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The influence of oxygen tension on theophylline clearance in the rat isolated perfused liver

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The effect of changes in the rate of perfusion and oxygen tension (Po_2) on theophylline clearance was determined in the rat isolated perfused liver. Changes in rate of perfusion had no effect on the extraction ratio of theophylline while a decrease in the Po_2 significantly increased the half life of theophylline. These results suggest that changes in Po_2 whether due to disease states or oxygen administration may necessitate theophylline dosage adjustment.

There is a large interpatient variability in theophylline clearance which hinders effective dosing with this drug. This variability appears to be due to differences in the rate of hepatic biotransformation which is modified by diet, smoking, concurrent illness, age and other drugs (Jusko et al 1979).

The hepatic extraction ratio for theophylline is only about 10% (Ogilvie 1978) which implies that the rate of metabolism is not sensitive to liver blood flow (Nies et al 1976) but rather to the ability of the liver to metabolize the drug (Wilkinson & Shand 1975).

Theophylline clearance can decrease with increasing congestive cardiac failure (Piafsky et al 1977) as well as acute lung disease (Vozeh et al 1978). Such conditions are associated with a reduction in oxygen tension (Po_2) in the blood. The mechanism responsible for decreased theophylline clearance in congestive heart failure is unknown but explanations have been proposed. It has been suggested that passive congestion may alter the metabolic activity of the liver by causing hepatocellular damage (Benowitz & Meister 1976). Decreased blood flow to the liver has also been said to decrease clearance, although the low extraction ratio of theophylline clearance in certain disease states, the present study was undertaken to determine the influence of changes in Po_2 and flow rate on the clearance of theophylline clearance (Vozeh et al 1978).

In an attempt to elucidate the mechanism of change in theophylline clearance in certain disease states the present study was undertaken to determine the influence of changes in Po_2 and flow rate on the clearance of theophylline in the rat isolated perfused liver.

Materials and methods

Apparatus. The perfusion system used by Pang & Rowland (1977) was modified to allow discrete changes in perfusate Po_2 (see Fig. 1). The main modification is that the oxygenator consists of two separate lengths of silastic tubing (Dow Corning, 0·058 inch i.d. and 0·077 inch o.d.). Perfusate flow could be directed through either length of tubing by means of three-way stopcocks directly before and after the oxygenator.

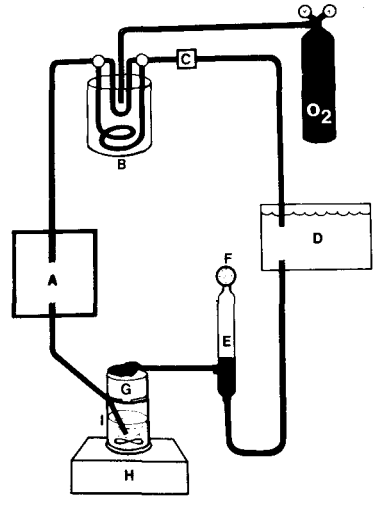

FIG. 1. Isolated rat liver perfusion system: A = peristaltic pump, B = oxygenator with different lengths of silastic tubing over which oxygen is blown, C = filter, D = water bath, E = bubble trap, F = pressure gauge, G = organ tray with water jacket, H = magnetic stirrer, I = reservoir containing perfusate.

The perfusate consisted of 20% washed, outdated, human red cells, 300 mg% glucose, 250 mg% bovine serum albumin, heparin 1 u ml^{-1} in a mixture of equal parts of human plasma and Krebs-Ringer-bicarbonate solution. A recirculating volume of 50 ml of perfusate was used.

Livers (12 ± 2 g s.d.) from male Sprague-Dawley rats, 350–400 g, were dissected free under ether anaesthesia according to Bartosek et al (1973). Cannulae were placed in the portal vein (inlet) and the inferior vena cava (outlet) and oxygenated saline containing heparin 5 u ml^{-1} was used to flush and then perfuse the liver during the dissection. The bile duct was also cannulated. The liver was removed and placed on a heated organ tray. Perfusion was quickly switched from saline to perfusate at a control rate of 12·5 ml min^{-1} (Pang & Rowland 1977) and the liver allowed to stabilize for 10–15 min. The experiment was only continued if: (i) the colour of the tissue was an even natural red without blotches, (ii) pressure measured at the bubble trap was

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negligible, (iii) bile flow was constant, and (iv) there were no leaks from the liver.

Theophylline was added to 50 ml of fresh perfusate at a concentration of 80 μg ml^{-1}. At the start of the experiment this perfusate replaced that used to stabilize the tissue. To ensure even mixing, the 20 ml of theophylline-free perfusate in the tubing was allowed to run to waste before recirculation was begun. Finally the whole system contained 50 ml of perfusate with theophylline evenly distributed.

Methods

As the elimination half life ($t_{\frac{1}{2}}$) of theophylline in the rat is 6 ± 1·5 h (Lohman & Miech 1976) it is necessary to pretreat rats with an enzyme inducer to allow completion of an experiment in the 2 to 3 h that the isolated liver remains viable. We found no decline in theophylline concentrations in the perfusate from livers of untreated rats. Pretreatment with an aqueous solution of phenobarbitone (37 mg kg^{-1}) twice daily or 3-methylcholanthrene (20 mg kg^{-1}) in olive oil once daily for 3 days i.p. gave a theophylline $t_{\frac{1}{2}}$ of 223 min (CV 29%, n = 6) or 59·8 min (CV 20%, n = 14), respectively. 3-Methylcholanthrene, therefore proved the most suitable inducing agent and was used throughout.

Rate of perfusion. The influence of perfusion rate was examined by changing the flow rate every 25 min in the following order: 12·5, 15·0, 12·5, 8·0 and 12·5 ml min^{-1}. Theophylline concentrations were determined in perfusate samples (170 μl) taken before (Ci) and after (Co) the perfusion at the following times: 0, 5, 15, 25, 50, 75, 100 and 125 min.

Oxygen tension. The oxygen tension (Po_2) was altered in the perfusate by shunting through the oxygenator via a control length, or a shorter length, of silastic tubing by means of the three way stopcocks (see Fig. 1) to give a Po_2 of 115 mmHg (CV 3%) and 50 mmHg (CV 10%), respectively. Oxygen tension in the perfusate was determined by means of a Clark oxygen electrode (type E5046) coupled to an acid-base analyser (model PHM 73) (Radiometer Denmark). Oxygen tension was measured during each experiment and after each change in Po_2. The experiment was carried out in 3 stages: stage I at a Po_2 of 115 mmHg; stage II at a Po_2 of 50 mmHg; stage III at a Po_2 of 115 mmHg. During each stage a 10 min stabilization period preceded a switch to theophylline-containing perfusate (80 μg ml^{-1}). Samples of 170 μl were taken from the reservoir at 0, 10, 20, 30 and 45 min after the switch to theophylline.

Analysis of theophylline. Theophylline in the perfusate was determined by enzyme-immunoassay (Rubenstein et al 1972); reagents were from Syva Corporation, Palo Alto, California, USA and used according to the instructions of the manufacturer. Absorbance was measured at 340 nm.

Data analysis. The extraction ratio (ER) of theophylline used to examine the influence of rate of perfusion was determined using the equation: ER = (Ci − Co)/Ci, where Ci = theophylline concentration in the perfusate before passage through the liver and Co = theophylline concentration after perfusion.

The elimination half life of theophylline at different Po_2 values was determined by fitting the perfusate theophylline concentration versus time profile to a mono-exponential function by means of the non-linear regression computer programme NONLIN (Metzler 1969).

Results

Rate of perfusion. The relation between rate of perfusion and theophylline extraction ratio is shown in Fig. 2. One way analysis of variance showed no significant difference in extraction ratios at different flow rates ($P > 0·05$).

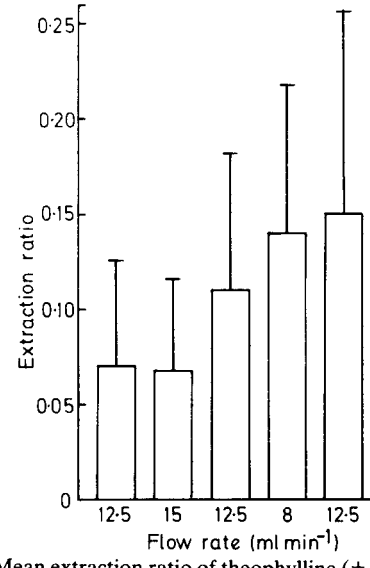

FIG. 2. Mean extraction ratio of theophylline (± s.d.) in the rat isolated perfused liver after serial changes in perfusate flow rate. One way analysis of variance showed no significant differences in extraction ratios ($P > 0·05$).

Oxygen tension. A monoexponential function adequately described the theophylline perfusate concentration data and in all cases an r value greater than 0·99 was obtained with the computer fit. Theophylline elimination half lives were calculated from the least squares estimate of the elimination rate constant and are presented in Fig. 3.

One way analysis of variance showed a significant difference in half lives between treatments. With the Newman-Keuls multiple range test a significant difference in $t_{\frac{1}{2}}$ was shown between treatment I and II and between treatment II and III ($P = 0·01$). There was no significant difference in $t_{\frac{1}{2}}$ between treatments I and III ($P > 0·05$).

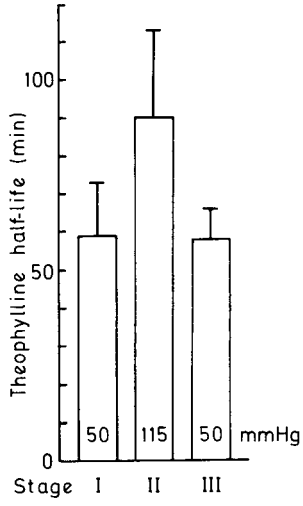

FIG. 3. Mean theophylline elimination half lives ($t_{\frac{1}{2}}$) (± s.d.) in the rat isolated perfused liver after serial changes in oxygen tension. One way analysis of variance followed by the Newman-Keuls multiple range test showed a significant difference in $t_{\frac{1}{2}}$ between stage I and II and between stage II and III ($P = 0{\cdot}01$).

Discussion

Changes in flow rate had no significant effect on the extraction ratios of theophylline following perfusion of the rat isolated liver; this is consistent with theoretical considerations related to drugs having a low extraction ratio (Nies et al 1976).

Reduction of Po_2 resulted in an increased $t_{\frac{1}{2}}$ of theophylline in the preparation. There was no significant differance between theophylline $t_{\frac{1}{2}}$ during stage I and III. This indicates that any change in $t_{\frac{1}{2}}$ was not due to irreversible change in hepatocellular function. Elimination half life is directly proportional to volume of distribution and inversely proportional to clearance (Gibaldi & Perrier 1975). Since volume of distribution is unlikely to change significantly, elimination half life was assumed to reflect changes in clearance.

Theophylline is catabolized chiefly by oxygen-dependent enzymes (Cooper et al 1975). A reduction in oxygen supply to the liver, therefore, could possibly decrease the optimal function of these enzymes, resulting in a decreased theophylline clearance and thus increased $t_{\frac{1}{2}}$. In so far as results from an animal model can be applied to man, the findings suggest that in conditions giving rise to a decrease in Po_2 (e.g. congestive heart failure and chronic obstructive bronchial disease) there could be a decreased clearance of theophylline. A decrease in arterial Po_2 has been reported in patients with low-output congestive cardiac failure (Westerfield et al 1981) and Vozeh et al (1978) showed a more than 2-fold increase in clearance of theophylline in one patient with improvement of congestive heart failure which paralleled increased Po_2 from 24 to 78 mmHg. They also showed an increase in theophylline clearance of 3-fold in a patient with resolving pneumonia accompnied by a change in Po_2 from 45 to 70 mmHg. In a third patient a 2-fold decrease in theophylline clearance was accompanied by worsening airway obstruction. The results suggest that availability of molecular oxygen in certain conditions could be a rate limiting factor in the oxidation of theophylline. Where there is reduced Po_2 any increase in it, may result in an increase in theophylline clearance thereby necessitating adjustment of theophylline dosage.

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