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Review

Human papillomavirus, cervical carcinogenesis and chemoprevention with Indole derivates – a review of pathomechanisms

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Cervical cancer is the second most common female cancer worldwide with high risk Human Papillomavirus (HPV) infection playing an essential aetiological role. Oestrogen interacts with HPV at a cellular level causing cell growth and inhibition of apoptosis. Indole derivatives, formed during digestion of cruciferous vegetables, have been shown to have chemopreventative properties inhibiting HPV transcription and influencing oestrogen metabolism. This review describes the interactions between HPV, oestrogen and indole derivatives. Further clinical research is required to evaluate the chemopreventative properties of these agents.

Keywords: Cervical cancer / Chemoprevention / HPV / Indole derivates / Oestrogen

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1 Introduction

Cervical cancer is the second commonest female cancer worldwide with Human Papillomavirus (HPV) playing a central aetiological role [1, 2]. HPVs can be classified according to their carcinogenic potential; high-risk types being associated with cervical cancer and cervical intraepithelial neoplasia, whereas low-risk HPVs are rarely associated with anogenital neoplasia [3–8]. High-Risk (HR)-HPV DNA is found in 99.7% of cervical tumours [9]. A multicentre study showed that infection with HR-HPV has an odds ratio (OR) of 158.2 (95%CI, 113.4–220.6) for cervical cancer [3]. HPV is also associated with other diseases including head and neck cancers [10, 11] and recurrent respiratory papillomatosis [11, 12]. Other factors such as smoking [14], oral contraceptive pill usage [15, 16], multiparity [17, 18] and chlamydial infection [19–22] are weak independent risk factors for cervical cancer.

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Abbreviations: CIN, cervical intraepithelial neoplasia; **DIM,** diindolylmethane; **HR,** high risk; **HRT,** hormone replacement therapy, **HVP,** human papillomavirus; **IARC,** international agency for research on cancer; **I3C**, indole-3-carbinol; **OR**, odds ratio; **Rb**, retinoblastoma

2 HPV and oestrogen in cervical carcinogenesis

Genital HPV infection is usually transmitted through sexual contact, although other HPV viruses can be transmitted by skin to skin contact, *e.g.* verruca-causing HPVs [23]. Infection with HPV may occur more easily in the cervical transformation zone where columnar epithelium undergoes squamous metaplasia, the epithelium being thinner and therefore more susceptible to HPV infection [24].

The transformation zone, the most oestrogen sensitive region of the cervix, is where the majority of cervical cancers develop [25]. Animal studies confirm the sensitivity of the cervical transformation zone to oestrogen-induced carcinogenesis [26, 27]. Expression of cervical oestrogen receptors is highest in the transformation zone, with receptor-positive cells localised mainly in the parabasal and intermediate cell layers [28]. It has been suggested that there is a difference in the oestrogen and/or progesterone receptor status between the normal cervix and the HPV infected cervix [29–33], although this was not confirmed in all studies [34]. Cells in cervical intraepithelial neoplasia (CIN) appear to loose their oestrogen expression [32, 35–37], whilst on the other hand, increased levels of 2-OH-oestrone may increase apoptosis in HPV-infected cells, thereby having a protective effect against the development of cervical cancer [38]. Women who express higher levels of oes-

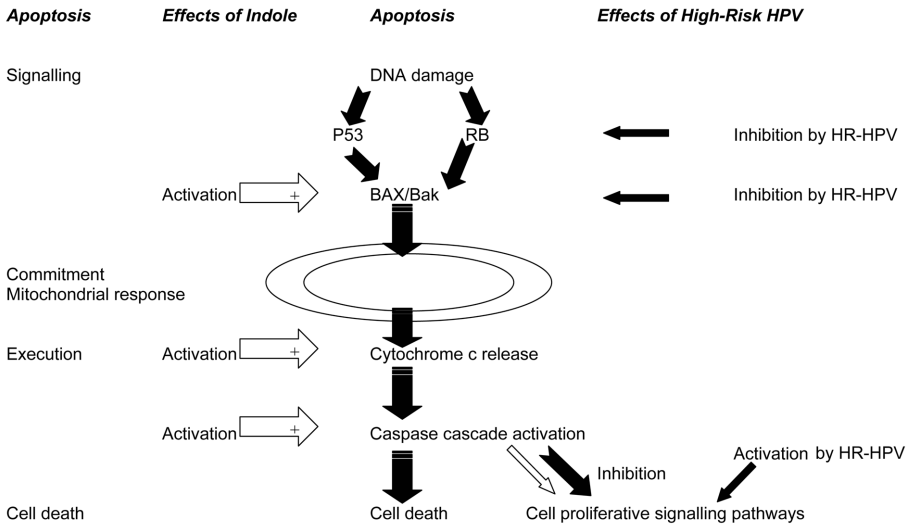

Figure 1. Simplified figure of apoptosis under influence of HR-HPV and chemopreventive agents such as Indoles. In general, DNA damage will activate tumour suppressor families such as p53 and Rb. They will prevent the cell from proceeding into S-phase. If the DNA damage cannot be repaired then pro-apoptotic factors such as Bax/Bak will be activated. This causes release of Cytochrome c from the mitochondria and the endoplasmic reticulum. Released cytochrome c activates the Caspase system, which finally executes cell death. High-Risk HPV (HR-HPV) inhibits tumour suppressors such as Rb thus releasing transcription factor E2F, which finally supports cell proliferation. HPV also inhibits apoptosis by activating anti-apoptotic factors and by inhibiting pro-apoptotic factors. Additionally, HPV activates cell signalling pathways, which cause cell proliferation. Indole derivates on the other hand support apoptosis by stimulation of tumour suppressor genes such as BRCA, by activation of pro-apoptotic factors and by inhibition of proliferative cell signalling pathways.

trogen receptors are more likely to have cervical HPV infection suggesting that oestrogen receptors may play an important part in cervical HPV infection [31].

HPV related carcinogenesis occurs at several points in the cell cycle. HR-HPVs inhibit tumour suppressor families such as p53 and retinoblastoma protein (Rb) and inhibit cell death (apoptosis) at various steps in the apoptotic process (see Fig. 1).

The Rb tumour suppressor protein family function as negative regulators of the cell cycle. In its active state it binds to E2F transcription factors. Responding to cell growth-promoting factors, cyclin-dependent kinases inactivate the Rb-E2F complex through phosphorylation of the Rb protein releasing E2F, which induces progression through the cell cycle. The HPV oncoprotein E7 binds to the Rb-E2F complex, thereby inactivating the Rb tumour suppressor, releasing E2F transcription factors. This leads to stimulation of other genes such as p16 and p14. Both regulate an ubiquitin ligase which controls levels of the tumour suppressor p53 (see review by Doorbar 2006 [39]). The HR-HPV protein E6 associates with p53, mediating p53 ubiquitation and degradation [40]. E6 can also inhibit apoptosis by binding to Bak [41] and Bax [42, 43].

Other HPV proteins, such as E5, may contribute to the carcinogenesis of cervical cancer but the exact role is poorly understood. E5 probably plays a role during the productive stage of the HPV-16 life cycle [44]. The E5 protein may contribute to cellular transformation and prevent cell death at the early stages of viral infection [45, 46]. It may also increase the carcinogenic activity of E7 [47, 48].

Oestrogen also activates cyclin-dependent kinases, supporting cell growth. Indole derivates on the other hand, inhibit cyclin-dependent kinases and change the ratio of oestrogen metabolites in favour of 2-hydroxy-oestradiol, with resultant inhibition of cycle progression (see Fig. 2).

At a cellular level oestrogen appears to interact with HPV although little is known about the mechanisms at this level. Oestrogen inhibits apoptosis in cervical cancer cells [49, 50]. HPV oncogene expression may be increased in response to oestrogen and progesterone stimulation, thus causing cell growth [49, 51–57]. However, a study on HPV-16 positive CaSki and SiHa cells showed increased cell proliferation under the influence of oestrogen as well as progesterone but no change in HPV-16 E6/E7 transcription levels [50]. The authors suggested that the anti-apoptotic effect of oestrogen might allow a growth advantage for HPV infected cells [50]. In a model using luciferase reporter constructs under control of oestrogen response element promoter it was shown that HPV-16 E7 can dysregulate and reduce the function of steroid receptor coactivator 1, which may explain the role of steroid hormones as co-factors in the carcinogenesis of HR-HPV related disease [58]. In specimens from HPV positive cervical carcinomas increased levels of aromatase, an enzyme which converts androgens to oestrogen, were identified. This was associated with increased oestrogen receptor levels as well as

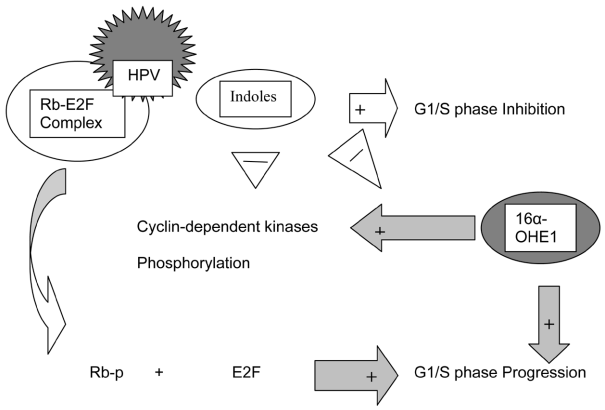

Figure 2. Schematic Illustration of the Effects of Oestrogen in HPV Infected Cells on the Tumour Suppressor Rb and Chemopreventive Action of Indole Derivates. HPV E7 oncoprotein binds to the tumour suppressor protein pRb and releases the E2F-transcritpion factor. Degraded pRb and free E2F can mediate the cell progress into the S phase. The released transcription factor E2F activates factors which promote cell-cycle progression.The oestrogen, 16a-hydroxy-oestrone, also activates the cyclin-dependent kinases (CDKs) and thus supports cell progression into the S phase. Indole derivates on the other hand reduce the 16α-hydroxy-oestrone level and inhibit the cyclin-dependent kinases. Thus, Rb remains in an active state and cell progression is inhibited. Activation is symbolised by ⇨, inhibition is symbolised by △. Gray: potential malignant convertion, white: potential anti-proliferative effect.

expression of the HPV oncogenes E6 and E7 [59]. However, in specimens from women with cervical intraepithelial neoplasia a down-regulation of oestrogen-receptor has been observed [34, 60]. Other researchers suggest that oestrogen can increase the level of apoptosis associated with HPV-16 E2 protein [38]. Studies using HPV-transgenic mice suggested a permissive effect of oestrogen on cervical carcinogenesis as well as on tumour persistence and growth [27, 61–64]. A case-control study in 390 postmenopausal women suggested an increased risk of any HPV infection in past-users of combined hormone replacement therapy (HRT) depending on the duration of treatment [65]. However, the incidence of HR-HPV infection was low at 2.8% and there was no association between HPV infection and duration of treatment in current HRT users. Larger HRT studies did not demonstrate an increase in cervical cancer in HRT users [66, 67]. A meta-analysis suggested that the oral contraceptive pill could be a co-factor in HPV-related cervical carcinogenesis with an OR for the use of oral contraception and increased risk of cervical cancer of 2.82 (95%CI, 1.46–5.42) for five to nine years of use and 4.03 (95%CI, 2.09–8.02) for ten years of use and longer [16]. However, the ORs are low compared to the ORs of HR-HPV infection.

3 Chemoprevention with indoles

A variety of potential anticarcinogenic and chemopreventive agents have been examined over the last 30 years [68, 69]. Several epidemiological studies have demonstrated health benefits and cancer prevention for a "green" diet (for review see [69–71]). The evidence from epidemiological studies show conflicting results for an association between indole-derivates and cervical cancer [69]. Indoles, found in Brassicas (cruciferous vegetables such as cabbage, cauliflower, Brussels sprouts, kali, broccoli) have been shown to have chemopreventive properties. Indole-3-Carbinol (I3C) forms condensation products during acid digestion, of which diindolylmethane (DIM) appears to be one of the most active [72, 73].

Various antitumour and chemopreventive properties for indoles have been described *in vivo* and *in vitro* [69] including induction of apoptosis [74–76], cytotoxic effects [77, 78], cell cycle arrest [79–81], antioxidant effects [82–85], antiproliferative characteristics [82, 86], modulation of cell cycle parameters, activation of tumour suppressor genes such as BRCA1 and BRCA2 [87] and various cell signaling pathways, as well as causing a change in androgen and oestrogen metabolism. Micro-array analysis has demonstrated that I3C and DIM change the function of around 700 genes [88], some of which apparently reduce the expression of HPV oncogenes [85]. As HPV cannot be grown *in vitro,* experiments have been performed with cancer cell lines containing integrated HPV DNA rather then episomal DNA. Therefore extrapolation of the results has to be undertaken with caution when considering the effects of indole derivates on HPV in an organic system.

4 Indoles and oestrogen metabolism

Degradation of 17β-oestradiol, results in either 2-hydroxy-oestradiol, 16α-hydroxy-oestrone or 4-hydroxy-oestrone metabolites (see Fig. 3). 2-hydroxy-oestradiol is anti-oestrogenic and anti-proliferative, whereas 16a-hydroxy-oestrone and 4-hydroxy-oestrone cause cell proliferation and have carcinogenic potential (see reviews in [89, 90]).

Indole derivates appear to influence oestrogen metabolism and control the growth of oestrogen-dependent cancer cells [54, 91–98], blocking abnormal proliferation and malignant transformation caused by oestradiol and 16a-hydroxy-oestrone [99, 100]. Additionally, Indoles modulate oestrogen-receptor activities [87, 94, 101–104] as noted in breast cancer cell lines [94, 105], HPV-immortalised cell lines, cervical cancer cell lines [75, 98] and in a mouse model [61]. Mice expressing transgenes for HPV-16 develop cervical cancer when given 0.165 mg daily of 17-beta-estradiol over two months, whereas co-administration of 2000 ppm 13C results in a significant reduction in cervical cancers [61, 75]. The oestrogenic effects in this model

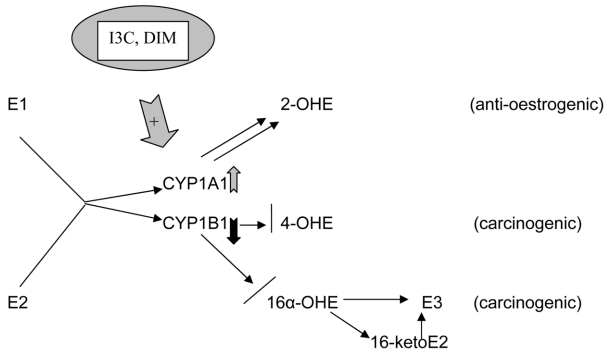

Figure 3. Schematic Simplified Illustration of Effect of Indole Derivates on Oestrogen Metabolism. Degradation of 17β-oestradiol (E2) and oestrone (E1) by CYP1A1 results in 2-hydroxy-oestradiol (2-OHE) which has anti-oestrogenic and anti-proliferative properties. Degradation by CYP1B1 results in 16a-hydroxy-oestrone (16-OHE) or 4-hydroxy-oestrone (4-OHE). Both cause cell proliferation and have carcinogenic potential. Indole derivates activate CYP1A1 but inhibit CYP1B1. This changes the balance in favour of 2-OHE production. At the same time, I3C can down-regulate the oestrogen-receptor alpha. Increase in metabolizing enzymes CYP1A1 is symbolized by ⇧, decrease in metabolizing enzymes CYP1B1 is symbolized by ⇩. E1, estrone; E2, 17β-oestradiol; E3 oestriol; 4-OHE, 4-hydroxy-estrone; 16α-OHE, 16α-hydroxyestrone.

have to be interpreted with some caution as a keratin 14 promoter directed the HPV-16 gene expression rather then the native HPV promoter (K14-HPV 16 mice).

It is thought that the effects of Indoles on oestrogen metabolism are partially due to induction of enzymes (CYP1A1 family), critical for the metabolic detoxification of carcinogens [94, 106–111]. Activation of these liver enzymes increases hydroxylation of 17β-oestradiol, leading to the protective 2-hydroxy oestradiol [54, 112–114].

In humans, excretion of 2-hydroxy oestradiol relative to that of 16α-hydroxy oestrone was significantly increased by I3C [90, 100, 115–121]. Indole derivates also decrease CYP1B1 activity, thereby decreasing the formation of the carcinogenic 4-hydroxy oestrone (see review [89]). Not all humans respond to Indole derivates possibly explained by genetic polymorphic variation in CYP1A1 [122, 123].

5 Indoles and HPV

Indoles appear to have antiviral activity, reducing endogenous transcription of HPV16 oncogenes *in vitro* [85] and in K14-HPV16 mice [61].

A prospective trial utilising 400 mg I3C daily as treatment for recurrent respiratory papillomatosis in 33 patients showed that one third of patients experienced remission of papillomatous growth and did not require surgery whilst on I3C; one third had a reduction in papillomatous growth that resulted in less frequent surgery and one third had no clinical response. No long-term side effects were observed [124]. Indole derivates are currently used as adjuvant treatment for recurrent respiratory papillomatosis.

A randomised placebo-controlled study of thirty patients with biopsy proven CIN II–III showed a 50% regression rate in women in the I3C arm (200 mg or 400 mg daily for three months), compared to none of the patients in the placebo arm (95% CI, 0. 25 to 0.99; $p = 0.023$) [90].

6 Critics of indoles

Some researchers have noted oestrogenic properties for DIM [102, 105, 125], with DIM exhibiting both oestrogenic as well as anti-proliferative and cytotoxic activity [91, 102]. In rodent models I3C but not DIM appeared to enhance the hepatotoxicity of tamoxifen [114, 126]. Long-term intake of indole derivates may cause bone loss (unpublished data [127]).

Under certain conditions in cell and in animal studies indoles have been found to promote or enhance effects on tumourgenesis [128–136]. Some of the most critical voices come from studies with rainbow trout where indole derivates act as oestrogen agonists [125, 136] as in rat models [126, 137]. High indole intake appears to reduce immune responses by reducing natural killer cell activity [138, 139].

7 Safety and side effects

Recent animal safety studies have demonstrated increased safety for formulated DIM compared to I3C with no evidence of teratogenicity [140–144]. The doses given in animal studies are significantly higher then the relative doses given in human research [144]. Known side effects in human subjects are mild, reversible and uncommon and include nausea, dyspepsia and flatulence. Harmless darkening of the urine due to excretion of coloured DIM metabolites in those with low fluid intake is reported. There is also a suggestion that DIM may increase the severity of headaches in women who suffer migraine [145]. Long-term studies of the effects of indoles in humans are outstanding.

8 Conclusion

Many studies have investigated the potential anti-carcinogenic properties of indoles including their effects upon apoptosis, oestrogen and androgen metabolism. I3C modulates the oestrogen-receptor and enhances the activity of detoxifying enzymes responsible for 2-hydroxylation of oestrogen, inducing the formation of 2-hydroxy oestradiol, which in itself has anti-oestrogenic activities. The accumulated evidence supports a role for indoles as chemopreven-

tive agents in oestrogen-dependent tissue. Further long-term studies of chemoprevention for hormone-dependent tumours such as breast, cervical and prostate cancer in humans are warranted.

Both authors are involved in a chemoprevention study using DIM in women with low grade cervical cytological abnormalities. The study is supported by Cancer Research UK and BioResponse DIM®. Neither organisation has had an input into the present review.

Conflict of interest statement: Both authors are involved in a chemoprevention study using Diindolylmethane in women with low grade cervical cytological abnormalities. The study is supported by Cancer Research UK and BioResponse DIM®. Neither organisation has had an input into the present review.

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