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High-dose and Low-dose Combined Oral Contraceptives: Protection Against Epithelial Ovarian Cancer and the Length of the Protective Effect

Karin A. Rosenblatt, David B. Thomas, Elizabeth A. Noonan and the WHO Collaborative Study of Neoplasia and Steroid Contraceptives*

The relations between use of high-dose and low-dose combined oral contraceptives and epithelial ovarian cancer were compared in an international hospital-based case–control study. 393 cases from seven countries were compared with 2561 matched controls. The odds ratio (OR) was somewhat lower for women who used high-dose oestrogen oral contraceptives (OR = 0.68) than for women who used low-dose preparations (OR = 0.81) although the difference could have occurred by chance. After controlling for time since last use, risk was slightly lower for long-term users of high-dose preparations than for long-term users of low-dose pills. Both high-dose and low-dose oral contraceptives protect against ovarian cancer, but the degree of protection may be slightly weaker for the newer, low-dose products.
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INTRODUCTION

PREVIOUS FINDINGS from this [1] and other studies [2] have indicated that use of combined oral contraceptives is protective for epithelial ovarian cancer. This study [1] and those of Booth *et al.* [3] and the CASH group [4] have demonstrated that the protective effect conferred by oral contraceptives lasts over 10 to 15 years.

During the past decade, the amounts of oestrogen and progestin in oral contraceptives have decreased in the United States [5] and in many developing countries [6]. The results of most previous studies pertain to high-dose products, and Van Leewen and Rookus [7] have raised the concern that lower dose oral contraceptives may not provide as strong and as long a protective effect against ovarian cancer as high-dose contraceptives. This is a report of results of analyses performed to address this hypothesis using data from a multinational hospital-based case–control study.

PATIENTS AND METHODS

The methods used in this study have been described previously [1]. Incident cases of breast, uterine cervix, uterine corpus, ovarian, and hepatobiliary cancers were identified through records of hospital admissions, outpatient clinics, and pathology departments. Findings from the analysis of data from nine centres in seven countries are included in this report (Australia, Chile, China, Israel, Mexico, the Philippines and Thailand). To be eligible for inclusion in the study, the subjects must have been older than age 15 and been at risk of exposure to steroid contraceptives during their fertile years (born after 1925 or 1930 depending on when steroid contraceptives became available locally). They must also have lived in a defined area served by the hospital of ascertainment for at least 1 year. Dates for recruitment of subjects varied by centre. Ascertainment

Correspondence to D.B. Thomas.
K.A. Rosenblatt, D.B. Thomas and E.A. Noonen are at the Fred Hutchinson Cancer Research Center, 1123 Columbia Street, Seattle, Washington 98104, U.S.A.
*The data collection centers, and the principal investigator (PI), co-investigator (CI), and pathologist (P) at each participating center in alphabetical order by country are listed in Participants at the end of the paper.
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began in October, 1979 and continued until 1988 in some centres.

Of 541 women with ovarian tumours identified by a local pathologist, 516 (95.4%) agreed to be interviewed. Cases consisted of women with histologically confirmed epithelial tumours that were of definite or borderline malignancy [8]. 26 potential cases were excluded because they were not confirmed by the reference pathologist as borderline or malignant. 74 cases were excluded because they were not epithelial.

Approximately two controls were selected for each case of the five neoplasms investigated in this study from the same hospital as the case, by periodic ascertainment of women admitted to specified hospital wards in the previous 24 h. Women were not eligible for control selection if they had been admitted to the hospital for selected conditions that might have altered their use of steroid contraceptives, including: circulatory or cardiovascular diseases, diabetes, chronic renal disease or renal transplant, benign breast disease, previously diagnosed cancer, and any gynecological or obstetrical condition. Of the 19844 controls identified, 18999 (95.7%) were successfully interviewed. Since controls were not matched to individual cases, these controls constituted a pool from which controls for the present analyses were drawn. 10 potential controls with previous ovarian cancer and 407 women with a previous bilateral oophorectomy or an unknown number of ovaries were excluded from the control group. Up to eight controls were then individually matched to each case on age (same 3-year interval) hospital, and date of diagnosis (same 2-year interval), resulting in the inclusion of 2561 controls in the analyses. No matches were available for 23 cases, and these have been excluded leaving 393 cases available for inclusion in the study. Controls with admission diagnoses in the following disease categories were included in the study [infections (2.2%), neoplasms (2.9%), endocrine and metabolic (7.9%), blood and blood-forming organs (0.7%), nervous system (15.0%), circulatory system (2.5%), respiratory system (8.2%), digestive system (37.3%), genitourinary system (3.5%), skin disorders (1.8%), musculoskeletal system (4.6%), injury or poisoning (5.8%), and other conditions (6.8%)].

Standardised questionnaires were administered in the local language, largely in hospitals, by trained study personnel, to obtain information on demographic and social factors, previous medical conditions, gynecological surgeries, reproductive and contraceptive practices, and use of other medications and hormones. Samples of hormonal contraceptives used in each country, and a calendar were used to facilitate recall of previous oral contraceptive use.

Pathologists at each centre recorded their provisional histological diagnosis, whether the tumour was invasive or of borderline malignant potential, the tumour size, and the stage at diagnosis.

Histological slides were sent to a reference pathology laboratory for review by one of the two reference pathologists. Table 1 shows a distribution of the cases by histological type and tumour behaviour, as recorded by the reference pathologist; 26.4% of the tumours were classified as borderline.

Mestranol is metabolised to ethinyl oestradiol and it takes two times the dose of mestranol to achieve the same level of hormonal activity in the bloodstream and the same cortisol binding capacity as ethinyl oestradiol [9, 10]. Therefore, it was assumed that mestranol was half as potent as ethinyl oestradiol. Combined oral contraceptives were considered to be 'low dose' if they contained less than 50 μl of ethinyl oestradiol or less than 100 μl of mestranol.

Table 1. Number of epithelial ovarian cancer cases by histological type and tumour behaviour

	Tumour Behaviour		
Histological type	Borderline	Malignant	Total
Serous	48	128	176
Mucinous	52	55	107
Endometriod	3	48	51
Clear cell	0	35	35
Other	1	23	24
Total	104	289	393

Women who had used both high-dose and low-dose preparations (4 cases and 60 controls) were excluded from those analyses that addressed separate effects of high-dose and low-dose preparations on risk of ovarian cancer. Also excluded from those analyses were subjects who had used sequential contraceptives, continuous progestin contraceptives, and triphasic and quinoestrol containing combined oral contraceptives (4 cases and 44 controls) as well as subjects who could not name the brand of all of the pills that they had used (28 cases and 197 controls).

Conditional logistic regression [11] was used to calculate odds ratios and 95% confidence intervals as estimates of relative risks. Multivariate models were used to control for the effects of confounding variables. Assessment of interaction was performed either by testing for significant heterogeneity in an unmatched stratified exact analysis when the data were sparse [12], or by performing a likelihood ratio test with conditional logistic regression. Comparisons were made between the risk associated with high-dose and low-dose oral contraceptives by estimating the ratio of their odds ratios [13]. Estimates from the variance–covariance matrix were used to derive the standard error of this measure.

RESULTS

Table 2 shows odds ratios of ovarian cancer in women who ever used high-dose and low-dose oral contraceptives, based on unmatched analyses of data from each participating centre. Exact tests for a common odds ratio indicated that there was no significant heterogeneity of results across centres for either high-dose ($P = 0.83$) or low-dose ($P = 0.27$) users. Data from all centres were therefore combined in further analyses.

40 variables were evaluated as possible confounders of the relationship between oral contraceptive use and ovarian cancer. These were related to reproductive status, contraceptive use, lactation, socioeconomic status, hormone use and infertility. Number of live births was most strongly related (inversely) to risk of ovarian cancer and controlling for this variable changed the odds ratio for users of high-dose pills from 0.53 to 0.68 and for users of low-dose pills from 0.61 to 0.81 (conditional logistic regression analyses). Other variables did not greatly influence the level of association after controlling for number of live births, and all odds ratios presented subsequently in this paper were adjusted for age, centre, year of diagnosis, and the number of live births.

As shown in Table 3, the odds ratio for ovarian cancer was lower in users of high-dose than low-dose products, although the difference between the two estimates was not statistically

Table 2. Odds ratios for epithelial ovarian cancer according to use of high-dose or low-dose oral contraceptives by centre

Centre	Cases	Controls	High-dose OR*	High-dose 95% CI	Low-dose OR*	Low-dose 95% CI
Australia	15	98	0.80	0.07–5.04	3.47	0.26–31.32
Chile	46	209	0.62	0.11–2.29	0.44	0.05–1.98
China	13	65	NU†		1.50	0.13–9.43
Israel	131	709	0.53	0.25–1.04	1.17	0.28–3.37
Mexico	17	136	1.37	0.13–7.41	0	0–2.69
Philippines	20	150	0	0–3.50	1.18	0.12–6.02
Thailand						
Chiang Mai	55	440	0.75	0.22–2.06	0.47	0.17–1.12
Chulalongkorn	44	344	0.41	0.08–1.40	0.81	0.26–2.14
Siriraj	52	410	0.36	0.07–1.18	0.38	0.07–1.25
Total	393	2561	0.54‡	0.35–0.82	0.65‡	0.41–1.00
P value of test§ for common OR				0.83		0.27

* Unmatched odds ratios.
† No use of this type of oral contraceptives at this centre.
‡ Stratified by study centre.
§ Exact test for common odds ratio.

Table 4. Odds ratios for epithelial ovarian cancer according to duration and time since last use of high-dose and low-dose contraceptives and comparison of high-dose and low-dose use

Type of comparison	Months of use	Months since last use 1–36	37–120	> 120
High-dose	1–18	1.38* (0.42–4.49) [4,21]†	0.99 (0.40–2.48) [6,52]	0.58 (0.26–1.29) [7,82]
	> 18	0.66 (0.24–1.76) [5,46]	0.40 (0.12–1.36) [3,56]	0.35 (0.081–1.53) [2,33]
Low-dose	1–18	1.05* (0.36–3.08) [5,32]	0.73 (0.26–2.01) [5,54]	0.55 (0.19–1.64) [4,60]
	> 18	1.52 (0.68–3.42) [8,49]	0.98 (0.33–2.87) [4,36]	0.44 (0.056–3.36) [1,21]

* Odds ratio comparing risk in users of specified type of pill with non-users. Odds ratios adjusted for age, centre, year of diagnosis and number of live births.
† [Cases,Controls].

significant (ratio of odds ratios = 0.84, 95% CI = 0.47–1.50). Table 3 also shows that there was no relation between risk and duration of use among women who had used low-dose preparations but there was evidence of a reduction in risk after 18 months of use of high-dose preparations. Weak decreasing trends in risk with time since first and last use were observed both in women who had used high-dose and low-dose preparations.

After stratifying on duration of use (Table 4), there appears to be little evidence for a substantial protective effect until 10 years after cessation of use, except for a possible earlier reduction in risk in long-term (> 18 months) users of high-dose products. For each category of months since last use, the odds ratios in high-dose users are less for users of over 18 months duration than for women who were exposed for less than 18 months, but

Table 3. Odds ratios for epithelial ovarian cancer according to high-dose or low-dose use and comparisons of risks for different types of oral contraceptives

	High-dose Cases	Controls	OR*	95% CI	Low-dose Cases	Controls	OR*	95% CI
Non-users	301	1688	1.00		301	1688	1.00	
Ever use	30	304	0.68	0.44–1.05	27	268	0.81	0.51–1.29
Months of use†								
1–6	8	94	0.60	0.28–1.28	6	100	0.45	0.18–1.10
7–18	9	61	1.07	0.50–2.29	8	46	1.36	0.59–3.10
19–60	6	81	0.48	0.20–1.18	9	57	1.47	0.68–3.18
61+	4	54	0.49	0.17–1.43	4	49	0.75	0.26–2.19
Months since first use‡								
1–72	7	43	1.24	0.51–3.00	13	64	1.56	0.78–3.11
73–132	8	84	0.78	0.35–1.70	6	80	0.75	0.31–1.80
133–180	5	80	0.48	0.18–1.22	4	56	0.60	0.21–1.75
181+	9	87	0.65	0.30–1.39	4	55	0.57	0.20–1.64
Months since last use§								
1–24	5	50	0.69	0.26–1.82	13	77	1.45	0.74–2.85
25–84	8	78	0.76	0.35–1.68	6	64	0.70	0.28–1.75
85–132	7	67	0.88	0.38–2.05	4	49	0.77	0.27–2.21
133+	7	99	0.44	0.20–0.99	4	68	0.48	0.16–1.39

* Adjusted for age, centre, year of diagnosis and number of live births.
† Excluding 3 cases and 30 controls whose duration was unknown.
‡ Excluding 1 case and 23 controls whose latency was unknown.
§ Excluding 3 cases and 30 controls whose recency was unknown.

this pattern, with respect to duration of use, was not observed for users of low-dose products. It must be emphasised, however, that all odds ratios are based on small numbers, are not significantly less than one, and are not significantly different from each other.

When we restricted our analyses to the centres that contributed 20 or more cases to the analysis we did not observe any appreciable differences from the findings presented in this report.

DISCUSSION

The odds ratio of 0.68 for high-dose users observed in this study is remarkably close to the summary odds ratio of 0.6 estimated by Prentice and Thomas [2] from published reports of 10 previous case–control studies. Nine of these reports were published before 1984 and largely pertain to users of high-dose products. The odds ratio of 0.81 observed in users of low-dose preparations in this study is higher than that observed in all but one of these 10 prior studies. In addition, an odds ratio of 1.0 was observed in a more recent study in the USA [14] which presumably included a higher proportion of users of low-dose products than prior studies, although an odds ratio of 0.5 was reported from another recent study in the UK [3] where the proportion of low-dose oral contraceptive users has also been increasing over time. Also, in the present study a non-significant decreasing risk with increasing duration of use was observed only for users of high-dose preparations, after stratifying on time since last use. A trend of decreasing risk with duration of use was also observed in the earlier studies of Rosenberg *et al.* [15], La Vecchia *et al.* [16], CASH [4] as well as one later study [17], but not in the recent study of Hartge *et al.* [14].

The findings from the present investigation for users of high-dose and low-dose products are thus generally compatible with results from other studies and suggest a slightly weaker protective effect of low-dose than high-dose oral contraceptives on risk of ovarian cancer.

Many previous studies have noted that the length of the protective effect of oral contraceptives is delayed and rather long. For all strengths combined, this study [1] and Booth *et al.* [3] reported a decline in risk with time since last use and the apparent protective effect to be strongest after 10 years since last use; and the CASH study [4] found a reduced risk to be present 15 years since last use. We have now provided results to suggest that the apparent protective effect may occur somewhat earlier after last use in long-term users of high-dose preparations than in users of low-dose products (Table 4).

Two theories on the genesis of ovarian cancer currently prevail in the epidemiological literature. The pituitary gonadotropin hypothesis assumes that excess production of pituitary gonadotropins, perhaps as a result of premature depletion of ovarian follicles, causes ovarian cancer [18], while the incessant ovulation hypothesis assumes that ovulation or mechanical trauma from ovulation is related to the development of ovarian cancer [18–20]. Since high-dose oral contraceptives appear to have a greater suppressive effect on the release of pituitary gonadotropins than low-dose contraceptives [21–23] but probably do not differ greatly in their effect on ovulation [24, 25], a greater protective effect of high-dose than low-dose oral contraceptives would support the pituitary gonadotropin hypothesis. However, since the difference in the apparent protective effect between high-dose and low-dose oral contraceptives is not great in this study, and since there could be some small difference in ovulation inhibition between the two types of oral contraceptives, our findings do not provide strong support for one hypothesis over the other. We encourage other clinical investigators to confirm that the level of ovulation suppression is the same in users of high-dose and low-dose pills, and we urge other epidemiologists to perform additional analyses of existing data to confirm or refute our findings.

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Participants—University of Sydney, Department of Public Health, Sydney, Australia: Geoffrey Berry (PI), Robert MacLennan (CI), Rodney Shearman (CI), Tatiana Jelihovsky (P), Joan Cooper Booth (P); University of Chile, Faculty of Medicine, Hospital Jose Joaquin Aguirre, Department of Obstetrics and Gynecology, and the Ministry of Health, Hospital Salvador, Department Obstetrics and Gynecology, Santiago, Chile: Ramiro Molina (PI), Luis Martinez (CI), Oriana Salas (CI), Alfredo Dabancens (P); Shanghai Institute of Planned Parenthood Research, Shanghai, China: Chen Zhiheng (PI), Tao Yun (CI), Hu Yong Wei (P); Chiam Sheba Medical Center, Department of Clinical Epidemiology, Tel Hashomer, Israel: Baruch Modan (PI), Elaine Ron (CI), Esther Alfandary (CI); Hospital General de Mexico, Mexico City: Hector Rodriguez Cuevas (PI), Socorro Benavides Salazar (CI), Antonio Palet (P), Patricia Ontiveros (P); University of the Philippines, College of Medicine, Manila, Philippines: Ruben A. Apelo (PI), Julietta R. de la Cruz (CI), Jose Baens (CI), Benita Javier (P); Chiang Mai University, Faculty of Medicine, Chiang Mai, Thailand: Suporn Silpisornkosol (PI), Tieng Pardthaisong (CI), Nimit Martin (CI), Choti Theetranont (P); Chulalongkorn University, Faculty of Medicine, Department of Obstetrics and Gynecology, WHO Collaborating Centre for Research in Human Reproduction, Bangkok, Thailand: Banpot Boosiri (PI), Supawat Chutivongse (PI), Pramuan Virutamasen (CI), Chansuda Wongsrichanalai (CI), Prasarn Jimakorn (P); Mahidol University, Faculty of Medicine, Siriraj Hospital, Department of Obstetrics and Gynaecology, Family Planning Research Unit, Bangkok, Thailand: Suporn Koetsawang (PI), Daungdao Rachawat (CI), Nivat Chantarakul (P); Massachusetts General Hospital, Department of Pathology, Boston, Massachusetts, USA: Robert E. Scully (Reference Pathologist), Robert H. Young (Reference Pathologist); Fred Hutchinson Cancer Research Center, Division of Public Health Sciences, Seattle, Washington, USA; Coordinating Center: David B. Thomas (Study Coordinator), Karin A. Rosenblatt (Epidemiologist), Elizabeth A. Noonan (Statistician); and World Health Organization: Susan Holck, Tim Farley, Olav Meirik, Special Programme of Research, Development and Research Training in Human Reproduction, Geneva, Switzerland.

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Allelic Loss on Chromosome 11p is a Less Frequent Event in Bilateral than in Unilateral Wilms' Tumours

M.H. Little, J. Clarke, J. Byrne, R. Dunn and P.J. Smith

Analyses to detect loss of heterozygosity (LOH) were performed at 11 polymorphic loci on chromosome 11 and, using a polymorphic CA repeat sequence in the WT1 gene, on a series of 39 tumours from 28 unilateral and 10 tumours from 6 bilateral Wilms' tumour (WT) patients. LOH was seen in 13 out of 35 patients including 12 out of 29 unilateral tumours, but only one of 10 bilateral tumours. This suggests that bilateral WT represents a subgroup of WT in which tumour initiating events less frequently involve LOH on chromosome 11 and that either epigenetic events, point mutations or another non-chromosome 11p locus are important in bilateral tumours. The observation of LOH in one WT but not another WT in a bilateral WT patient provides evidence that these tumours arising in the same patient are not monoclonal proliferations and most likely arise via different molecular pathways.
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INTRODUCTION

WILMS' TUMOUR (WT) is a paediatric neoplasm of the kidney which is hypothesised to require at least two mutational steps for tumour development. The first may be prezygotic and acquired from the germinal cells of one parent in hereditary cases of the disease. Thus, some patients will carry this first mutation constitutionally. The second mutation is hypothesised to be postzygotic, resulting in the loss or effective loss of the remaining normal copy of a tumour-suppressor gene. At least one of the genes responsible for WT is known to reside on the short arm of chromosome 11, at 11p13, due to observations of 11p13 deletions in Wilms' tumour/aniridia/genitourinary dysplasia/mental retardation (WAGR) patients and the tumours of sporadic WT patients. Recently WT1, a zinc finger gene, has been cloned from the region of interest at 11p13 [1, 2].

Restriction fragment length polymorphism (RFLP) studies on WT have shown that regions along the short arm of chromosome 11 that are constitutionally heterozygous become homozygous in some tumours [3–6]. This loss of heterozygosity (LOH) for 11p loci corroborated the cytogenetic evidence that

Correspondence to P.J. Smith.
The authors are at the Queensland Cancer Fund Research Unit, Department of Pathology, University of Queensland, Herston Rd., Herston 4006, Brisbane; M.H. Little, J. Clarke and P.J. Smith are also at the Queensland Institute of Medical Research, Bramston Terrace, Herston 4006, Brisbane; P.J. Smith is at the Royal Children's Hospital, Herston Road, Herston 4029, Brisbane, Australia. M.H. Little is currently at the MRC Human Genetics, Western General Hospital, Crew Road, Edinburgh EH4 2XU, U.K.
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