J. Pharm. Pharmacol. 1990, 42: 511–512
Communicated January 19, 1990

© 1990 J. Pharm. Pharmacol.

The in-situ absorption of antipyrine as a measure of intestinal blood flow in Fluosol-DA haemodiluted rats

ROBERT P. SHREWSBURY, LISA G. WHITE,* *School of Pharmacy, University of North Carolina, Chapel Hill, NC 27599, *College of Veterinary Medicine, North Carolina State University, Raleigh, NC 27606, USA*

Abstract—The effect of moderate Fluosol-DA haemodilution on intestinal blood flow has been investigated in the rat. Antipyrine in-situ intestinal absorption is blood flow limited, and did not alter 0·5, 24, 48, or 72 h after haemodilution with 40 mL kg^{-1} Fluosol. Thus the intestinal blood flow rate is maintained as part of the physiological response to ensure adequate perfusion of the vital organ.

Fluosol-DA (Fluosol) is a proprietary perfluorochemical (PFC) emulsion which is an acellular oxygen carrying substance. Preclinical studies have shown that Fluosol haemodilution increases the cerebral (Nagasawa et al 1981), myocardial (Biro et al 1987; Biro 1988) and hepatic arterial (Haneda et al 1983) blood flow rates in different animal species excluding the rat. Ohyanagi et al (1988) showed that Fluosol infusion maintained the gastroduodenal arterial and superior pancreatic duodenal venous blood flow rates, despite decreased cardiac ouput in dogs with induced acute haemorrhagic necrotizing pancreatitis. Lutz & Metzenauer (1980) and Bizot & Rink (1985) however reported that Fluosol administration decreased hepatic blood flow in rats. In these experiments though, indocyanine green was used as the dye reported by Shrewsbury et al (1987) as having a low hepatic extraction ratio in the rat, which led them to study the clearance of (+)-propranolol which has an extraction ratio greater than 0·9. In that study, hepatic blood flow was not altered by moderate haemodilution for 48 h but was significantly decreased at 72 h.

The influence of Fluosol administration on the intestinal blood flow in the rat has not been reported. This investigation determines if Fluosol haemodilution alters intestinal blood flow, using antipyrine in-situ intestinal absorption as the marker. Antipyrine intestinal absorption has been shown to be flow limited in unexchanged (Winne 1978; Schulz & Winne 1987) or Fluosol-43 perfused rats (Takahashi et al 1988). Antipyrine absorption was studied at various times after haemodilution since Fluosol is eliminated slowly (Lutz & Stark 1987) and hepatic blood flow was altered in a time-dependent manner in the rat (Shrewsbury et al 1987).

Materials and methods

Fluosol was donated by Alpha Therapeutic Corporation (Los Angeles, California), and prepared as directed within 0·5 h of use. Antipyrine and other chemical reagents were obtained from commercial sources and used without further purification. Sprague-Dawley, male, albino rats, 261–331g, were used.

Antipyrine absorption was determined in unexchanged rats (control) and rats haemodiluted with 40 mL kg^{-1} Fluosol using a reported procedure (Shrewsbury et al 1987). The antipyrine in-situ intestinal absorption was determined 0·5, 24, 48 and 72 h after haemodilution. Details of the in-situ procedure are reported by Shrewsbury et al (1982). Approximately 6 mL of antipyrine solution (25 mg L^{-1}, pH = 6·4, 300 mOsm kg^{-1}, prewarmed to 37°C) was instilled and passed through the cannulated intestinal segment three times to ensure mixing with the residual contents. A 0·1 mL zero time sample was collected, and the solution was immediately returned to the segment. Samples were collected at 5 min intervals for 30 min. Antipyrine concentrations in the luminal samples were determined by HPLC (Shrewsbury et al 1988). A semi-logarithmic plot of the luminal concentrations divided by the zero time concentration versus time was constructed, and the absorption half-life was determined from the slope of the line.

Correspondence to: R. P. Shrewsbury, School of Pharmacy, University of North Carolina, Chapel Hill, NC 27599, USA.

Results

Antipyrine absorption half-life ($t_{\frac{1}{2}}$) for the control and Fluosol haemodiluted groups are shown in Fig. 1. The absorption $t_{\frac{1}{2}}$ was not significantly different from the control in any group ($P \leqslant 0.05$, Student's t-test), although the lowest mean was found at 72 h.

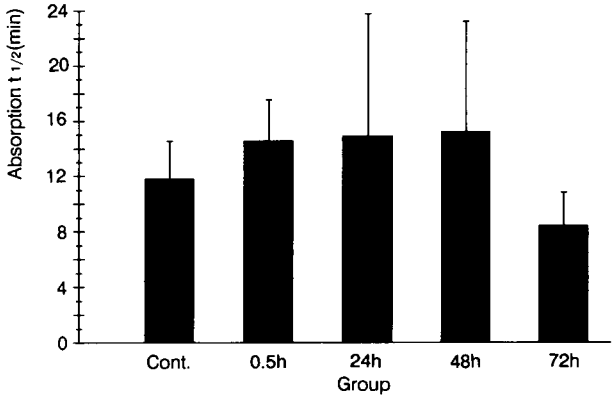

FIG. 1. Mean antipyrine intestinal absorption $t_{\frac{1}{2}}$ following haemodilution with 40 mL kg^{-1} of Fluosol (n = 3–4). Error bars represent 1 standard deviation.

Discussion

The in-situ procedure has been shown to be an appropriate method for determining intestinal drug absorption. Doluisio et al (1969) reported that water flux was less than 10%, absorption rates were reproducible regardless of the initial lumen concentration, and redistribution of drugs back into the lumen was undetectable. The procedure was also used by Takahashi et al (1988) who investigated antipyrine absorption as a function of flow using Fluosol-43 as a vascular perfusate.

Other investigations have studied the influence of PFC emulsions on intestinal morphology or absorption. Fluosol-43 is a PFC emulsion that contains perfluorotributylamine instead of perfluorodecalin and perfluorotripropylamine which are the PFCs in Fluosol. Intraluminal administration of Fluosol-43 provided protection for 2 h against acidosis and morphological changes in an ischaemic dog intestine preparation (Baba et al 1979). The first report of using Fluosol as an ex-vivo perfusate was in 1983, but its effects on intestinal morphology were not

studied at that time (Nakamura et al 1983). However, ex-vivo perfusion of human intestine for 6 h with either Fluosol or Fluosol-43 did not cause ischaemic changes in the microvilli and only minimal reversible mitochondrial changes (Baba et al 1988). When used as a vascular perfusate in-vitro, Fluosol-43 maintained the normal intestinal absorption barriers to tritiated water, salicylic acid, D-glucose, glutamic acid, and antipyrine (Takahashi et al 1988). The in-situ duodenal absorption of Ca^{2+} in rats was not altered using Fluosol as a vascular perfusate (Aso et al 1979). Thus, PFC emulsions appear to maintain the integrity of the intestinal membrane.

In the present study, there was no significant difference in antipyrine intestinal absorption in any Fluosol haemodiluted group compared with the control. This implies, in an indirect manner, that moderate Fluosol haemodilution does not change intestinal blood flow in the rat for 72 h. In another study, hepatic blood flow was maintained for 48 h after Fluosol haemodilution, but significantly decreased at 72 h (Shrewsbury et al 1987). Although the lowest mean antipyrine absorption $t_{\frac{1}{2}}$ was at 72 h in the current study, it was not significantly different from the control. These results may indicate that hepatic and intestinal blood flows decrease at or beyond 72 h. However, the intestinal blood flow is maintained for at least 72 h after haemodilution, presumably as part of the physiological response to ensure adequate perfusion of the visceral organs.

This study was funded by the Biomedical Research Support Grant Program (RR07072).

References

Aso, Y., Yamada, Y., Mishima, K. (1979) Duodenal calcium absorption in rats with transfusion of Fluosol-DA: rapid action of 1,25-dihydroxyvitamin D_3. In: Naito, R. (ed.) Proceedings of the IVth International Symposium on Perfluorochemical Blood Substitutes. Excerpta Medica, Amsterdam, pp 267–271

Baba, S., Mitzutani, K., Kamiya, T., Nakamura, S., Seki, A., Tokura, Y., Sakaguchi, S. (1979) The intraluminal administration of Fluosol-43 (FC-43) to the ischemic intestine. In: Naito, R. (ed.) Proceedings of the IVth International Symposium on Perfluorochemical Blood Substitutes. Excerpta Medica, Amsterdam, pp 307–318

Baba, S., Nakai, K., Mizutani, K. (1988) Ex-vivo perfusion of surgically removed organs. Biomat. Art. Cells Art. Org. 16: 623–624

Biro, G. P. (1988) Blood substitutes and the cardiovascular system. Ibid. 16: 595–606

Biro, G. P., White, F. C., Guth, B. D., Breish, E. A., Bloor, C. M. (1987) The effect of hemodilution with fluorocarbon or dextran on regional myocardial flow and function during acute coronary stenosis in the pig. Am. J. Cardiovasc. Path. 1: 99–114

Bizot, W. B., Rink, R. D. (1985) Effects of exchange transfusion with perfluorochemical emulsions on hepatic oxygen supply and blood flow in the rat. Experientia 41: 1127–1129

Doluisio, J. T., Billups, N. F., Dittert, L. W., Sugita, E. T., Swintosky, J. V. (1969) Drug absorption I: An in-situ rat gut technique yielding realistic absorption rates. J. Pharm. Sci. 58: 1196–1200

Haneda, K., Honda, K., Kanno, M. (1983) The use of Fluosol (TM)-DA 20% (FDA) infusion for hemorrhagic shock. In: Bolin, R. B., Geyer, R. P., Nemo, G. J. (eds) Advances in Blood Substitutes Research: Progress in Clinical and Biological Research, Vol. 122. Alan R. Liss, New York, p 438

Lutz, J., Metzenauer, P. (1980) Effects of potential blood substitutes (perfluorochemicals) on rat liver and spleen. Ibid. 387: 175–181

Lutz, J., Stark, M. (1987) Half life and changes in the composition of a perfluorochemical emulsion within the vascular system of rats. Pflugers Arch. 410: 181–184

Nagasawa, S., Handa, H., Naruo, Y., Oda, Y., Yonekawa, Y., Takebe, Y. (1981) Effects of a transfusion with a blood substitute (Fluosol-DA, 20%) on cerebral circulation in the monkey. Neurologic. Res. 3: 237–250

Nakamura, S., Kino, I., Baba, S. (1983) Ex vivo autoradiography of the human gastrointestinal tract: a new approach to cell kinetic studies of surgically removed tumor-bearing organs. Gann 74: 116–121

Ohyanagi, H., Ohashi, O., Nakayama, S., Yamamoto, M., Okumura, S., Saitoh, Y. (1988) Experimental studies on Fluosol-DA administration in acute pancreatitis. Biomat. Art. Cells Art. Org. 16: 585–594

Schulz, R., Winne, D. (1987) Relationship between antipyrine absorption and blood flow rate in rat jejunum, ileum and colon. Naunyn-Schmiedeberg's Arch. Pharmacol. 335: 97–102

Shrewsbury, R. P., Metcalf, T. B., Weiss, D. L., Dittert, L. W. (1982) Influence of disodium etidronate on salicylic acid absorption in the rat. J. Pharm. Pharmacol. 34: 823–825

Shrewsbury, R. P., Lewis, L. M., Oliver, S. R., Womble, C. L. (1987) Effect of moderate haemodilution with Fluosol-DA or normal saline on indocyanine green and (+)-propranolol kinetics. Ibid. 39: 592–598

Shrewsbury, R. P., Oliver, S. R., Anderson, W. T., Lewis, L. M., White, L. G. (1988) The effect of varying percentages of haemodilution with Fluosol-DA or normal saline on antipyrine metabolism in the rat. Ibid. 40: 392–398

Takahashi, H., Nishikawa, M., Hayashi, M., Awazu, S. (1988) The use of perfluorochemical emulsion as a vascular perfusate in drug absorption. Ibid. 40: 252–257

Winne, D. (1978) Blood flow in intestinal absorption models. J. Pharmacokinet. Biopharm. 6: 55–78