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NEPHROTOXIC EFFECTS OF OXYGEN TRANSPORT MEDIA IN THE ISOLATED RAT KIDNEY

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Abstract. Perfusion of isolated kidneys from rats demonstrated the following nephrotoxic effects of Fluosol-DA: decreased glomerular filtration rate (GFR), urine flow rate (UFR), and fractional reabsorption of potassium (Fr_{K+}) ($P < 0.01$). Fluosol-DA perfusions were at flow rates about equal to the physiologically normal rodent renal plasma flow rate of 4 ml/min. Stroma-free hemoglobin (SFH) perfusions, also at 4 ml/min, were associated with physiologically normal renal functions, as were those of control Krebs-Ringer bicarbonate (KRB) perfusions at 32 ml/min.

The kidney is highly susceptible to hypoxic tissue injury (1). Isolated kidney preparations have been used as models for studying the adequacy of synthetic oxygen transport media as blood substitutes for the treatment of hypoxia (2). Stroma-free hemoglobin (SFH) solutions have been shown to unload oxygen and maintain fluid balance during perfusions (3). Perfluorochemical solutions have been shown to maintain tissue oxygenation and fluid balance, although accumulating in tissues (4) and reducing urine flow (2).

Methods. Kidneys from male rats (Sprague-Dawley, 300-400 g) were isolated by a modification of the method described by Bowman (5), as follows. Animals were anesthetized with pentobarbital sodium (50 mg/kg, i.p.), the right kidney was exposed, and the right ureter was catheterized. After heparinization with heparin sodium (1,000 U/kg), a 20-gauge cannula connected to a perfusion apparatus was inserted into the left mesenteric artery, passed retrograde across the abdominal aorta into the right renal artery, and secured by ligature. The kidney was perfused from an open washout reservoir system at constant perfusion pressure (100-110 mm Hg), excised, and transferred to the top of a vertical glass water-jacketed collection column. Perfusion was then switched by stopcock to a pump-driven recirculating system in which effluent was collected and returned to the kidney through a filter. Perfusion temperature was maintained at 37 C. Urine was collected in weighed vials at five successive 15-min intervals.

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The Krebs-Ringer bicarbonate (KRB) solutions used for perfusions in control experiments (n = 20) contained 6% dextran. KRB solutions were tonometered with a gas mixture of 95% oxygen and 5% carbon dioxide, adjusted to pH 7.40, and passed through a series of millipore filters to minimum pore size of 0.22 um. In 10 preparations, KRB perfusion flow rate of 32 ml/min was required to produce a glomerular filtration rate (GFR) >500 ul/min, a range considered physiologically normal. In the other 10 preparations, the KRB perfusion flow rate was the physiologically normal in vivo renal plasma flow rate of 4 ml/min.

SFH was prepared from human erythrocytes by a modification of DeVenuto's crystallization method (3). Washed crystals were dissolved in distilled water and dialyzed (25% v/v) against KRB solution. Hemoglobin and methemoglobin concentrations were determined spectrophotometrically. Adjusted to a hemoglobin concentration of 7.0 g/dl, the SFH solutions were then tonometrically equilibrated with a gas mixture of 95% oxygen and 5% carbon dioxide at pH 7.4. The perfluorochemical emulsion Fluosol-DA (20%) was commercially manufactured by Green Cross Co., Osaka, Japan. Emulsion pH was 7.3. Perfusions with both SFH and Fluosol-DA were performed at flow rates of 4 ml/min.

Renal function was evaluated by measuring GFR, urine flow rate (UFR), and fractional reabsorptions of sodium and potassium (Fr_{Na^+} and Fr_{K^+}, respectively). GFR was determined by the inulin clearance method. Following addition of tritiated inulin (50-100 uCi) to perfusates, inulin concentrations were determined by liquid scintillation spectrophotometry at 15-min intervals on samples of perfusate and urine. Na^+ and K^+ concentrations were measured polarographically every 15 min in perfusate and urine samples using ion-selective electrodes. The coefficients Fr_{Na^+} and Fr_{K^+} were given by the expression $100 \times (F-E)/F$, where F, the filtration in uEq/min, was the product of GFR and perfusate electrolyte concentration; and E, the excretion in uEq/min, was the product of UFR and urinary electrolyte concentration.

The significance of differences in renal function variables between kidneys perfused with media of low oxygen capacity and media of high oxygen capacity was determined using the two-tailed Student t-test for independent samples with statistical significance at the $P < 0.01$ level.

Results. In kidney preparations perfused with KRB at 4 ml/min, GFR was 318 ± 43 ul/min, UFR was 23 ± 3 ul/min, Fr_{Na^+} was 91.0% ± 0.8%, and Fr_{K^+} was 73.2% ± 2.0%. Because values of GFR, UFR, and Fr_{K^+} were not physiologically normal at this perfusion flow rate, KRB perfusions were performed at a flow rate of 32 ml/min.

Figure 1 depicts differences among renal function variables during perfusion with KRB, SFH, and Fluosol-DA solutions. Mean GFR values for KRB- and SFH-perfused kidneys were not significantly different. GFR of SFH-perfused kidneys could be maintained physiologically normal at a perfusion flow rate of 4 ml/min; however, a significant decrease in GFR, from 574 ± 16.5 ul/min to 192 ± 35.0 ul/min, was associated with the Fluosol-DA perfusions at a flow rate of 4 ml/min. Similarly, UFR values of 39.6 ± 10 ul/min to 43.0 ± 14 ul/min found during KRB and SFH perfusions decreased to 8.2 ± 0.4 ul/min during Fluosol-DA perfusions. Values for Fr_{K^+} were 85.9% ± 2.3% in kidneys perfused with KRB, 82.4% ± 2.5% in kidneys perfused with SFH, and 76.8% ± 4.5% in kidneys perfused with Fluosol-DA. Values for Fr_{Na^+} were physiologically normal (91%-94%) in all experiments.

Discussion. The use of the isolated kidney has been limited because of the technical difficulties in obtaining physiologically normal perfusions; however, previous workers have developed perfused kidney models that are biochemically viable (5, 6). Physiologists have preferred to study kidneys with capacity to

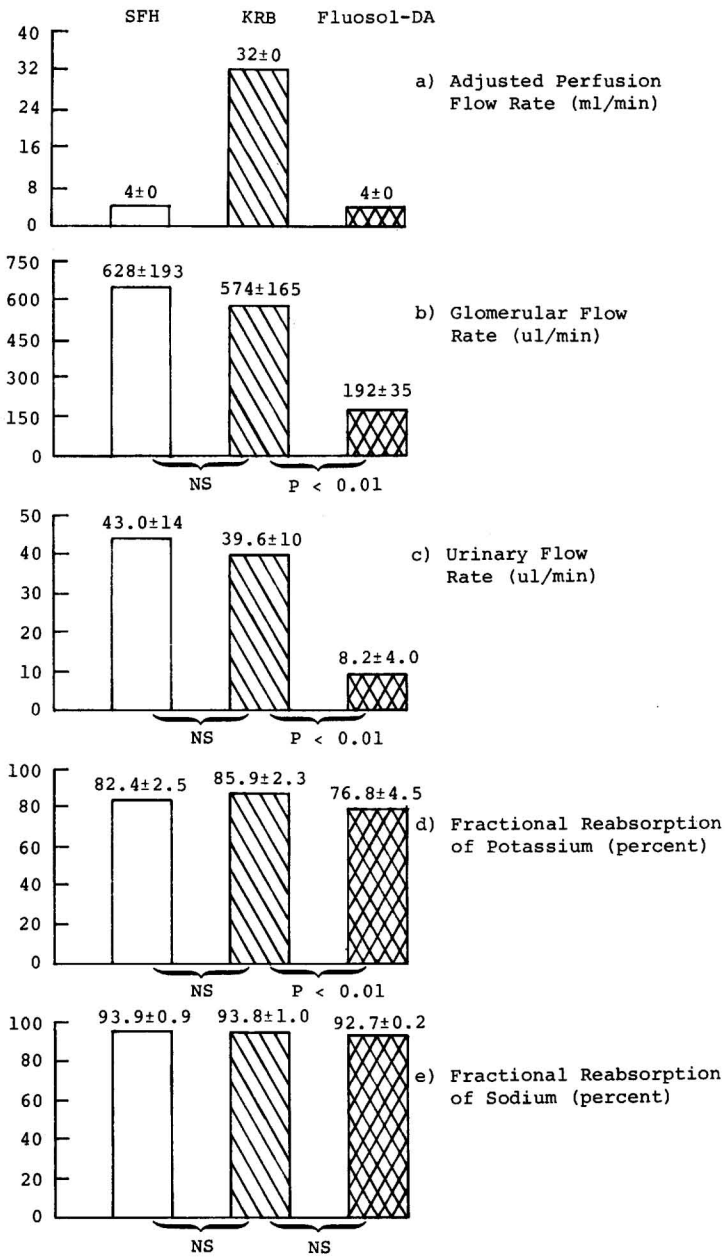

Figure 1. Renal function variables during isolated kidney perfusions. Effects of perfusions with KRB (of low oxygen capacity) are compared with perfusions with SFH and Fluosol-DA (of high oxygen capacity). NS = not significant.

reabsorb 98%-99% of filtered water and sodium, a level of performance difficult to obtain in vitro (7). Such high renal efficiency is not required for screening potential nephrotoxicants, only a system that yields reproducible results near physiological normalcy. Bowman (5) has found that isolated kidney is indeed

able to maintain physiologically normal GFR, UFR, Fr_{Na^+}, Fr_{K^+}, hormonal responses, and functional energy levels during perfusion. Similar results have been reported by Brunengraber (8), who measured isolated kidney function by urinary clearance and metabolism of mevalonate.

The present experiments demonstrated physiological use of an isolated rodent kidney preparation. Homeostatic glomerular filtration and urine flow required that kidney perfusions with a standard, oxygenated KRB solution of low oxygen transport capacity be performed at flow rates eight times greater than the physiologically normal in vivo renal plasma flow rate. KRB perfusions at the in vivo renal plasma flow rate of 4 ml/min showed significant decreases in GFR, UFR, Fr_{Na^+}, and Fr_{K^+}. Previous studies (2, 9) have also used perfusion flow rates between 20 and 50 ml/min to obtain functional integrity of isolated kidney preparations.

SFH perfusions at the in vivo renal plasma flow rate of 4 ml/min showed larger GFR and UFR values than KRB perfusions; however, K^+ reabsorption was reduced. Inhibition of K^+ reabsorption and its excretion may have been enhanced by the larger K^+ concentration (4.2 mM compared to 3.5 mM) found in SFH as compared to KRB.

Fluosol-DA perfusions at the in vivo renal plasma flow rate of 4 ml/min showed decreased values of GFR and UFR, as well as of Fr_{K^+}.

These findings suggest that Fluosol-DA is a potentially nephrotoxic oxygen transport medium. Despite a slight kaliuretic effect, SFH perfusions of isolated kidneys are essentially physiological, hence significantly less nephrotoxic than Fluosol-DA perfusions.

Numerous methods are available for further study of perfluorochemically induced nephrotoxicity. Renal micropuncture of selected regions of the nephron should distinguish among vascular, tubular, and glomerular effects (10). The renal cortical slice technique may demonstrate effects on organic ion transport (1). Study of the arteriovenous oxygen difference may provide a nonspecific indication of effects on renal metabolism; specific data concerning ATP production and metabolic substrates can be derived from NMR spectroscopy as used in muscle studies (11). Light microscope study of carefully dissected renal tubules has identified loci of nephrotoxicity (12). Ultrastructural histochemical examination of cytochrome oxidase activity of renal mitochondria may provide useful information on nephron oxygen utilization (13).

Conclusion. It is concluded that the isolated kidney preparation is a useful method for screening oxygen transport media for potential nephrotoxicity. Although Fluosol-DA shows significant potential nephrotoxicity, the specific loci of nephrotoxicity remain unclear.

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