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IN VITRO METABOLISM OF THE NITRIC OXIDE-DONATING DERIVATIVE OF ASPIRIN (NCX 4016) BY RAT LIVER: LC AND LC-MS STUDIES

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The metabolism of the nitroderivative of aspirin (NCX 4016), the lead compound of a new class of NO-NSAIDs, has been studied *in vitro* in rat liver subcellular fractions (S 9,000 *g*, microsomes, cytosol). After extraction and purification, samples were analyzed by a newly developed reverse-phase HPLC assay that allows the simultaneous determination of NCX 4016 and its metabolites. In the S 9,000 *g* fraction, NCX4016 undergoes rapid metabolization, with the formation of salicylic acid (SA) and [3-(nitrooxymethyl)phenol] (HBN). HBN is then rapidly metabolised to 3-hydroxybenzylalcohol (HBA), and mainly to a metabolic species, identified by LC-MS/MS analysis (Electrospray ionisation) as 1-(glutathion-S-yl)methylene-3-hydroxy-benzene, a conjugated product between GSH and the benzyl carbon atom of HBN, with the displacement of ONO_2^-. In rat liver cytosol HBN is completely metabolised to this thioether adduct within 30 min incubation; the process is enzymatically mediated by GSH transferase and strictly dependent on GSH. This new metabolic pathway, which seems specifically linked to the chemical structure of the spacer of NCX4016, is quite unusual and can be considered alternative to that commonly proposed for organic nitrates used in cardiovascular therapy (i.e. nitroglycerin), where the nitrate ester enzymatically trans-esterificate GSH with formation of $GSNO_2$ and of the corresponding alcohol. Studies are in progress to evaluate the hepatic metabolic profile of NCX4016 in human liver subcellular fractions, to verify whether the formation of the conjugated product with GSH takes place also in man.