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Evaluation of the antitumoral potential of different nitric oxide-donating non-steroidal anti-inflammatory drugs (NO-NSAIDs) on human urological tumor cell lines

Sandra Huguenin[a], Francis Vacherot[a], Jocelyne Fleury-Feith[a,b], Jean-Pierre Riffaud[c], Dominique K. Chopin[a,*], Manlio Bolla[c], Marie-Claude Jaurand[a]

[a]*Groupe de recherche INSERM E 03-37, Oncogenèse des Tumeurs Respiratoires et Urogénitales, Faculté de Médecine, 8, Rue du Général Sarrail, 94010 Créteil Cedex, France*
[b]*Service d'Histologie et de Biologie Tumorale, Hôpital Tenon, Rue de la Chine, 75020 Paris, France*
[c]*NicOx S.A., 2455 route des Dolines, 06906 Sophia-Antipolis Cedex, France*

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Abstract

Our work aimed at identifying the antitumoral potential of new nitric oxide (NO)-releasing non-steroidal anti-inflammatory drug (NSAID) derivatives on human prostate and bladder carcinoma cell lines. Among all molecules tested, two sulindac derivatives, NCX 1102 ((Z)-5-fluoro-2-methyl-1-[[4-(methylsulfinyl)phenyl] methylene]-1H-indene-3-acetic acid 4-(nitrooxy) butyl ester) and NCX 1105 ((Z)-5-fluoro-2-methyl-1-[[4-(methylsulfinyl)phenyl] methylene]-1H-indene-3-acetic acid 6-(nitrooxymethyl)-2-methylpyrydyl ester hydrochloride), were the most cytotoxic compounds. In contrast to its parent molecule sulindac, cell cycle analysis showed that NCX 1102 led to cell accumulation in the G2-M transition stage in all cell lines, and induced apoptosis in five out of the six cell lines. Thus, NO-NSAIDs may be useful for the elaboration of new therapeutic strategies in the management of bladder and prostate cancer.

Keywords: Bladder carcinoma; Prostate cancer; NO-NSAIDs; Cell cycle; Apoptosis; Nitric oxide

1. Introduction

Nitric oxide (NO)-donating non-steroidal anti-inflammatory drugs (NO-NSAIDs) represent a new class of compounds showing at least equivalent or enhanced anti-inflammatory, anti-pyretic, and analgesic potential compared to classical NSAIDs [1], but with less side effects on the gastrointestinal (GI) tract [2] and kidneys [3], due to the protective action of NO [4].

NO-NSAIDs are also antitumoral agents, combining the cyclooxygenase (COX) inhibiting property of their NSAID moiety with the antitumoral potential of their NO-donating part. NO is known to exert

* Corresponding author. Tel.: +33-1-49-81-3656; fax: +33-1-49-81-3533.
E-mail address: catherine.vaslin@im3.inserm.fr (D.K. Chopin).

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different effects on cancer cells, related to both direct and indirect ways of action. By direct action it can be cytostatic by inhibiting respiration (control of mitochondrial function), altering iron metabolism and interacting with several enzymes (e.g. caspases). On the other hand, interaction with oxygen derived free radicals may induce indirect effects by damaging cellular components. NO represents therefore a potential inductor of apoptosis in tumor cell, possibly through different mechanisms [5,6].

The antiproliferative and proapototic potential of NO-NSAIDs has already been demonstrated on colon cancer in vitro [7–9]. Moreover, their chemopreventive action was shown in vivo against the formation of aberrant colon crypt foci in a rat chemocarcinogenic model [10], and in the model of Min Mice [11].

As in colon cancer, the inducible isoform of cyclooxygenase (COX-2) is overexpressed in prostate and bladder cancers [12] and seems to be involved in the development and progression of these diseases [13,14]. Previous results on colon cancer and other tumoral cell lines [8] prompted us to test on prostate and bladder cancer cell lines the antitumoral potential of eight different NO-NSAIDs. These new chemical entities were obtained from six different NSAIDs (aspirin, indomethacin, sulindac, piroxicam, ibuprofen, and flurbiprofen) and their cytotoxic effects were compared using three human bladder carcinoma cell lines (T24, 647V, and 1207) and three human epithelial prostatic cell lines of varying transformation degrees (PNT1A, LNCaP, and PC3).

We discovered that NO-sulindac compounds were more active on the tested cell lines than all other derivatives. Moreover, NO-sulindac (NCX 1102: (Z)-5-fluoro-2-methyl-1-[[4-(methylsulfinyl)-phenyl] methylene]-1H-indene-3-acetic acid 4-(nitrooxy)butyl ester) was able to induce apoptosis in most cell lines and, depending on its concentration, to disturb the cell cycle of all cell lines. Finally, a new aspirin derivative, NCX 4040 (2-(acetyloxy)benzoic acid 4-(nitrooxymethyl)phenyl ester), revealed a much greater cytotoxic potential when compared to NO-sulindac. The goal of our study was to evaluate the potential of NO-NSAIDs as effective and safe compounds for the management of prostate and bladder cancers.

2. Materials and methods

2.1. Cell lines

The human bladder carcinoma cell lines T24 and 647V were obtained from the molecular biology department of University of California at Los Angeles and cell line 1207 was established in our laboratory. The human prostate cancer cell lines LNCaP and PC3 were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). Normal immortalized prostate epithelial cells PNT1A were a generous gift from Prof. O. Cussenot (Hôpital Tenon, Paris, France).

All cell lines were grown as monolayer in RPMI 1640 medium (Invitrogen, Cergy Pontoise, France) supplemented with 5% foetal bovine serum (FBS, Invitrogen) and 2 mM L-glutamine (Invitrogen). Cells were seeded at a density of 20,000 cells per cm^2 and incubated at 37 °C with 5% CO_2 and 95% relative humidity.

2.2. Reagents

Sodium nitroprusside (SNP) was purchased from (Sigma, Saint-Quentin Fallavier, France). The following compounds were provided by NicOx SA (Sophia-Antipolis, France): NO-aspirin [NCX 4040, 2-(acetyloxy)benzoic acid 4-(nitrooxymethyl)phenyl ester; NCX 2219, *trans*-3-[4-[2-acetyloxy-benzoyloxy]-3-methoxyphenyl]-2-propenoic acid 4-(nitrooxy)butyl ester]; NO-flurbiprofen [HCT 1026, 2-fluoro-α-methyl[1,1′-biphenyl]-4-acetic acid 4-(nitrooxy)butyl ester; NCX 2216, *trans*-3-[4-[2-fluoro-α-methyl-(1,1′-biphenyl)-4-acetyloxy]-3-methoxyphenyl]-2-propenoic acid 4-(nitrooxy)butyl ester]; NO-sulindac [NCX 1102, (Z)-5-fluoro-2-methyl-1-[[4-(methylsulfinyl)phenyl] methylene]-1H-indene-3-acetic acid 4-(nitrooxy)butyl ester; NCX 1105, (Z)-5-fluoro-2-methyl-1-[[4-(methyl-sulfinyl)phenyl] methylene]-1H-indene-3-acetic acid 6-(nitrooxymethyl)-2-methylpyrydyl ester hydrochloride]; NO-ibuprofen [NCX 2210, α-methyl-4-(2-methylpropyl)benzeneacetic acid 2-methoxy-4-[(1E)-3-[4-(nitrooxy)butyloxy]-3-oxo-1-propenyl] phenyl ester]; NO-piroxicam [NCX 1301, 4-hydroxy-2-methyl-N-2-pyridinyl-2H-1,2-benzothiazine-3-carboxamide 1,1-dioxide nitrate]; NO-indomethacin