Drug effects on melanoma

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Chapter 9

Does use of estrogens decrease the Breslow thickness of melanoma of the skin?

Oral Contraceptives and Hormonal Replacement Therapy

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Abstract

Background: Recently, we demonstrated there was a cumulative dose-dependent association between the use of estrogens and the incidence of melanoma (CM). This association was demonstrated for both oral contraceptives (OC) and hormonal replacement therapy (HRT). Some *in vitro* studies, however, have suggested a direct inhibitory effect on melanoma tumor growth. Therefore, the use of different types of estrogens, OC and HRT, may be associated with a decreased Breslow thickness. Consequently, the clinical impact of our previous findings may be limited. In this study, we investigate if estrogen use (≥ 0.5 year), OC or HRT, are associated with a decreased Breslow thickness.

Patients and Methods: For this study, we linked the national Dutch pathology database (PALGA) to a pharmacy database (PHARMO). Cases were women with a primary cutaneous melanoma between January 1st 1991 and December 14th 2004, aged ≥ 18 years and having ≥ 3 years of follow-up prior to diagnosis of cutaneous melanoma.

Results: In total, 687 women with melanoma were included. Univariable linear regression analysis suggested a decreased Breslow thickness with the use of OC and HRT. Statistically significant interaction was observed between age and estrogen use (p <0.01) suggesting effect modification by age. However, in stratified multivariable analyses for different age groups (< 45 years, 45-55 years, ≥ 55 years), no statistically significant associations between the use of OC or HRT and Breslow thickness were observed.

Conclusion: An association between use of OC and HRT and Breslow thickness could not be confirmed.

Introduction

Influences of estrogens on cutaneous melanoma (CM) are suggested by several observations. First, CM incidence rates in women mimic incidence rates of breast cancer. [1] Therefore, estrogens have been hypothesized to be involved in the development of CM in women. Second, women with a history of breast cancer have been reported to be at higher risk of CM and *vice versa*. [2] In addition, estrogen-binding receptors have been detected in melanomas and benign *nevi*. [3] Moreover, indicating effects on melanocyte proliferation, hyperpigmentation can occur as a side-effect of oral contraceptives (OC), during pregnancy (chloasma) or with the use of hormonal replacement therapy (HRT). [4]

Observational studies investigating the association between estrogen use and CM development are, however, conflicting. [1] Recently, in a large population-based case-control study, we demonstrated a cumulative dose-dependent association between the use of estrogens, for both OC and HRT, and the incidence of CM. [5] The clinical relevance of our findings, however, requires further study. In explanation, estrogens may specifically be associated with melanomas with a relative good prognosis such as superficial spreading melanomas. [6] More importantly, both *in vitro* and *in vivo* studies have demonstrated that estrogens may have a direct inhibitory effect on melanoma tumor growth. [7-10] It may be, therefore, that the clinical impact of estrogen effects on melanoma is more limited than would be expected. In addition, the effects of estrogens on melanomas may differ with the type of estrogen, OC or HRT, which is used. [8]

The objective of this study, therefore, is to investigate whether use of different types of estrogens, OC and HRT, is associated with a decreased Breslow thickness across women of different age groups.

Patients and methods

Setting

Data were extracted from PHARMO, a pharmacy database representing > 12% of the Dutch population who are included regardless of type of health insurance. [11] The core file of the PHARMO system is a patient-roster file that includes entry and exit dates for all patients. To this roster file, the drug dispensing records of all pharmacies, and pathology records are linked on a patient-centric level. Dispensing histories are virtually complete. [12] The drug dispensing histories contain all dispensed prescriptions and include type, quantity, dosage form, strength, dispensing date and

prescribed daily dose of the dispensed drug. PHARMO was linked to PALGA, the Dutch nationwide registry of histo- and cytopathology, with a variation of a reliable probabilistic algorithm. [13] PALGA represents all Dutch patients and forms the basis for the Netherlands Cancer Registry. Abstracts of all pathology reports are recorded in PALGA, and these have encrypted patient identification and include diagnostic terms in scope with SNOMED classification. In 2004, over 9 million patients were archived. Since 1990, the registration reached 100% participation. [14]

Study population

The study population has been described previously. [15] Briefly, cases had a primary CM diagnosis in PALGA between January 1st 1991 and December 14th 2004 and were included in PHARMO. The date of CM diagnosis was considered the end of follow-up. All records from the eligible cases in PALGA were read by one of two investigators (AJ, ERK). Final diagnosis, date, anatomical body location, continuous Breslow depth, regression and CM subtype according to WHO classification were recorded. Accordance was high (kappa values >0.85) in a random sample of 300 cases. [15]
Potential cases were excluded if, in PHARMO, a date of entry was unknown, gender was unknown, or if follow-up in the three years before CM diagnosis was incomplete. In addition, patients were excluded if, in PALGA, the date of CM diagnosis was before the age of 18 or before January 1 1991, the CM was not pathologically confirmed, the primary CM was not on the skin (e.g. in the eye) or if the CM was *in situ*.

Drug Exposure

All estrogens were included that were commercially available and approved in The Netherlands during the study period. Estrogen use was differentiated between OC (anatomical therapeutic chemical (ATC) codes: G03AAXX and G03ABXX) and HRT (ATC code: G03CAXX) and was expressed in defined daily doses (DDD) according to the WHO definitions. All administration routes were included. Locally applied HRT, however, was only included if the estimated systemic exposure was comparable with at least 0.25 DDD/day orally.
Exposure was defined as the use of one or more estrogens containing formulations for at least 0.5 year in the 3 years before CM. To further detail estrogen use, we also calculated the cumulative dispensed dose and the cumulative prescribed duration in the 3 years before CM. The cumulative dispensed dose was calculated as the sum of all dispensed estrogens in DDD. The cumulative prescribed duration was calculated as the time period between first dispense and last dispense plus the estimated time period in which the dispensed estrogens of the last dispense were used.

Potential confounders

Drugs possibly related to progression and development of CM were considered potential confounders. These included Non-steroidal Anti-Inflammatory Drugs (NSAIDs including COX-2-inhibitors) and statins. [16] As a proxy for health care consumption, we included from PHARMO the total number of unique medical diagnoses (International Classification of Disease 9th revision, clinical modification; ICD9-CM) in the 3 years before CM. As a second proxy we also considered the total number of unique prescriptions (the number of different drugs used expressed as ATC codes and excluding estrogens) in these 3 years. Age at CM diagnosis, calendar year of diagnosis, pathological subtype of CM, regression and body location of the primary tumor were also tested as potential confounders.

Statistical analysis

To test for statistical differences, χ^2 and Student's *t*-tests were used for categorical and continuous variables respectively. All statistical tests were performed two-sided with rejection of the null hypothesis at $p < 0.05$.

Multiple linear regression, using continuous log transformed Breslow thickness as dependent variable, was used to estimate the effect of estrogen use on local CM progression (adjusted coefficients and 95% confidence interval (CI)). Since differences on log scale correlate to proportions on normal scale (i.e., log A – log B = log A/B), the results will be presented as a percentage change. This estimated percentage change in Breslow Depth can be calculated with the following formula:

$(e^{coefficient} - 1.00) \times 100\ \%$

Estrogen, OC and HRT variables were divided in categories of equal distances to ease the interpretation of the findings. Potential confounders were included in the multivariable model if they influenced the estimate by 10% or more. [17] Separate analyses will be performed for OC and HRT. To test for effect modification, interaction terms of different variables for estrogen use with age were tested in the multivariable linear model. As effect modification was present, separate analyses will be presented for different age groups (< 45 years, 45-55 years, ≥ 55 years; i.e., premenopausal, menopausal and postmenopausal age groups).

All statistical analyses were performed using SPSS 16.0 (SPSS Inc. Chicago, IL). This publication is reported according to the STROBE guidelines. [18]

Results

Study Population and Melanoma Characteristics

Of the 778 included female cases in the baseline study [15], 91 were excluded because of missing data on Breslow thickness or body location of the primary melanoma. The demographics are summarized in Table 1. Mean age of eligible women was 53.3 years [standard deviation (SD) 16.6].

Table 1 Demographics of the study population and melanoma characteristics

	Cases (n = 687)
Age at diagnosis	
mean ± standard deviation	53.3 ± 16.6 yr
range	18 yr - 94 yr
Drug use *(n, %)*	
estrogen users [a]	178 (26 %)
OC users [a]	151 (22 %)
HRT users [a]	26 (3.8 %)
statin users [b]	39 (5.7 %)
NSAID users [b]	335 (49 %)
Melanomas	
Breslow thickness	
median	0.90 mm
interquartile range	0.5 mm - 1.8 mm
Body location *(n, %)*	
extremities	393 (57 %)
trunk	220 (32 %)
head & neck	74 (11 %)
Pathological subtype [c] *(n, %)*	
superficial spreading	469 (68 %)
nodular	91 (13 %)
lentigo maligna	33 (4.8 %)
other	94 (14 %)
Regression [d] *(n, %)*	49 (7.1 %)

[a] At least 6 months of drug use.
[b] Ever drug use.
[c] Pathological CM subtype according to WHO classification.
[d] Regression mentioned in the pathologist report.
OC = oral contraceptive, HRT = hormonal replacement therapy, NSAID = non-steroidal anti-inflammatory agent, CM = cutaneous melanoma, WHO = world health organization.

Table 2 Pathological subtype and Breslow depth in different age groups

	Age group (Total: n= 687)					
	< 45 yrs		45 – 55 yrs		≥ 55 yrs	
Breslow thickness[a]	OC users (n=118)	Non-users (n=119)	OC/HRT users (n=33)	Non-users (n=101)	HRT users (n=19)	Non-users (n=289)
< 1.01 mm	78 (66%)	75 (63%)	21 (64%)	63 (62%)	16 (84%)	123 (43%)
1.01 – 2.0 mm	22 (19%)	30 (25%)	9 (27%)	18 (18%)	0 (0%)	80 (28%)
2.01 – 4.0 mm	14 (12%)	12 (10%)	2 (6%)	16 (16%)	2 (11%)	56 (56%)
≥ 4.01 mm	4 (3%)	2 (2%)	1 (3%)	4 (4%)	1 (5%)	30 (10%)
Pathological subtype[b]						
superficial spreading	100 (85%)	92 (77%)	25 (76%)	71 (70%)	13 (68%)	161 (56%)
nodular	7 (6%)	12 (10%)	4 (12%)	11 (11%)	1 (5%)	56 (19%)
lentigo maligna	0 (0%)	1 (0.8%)	0 (0%)	2 (2%)	2 (11%)	28 (10%)
other	11 (9%)	14 (12%)	4 (12%)	17 (17%)	3 (16%)	44 (15%)

[a] Breslow thickness in AJCC categories.
[b] Pathological CM subtype according to WHO classification.

CM = cutaneous melanoma, WHO = world health organization.

The melanoma characteristics are summarized in Table 1. Breslow thickness was non-normally distributed and therefore log-transformed. The distribution of Breslow thickness in AJCC categories was: 0-1 mm: 381 (56%), 1.01-2 mm: 161 (23%), 2.01-4 mm: 103 (15%) and > 4 mm: 42 (6%). In Table 2, the Breslow thickness in AJCC categories and pathological subtype of melanoma are summarized for different women < 45 years, 45-55 years and ≥ 55 years of age, separately.

Estrogen use

Estrogens (OC and/or HRT) were used by 178 women (26%) for more than half a year in the 3 years before diagnosis of CM. Of the estrogens used, 78% was ethinylestradiol. Cases using estrogens were prescribed an average estrogen day dose of 0.94 DDD per day [SD 0.21]. The results of univariable analysis are presented in Table 3.

Table 3 Univariable linear regression on log-transformed Breslow thickness and estrogen use

Variables	Coefficient	95% CI	*p*	Change in independent variable	Estimated % Change in Mean Breslow [a]	95% CI
Demographics						
Age at diagnosis	0.116	0.078 to 0.154	<0.001	10 years	12.3	8.1 to 16.6
Calendar year of diagnosis	-0.001	-0.021 to 0.018	0.89	1 year	-0.1	-2.1 to 1.8
Total unique diagnoses	0.064	0.017 to 0.111	0.01	1 diagnosis	6.6	1.7 to 11.7
Total unique prescriptions	0.008	-0.002 to 0.018	0.13	1 prescription	0.8	-0.2 to 1.8
Tumor characteristics						
Type of melanoma						
Superficial Spreading Melanoma	*reference*	*reference*	-	-	-	-
Nodular Melanoma	1.172	1.002 to 1.342	<0.001	*vs. reference*	223	172 to 283
Lentigo Maligna Melanoma	-0.082	-0.349 to 0.185	0.55	*vs. reference*	-7.8	-29.5 to 20.3
Other subtypes	0.338	0.171 to 0.506	<0.001	*vs. reference*	40.2	18.6 to 65.9
Location of primary tumor						
Extremities	*reference*	*reference*	-	-	-	-
Trunk	-0.081	-0.222 to 0.060	0.26	*vs. reference*	-7.8	-20.0 to 6.2
Head & Neck	0.092	-0.120 to 0.305	0.39	*vs. reference*	9.6	-11.3 to 35.7
Regression of primary tumor	-0.223	-0.470 to 0.025	0.08	No regression/regression	-20.0	-37.5 to 2.5

Drug use						
NSAIDs [b]	0.024	-0.104 to 0.152	0.71	non-user/user	2.4	-9.9 to 16.4
Statins [c]	0.163	-0.113 to 0.493	0.25	non-user/user	17.7	-10.7 to 63.7
Estrogen use [c]	-0.276	-0.421 to -0.132	<0.001	non-user/user	-24.1	-34.4 to -12.4
Oral Contraceptives [c]	-0.256	-0.409 to -0.103	0.001	non-user/user	-22.6	-33.6 to -9.8
Hormonal Replacement Therapy [c]	-0.293	-0.627 to 0.041	0.09	non-user/user	-25.4	-46.6 to 4.2

[a] Since differences on log scale correlate to proportions on normal scale (i.e., log A – log B = log A/B), the Estimated % Change in Breslow Depth is calculated as $(e^{coefficient} - 1.00) \times 100\ \%$.
[b] NSAID use was defined as ever use in the 3 years before CM
[c] Estrogen use (HRT or OC), HRT use, OC use and statin use defined as use ≥ 0.5 year in the 3 years before CM.

CI = confidence interval, NSAID = non-steroidal anti-inflammatory agent, OC = oral contraceptive, HRT = hormonal replacement therapy, CM = cutaneous melanoma.

Oral Contraceptives (OC) and Hormonal Replacement Therapy (HRT)

OC were used in the study period for more than half a year by 22% of the cases. Of the OC used, 99% was ethinylestradiol. During the study period, 3.8% of the cases used HRT (≥ 0.5 year). Of the HRT used, 57% was estradiol and 33% were conjugated estrogens.

Multivariable analysis

We estimated the effect of estrogen use, OC or HRT on Breslow depth of CM with multiple linear regression, using continuous log transformed Breslow thickness as dependent variable. If an interaction term between age and estrogen use was added to the model, this resulted in a statistically significant term in the model suggesting effect modification by age (p<0.01). For this study, we therefore subsequently stratified all analyses for different age groups (< 45 years, 45-55 years, ≥ 55 years; i.e., premenopausal, menopausal and postmenopausal age groups) (Table 4).

Women younger than 45 years

Among women aged younger than 45 years, use of OC (≥ 0.5 year) was not associated with a clinically relevant decrease in Breslow thickness after adjusting for age at diagnosis, pathological CM subtype and the total number of different diagnosis in the 3 years before CM (–1.4%; 95% CI= –18.5 to 19.5%). There was also no statistically significant effect of OC with increasing cumulative prescription duration or increasing cumulative dose (respectively 1.2%; 95% CI: –6.9 to 10.1% and 0.0%; 95% CI: –7.9 to 8.5%).

Table 4 Multivariable linear regression on log-transformed Breslow thickness and estrogen use for different age groups

Variables	Coefficient [a]	95% CI	*p*	Change in independent variable	Estimated % Change in Mean Breslow	95% CI
WOMEN AGED < 45 YEARS [b] Total n=237; OC users n=118						
Estrogen use for at least 0.5 year	- 0.014	- 0.205 to 0.178	0.89	Yes/No	-1.4	-18.5 to 19.5
Cumulative duration of prescriptions	0.012	- 0.071 to 0.096	0.77	1.5 years	1.2	-6.9 to 10.1
Cumulative estrogen dose	- 0.00009	- 0.082 to 0.082	0.99	500 DDD	0.0	-7.9 to 8.5
WOMEN AGED 45-55 YEARS Total n=134; Estrogen users n=33 (26 OC users, 6 HRT users and 1 used both)						
Estrogen use for at least 0.5 year	- 0.196	- 0.488 to 0.096	0.19	Yes/No	-17.8	-38.6 to 10.1
Cumulative duration of prescriptions	- 0.087	- 0.210 to 0.036	0.17	1.5 years	-8.3	-18.9 to 3.7
Cumulative estrogen dose	- 0.074	- 0.197 to 0.048	0.23	500 DDD	-7.1	-17.9 to 4.9
WOMEN AGED ≥ 55 YEARS [c] Total n=308; HRT users n=19						
Estrogen use for at least 0.5 year	- 0.165	- 0.529 to 0.199	0.37	Yes/No	-15.2	-41.1 to 22.0
Cumulative duration of prescriptions	- 0.067	- 0.256 to 0.131	0.51	1.5 years	-6.5	-22.6 to 14.0
Cumulative estrogen dose	- 0.050	- 0.222 to 0.122	0.57	500 DDD	-4.9	-19.9 to 13.0

[a] Adjusted for age, pathological subtype of CM and total unique diagnoses in the 3 year before CM.
[b] Users of Hormonal Replacement Therapy excluded (i.e., post menopausal women excluded).
[c] Users of Oral Contraceptives excluded (i.e., pre menopausal women excluded).

CI = confidence interval, OC = oral contraceptive, HRT = hormonal replacement therapy, CM = cutaneous melanoma.

Women aged 45 to 55 years

In multiple linear regression analysis adjusting for the confounders mentioned before, use of OC or HRT (≥ 0.5 year) among women aged 45 tot 55 years was associated with an average decrease in Breslow depth of 17.8 percent (95% CI= –38.6 to 10.1%). This association, however, did not reach statistical significance. In accordance, cumulative prescription duration and dose of OC or HRT did not statistical significantly affect melanoma thickness (respectively –8.3%; 95% CI: –18.9 to 3.7% and –7.1%; 95% CI: –17.9 to 4.9%).

Women 55 years and older

Among women aged 55 years and older, use of HRT (≥ 0.5 year) after adjusting for relevant confounders was associated with a statistically non-significant decrease in Breslow thickness of 15.2% (95% CI= –41.1 to 22.0%). The results for increasing cumulative prescription duration of HRT or increasing cumulative dose of HRT were similar (respectively –6.5%; 95% CI: –22.6 to 14.0% and –4.9%; 95% CI: –19.9 to 13.0%).

Discussion

The results of this study could not confirm that the use of OC or HRT (> 0.5 year in the 3 years before CM) has a significant influence on Breslow thickness of CM. However, the association between estrogen use and Breslow thickness of CM seems to be modified by age (Table 2 and 4). Thus, also because of limited sample size, we cannot exclude decreased Breslow depth of CM among patients aged 55 years and older and using HRT (i.e. possible type II error due to lack of power in the subgroup analyses). Moreover, because of collinearity, it is not possible to differentiate between effect modifying by age or different effects of ethinylestradiol (OC) and estrogens used in HRT (estradiol, conjugated estrogens). In explanation, synthetic estrogens may have no effect on Breslow depth whereas natural estrogens do or estrogens might only have effects on Breslow depth in postmenopausal women.

Nevertheless, the observed effects may be caused by an increased incidence of thin melanomas in this population instead of growth inhibition of CM. [5] Thus, the observed effects could also support a specific increase in the incidence of superficial spreading melanomas.

This nested case-control study is large and population-based. Pharmacy data are gathered prospectively, and therefore avoid recall bias. As we had detailed drug-dispensing information, we were able to study dose-effect responses, differentiate between OC and HRT, and could also exclude low-dosed topically applied estrogens.

Although we corrected for the pathological subtypes, we cannot exclude residual confounding. PHARMO and PALGA data, for instance, do not include data on most risk factors of (thick) melanomas such as family history of melanoma, skin type and other phenotypic characteristics, sun exposure history or socioeconomic status. As these factors are unlikely to affect the likelihood of estrogen prescription, confounding by indication seems unlikely, except possibly socioeconomic status. In addition, there is no biologic basis to assume that menopausal complaints (e.g., flushing and vaginal atrophy) or possible causes of menopause (e.g., hysterectomy) are associated with the Breslow depth of melanomas of the skin.

Conclusion

Our study does not show a statistically significant association between the use of oral contraceptives or hormonal replacement therapy and the Breslow thickness of cutaneous melanoma. However, we cannot exclude decreased Breslow thickness among women using estrogens, especially among older women.

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