clearance method is also easily applicable to clinical situations (7).

Plasma activity of alanine amino transferase is widely used to evaluate liver damage, since it was demonstrated that hepatic damage leads to increased activity in serum (12). The basis for this increased activity is largely unknown. The most common view is that necrotic cells leak enzymes to the plasma; increased plasma activity, however, has been demonstrated in diseases with little or no tissue necrosis, e.g. in anoxia (12). In the present study we found elevated plasma activity during the first 2 days after hepatectomy. The technique used for removing 90% of the liver is performed by removing one lobe at a time, and ligations have to be performed so that almost no non-viable liver tissue is left. This makes it unlikely that the origin of the increased plasma activity is necrotic tissue. Recently it has been suggested that the basis for the increased plasma activity after chemical hepatic damage is increased activity in viable cells rather than leakage from necrotic cells (13). Our study supports this hypothesis. Furthermore, it clearly demonstrates the non-quantitative nature of plasma alanine amino transferase activity as a measure of hepatic function.

Hepatic glutathione is considered crucial in many detoxification processes in the liver. The level in the regenerating liver is maintained constant, supporting the hypothesis that regenerating liver is not more susceptible to toxic injury than normal liver.

In conclusion, this study demonstrates that the one-sample antipyrine saliva clearance method and in vitro estimation of the antipyrine metabolizing cytochrome P450 enzymes are closely related, and related to liver weight, demonstrating that antipyrine is a quantitative in vivo measure of the functional hepatic mass.

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References


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