Antihistamines and Ovarian Cancer Survival

Nationwide Cohort Study and in Vitro Cell Viability Assay

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Brief communication

Antihistamines and ovarian cancer survival: nationwide cohort study and *in vitro* cell viability assay

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Abstract

Antihistamines with cationic amphiphilic drug (CAD) characteristics induce cancer-specific cell death in experimental studies. Epidemiologic evidence is, however, limited. In a Danish nationwide cohort of ovarian cancer patients diagnosed during 2000-2015 (n=5075), we evaluated the association between antihistamine prescriptions and cancer mortality. We used Cox regression models to estimate hazard ratios (HRs) with 95% confidence intervals (CIs) for ovarian cancer mortality. In an *in vitro* cell viability assay, we evaluated cell-death in three ovarian cancer cell lines after treatment with clinically relevant doses of eight antihistamines. In our cohort study, CAD antihistamine use (≥1 prescription; n=133) was associated with a HR of 0.63 (95% CI: 0.40 to 0.99) compared to use of non-CAD antihistamines (n=304), and we found a tendencytowards a dose-response association. In our cell viability assay, we found consistent and dose-dependent cytotoxicity for all CAD but not non-CAD antihistamines. In this nationwide cohort study, use of antihistamines with CAD characteristics is associated with a prognostic benefit in ovarian cancer patients.

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Despite advances in ovarian cancer treatment, survival rates remain low and the identification of strategies to improve outcomes has high clinical priority.[1] Antihistamines are used for relief of allergic symptoms[2]. Repurposing these drugs for cancer therapy has gained considerable attention, following laboratory studies reporting anti-neoplastic effects.[3,4,5] Recently, we found that use of antihistamines with cationic amphiphilic drug (CAD) characteristics was associated with reduced mortality among patients with advanced stage cancer.[6] This prompted us to evaluate the potential of prescribed CAD antihistamines to improve prognosis in a large nationwide cohort of ovarian cancer patients, linking data from the nationwide Danish cancer, prescription and other registries. Additionally, we evaluated cytotoxicity of commonly used antihistamines on ovarian cancer cell lines.

We identified all women in Denmark aged 30-84 years with an incident diagnosis of epithelial ovarian cancer during 2000-2015. Antihistamine use was defined as ≥1 filled prescription within 6 months prior to cancer diagnosis and start of follow-up. Follow-up started one year (1-year baseline) or three years (3-year baseline) after the cancer diagnosis, and ended at time of death, emigration or end of the study (December 31st, 2016), whichever came first (**Supplementary Figure 1**). We used Cox proportional hazard regression models to estimate multivariable-adjusted hazard ratios (HRs) and two-sided 95% confidence intervals (CIs) for the association between antihistamine prescriptions and ovarian cancer mortality. Other-cause mortality was evaluated as a secondary outcome to estimate the impact of competing events. The proportional hazards assumption was tested using scaled Schoenfeld residuals. We tested the robustness of our findings by repeating the analyses using inverse probability of treatment weighting with propensity scores.[7] Results were considered statistically significant that if the 95% CIs of the HR did not cross

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1.00. Methods are described in more detail in the **Supplementary Materials** (**Supplementary Methods**, **Supplementary Table 1**, **Supplementary Figure 1**).

In our cohort of 5075 ovarian cancer patients (population characteristics, **Supplementary Tables 2-3**), use of CAD antihistamines compared to non-CAD antihistamines was associated with reduced ovarian cancer mortality in analyses with baseline at 1 year (HR: 0.74, 95% CI: 0.51 to 1.06) or 3 years (HR: 0.63, 95% CI: 0.40 to 0.99) (**Table 1**). We found some evidence of a more pronounced effect with increasing cumulative amount. Compared to non-use of any antihistamine, a tendency towards reduced mortality was found for CAD antihistamines, but not for non-CAD antihistamines. We found no strong evidence for effect modification by chemotherapy (**Supplementary Table 4**). Results were not meaningfully different in sensitivity analyses, restricting to serous ovarian cancer patients (**Supplementary Table 5**), including clemastine among CAD antihistamines (**Supplementary Table 6**), or using propensity score weighted Cox models (**Supplementary Table 7**). We also found no indication of a meaningful influence of competing events (**Supplementary Table 8, Supplementary Figure 2**).

To evaluate biologic plausibility of our findings, we tested the effect of frequently used antihistamines on cell viability in three high-grade serous ovarian cancer cell lines (OVCAR-3, UWB1.289, and ovc316). All statistical tests were two-sided and a P value of less than 0.05 was considered statistically significant (**Supplementary Methods**). We found a clear and consistent dose response for all CAD antihistamines (one-way ANOVA, p<0.03 for each drug), but not for non-CAD antihistamines (one-way ANOVA, p>0.37 for each drug) (**Figure 1**). Terfenadine was the most potent CAD antihistamine with 34.8%-70.4% cell death at low concentration (6µM). In contrast, treatment with non-CAD

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antihistamines at the highest concentration (50µM) resulted in 2.3%-3.3% cell death, not markedly different from control.

In our cohort study, use of CAD antihistamines was associated with a reduction of around 20%-35% in ovarian cancer mortality, whereas no association was found for non-CAD antihistamines. In our *in vitro* experiments, we confirmed the biological plausibility of these findings.

CADs are a diverse group of compounds, which due to their amphiphilic and weak basic properties accumulate in acidic lysosomes, where they can induce permeabilization of the lysosomal membrane, leading to cell death.[8] Several CADs have shown cancer-specific cytotoxicity, *in vivo* and *in vitro*[8,9,10,11,12], with some evidence in ovarian cancer[8,13,14]. The molecular basis for this specificity is that in cancer cells, as opposed to normal cells, lysosomes are more abundant, larger, and particularly susceptible to membrane permeabilization.[8,15] CADs accumulate in acidic tumors, particularly in tumor lysosomes (up to 1000-fold)[16], therefore the dose range used in our *in vitro* experiments may be relevant for the concentrations achieved after oral anthistamine use. CADs are also hypothesized to revert multidrug resistance in cancer cell lines[6,8,17,18], including ovarian cancer cells[19,20]. Previously, we found more pronounced inverse associations between CAD antihistamine use and mortality among patients who had received chemotherapy compared to patients who did not.[6] In the current study, a similar risk pattern did not emerge, however, the number of patients not receiving chemotherapy was low.

A potential limitation of our study is exposure misclassification due to over-the-counter use of antihistamines, which is around 40% of total antihistamine use in the general population in Denmark.[21] In our study, however, the proportion of antihistamines on

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prescription may expectedly be higher due to the increased medical surveillance of cancer patients. Nonetheless, such misclassification may have biased our estimates towards no observed association, particularly in analyses with non-use as the reference group. We also had limited statistical power in analyses of CAD antihistamines, which prohibited an evaluation of histology-specific associations for non-serous ovarian cancer types and testing of drug-mortality associations for individual CAD antihistamines. Loratadine and its metabolite desloratadine constituted the vast majority (>80%) of CAD antihistamine use (**Supplementary Table 9**). Finally, we cannot exclude residual confounding, which may be related to the indication for antihistamine use and selective prescribing. However, to the best of our knowledge, clinician's preferences to prescribe a specific antihistamine is not related to its CAD characteristics. Thus, by using non-CAD antihistamines as an active comparator, we were able to minimize such biases[22], and the specificity of the inverse association to CAD antihistamines but not non-CAD antihistamines suggests that our results are not driven by confounding.

In conclusion, in a nationwide cohort study we provide epidemiologic evidence suggesting that antihistamines with CAD characteristics at current doses may provide a prognostic benefit in ovarian cancer patients. The plausibility of this finding was confirmed *in vitro* in ovarian cancer cell lines. Further efforts are required to confirm our results in other study populations, and to elucidate the precise biological mechanism. Given that current antihistamines are well-tolerated[2], inexpensive, and already commonly used in cancer patients, CAD antihistamines may become promising candidates as adjuvants to standard ovarian cancer treatment and merit further research.

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Table 1: Association between antihistamine use and ovarian cancer-specific mortality: use of CAD antihistamines compared with non-CAD antihistamine use as an active comparator (upper panel), and compared with non-use of any antihistamines (lower panel).

Analysis	Antihistamine use	N	Events	HR (95% CI)*	HR (95% CI)[†]
1y-baseline	Non-CAD antihistamine[‡]	346	168	1 (ref)	1 (ref)
	CAD antihistamine[§]	138	58	0·77 (0·57 to 1·05)	0·74 (0·51 to 1·06)
	Cumulative amount (/100 DDD)[‖]				0·92 (0·76 to 1·12)
3y-baseline	Non-CAD antihistamine[‡]	304	111	1 (ref)	1 (ref)
	CAD antihistamine[§]	133	37	0·74 (0·51 to 1·09)	0·63 (0·40 to 0·99)
	Cumulative amount (/100 DDD)[‖]				0·95 (0·85 to 1·05)
1y-baseline	Non-use	4591	2213	1 (ref)	1 (ref)
	Antihistamine (any)[¶]	484	226	1·06 (0·92 to 1·22)	0·97 (0·84 to 1·12)
	CAD antihistamine[§]	138	58	0·86 (0·66 to 1·11)	0·80 (0·61 to 1·04)
	Non-CAD antihistamine[‡]	346	168	1·15 (0·99 to 1·35)	1·05 (0·89 to 1·23)
3y-baseline	Non-use	2524	863	1 (ref)	1 (ref)
	Antihistamine (any)[¶]	437	148	1·05 (0·88 to 1·25)	0·90 (0·75 to 1·08)
	CAD antihistamine[§]	133	37	0·81 (0·58 to 1·12)	0·71 (0·50 to 0·99)
	Non-CAD antihistamine[‡]	304	111	1·16 (0·96 to 1·42)	1·00 (0·81 to 1·23)

Abbreviations: CAD, cationic amphiphilic drug; CI, confidence interval; DDD, defined daily dose; HR, hazard ratio.

*Adjusted for age at diagnosis and year of diagnosis.

[†]Adjusted for clinical stage, tumor histology, chemotherapy, comorbid conditions, use of other prescription drugs, and socio-economic factors, including highest achieved education, income, and marital status (see **Supplementary Methods** E, **Supplementary Table 1**).

[‡]≥1 filled prescription for non-CAD antihistamines and no prescriptions for CAD antihistamines within 6 months prior to diagnosis and start of follow-up (baseline).

[§]≥1 filled prescription for a CAD antihistamine (including ebastine, loratatadine, desloratadine, astemizole, terfenadine, and cyproheptadine) within 6 months prior to diagnosis and start of follow-up (baseline).

[‖]Association according to cumulative amount by including separate linear terms for CAD and non-CAD antihistamine use in the model. Presented as the change in the HR per increment of 100 DDDs, i.e. comparing CAD and non-CAD antihistamine users with the same cumulative amount. Note that the reference group, therefore, is not the entire group of non-CAD antihistamine users.

¶≥1 filled prescription for any antihistamines within 6 months prior to diagnosis and start of follow-up (baseline).

*, †, ‡, §, ||, ¶,

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Figure 1: *In vitro* assessment of cytotoxicity of CAD and non-CAD antihistamines in ovarian cancer. Cell death (exclusion of propidium iodide) induced by treatment with antihistamines at different concentrations (1, 3, 6, 12, 25, 50 µM) for 48 hours in three ovarian cancer cell lines, presented as means ± standard deviation (n=3): (A) OVCAR-3 , (B) ovc316, (C) UWB1.289. P-values derived from one-way anova after logit transformation, present comparison of group means by dose.
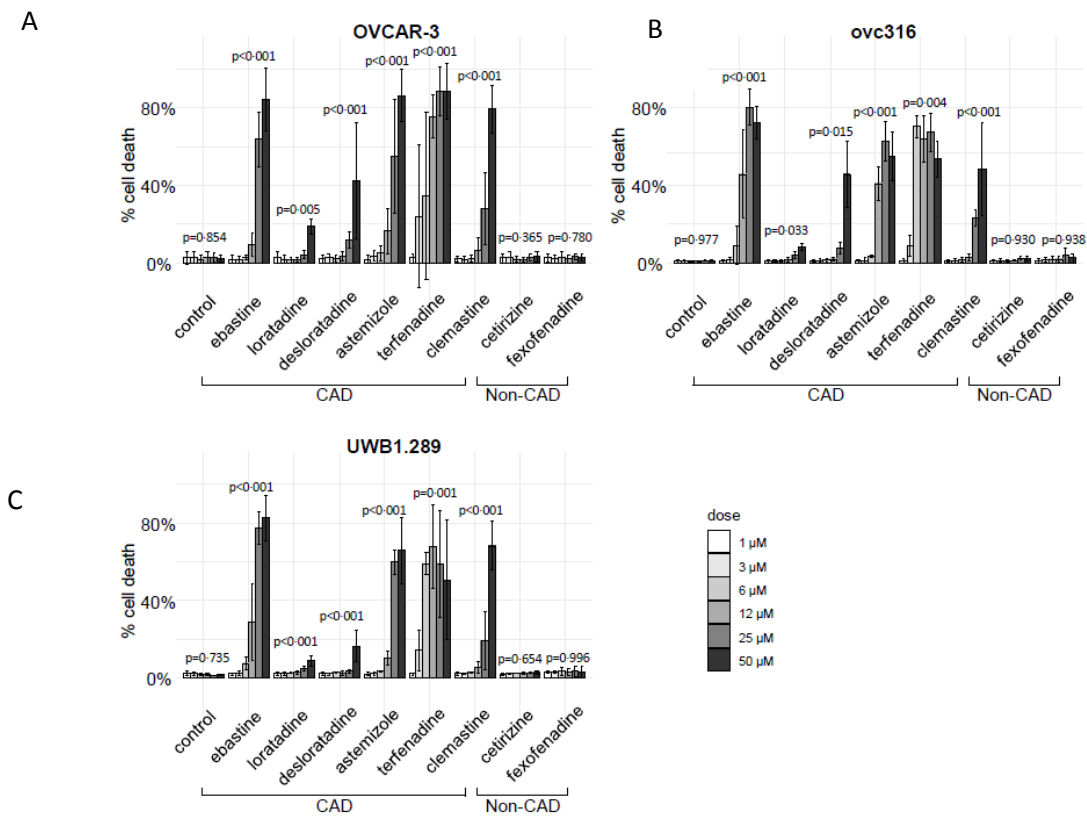
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Figure 1--FINAL

A

B

C

Figure 1