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

HENRIK E. POULSEN

Department of Medicine A, Division of Hepatology, and Laboratories of Experimental Pathology, Rigshospitalet, Copenhagen, Denmark

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 Rostock® rat pellets and tap water *ad libitum*, and housed in individual cages with constant temperature and humidity. Between 9 and 11 a.m., either a 90% partial hepatectomy (5) or a sham operation consisting of laparotomy 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 pilocarpine 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 (Autoanalyser, 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 KCl (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 $\pm$ 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 initial values.

Self-induction by the antipyrine doses administered in this study, i.e. 10 mg administered 8 times over 312 h, increased the antipyrine clearance by 0.10 ± 0.03 ml/min (mean $\pm$ 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 $\pm$ SEM. Hepatectomy reduced the total hepatic cytochrome P450 from 377 ± 18 to 49 ± 2 nmol (mean $\pm$ 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 $\pm$ SEM), i.e. 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

Table 1

Antipyrine clearance (ml/min) before and after (hours) 90% partial hepatectomy in nine rats

Before	0	48	144	168	216	240	312 h
0.73 ± 0.02	0.14 ± 0.01	0.25 ± 0.03	0.40 ± 0.02	0.60 ± 0.03	0.59 ± 0.02	0.88 ± 0.04	0.62 ± 0.03

Table 2

Total hepatic cytochrome P450 content (P450, nmol), liver weight (LW, g) serum alanine transferase activity (ALAT, I.U.), and hepatic glutathione concentration (GSH, nmol/mg protein) at various intervals after 90% partial hepatectomy

	Before	0	24	36	52	72	120	168	216	360 h
P450	376 ± 18	49 ± 2	49 ± 5	49 ± 5	64 ± 4	90 ± 12	134 ± 13	185 ± 15	234 ± 15	266 ± 13
LW	8.66 ± 0.42	1.20 ± 0.05	1.32 ± 0.09	1.51 ± 0.11	1.93 ± 0.06	2.53 ± 0.25	3.69 ± 0.36	4.60 ± 0.23	6.17 ± 0.33	6.80 ± 0.70
ALAT	23.8 ± 4.1	20.0 ± 4.6	370 ± 45	226 ± 66	47.3 ± 4.9	21.8 ± 2.1	18.3 ± 5.7	21.8 ± 6.4	16.0 ± 2.3	10.3 ± 2.4
GSH	34.1 ± 1.5	35.5 ± 6.2	44.0 ± 4.6	43.2 ± 3.3	48.5 ± 3.6	38.8 ± 2.4	36.9 ± 9.4	37.9 ± 5.9	33.3 ± 4.6	42.3 ± 5.2

Values indicate mean ± SEM of groups of four animals.

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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Address:
Henrik E. Poulsen
Department of Medicine A2152
Rigshospitalet
9 Blegdamsvej
DK-2100 Copenhagen Ø
Denmark