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Research Paper

Repurposing Cationic Amphiphilic Antihistamines for Cancer Treatment

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ABSTRACT

Non-small cell lung cancer (NSCLC) is one of the deadliest cancers worldwide. In search for new NSCLC treatment options, we screened a cationic amphiphilic drug (CAD) library for cytotoxicity against NSCLC cells and identified several CAD antihistamines as inducers of lysosomal cell death. We then performed a cohort study on the effect of CAD antihistamine use on mortality of patients diagnosed with non-localized cancer in Denmark between 1995 and 2011. The use of the most commonly prescribed CAD antihistamine, loratadine, was associated with significantly reduced all-cause mortality among patients with non-localized NSCLC or any non-localized cancer when compared with use of non-CAD antihistamines and adjusted for potential confounders. Of the less frequently described CAD antihistamines, astemizole showed a similar significant association with reduced mortality as loratadine among patients with any non-localized cancer, and ebastine use showed a similar tendency. The association between CAD antihistamine use and reduced mortality was stronger among patients with records of concurrent chemotherapy than among those without such records. In line with this, sub-micromolar concentrations of loratadine, astemizole and ebastine sensitized NSCLC cells to chemotherapy and reverted multidrug resistance in NSCLC, breast and prostate cancer cells. Thus, CAD antihistamines may improve the efficacy of cancer chemotherapy.

1. Introduction

Non-small cell lung cancer (NSCLC) is one of the most common cancers and the leading cause of cancer death worldwide (Siegel et al., 2015). The majority of patients are diagnosed only after the disease has spread beyond the primary site. Thus, systemic chemotherapy, usually with combinations containing platinum-based and microtubule-disturbing drugs, forms the foundation of the treatment of these patients. As is the case for most advanced cancers, acquired apoptosis and therapy resistance pose, however, major challenges for the treatment of NSCLC (Chang, 2011). During cancer development, cells accumulate numerous genetic and epigenetic alterations to escape apoptosis initially induced by the transformation process itself, later by the hostile tumor environment and finally by cancer treatment (Groth-Pedersen and Jäättelä, 2013, Hanahan and Weinberg, 2011). Moreover, chemotherapy-treated cancer cells often acquire an ability to efflux the chemotherapeutic drugs by increasing the expression of multidrug resistance (MDR)-associated P-glycoproteins of the ATP-binding cassette transporter family (Gottesman et al., 2002, Chang, 2011). Importantly, cells harbor alternative cell death pathways that remain functional even in otherwise therapy-resistant cancer cells (Fulda, 2014, Kallunki et al., 2013). Of special interest in this context is lysosomal cell death. Cancer progression to metastatic disease depends on the activation of the lysosomal compartment, which is manifested by increased lysosomal biogenesis and acidification (Kallunki et al., 2013, Perera et al., 2015). Besides being tumor-promoting, these lysosomal changes associate with reduced lysosomal membrane stability (Fehrenbacher et al., 2008, Fehrenbacher et al., 2004). This frailty of cancer cell lysosomes can be targeted by several cationic amphiphilic drugs (CADs) that accumulate in the acidic lysosomes and induce lysosomal damage preferentially in cancer cells (Ostenfeld et al., 2008, Petersen et al., 2013, Sukhai et al., 2013, Jahchan et al., 2013, Shchors et al., 2015).

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CADs include hundreds of pharmacologic agents used to treat a broad spectrum of common diseases, *e.g.* psychiatric disorders, allergies, heart diseases and infections (Kornhuber et al., 2010). They are characterized by a hydrophobic ring structure and a hydrophilic side chain with a cationic amine group. In acidic milieu, the basic amine groups are protonated allowing an up to 1000-fold drug accumulation inside acidic lysosomes (Trapp et al., 2008). The incorporation of CADs into membranes in the lysosomal lumen neutralizes the negative membrane charge thereby inhibiting the function of several lysosomal lipases, including acid sphingomyelinase (Kolzer et al., 2004). Cancer cells are especially sensitive to the accumulation of sphingomyelin (Barcelo-Coblijn et al., 2011, Teres et al., 2012, Petersen et al., 2013), which may explain why CADs that are effective acid sphingomyelinase inhibitors display selective cytotoxicity towards transformed cells (Petersen et al., 2013, Sukhai et al., 2013, Jahchan et al., 2013, Shchors et al., 2015).

Repurposing of well-characterized and well-tolerated drugs for cancer therapy has emerged as an attractive alternative for a long and costly process of drug development. Encouraged by the well-documented anti-cancer activity of several CADs, we searched systematically for CADs with highest anti-NSCLC potential by screening a CAD library for cytotoxicity against A549 NSCLC cells. Prompted by the enrichment of antihistamines among the hits, we performed a more detailed study of their cytotoxic activity alone and in combination with chemotherapy, and conducted a pharmacoepidemiological register-based cohort study of the association between CAD antihistamine use and mortality among Danish cancer patients.

2. Materials and Methods

2.1. Pharmacoepidemiological Study

To evaluate the association between use of antihistamines and mortality among all Danish residents above 30 years of age diagnosed with any non-localized cancer (defined based on either regional or distant metastases) during 1995–2011 or non-localized NSCLC during 2004–2011 (Supplemental Table S1), we linked data from six nationwide sociodemographic or health registries described below and in the Supplemental Table S2 using the personal identification number assigned to all Danish residents (Thygesen et al., 2011). From the Danish Prescription Registry, we retrieved information on prescriptions dispensed during 1995–2011 for systemic CAD (astemizole, clemastine, desloratadine, ebastine, loratadine and terfenadine) and non-CAD (cetirizine and fexofenadine) antihistamines (Supplemental Table S2). Ebastine, loratadine, cetirizine and fexofenadine became available over-the-counter during the study period. The majority of the antihistamine sale (ebastine >75%, loratadine >65%, cetirizine >55% and fexofenadine >97%) was, however, by prescription (Sundhedsdatastyrelsen, 2016). We defined antihistamine (CAD or non-CAD) use as one or more prescriptions within 0–6 month following the diagnosis of any non-localized cancer and from three months before until three months after the non-localized NSCLC diagnosis. The patients were followed from six (all non-localized cancers) or three (non-localized NSCLC) months after the diagnosis until death, emigration, or end of study (31 December 2013), whichever occurred first. Cox proportional hazards regression was used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) for all-cause mortality associated with the use of antihistamines. The time since baseline was used as the underlying time-scale. We compared users of CAD antihistamines with non-users, as well as with users of either of the two non-CAD antihistamines fexofenadine or cetirizine, while adjusting for covariates identified from prescription and patient registries (Tables S3 and S4). We repeated the analyses stratified according to records of chemotherapy (yes/no) during the first six months following the diagnosis, which were available only for patients diagnosed between 2002–2011.

The HR estimates for all-cause death associated with use of antihistamine were adjusted for age, year of cancer diagnosis, highest achieved education, disposable income, Charlson Comorbidity Index score and drugs as described below. From the Prescription Registry (Kildemoes et al., 2011), we obtained information on prescriptions of aspirin, non-aspirin nonsteroidal anti-inflammatory drugs (NA-NSAID), statins and inhibitors of the renin-angiotensin system (including angiotensin converting enzyme inhibitors (ACEi) and angiotensin-receptor blockers (ARB)). Use of the 'confounder drugs' was defined as ≥1 prescriptions within the exposure period for antihistamines. From the Danish National Patient Registry (Schmidt et al., 2015), we retrieved information on history (at baseline) of diagnoses of chronic conditions included in the validated Charlson Comorbidity Index (Charlson et al., 1987) and computed Charlson Comorbidity Index score, categorized as 0, 1 or ≥2. Socio-economic status one year prior to the cancer diagnosis was estimated by the highest achieved education and the disposable income retrieved from registers at Statistics Denmark (Jensen and Rasmussen, 2011, Baadsgaard and Quitzau, 2011).

The local institutional review board and the Danish Data Protection Agency approved the study and waived the requirement for individual informed consent. Ethical approval is not required for registry-based studies in Denmark.

2.2. Danish Registries

The *Danish Cancer Registry* has recorded detailed nationwide information on cancer incidence since 1943 and offers an accurate and almost complete record of cancer cases (Storm et al., 1997, Gjerstorff, 2011). Cancer diagnoses are recorded according to the *International Classification of Diseases*, Eighth (ICD-8) or Tenth Revision (ICD-10), and the *International Classification of Diseases for Oncology* (ICD-O) is used for coding of topography and morphology (Gjerstorff, 2011). The Cancer Registry also contains data on clinical stage, categorized as localized, regional, distant, or unknown until 2003 and according to the tumor-node-metastasis (TNM) system from 2004 to the present (Storm et al., 1997, Gjerstorff, 2011, Edge and Compton, 2010).

The *Danish Prescription Registry* consists of records of all drug prescriptions dispensed at pharmacies in Denmark since 1995 (Kildemoes et al., 2011). The data include the type and amount of drug prescribed according to the Anatomical Therapeutical Chemical (ATC) classification system (WHO, 2013), number of packages, and the date of dispensing at the pharmacy. The dosing schedule and indication(s) are not recorded, and no information is available on drug use dispensed at hospital level.

The Danish National Patient Registry contains detailed individual data on all somatic hospitalizations in Denmark since 1977 and on ambulatory hospital contacts and psychiatric admissions since 1995 (Schmidt et al., 2015). Discharge and contact diagnoses are coded according to ICD-8 from 1977 to 1993 and ICD-10 from 1994 to the present. Information on main types of oncological therapy (chemotherapy, radiotherapy, endocrine therapy, *etc.*) is available from 2002.

The *Danish Register of Causes of Death* contains information on date and cause of death of all inhabitants of Denmark, classified according to ICD-8 until 1993 and to ICD-10 from 2004 (Helweg-Larsen, 2011).

Statistics Denmark administers registries on socio-economic data, including education and income, of all Danish residents (Jensen and Rasmussen, 2011, Baadsgaard and Quitzau, 2011).

The *Population Education Register* holds information on the highest completed level of education, derived from type and duration of schooling (Baadsgaard and Quitzau, 2011).

The *Danish Civil Registration System* maintains the civil registry number (encoding gender and date of birth) assigned to all Danish residents since 1968 and contains continuously updated address, date of death, and migration to and from Denmark. Use of the civil registration number ensures unambiguous linkage between population-based registries (Thygesen et al., 2011, Schmidt et al., 2014).