METABOLIC PROFILE OF NITROFLURBIPROFEN (HCT 1026) IN RAT PLASMA AND BRAIN: A LC-MS STUDY

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The nitrooxybutyl ester derivative of flurbiprofen (HCT 1026), a new nitric oxide-releasing non steroidal anti-inflammatory drug (NO-NSAIDs), has been recently demonstrated to attenuate the extent of lipopolysaccharide-induced brain inflammation in rats with a potency comparable to that of flurbiprofen but with a markedly reduced gastrointestinal toxicity. Since it is not known whether HCT1026 exerts its anti-inflammatory activity within the brain as such or via its main metabolic product flurbiprofen, the aim of this work was to develop an analytical methodology (a combination of LC-UV-DAD with LC-MS/MS techniques) for identification and quantitation of HCT1026 and its potential metabolic species in brain and blood of rats after acute administration of the drug. Male rats were p.o. or i.p. treated with HCT 1026 (15 and 100 mg/Kg respectively) and plasma and brain levels of the parent drug and its potential metabolites (HCT 1027 and flurbiprofen) determined at different times post-dosing by a validated HPLC method (UV-DAD detection) at 0.13 nmoles/ml (plasma) and 0.3 nmoles/g (brain tissue) levels. Structure confirmation of the analytes was achieved by MS monitoring of their deprotonated (negative ion mode) or cationized/protonated (positive ion mode) molecular ions and of the relative fragment ions obtained by collision-induced dissociation (CID) experiments. Following the p.o. or the i.p. routes, flurbiprofen is the only metabolite found at measurable levels in both plasma and brain, while HCT 1026 or its denitrated metabolite HCT 1027 were always below the limit of detection at all the observation times. This indicates that HCT 1026 in the brain exerts its anti-inflammatory activity through its main metabolic product flurbiprofen and indirectly that the bulk of NO molecule is released from the parent drug prior to entry into the brain.