International Journal of Pharmaceutics, 51 (1989) 259–261
Elsevier

IJP 01777

Studies on curcumin and curcuminoids. XVI. Effect of curcumin analogs on hyaluronic acid degradation in vitro

Hanne Hjorth Tønnesen

Institute of Pharmacy, University of Oslo, Oslo (Norway)

(Received 21 November 1988)
(Accepted 1 December 1988)

Key words: Curcumin analog; Catalytic effect; Hyaluronic acid degradation; Hydroxyl radical

Curcumin is a naturally occurring compound with possible anti-inflammatory activity (Mukopadhyay et al., 1982; Rao et al., 1982; Deodhar et al., 1980; Satoskar et al., 1986; Yegnanarayan et al., 1976; Srimal and Dhawan, 1973; Arora et al., 1971; Sharma and Chandra, 1987; Ghatak and Basu, 1972). In a previous work (Tønnesen, 1989) the action of pure, synthetic curcumin on the depolymerization of hyaluronic acid in vitro was investigated. Anti-inflammatory drugs are postulated to act partly by prevention of the synovial fluid degradation, which might be ascribed to a quenching effect on free radicals (Puig-Parellada and Planas, 1978). In vitro results indicate that the viscosity of hyaluronic acid is reduced in the presence of free radicals (Greenwald and Moy, 1980; McCord, 1974). Hyaluronic acid degradation studies could therefore be useful in the evaluation of reaction mechanisms for anti-inflammatory drugs.

Curcumin was shown to have a catalytic effect on the depolymerization of hyaluronic acid under the given conditions (Tønnesen, 1989). The effect of curcumin was inhibited by addition of a hydroxyl radical quencher (mannitol). These results indicate that curcumin affects the formation of hydroxyl radicals in the depolymerization process.

Commercially obtained curcumin isolated from the rhizomes of the plant *Curcuma longa* L. (turmeric) contains, in addition to curcumin, about 6% of demethoxycurcumin and about 0.3% of bisdemethoxycurcumin (Tønnesen, unpublished work). Experiments made on animals indicate that the anti-inflammatory effect of pure, synthetic curcumin is less than what is observed when commercially available curcumin is used (Srimal and Dhawan, personal communication). This might be ascribed to a synergistic effect of the three curcumin analogs in vivo. For further evaluation of the reaction mechanism of curcumin as a potential anti-inflammatory drug, hyaluronic acid degradation studies in vitro were performed with pure, synthetic demethoxy- and bis-demethoxycurcumin and with commercially obtained curcumin.

The curcumin analogs were synthesized according to the method of Pabon (1964). Commercially available curcumin was provided by Chr. Hansen's Laboratories, Denmark. The experimental conditions were described previously (Tønnesen, 1989). As a superoxide source hypoxanthine/xanthine oxidase was used (Greenwald, 1986). The effect of oxygen radicals on hyaluronic acid can be followed directly by monitoring the viscosity of the solution as a function of time.

The solubility of the curcuminoids in buffer at pH = 7.3 is low. Saturated solutions of the

Correspondence: H.H. Tønnesen, Institute of Pharmacy, University of Oslo, P.O. Box 1068, Blindern, 0316 Oslo 3, Norway.

TABLE 1

Effect of the curcuminoids

Reaction time	RS + $FeCl_3$	RS	RS + pure demethoxy-curcumin	RS + bisdemethoxy-curcumin	RS + pure curcumin	RS + commercially obtained curcumin
1.5 min	4.6%	3.2%	4.6%	4.6%	7.4%	3.2%
5.0 min	6.0%	3.2%	7.4%	7.4%	7.4%	6.0%
10.0 min	7.4%	4.6%	7.4%	7.4%	64.9%	6.0%
15.0 min	64.9%	7.4%	7.4%	7.4%	66.3%	7.4%
20.0 min	67.7%	7.4%	66.3%	64.9%	66.3%	7.4%

Depolymerization of hyaluronic acid in the hypoxanthine/xanthine oxidase system demonstrated by the reduction of specific viscosity (%) as a function of time. RS, reaction substrate.

curcumin analogs were used in these experiments. The solubility of the curcuminoids in the solvent was determined by means of HPLC (Tønnesen and Karlsen, 1983) after extraction of the saturated samples with ethyl acetate. The results are given in Tables 1 and 2. During the depolymerization of hyaluronic acid, enzymatic generation of superoxide by xanthine/xanthine oxidase is followed by dismutation of superoxide which leads to formation of hydrogen peroxide. If iron is present in the medium, hydroxyl radicals will be formed by the iron catalysed Haber–Weiss reaction (Halliwell and Gutteridge, 1985). The depolymerization of hyaluronic acid caused by free radicals will then consist of two steps. This is clearly demonstrated in Table 1. In the presence of iron, a reduction in specific viscosity of the reaction substrate of 7.4% is observed after 10 min under the given conditions due to the generation of superoxide by hypoxanthine/xanthine oxidase. After 20 min, a reduction in viscosity of 67.7% is observed due to the following formation of hydroxyl radicals. Without addition of iron to the reaction substrate, the reduction in specific viscosity does not exceed 7.4% after 20 min reaction time.

As previously shown, pure curcumin has a catalytic effect on the depolymerization of hyaluronic acid in this system (Tønnesen, 1989). The results indicate that the catalytic effect of this compound on hyaluronic acid depolymerization affect the second step of the oxygen radical formation. To investigate further this postulation, the reactions in the following experiment were performed without any addition of iron. If the curcuminoids affect the formation of hydroxyl radicals, the change in specific viscosity of the samples should reach about 67% independent of iron present. This proved to be the case for the 3 curcuminoids when tested separately, although the reaction seems to proceed somewhat slower with demethoxycurcumin and bisdemethoxycurcumin compared to curcumin (Table 1). Commercially obtained curcumin, however, did not seem to influence the formation of hydroxyl radicals, as the change in specific viscosity reached only 7.4% after 20 min. The results obtained can not easily be related to the observed differences in solubility of the curcumin analogs in the system used (Table 2).

To explain the observations made in this experiment, further investigations on curcumin and the curcuminoids as potential sources of oxygen radicals have to be carried out. The different behaviour of pure samples compared to a mixture of the curcuminoids, however, might partly be related to the difference in anti-inflammatory activity observed with the same compounds in vivo (Srimal and Dhawan, personal communication).

TABLE 2

Solubility of the curcuminoids in 0.05 M phosphoric acid buffer, pH = 7.3, with addition of 0.2 mM EDTA

Sample	Solubility
Curcumin	0.0004 mg/ml
Demethoxycurcumin	0.0015 mg/ml
Bisdemethoxycurcumin	0.000032 mg/ml

References

Arora, R.B., Basu, N., Kapoor, V. and Jain, A.P., Anti-inflammatory studies on *Curcuma longa* (turmeric). *Indian J. Med. Res.,* 59 (1971) 1289–1295.

Deodhar, S.D., Sethi, R. and Srimal, R.C., Preliminary study on antirheumatic activity of curcumin (diferuloyl methane). *Indian J. Med. Res.,* 71 (1980) 632–634.

Ghatak, N. and Basu, N., Sodium curcuminate as an effective anti-inflammatory agent. *Indian J. Exp. Biol.,* 10 (1972) 235–236.

Greenwald, R.A. and Moy, W.W., Effect of oxygen-derived free radicals on hyaluronic acid. *Arth. Rheum.,* 23 (1980) 455–463.

Greenwald, R.A., *Handbook of Methods for Oxygen Radical Research.* CRC, FL 1986.

Halliwell, B. and Gutteridge, J.M.C., *Free Radicals in Biology and Medicine,* Clarendon, Oxford 1985.

McCord, J.M., Free radicals and inflammation: protection of synovial fluid by superoxide dismutase. *Science,* 185 (1974) 529–530.

Mukhopadhyay, A., Basu, N., Ghatak, N. and Gujral, P.K., Anti-inflammatory and irritant activities of curcumin analogues in rats, *Agents Actions,* 12 (1982) 508–515.

Pabon, H.J.J., A Synthesis of curcumin and related compounds. *Rec. Trav. Chim. Pays-Bas,* 83 (1964) 379–386.

Puig-Parellada, P. and Planas, J.M., Synovial fluid degradation induced by free radicals. In vitro action of several free radical scavengers and anti-inflammatory drugs. *Biochem. Pharm.,* 27 (1978) 535–537.

Rao, T.S., Basu, N., Siddiqui, H.H., Anti-inflammatory activity of curcumin analogues. *Indian J. Med. Res.,* 75 (1982) 574–578.

Satoskar, R.R., Shah, S.J., Shenoy, S.G., Evaluation of anti-inflammatory property of curcumin (diferuloyl methane) in patients with postoperative inflammation. *Int. J. Clin. Pharmacol. Ther. Toxicol.,* 24 (1986) 651–654.

Sharma, K.K. and Chandra, S., Synthesis and antiarthritic study of a new orally active diferuloyl methane (curcumin) gold complex. *Inorg. Chim. Acta,* 135 (1987) 47–48.

Srimal, R.C. and Dhawan, B.N., Pharmacology of diferuloyl methane (curcumin), a non-steroidal anti-inflammatory agent. *J. Pharm. Pharmacol.,* 25 (1973) 447–452.

Tønnesen, H.H. and Karlsen, J., High-performance liquid chromatography of curcumin and related compounds. *J. Chromatogr.,* 259 (1983) 367–371.

Tønnesen, H.H., Studies on curcumin and curcuminoids. XIV. Effect of curcumin on hyaluronic acid degradation in vitro. *Int. J. Pharm.,* 50 (1989) 91–95.

Yegnanarayan, R., Saraf, A.P. and Balwani, J.H., Comparison of anti-inflammatory activity of various extracts of *Curcuma longa* (L.). *Indian J. Med. Res.,* 64 (1976) 601–608.