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NITRIC OXIDE RELEASE AND DISTRIBUTION FOLLOWING ORAL ADMINISTRATION OF NITROASPIRIN (NCX4016) IN THE RAT

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The metabolic fate of nitric oxide (NO) released from nitroaspirin, benzoic acid, 2-(acetyloxy)-3-[(nitrooxy)methyl]phenyl ester (NCX4016), the lead compound of a new class of NO-releasing non steroidal anti-inflammatory drugs (NO-NSAIDs) has been studied in the rat following p.o. administration of 100 mg/Kg, by monitoring in blood and plasma the bioactive storage forms of NO (nitrosylhemoglobin and nitrosothiols) and its oxidation products (nitrites/nitrates). In parallel plasma samples were analyzed for unchanged drug and metabolites by reverse-phase HPLC. Electron Spin Resonance spectroscopy (ESR) was applied for the detection/quantitation of nitric oxide (NO) as nitrosylhemoglobin HbFe(II)NO: the paramagnetic complex was detected at 100 K in the venous blood of the rat (microwave power 20 mW) and characterized by a three-lines hyperfine structure with coupling constants (A_x and A_z) of 17 G at g_x = 2.066 and g_z = 2.009. The concentration of HbFe(II)NO was determined by double integration of the signal, using Cu^{2+}-EDTA as standard, and the limits of detection (LOD) and quantitation (LOQ) were 0.2 µM and 0.5 µM respectively. Plasma levels of nitrite/ nitrate (NOx) and S-nitrosothiols (RS-NO) were determined by an ozone-based chemiluminescent assay with a Nitric Oxide Analyzer. Plasma samples (1 ml aliquots) were deproteinized with cold ethanol and centrifuged (14,000 rpm; 5 min): 10 µl aliquots were used for NOx and 200 µl for RS-NO determination. The calibration curves were prepared by adding known amounts of sodium nitrate or nitrosoglutathione to plasma blanks in the concentration ranges 1-100 µM and 0.05-5.0 µM (LOQs 0.3 µM for NOx and 0.06 µM for RS-NO). No unchanged drug is observed in the 0-24 h interval post-dosing, but a linear increase of salicylic acid (plateau at the 6^{th} h). Nitrosylhemoglobin is detectable at 1 h, peaks between 4-6 h post-administration (no detectable levels at 24 h) and the time-course of its formation parallels that of plasma NOx and RS-NO.