and the sedative actions of the benzodiazepines seems to have been prematurely dismissed and further studies combining behaviour with biochemistry are needed to clarify the role of this neurotransmitter in benzodiazepine-induced sedation.

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REFERENCES

Chase, T. N., Katz, R. I., Kopin, I. J. (1970) Neuropharmacology 9: 103–108

Cook, L., Sepinwall, J. (1975) Advances in Biochemical Psychopharmacology, Vol. 14, 1–28, Raven, New York

Crawley, J. N., Marangos, P. J., Stivers, J., Goodwin, F. K. (1982) Neuropharmacology 21: 85–89

Curzon, G., Kantamaneni, B. D., Tricklebank, M. D. (1981) Br. J. Pharmacol. 73: 555–561

Dominic, J. A., Sinha, A. K., Barchas, J. D. (1975) Eur. J. Pharmacol. 32: 124–127

File, S. E. (1982) Ibid. 81: 637–643

File, S. E., Vellucci, S. V. (1978) J. Pharm. Pharmacol. 30: 105–110

File, S. E., Wardill, A. G. (1975) Psychopharmacology 44: 53–59

Greenblatt, D. J., Shader, R. I. (1974) Benzodiazepines in clinical practice Raven Press, New York

Greenblatt, D. J., Shader, R. I. (1978) Drug Metab. Rev. 8: 13–28

Jenner, P., Chadwick, D., Reynolds, E. H., Marsden, C. D. (1975) J. Pharm. Pharmacol. 27: 707–710

Pratt, J., Jenner, P., Reynolds, E. H., Marsden, C. D. (1979) Neuropharmacology 18: 791–799

Rastogi, R. B., Lapierre, Y. D., Shinghal, R. L. (1978) Can. J. Physiol. Pharmacol. 56: 777–784

Wise, C. D., Berger, B. D., Stein, L. (1972) Science 177: 180–183

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Biological activity of indoprofen and its optical isomers

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Indoprofen, (α[4-(2-isoindolinyl-1-one)-phenyl] propionic acid) a non-steroidal analgesic anti-inflammatory drug (NSAID) (Buttinoni et al 1973; Bruni et al 1980) has an asymmetric carbon atom and can therefore occur either as the (+)- or (−)-isomer. The optically active indoprofen enantiomers have been resolved (Tosolini et al 1974) and their absolute configurations determined (De Munari et al 1980). Several workers have claimed that biological activity in some substituted phenylpropionic acids is due almost entirely to the (+)-isomer (Shen 1967; Ham et al 1972; Takeguchi & Sih 1972; Tomlinson et al 1972; Greig & Griffin 1975) believed to have the *S*-configuration (Wechter et al 1974; Simmonds et al 1980; Tamura et al 1981). The aim of this study was to determine the contribution of the enantiomers to the activity and toxicity of racemic indoprofen. We also investigated their effect on prostaglandin biosynthesis, as the biological activity of this class of compounds is closely linked to inhibition of the prostaglandin system.

Methods

Anti-inflammatory activity was studied in male ICEM:CER (SPF Caw) rats on acute and subchronic models, carrageenan oedema (Winter et al 1963) and granuloma pouch (Boris & Stevenson 1965); analgesic activity was assayed in ICEM:CET (SPF Caw) mice on phenylquinone writhing (Siegmund & Cadmus 1957). Drugs were given orally, suspended in 0·5% Methocel (hydroxypropyl-methyl cellulose 400). Acute toxicity was determined in rats 7 days after oral and intravenous treatment (drugs given as sodium salts). Biological activity was determined and evaluated as previously described (Buttinoni et al 1973).

In-vitro inhibition of prostaglandin synthesis was studied according to Ceserani et al (1979). The concentration used were 0·6, 1·2, 2·4 ng ml^{-1} for racemic indoprofen (n = 11–13) 0·3, 0·6, 1·2 ng ml^{-1} for (*S*)-(+)-enantiomer (n = 11–12) and 24, 48, 96 ng ml^{-1} for (*R*)-(−)-enantiomer (n = 3). The findings were analysed statistically as previously described (Ceserani et al 1979) to obtain KB (KB = dose of indoprofen which reduces the activity of arachidonic acid by 50%, see Furchgott 1972).

Results

Tables 1 and 2 show the anti-inflammatory and analgesic activity and the acute toxicity of racemic indoprofen and its enantiomers. The (*S*)-(+)-isomer is twice as effective and toxic as racemic indoprofen, but the (*R*)-(−)-isomer displayed very little activity and toxicity.

Similar potency ratios were obtained in-vitro. Racemic indoprofen and the (*S*)-(+)-isomer inhibited prostaglandin synthesis with a KB = 2·29 (confidence limits for $P = 0.95$ (1·56–3·37) and 1·27 (0·43–3·74)

* Correspondence.

Table 1. Anti-inflammatory and analgesic activity of indoprofen and its enantiomers after oral administration.

Compound	Carrageenan oedema in rats ED50 mg kg^{-1}	Granuloma pouch in rats ED25 mg kg^{-1}	Phenylquinone writhing in mice ED50 mg kg^{-1}
Indoprofen	1·26 (1·12–1·44)	0·72 (0·44–0·99)	0·49 (0·43–0·55)
(*S*)-(+)-Enantiomer	0·67 (0·59–0·75)	0·36 (0·27–0·51)	0·27 (0·24–0·29)
(*R*)-(−)-Enantiomer	12·23 (8·22–21·40)	11·30 (9·23–13·59)	6·25 (5·61–6·88)

95% confidence limits in parentheses.

Table 2. Acute toxicity of indoprofen and its enantiomers in rats.

Compound	LD50 mg kg^{-1} oral	LD50 mg kg^{-1} i.v.
Indoprofen	60·83 (51·00–72·55)	58·66 (53·41–64·42)
(*S*)-(+)-Enantiomer	33·75 (30·19–37·72)	31·98 (29·49–34·68)
(*R*)-(−)-Enantiomer	538·02 (460·67–628·37)	555·39 (518·51–594·89)

95% confidence limits in parentheses.

respectively. (*R*)-(−)-Indoprofen was virtually ineffective at more than 100 times higher concentrations.

The results of in-vivo experiments show that the (*S*)-(+)-isomer has twice the anti-inflammatory and analgesic activity of the racemic mixture, and about 20 times that of the (*R*)-(−)-isomer. Similar results were obtained in toxicity trials; in the rat the (*S*)-(+)-isomer was 16–17 times as toxic as the (*R*)-(−)-isomer and twice as toxic as the racemic mixture. Racemic indoprofen had about the same toxicity that would have been expected for a 1:1 mixture of (*S*)-(+)- and (*R*)-(−)-enantiomers on the basis of additive effects. The ratio of toxicity found/toxicity expected, calculated by Finney's method (Finney 1952) on the basis of the hypothesis of additivity, was 1·038 (0·827–1·302) for the rat after oral administration and 1·025 (0·913–1·151) after intravenous administration.

Discussion

The in-vitro findings confirm that the inhibitory effect on prostaglandin synthetase resides in the (*S*)-(+)-isomer. The (*R*)-(−)-isomer was practically ineffective as an inhibitor of prostaglandin synthesis. It is thus evident that the (*R*)-(−)-isomer is practically inert and that the pharmacological effects of the racemic mixture are largely due to the (*S*)-(+)-isomer. The same highly stereospecific anti-inflammatory effect was observed for several pairs of enantiomers of α-methyl-uryl-acetic acids and for (+)-naproxen (Harrison et al 1970; Shen 1972). For other NSAID, such as ibuprofen, benoxaprofen and clindanac, evidence for an optical inversion mechanism in animals and man is reported (Adams et al 1976; Simmonds et al 1980; Tamura et al 1981). Our results indicate that only a small degree of inversion of the (*R*)-(−)- to the (*S*)-(+)-enantiomer occurs in the rat and mouse.

REFERENCES

Adams, S. S., Bresloff, P., Mason, C. G. (1976) J. Pharm. Pharmacol. 28: 256–257

Boris, A., Stevenson, R. H. (1965) Arch. Int. Pharmacodyn. 153: 205

Bruni, G., Groppi, W., Fanfani, A., Sacchetti, G. (1980) Clinics in Rheumatic Diseases vol. 6, no 3, chapter 2

Buttinoni, A., Cuttica, A., Franceschini, J., Mandelli, V., Orsini, G., Passerini, N., Turba, C., Tommasini, R. (1973) Arzneim.-Forsch. (Drug Res.) 23: 1100–1107

Ceserani, R., Colombo, M., Mandelli, V. (1979) Prostaglandins Med. 2: 337–342

De Munari, S., Marazzi, G., Forgione, A., Longo, A., Lombardi, P. (1980) Tetrahedron Lett. 21: 2273–2276

Finney, D. J. (1952) Statistical Method in Biological Assay, C. Griffin, London, p. 222

Furchgott, R. F. (1972) Handbook of experimental Pharmacology, Berlin Springer Vol 23: 283–335

Greig, M. E., Griffin, R. L. (1975) J. Med. Chem. 18: 112–116

Ham, E. A., Cirillo, V. J., Zanetti, M., Shen, T. Y., Kuehl, F. A. (1972) in: Ramwell, P. W. (ed.) Proc. ALZA Conf. 1971: Prostaglandins in cellular biology and the inflammatory processes. New York, Plenum, pp 345–352

Harrison, T. I., Lewis, B., Nelson, P., Rooks, W., Roszkowski, A., Tomolonis, A., Fried, J. H. (1970) J. Med. Chem. 13: 203

Shen, T. Y. (1967) Ann. Rep. Med. Chem. p. 215

Shen, T. Y. (1972) Angew Chem. Internat. Edit. vol. II (no 6) p. 460

Siegmund, E., Cadmus, R. (1957) Proc. Soc. Exp. Biol. Med. 95: 729

Simmonds, R. G., Woodage, T. J., Duff, S. M., Green, N. (1980) Eur. J. Drug Metab. Pharmacokinet. 5: 169–172

Takeguchi, C., Sih, C. J. (1972) Prostaglandins 2: 169–184

Tamura, S., Kuzuna, S., Kawai, K., Kishimoto, S. (1981) J. Pharm. Pharmacol. 33: 701–706

Tomlinson, R. V., Ringold, H. J., Qureshi, M. C., Forchielli, E. (1972) Biochem. Biophys. Res. Commun. 46: 552–558

Tosolini, G. P., Moro, E., Forgione, A., Ranghieri, M., Mandelli, V. (1974) J. Pharm. Sci. 63: 1072–1076

Wechter, W. J., Loughead, D. G., Reischer, R. J., Van Giessen, G. J., Kaiser, D. G. (1974) Biochem. Biophys. Res. Commun. 61: 833–837

Winter, C. A., Risley, E. A., Nuss, G. W. (1963) J. Pharm. Exp. Ther. 141: 369