African Journal of Biotechnology Vol. 8 (15), pp. 3610-3614, August, 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1996-0786 © 2009 Academic Journals

Full Length Research Paper

Bioconversion of meloxicam by bacteria

G. Shyam Prasad*, K. Narasimha Rao, S. Girisham and S. M. Reddy

Department of Microbiology, Kakatiya University, Warangal- 506009, Andhra Pradesh, India.

Accepted 26 January, 2009

Bioconversion of an anti-inflammatory drug meloxicam was investigated by employing twelve bacterial strains representing six genera. Among the bacterial cultures screened, *Bacillus subtilis* MTCC 441 and *Pseudomonas putida* NCIM 2782 could transform meloxicam to 5-hydroxy methyl meloxicam. Whereas, other bacterial cultures under study failed to transform meloxicam. The progress of the transformation was confirmed by HPLC, and based on LC-MS data and previous reports, the metabolite was predicted to be 5-hydroxy methyl meloxicam.

Key words: Biotransformation, meloxicam, 5-hydroxy methyl meloxicam, bacteria.

INTRODUCTION

Meloxicam (4-hydroxy-2-methyl-N-(5-methyl-2-thiazolyl)-2H-1,2-benzothia zine-3-carboxamide-1,1-dioxide) is a non steroidal anti-inflammatory drug (NSAIDS) with a selective inhibition of cyclooxygenase-2(Cox-2) (Noble et al., 1996; Mitchell et al., 1993). It is effective in the treatment of rheumatoid arthritis (Reginster et al., 1996) and osteoarthritis (Hosie et al., 1996) and appears to be well tolerated due to its preferential inhibition of cyclo-oxygenase (cox-2) (Engelhardt et al., 1995). Some of the reported side effects of the meloxicam include signs of bleeding, allergic reactions, blurred vision, difficulty in swallowing, severe heart burning, pain in throat, pain or difficulty passing urine, stomach pain, swelling of feet or ankles, unexplained weight gain or edema, yellowing of eyes, of skin, diarrhea, dizziness, gas or heart burn, nausea or vomiting (Mobic, Boehringer Ingelheim, Taiwan). Meloxicam is practically insoluble in water. The poor solubility and wettability of meloxicam leads to poor dissolution and thereby variation in bioavailability. Thus increasing the aqueous solubility and dissolution of meloxicam is of paramount therapeutic importance (Guruswamy et al., 2006).

Biotransformation is a process involving the use of boil-ogical agents as catalysts to perform transformations of chemical compounds and is useful technique for producing medicinal and agricultural chemicals from both active and inactive products. Biotransformation proce-sses that involve enzymatic or microbial biocatalysts, when compared to their chemical counterparts, offer the advantages of high regioselectivity, stereo specificity and mild operating conditions. Microbial transformation is one of the most attractive approaches for introducing func-tional groups into various positions of organic compounds (Smith, 1984). Microbial transformation is one of the simplest and most direct methods for the preparation of a range of optically active compounds of moderate to high enantiomeric purity.

Therefore the present paper deals with the biotrans-formation of meloxicam by using bacteria with an aim to produce novel metabolites which possess increased solu-bility and efficacy than that of the parent compound meloxicam.

MATERIALS AND METHODS

Chemicals

Meloxicam was gifted by Unichem laboratories, Mumbai, India. Methanol of HPLC grade was obtained from Ranbaxy, New Delhi, India. Peptone, yeast extract, potato dextrose agar, glucose and all other chemicals of highest available purity were obtained from Himedia, Mumbai, India.

*Corresponding author. E-mail: shyamprasad1919@yahoo.com. Phone: +91 970 503 7655.

Table 1. Biotransformation of meloxicam by bacteria.

Bacteria	Metabolite formed (%)	
	5-hydroxy methyl meloxicam	Meloxicam remaining
Bacillus cereus NCIM 2155	0.00	100
Bacillus subtilis MTCC 619	0.00	100
Bacillus subtilis MTCC 441	27.12	72.88
Bacillus subtilis NCIM 2162	0.00	100
Enterobacter aerogenes NCIM 2695	0.00	100
Escherichia coli MTCC 118	0.00	100
Klebsiella aerogenes NCIM 2258	0.00	100
Klebsiella pneumoniae MTCC 109	0.00	100
Proteus mirabilis NCIM 2241	0.00	100
Proteus vulgaris NCIM 2813	0.00	100
Pseudomonas aeruginosa NCIM 2074	0.00	100
Pseudomonas putida NCIM 2782	41.15	58.85

Microorganisms

The bacterial cultures were procured from National Collection of Industrial Microorganisms (NCIM), Pune or Microbial Type Culture Collection (MTCC) Chandigarh. The procured cultures include *Bacillus cereus* NCIM 2155, *B. subtilis* MTCC 619, *B. subtilis* MTCC 441, *B. subtilis* NCIM 2162, *Enterobacter aerogenes* NCIM 2695, *Escherichia coli* MTCC 118, *Klebsiella aerogenes* NCIM 2258, *K. pneumoniae* MTCC 109, *Proteus mirabilis* NCIM 2241, *P. vulgaris* NCIM 2813, *Pseudomonas aeruginosa* NCIM 2074 and *P. putida* NCIM 2782. Stock cultures were maintained on nutrient agar slants at 4°C and sub cultured for every 3 months.

Biotransformation

Biotransformation was performed using a two-stage fermentation protocol. In the first stage, fermentation was initiated by inoculating a 250 ml culture flask consists of 50 ml of liquid broth. The liquid broth used contains (per litre) peptone (5.0 g), beef extract (3.0 g), yeast extract (5.0 g) and sodium chloride (5.0 g). The pH of the broth was adjusted to 6.0 with 0.1 N HCl or 0.1 N NaOH. The prepared media was autoclaved and cooled to room temperature. The media was inoculated with a loopful of culture obtained from freshly grown nutrient agar slants. The flasks were incubated at 120 rev/min and 37°C for 24 h. Second stage cultures were initiated in the same media using an inoculum of 1 ml of first stage culture per 20 ml of medium in a 100-ml culture flask. The second stage cultures were incubated for 24 h and the substrate meloxicam in dimethyl formamide was added to give a final concentration of 20 mg/l. The flasks were incubated under similar conditions for 2 days. Culture controls consisted of a fermentation blank in which the microorganism was grown under identical conditions and no substrate was added. Substrate controls comprised of meloxicam added to the sterile medium were incubated under similar conditions. Each culture was studied in triplicate. The cultures were extracted with three volumes of ethyl acetate, the combined organic extracts were evaporated using a rotary vacuum evaporator and dried over a bed of sodium sulfate. The resultant residues were analyzed by HPLC and LC-MS for identification of metabolites.

Analysis

The transformation was identified by HPLC analysis according to the method described by Elbary et al. (2001) with a slight modification. The samples were analyzed using an LC-10AT system (Shimadzu, Japan) by injecting 20 µl of sample into the syringe-loading sample injector (Model 7725i, Rheodyne, USA). The column used was Wakosil II, C18, 250 x 4.6 mm and 5 µm (SGE, Australia). The mobile phase consisted of a mixture of methanol-water (pH adjusted to 3.0 with orthophosphoric acid) in the ratio of 60:40. The analysis was performed isocratically at a flow rate of 1 ml/min and the analytes were detected at 364 nm using a photodiode array detector (Model SPD M10Avp, Shimadzu, Japan). LC-MS analysis was carried out using a Waters system, column X Terra C18, 25 x 0.46 cm, 5 µm and a mobile phase consisting of methanol and water (pH adjusted to 3.0 with formic acid) in 60:40 ratios. The ESI detection was set to positive mode. A temperature of 300°C and a scan range of 50 - 500 were set for the analysis. The transformed compounds were identified from the masses of the fragmentation products obtained.

RESULTS AND DISCUSSION

In order to find the bacteria capable of performing biotransformation of meloxicam, twelve bacterial strains representing six genera were tested. Among the three strains of *B. subtilis* screened, only one strain *B. subtilis* MTCC 441 could transform meloxicam to 5-hydroxy methyl meloxicam (Table 1). While, *B. subtilis* MTCC 619, *B. subtilis* NCIM 2162 failed to transform meloxicam. Whereas, *B. cereus* NCIM 2155 studied under investigation also failed to transform meloxicam. Among the two *Pseudomonas* species tested, 5-hydroxy methyl meloxicam was detected in culture broth of *P. putida* NCIM 2782 while *P. aeruginosa* NCIM 2074 could not transform meloxicam. Two *Klebsiella* species, *K. aerogenes* NCIM

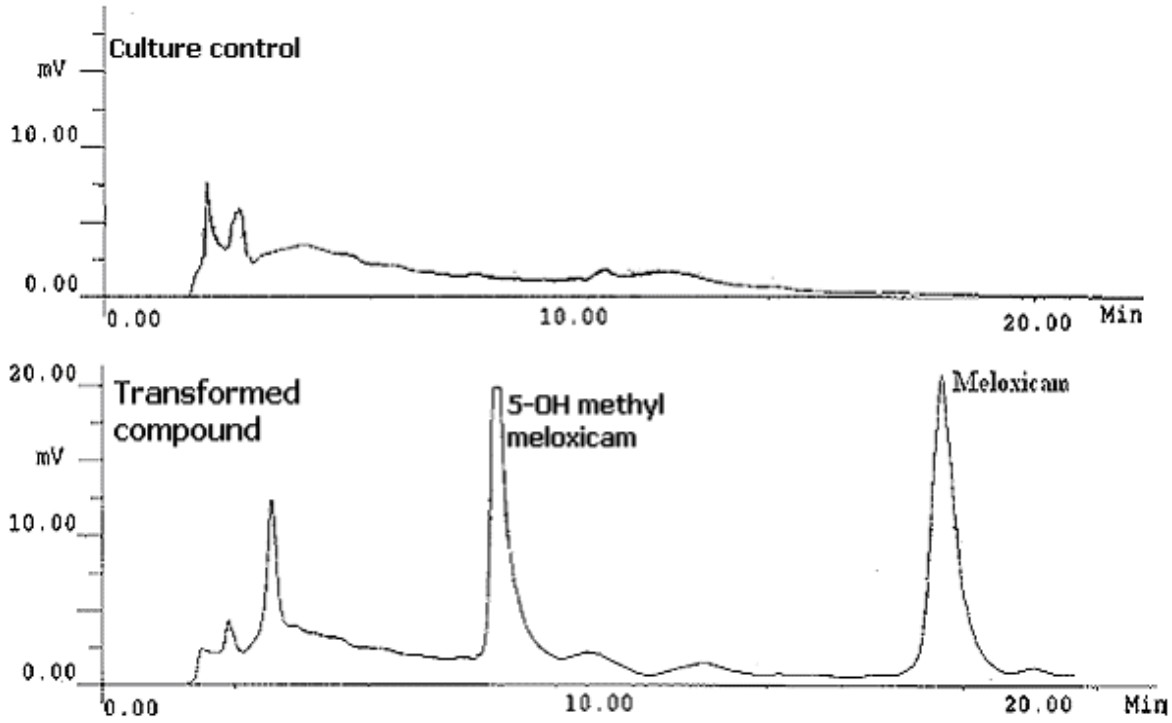

Figure 1. HPLC chromatograms showing culture control and transformed compounds obtained in *Pseudomonas putida*.

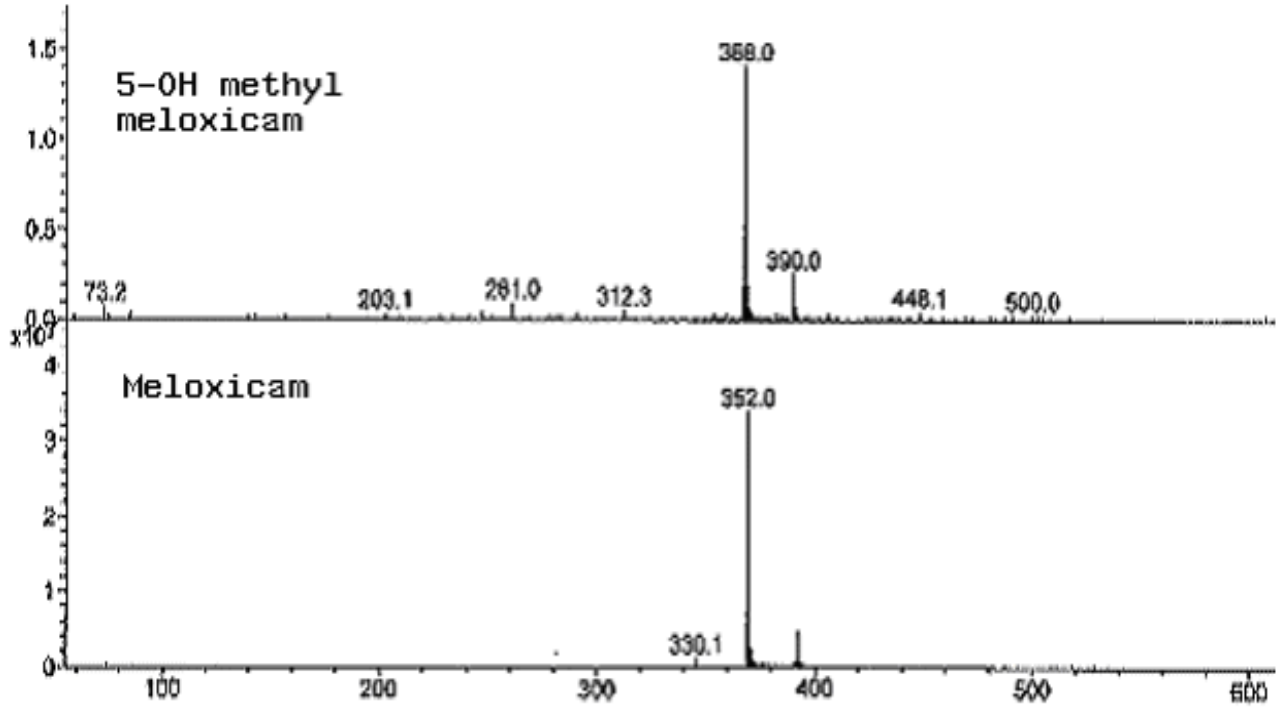

Figure 2. LC-MS spectra of metabolites detected in meloxicam fed culture broth of *Pseudomonas putida*.

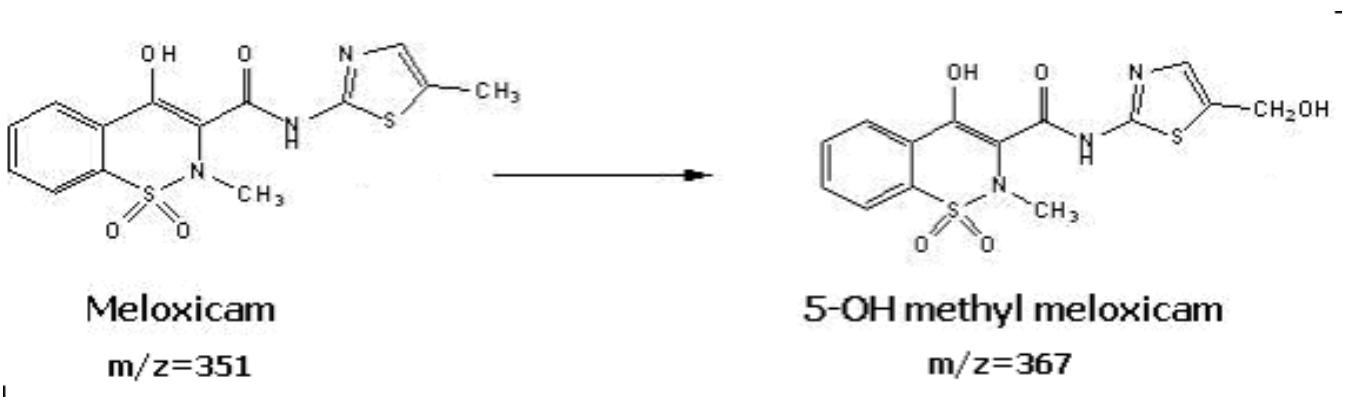

Figure 3. Proposed metabolic pathway of meloxicam in culture broth of *Pseudomonas putida*.

2258, *K. pneumoniae* MTCC 109 screened under the study could tolerate and grow in the medium containing meloxicam without performing any transformation. Similarly, the two *Proteus* species; *Pr. vulgaris* NCIM 2813 and *Pr. mirabilis* NCIM 2241 also failed to transform meloxicam. The rest of the bacteria under investigation, *E. coli* MTCC 118, *En. aerogenes* NCIM 2695 could not perform any transformation of meloxicam (Table 1).

The metabolites formed were identified basing on observation of new peaks in HPLC (Figure 1) and characterized with the help of the mass values of fragmentation ions obtained in LC-MS analysis. Mass spectrometric analysis of the metabolite showed a molecular ion at m/z 368 (an increase of 16 units) indicating addition of single oxygen atom to meloxicam which results in formation of 5-hydroxymethyl meloxicam (Figure 2). These analyses indicated that the metabolite is 5-hydroxy methyl meloxicam (9.78 min) where the substrate meloxicam was eluted at 19.10 min in HPLC analysis. The metabolites were quantified based on the peak areas obtained in HPLC analysis taking the drug and metabolites peak areas together as 100%. The pathway of the metabolite formation is shown in Figure 3.

Present studies on the transformation of meloxicam revealed that *B. subtilis* MTCC 441 and *P. putida* NCIM 2782 could transform meloxicam to 5-hydroxy methyl meloxicam while, rest of the cultures under investigation failed to transformation meloxicam. However, these cultures produced visible growth indicating the non-toxicity of meloxicam.

The pharmacokinetics of meloxicam have been investigated in a number of animal species, including mice, rats, dogs, mini-pigs, and baboons, to provide comprehensive profiles and to determine which animal species exhibits a profile most closely resembling the pharmacokinetic profile in humans. The main metabolites of meloxicam in humans, rats, mice, and mini-pigs were a 5-hydroxymethyl derivative and a 5-carboxy derivative. Other metabolites have also been detected in rats and humans (Schmid et al., 1995a, b); the major metabolite is (5-methyl-2-thiazolyl) aminooxoacetic acid, which is typical for oxicams (Woolf and Radulovic, 1989).

In the present investigation, it is interesting to note that the transformation of meloxicam was found to be oxidative in nature and the metabolite 5-hydroxy methyl meloxicam detected in bacteria was also observed among all other experimental animals studied, which states the presence of similar enzyme system in microorganisms. However, further investigations are needed to confirm the enzyme system involved in production of 5-hydroxy methyl meloxicam in bacteria.

In our search to find and develop an efficient microbial conversion of meloxicam in an effort to obtain novel derivatives with increasing activity or differing pharmacological properties, *P. putida* NCIM 2782 and *B. subtilis* MTCC 441 could oxidize meloxicam while *B. subtilis* MTCC 619, *E. coli* MTCC 118 and *K. pneumoniae* MTCC 109, *En. aerogenes* NCIM 2695, *K. aerogenes* NCIM 2258 and *P. aeruginosa* NCIM 2074 could not perform any transformation of meloxicam.

Conclusion

From the present study it is concluded that meloxicam could be transformed to 5-hydroxy methyl meloxicam by employing bacteria. However, a detailed parametric study is needed to optimize the conditions necessary for production of novel metabolites in large quantities by exclusively directing the transformation towards increased activity or decreased toxicity.

ACKNOWLEDGEMENTS

The authors are thankful to Head, Department of Microbiology, Kakatiya University for providing necessary facilities and University Grants Commission, New Delhi for financial assistance.

REFERENCES

Elbary AA, Foda N, Elkhateeb M (2001). Reversed phase liquid chromatographic determination of meloxicam in human plasma and its pharmacokinetic application. Anal. Lett. 34:1175-1187.

Engelhardt G, Homma D, Chilegel K, Utzmann R, Schnitzler C (1995). Anti-inflamatory, analgesic, anti pyretic and related properties of meloxicam, a new non-steroidal anti-inflammatory agent with favourable gastrointestinal tolerance. Inflammation. Res. 44: 423-433.

Guruswamy S, Kumar V, Mishra DN (2006). Preparation and evaluation of solid dispersion of meloxicam with skimmed milk. Pharm. Soc. Japan. 126: 93-97.

Hosie J, Distel M, Bluhmki E (1996). Meloxicam in osteoarthritis: a 6-month double blind comparision with dielofenac sodium. Br. J. Rheumatol. 35:39-43.

Mitchell JA, Akarasereenont P, Thiemumann C, Flower RJ, Vane JR (1993). Selectivity of non-steroidal anti-inflammatory drugs as inhibitors of constitutive and inducible cyclooxygenase. Proc. Natl. Acad. Sci. USA 90: 693-697.

Mobic ®. Product Information, Boehringer Ingelheim Taiwan Ltd., Taiwan. Noble S, Balfour JA (1996). Meloxicam. Drugs, 51: 424-430.

Reginster JY, Distel M, Bluhmki E (1996). A double blind three-week study to compare the efficacy and safety of meloxicam 7.5 mg and meloxicam 15 mg in patients with rheumatoid arthritis. Br. J. Rheumatol. 35:17-21.

Schmid J, Busch U, Henizel G, Bozler G, Kasehke S, Kummer M (1995a). Meloxicam pharmacokinetics and metabolic pattern after intravenous infusion and oral administration to healthy subjects. Drug Metab. Dispos. 23:1206-1213.

Schmid J, Busch U, Trummitz G, Prox A, Kaschke S, Wachsmuth (1995b). Meloxicam: metabolic profile and biotransfomation products in the rat. Xenobiotica. 25:1219-1236.

Smith LL (1984). Steroids. In-Biotechnology, ed. H.J. Rehm, G. Reed, Vol. 6a, Chrmie GmbH, Weinheim, pp. 31-78.

Woolf TF, Radulovic LL (1989). Oxicams: Metabolic disposition in man and animals. Drug Metab. Rev. 21: 255-276.