of the nicotinamide in the heavily contaminated batch was such that its solubility was a problem.

The reason for the particulate contamination in the preparations containing ergometrine has not been elucidated but it is possible that it is also a formulation problem.

The analysis of particulate contamination by Taylor & Spence (1983) does not give a comparable indication of the number of particles present in the solutions because they snap-opened the ampoules and did not use an ultrasonic bath to remove any air bubbles. A comparison between the level of contamination in their ampoules compared with locally manufactured ampoules would be interesting. Certainly the latter showed a commendably low level of contamination (Table 5).

Conclusion

It can be concluded that, in most instances, the level of particulate contamination in locally manufactured ampoules is low. In this limited study it appeared that where problems arise they appear to be due to the formulation or the quality of the raw materials rather than to the manufacturer of the ampoule solutions or the supplier of the ampoules.

With regard to setting a standard for limiting contamination in ampoules, the problem is complicated by lack of knowledge about the size of particle or the number of particles that constitute a danger. The USP Sub-Committee on Parenteral Products (1983, 1984) favours limiting the number of particles per injection rather than per ml. This appears to be a realistic approach and takes into account the problem of increasing the particulate contamination of LVPs when relatively large volumes of SVPs are included as additives. However it is obvious that it is possible to produce ampoules with a low level of particulate contamination and that particle numbers would appear to be related to the formulation, the purity of the raw material and to GMP; therefore a reasonable limit would help to ensure a well formulated high quality product.

REFERENCES

Alexander, D. M. (1983) Paper delivered to the Congress of S.A. Academy of Pharmaceutical Sciences.

Boddapati, S., Butler, L. D., Im, S., DeLuca, P. P. (1980) J. Pharm. Sci. 69 (5): 608–610

Groves, J. J. (1969) Analyst 94: 992–1001

Groves, M. J., Wana, D. (1977) Powder Technology 18: 215–223

Hailey, D. M., Lea, A. R., Kendall, C. E. (1982) J. Pharm. Pharmacol. 34: 615–620

Haines-Nutt, R. F. (1983) Pharm. J. 230 (6220): 373

Hayashi, T. (1980) Yakuzaigaku 40: 133–136

Spence, J. (1981) Anal. Proc. 18: 522–525

Taylor, S. A., Spence, J. (1983) J. Pharm. Pharmacol. 35: 769–773

United States Pharmacopeial Convention inc. 1983 (Nov–Dec) Pharmacopeial Forum 3729–3742

United States Pharmacopeial Convention inc. 1984 (Jan–Feb) Pharmacopeial Forum 3937–3939

United States Pharmacopeial Convention inc. 1984 (July–Aug) Pharmacopeial Forum 447–4470

van Wyk, C. J., Goossens, A. P. G. (1980) S.A. Pharm. J. 47: (12) 550–554

J. Pharm. Pharmacol. 1985, 37: 55–57
Communicated March 12, 1984

A carrier-mediated transport system for benzylpenicillin in isolated hepatocytes

AKIR TSUJI*, TETSUYA TERASAKI, IKUMI TAMAI, EMI NAKASHIMA, KAZUHIRO TAKANOSU, *Faculty of Pharmaceutical Sciences, Kanazawa University, Takara-machi, Kanazawa 920, Japan*

The transport mechanism of benzylpenicillin was studied in freshly prepared rat hepatocytes. The initial uptake rate followed both saturable and unsaturable transport processes. The Arrhenius plot of the initial uptake rate gave an activation energy of 16.8 kcal mol^{-1} (69 kJ mol^{-1}). The benzylpenicillin uptake by hepatocytes was significantly inhibited by antimycin A, sodium cyanide, rotenone, 2,4-dinitrophenol, phenoxymethylpenicillin, probenecid and taurocholic acid. No significant inhibition was observed by acetylaminohippuric acid and several kinds of amino acids and dipeptides. The present study provides the first evidence for the the existence of a carrier-mediated and energy-dependent transport system of benzylpenicillin in the liver.

Hepatic membrane permeation is the most important step in the process of metabolism and biliary secretion in the liver. Since some derivatives of β-lactam antibiotics are known to be rapidly and exclusively eliminated from the liver while others are eliminated from the kidney after administration (see Bergan 1978; Brogard et al 1978), the reason why these different elimination routes were characteristic of certain derivatives needs clarification. Though the mechanism of renal β-lactam antibiotic elimination has been studied (Hori et al 1982; Inui et al 1983), there is little knowledge about the hepatic transport process. In this communication, we describe the basic characteristics of the transport process of benzylpenicillin, the most fundamental β-lactam antibiotic, in hepatic parenchymal cells of rats. Our present study provides the first evidence for the existence of a carrier-mediated and energy-dependent transport

* Correspondence.

system for benzylpenicillin in the liver, which has a different nature from that of renal tubules.

Methods

Liver cells from Wistar rats (Sankyo Laboratory Co. Ltd., Toyama, Japan) weighing 270–320 g, fed on standard chow, were isolated by the method of Moldeus et al (1978). Benzylpenicillin uptake by rat isolated hepatocytes was studied at 37 °C within 4 h after completion of preparation. The transport reaction was terminated by the centrifugal filtration method (Schwarz et al 1977). The protein concentration of the cells was determined by the method of Lowry et al (1951) using bovine serum albumin (Fraction V, Sigma Chemical Co., St Louis, MO) as a standard. The extracellular fluid volume of hepatocytes was corrected from the total uptake by using the value of the inulin space. Viability of isolated hepatocytes was checked immediately after isolation. Cells were used only if they exhibited a value grater than 92% for the lactate dehydrogenase latency test (Moldeus et al 1978). [^{14}C]Benzylpenicillin and [^{14}C]inulin were purchased from Amersham International Ltd, Amersham, England. Collagenase (Clostridiopeptidase A) was obtained from Boehringer-Mannheim GmbH, Mannheim, F.R.G.

Results and discussion

The time course of benzylpenicillin uptake by isolated hepatocytes proceeded linearly for at least 1 min with equilibration at about 15 min. Fig. 1 shows the relationship between the slope of the linear part (0·25–1·0 min) of the time course of the uptake and the concentration of benzylpenicillin in the medium. A curvilinear plot was obtained, suggesting that benzylpenicillin uptake is a combination of saturable and unsaturable processes. Kinetic parameters for the apparent Michaelis constant, K_t, the apparent maximum transport rate, V_{max}, the apparent first-order transport rate constant, k_0, were estimated from the resulting curve in Fig. 1. The values of K_t, V_{max} and k_0 were calculated to be 473 ± 158 μM, 2·02 ± 0·48 nmol min^{-1} (mg protein)$^{-1}$ 0·580 ± 0·012 nmol min^{-1} (mg protein)$^{-1}$ mM, respectively. The apparent unsaturable process could represent either simple diffusion through the plasma membrane or the participation of a very low affinity system for benzylpenicillin uptake.

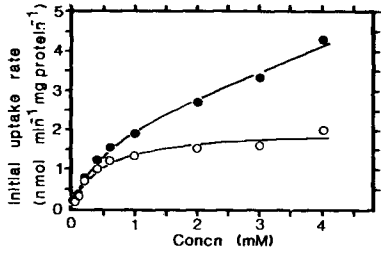

FIG. 1. Plot of initial uptake rate against benzylpenicillin concentration. Each point is the mean ± s.e.m. of three independent experiments. The upper line represents the overall uptake rate, while the lower line represents uptake due only to the saturable component of the uptake process.

Since the uptake experiments were carried out in Krebs-Henseleit buffer containing 1·0% bovine serum albumin (BSA) (Moldeus et al 1978), a binding experiment for benzylpenicillin with BSA was carried out at 37 °C using the equilibrium dialysis method (Tsuji et al 1983). The percentage value of the amount unbound to BSA was 90·8 ± 2·0% (n = 21) for the total concentration range of benzylpenicillin (0·05–4·04 mM), indicating that binding of benzylpenicillin to BSA could be neglected under the experimental conditions used. The temperature dependency of benzylpenicillin uptake (200 μM) was examined at 22, 27, 32 and 37 °C (n = 3 at each temperature). The Arrhenius plot exhibited a single straight line corresponding to an activation energy of 16·8 kcal mol^{-1} (69 kJ mol^{-1}). The evidence of saturability and temperature dependency suggests that a carrier-mediated transport system may participate in benzylpenicillin uptake by hepatocytes. As shown in Fig. 2, addition of antimycin A (10·0 μM), sodium cyanide (1·0 mM), rotenone (1·0 mM) and 2,4-dinitrophenol (1·0 mM) to the reaction medium diminished the initial uptake rate of benzylpenicillin at a concentration of 200 μM to about 38·0, 56·0, 55·0 and 36·0% of the control, respectively. These significant inhibitory effects on benzylpenicillin uptake suggest that the saturable uptake process is dependent on metabolic energy supplies.

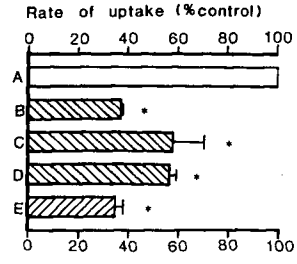

FIG. 2. Effect of metabolic inhibitors on the uptake rate of benzylpenicillin. Each bar represents the mean ±s.e.m. of three independent experiments. Benzylpenicillin (A) concentration was 200 μM. Metabolic inhibitors used were: antimycin A (10·0 μM) (B), sodium cyanide (1·0 mM) (C), rotenone (1·0 mM) (D), 2,4-dinitrophenol (1·0 mM) (E). Level of significance was set at $P < 0·05$ (*).

To clarify the specificity of the transport system, a benzylpenicillin uptake experiment for hepatocytes was performed in the presence of several organic anions. Fig. 3 shows the effects of phenoxymethylpenicillin (1·0 mM), taurocholic acid (100 μM), probenecid (5·0 mM), and *p*-acetylaminohippuric acid (5·0 mM) on benzylpenicillin uptake at a concentration of 200 μM by

hepatocytes. Phenoxymethylpenicillin, a structural analogue of benzylpenicillin, significantly reduced uptake, suggesting that benzylpenicillin is transported by a system specific to β-lactam antibiotics. Since probenecid and *p*-acetylaminohippuric acid are known to be eliminated from the liver via an organic anion transport system (Gigon & Guarino 1970; Kupferberg et al 1964), the effect of these anions on benzylpenicillin uptake was also examined. Although probenecid exhibited a significant inhibitory effect, *p*-acetylaminohippuric acid did not affect uptake under the experimental conditions employed. These results suggest that benzylpenicillin and *p*-acetylaminohippuric acid have different transport systems in the liver. It is noteworthy that taurocholic acid significantly inhibited benzylpenicillin uptake at a concentration of 200 μM. Since uptake of taurocholic acid in the sinusoidal membrane vesicle was significantly inhibited by probenecid (Inoue et al 1982), it is possible to assume that there is a common carrier system for β-lactam antibiotics, probenecid and taurocholic acid. Since β-lactam antibiotics contain amino acids in the molecule and some derivatives of β-lactam antibiotics were taken up by a carrier-mediated system

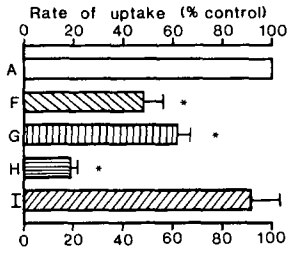

FIG. 3. Effect of organic anions on the uptake rate of benzylpenicillin. Each bar represents the mean ± s.e.m. of three independent experiments. Benzylpenicillin (A) concentration was 200 μM. Organic anions used were: phenoxymethylpenicillin (1·0 mM) (F), taurocholic acid (100 μM) (G), probenecid (5·0 mM) (H) and *p*-acetylaminohippuric acid (5·0 mM) (I). Level of significance was set at $P < 0{\cdot}05$ (*).

common to that of dipeptides in rat small intestine (Nakashima et al 1984; Nakashima et al in press) and renal brush border membrane vesicles (Hori et al 1982; Inui et al 1983), it is necessary to determine whether or not amino acids and peptides inhibit the uptake of benzylpenicillin. Several kinds of amino acids (leucine, histidine, phenylalanine, valine, alanine, glutamic acid and glycine) and peptides (prolyl-leucine, glycyl-glycine, glycyl-sarcosine, glycyl-leucine and γ-glutamyl-cysteinyl-glycine) did not influence uptake of benzylpenicillin at 200 μM when they were used at an inhibitory concentration of 5·0 mM. These results from the inhibition experiments indicated that the benzylpenicillin transport system has different characteristics from that in the small intestine and renal tubule (Hori et al 1982; Inui et al 1983; Nakashima et al 1984; Nakashima et al in press).

In conclusion, hepatocytes take up benzylpenicillin through an energy-dependent, carrier-mediated system. The present findings on the hepatic transport process should provide a valuable basis for the study of biliary secretion and metabolism of β-lactam antibiotics.

The authors wish to thank Dr Masaharu Hirata, Shionogi Research Laboratory, Shionogi & Co. Ltd, Osaka, Japan for his helpful advice on the preparation of rat hapatocytes and Miss S. Miki and Mr H. Hirooka for their skilful technical assistance.

REFERENCES

Bergan, T. (1978) in: Schönfeld, H. (ed.) Antibiotics and Chemotherapy, 25, Karger, S., Basel, pp 1–122

Brogard, J. M., Comte, F., Pinget, M. (1978) Ibid, pp 123–162

Gigon, P. L., Guarino, A. M. (1970) Biochem. Pharmacol. 19: 2653–2662

Hori, R., Ishikawa, Y., Takano, M., Okano, T., Kitazawa, S., Inui, K. (1982) Ibid. 31: 2267–2272

Inoue, M., Kinne, R., Tran, T., Arias, I. M. (1982) Hepatology 2: 572–579

Inui, K., Okano, T., Takano, M., Kitazawa, S., Hori, R. (1983) Biochem. Pharmacol. 32: 621–626

Kupferberg, H. J., Solomon, H. M., Schanker, L. S. (1964) Pharmacologist 6: 177

Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J. (1951) J. Biol. Chem. 193: 265–275

Moldeus, P., Hogberg, J., Orrenius, S. (1978) Methods in Enzymology 52: 60–71

Nakashima, E., Tsuji, A., Kagatani, S., Yamana, T. (1984) J. Pharm. Dyn. 7: 452-464

Nakashima, E., Tsuji, A., Mizuo, H., Yamana, T. (1984) Biochem. Pharmacol. in press

Schwarz, L. R., Schwenk, M., Pfaff, E., Greim, H. (1977) Ibid. 26: 2433–2437

Tsuji, A., Yoshikawa, T., Nishide, K., Minami, H., Kimura, M., Nakashima, E., Terasaki, T., Miyamoto, E., Nightingale, C. H., Yamana, T. (1983) J. Pharm. Sci. 72: 1239–1252