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Diénogest + extrait de Brocoli plus efficace dans l'Endométriose

Comparison of dienogest effects upon 3,3'-diindolylmethane supplementation in models of endometriosis and clinical cases
Morales-Prieto et al. 2018 (Reprod. Biol.)

Le **diénogest (DNG)** est un traitement bien défini chez les **femmes atteintes d'endométriose.** Le principal inconvénient est la **persistance de saignements irréguliers** et **d'autres effets secondaires tels que la prise de poids**, l'augmentation de la pression artérielle et la sensibilité des seins, qui entraînent parfois **une adhésion limitée au traitement**.

Les dérivés phytochimiques de **l'indole-3-carbinol, présents dans les choux comme le brocoli**, sont connus pour leurs propriétés préventives dans les pathologies hormonales. En tant que précurseur du 3,3'-diindolylméthane (DIM), ils ont déjà été **proposés pour le traitement de la mastalgie et de l'endométriose**.

En raison de la **différence de mécanisme d'action entre le DNG et le DIM**, les chercheurs de cette étude ont voulu **étudier l'effet d'une thérapie combinée** via des modèles *in vitro* et *ex vivo*, ainsi que son potentiel chez les **patientes atteintes d'endométriose**.

<u>Résultats et Conclusion</u>:

Une première étude *ex vivo* a examiné la viabilité du tissu endométrial de patientes, sous l'influence du DNG et du DIM. Les deux substances ont un effet antiprolifératif (DNG : -9%, DIM : -25%). Ces **effets ont été nettement augmentés par la combinaison de DNG et de DIM, diminuant la viabilité du tissu endométrial d'environ 40%**.

Une analyse du schéma des saignements chez les patientes atteintes d'endométriose (pendant 84 jours), après le traitement par DNG et la combinaison de DNG et DIM, n'a montré aucune différence dans le nombre d'épisodes de saignement.

Cependant, un **traitement combiné avec DNG et DIM** a conduit à une **diminution significative de la durée des épisodes de saignement**, entraînant une **augmentation significative du nombre de jours sans saignement** et une **réduction de la douleur associée à l'endométriose** (Figure 1).

On peut en conclure que la **thérapie DNG + DIM est efficace pour réduire les symptômes résiduels, tels que les schémas de saignement et les douleurs associées à l'endométriose**, qui peuvent encore fréquemment causer de l'inconfort chez les patientes sous thérapie DNG.

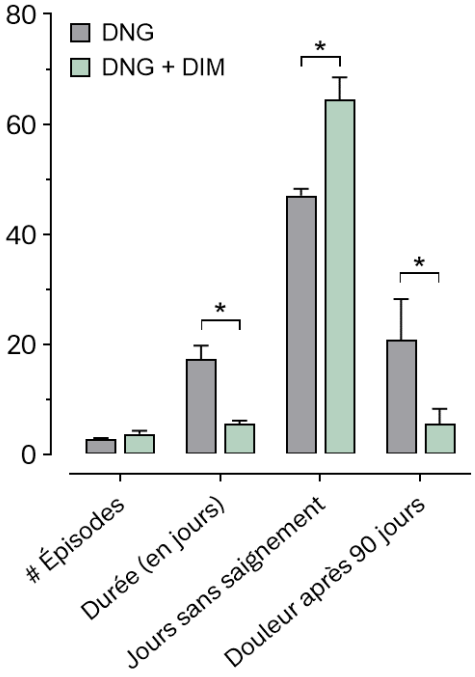

Figure 1 | Schémas de saignement chez les patients atteints d'endométriose. La comparaison des paramètres cliniques chez les patients sous traitement DNG ou DNG + DIM montre une amélioration significative avec l'ajout de DIM. * $p < 0.05$.

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Original article

Comparison of dienogest effects upon 3,3′–diindolylmethane supplementation in models of endometriosis and clinical cases

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ABSTRACT

Dienogest (DNG) administration is a well-established treatment for endometriosis but bleeding irregularities remain its main disadvantage. Changes in diet, mainly to vegetable consumption, are beneficial in the treatment of estrogen-related pathologies but their use for endometriosis has been poorly studied. In this study, addition of the phytochemical 3,3′-diindolylmethane (DIM) to DNG therapy has been investigated in *in vitro and ex vivo* models for endometriosis and in a small cohort of women with endometriosis. Endometrial Ishikawa cells were treated with DNG or DIM at dosages from 10^{-10} M to 10^{-5} M for up to 72 h. Cell proliferation was measured by assessing BrdU incorporation. Endometrial tissue from women with endometriosis and controls was incubated with DNG or a combination of DNG and DIM. Tissue viability was determined using a modified colorimetric MTS assay. 17β-estradiol secretion was quantified by an electro-chemiluminescence immunoassay. Finally, DNG as monotherapy or in combination with DIM was randomly administered to women with endometriosis (n = 8) over 3 months. Bleeding patterns and associated pelvic pain were assessed by Visual Analogue Scale (VAS). DNG and DIM significantly reduced cell proliferation in Ishikawa cells. *Ex vivo,* DIM reduced viability and estradiol secretion specifically in endometriotic but not in normal endometrial tissue. This effect was enhanced by combination with DNG. Endometriosis associated pelvic pain was significantly reduced in patients taking the DNG-DIM combination therapy compared to those taking DNG alone. Bleeding pattern (number and duration of episodes) was significantly improved by addition of DIM to the DNG treatment. In conclusion, addition of DIM enhances effects of DNG *ex vivo* and may ameliorate bleeding patterns in endometriosis patients.

1. Introduction

Dysmenorrhea, subfertility and chronic pelvic pain are amongst the most common symptoms of endometriosis, a condition that affects approximately 6–10% of women of reproductive age [1]. A recently published hypothesis of its pathogenesis proposes an aberrant placement of stem cells within the mesoderm. Additionally, alterations of the peritoneal microenvironment in regard to immune cells, adhesion molecules, extracellular matrix metalloproteinase and cytokines may promote proliferation and survival of ectopic endometrial cells [2].

According to the Practice Committee of the American Society for Reproductive Medicine (ASRM), "endometriosis should be viewed as a chronic disease that requires a life-long management plan with the goal of maximizing the use of medical treatment and avoiding repeated surgical procedures" [3]. There is limited evidence of the benefits of the available medical therapies. Therefore, the choice of an optimal long-term medical management is problematic.

Accumulating evidence has demonstrated an association between endometriosis and abnormal progesterone signaling in the endometrium. Several causes for progesterone resistance have been proposed including neonatal "preconditioning", genetic and epigenetic changes, repetitive retrograde menses and chronic inflammation (reviewed in [4]). The molecular basis for progesterone resistance relies, among others, in the downregulation of the progesterone receptor (PR) A isoform (PRA) and loss of the longer isoform B (PRB) in endometriotic tissue [5]. This effect has been reported also in stromal cells from women with endometriosis exposed to inflammatory cytokines [6]. This has led to the development of synthetic progestins that may alleviate

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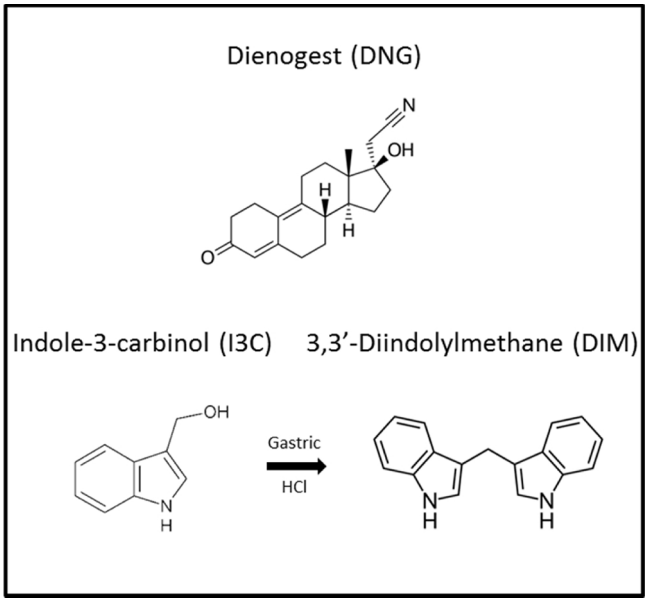


Fig. 1. Molecular structures of the analyzed drugs.

clinical symptoms of endometriosis by restoring progesterone function. Progestins have beneficial effects against pain and reduce lesions and risk of recurrence at high levels of safety and tolerability, making them valuable tools for long-term treatment [7–9]. The advantage of the progestin therapy is a favorable balance among benefits and potential harms in comparison with alternative medical therapies such as estrogen-progestin oral contraceptive pills (OCPs), gonadotropin-releasing hormone agonists (GnRH-a) and danazol [7,8,10].

Because progestins suppress, but do not cure ectopic foci, their effect is maintained only as long as they are used but symptoms recur generally after treatment discontinuation [8,10]. The main problem associated with the continuous progestin-only use is bleeding irregularities, which may lead to therapy discontinuation [1,11]. Therefore, pharmacological approaches are necessary to improve the therapeutic efficacy of the progestin therapy in endometriosis by reducing its associated bleeding problems.

Medroxyprogesterone acetate (MPA), dienogest (DNG) and levonorgestrel constitute the most commonly used progestins for the management of pain in patients with endometriosis [1]. DNG (17α-cyanomethyl-17β-hydroxy-4,9-estradien-3-one; STS 557; Fig. 1) was firstly synthesized and pharmacologically characterized in 1980 in Jena, Germany, upon extensive structure-efficacy studies [12]. DNG plays a role in regulating progesterone resistance by targeting PRB and suppressing secretion of inflammatory cytokines including interleukin (IL)-6, IL-8 in endometrial stromal cells from women with endometriosis [13,14]. Numerous publications have enlarged the knowledge about the preclinical and clinical profile of DNG including successful clinical studies [8,15–20]. The main reported advantage of the DNG therapy is the significant decrease in dysmenorrhea, dyspareunia, premenstrual and diffuse pelvic pain, as well as its long-term safety [8,20–22]. DNG as monotherapy improves quality of life assessed as general and mental health, vitality, emotional role, as well as the female physical, social and sexual function [22]. Similar effects were observed in a prospective study in women treated with a combination of DNG and ethinyl estradiol. Remarkably, the positive effects on quality of life and sexual function improvement were observed only in the group of continuous compared to the conventional 21/7 regimen [21,22]. Nevertheless, side effects such us weight gain, increased blood pressure, breast tenderness, nausea and irregular bleeding remain the main disadvantages of DNG treatment [8,23].

Recently, phytochemical derivatives from indole-3-carbinol (I3C), which are known for their preventive properties against carcinogenesis [24], have been proposed for the treatment of mastalgia, endometriosis and HPV-related disorders [25]. *Brassica* vegetables such as cabbage, Brussels sprouts, broccoli, and the spice cardamom are rich in I3C, an active phytonutrient and a precursor of many compounds, including 3,3′-diindolylmethane (DIM; Fig. 1). In an acidic environment, I3C undergoes acid-catalyzed dehydration and polymerization and converts into the more stable DIM [26,27]. DIM abrogates proliferation of human cancer cells of prostate, breast, colon, ovary and pancreas [28]. In estrogen-sensitive cells, DIM specifically reverses estrogen effects by inhibiting ER-α signaling leading to modification of growth arrest gene expression [29]. These capacities suggest a potential use of DIM also for the treatment of endometriosis. Beside its cytostatic effects on human endothelial cells [30], its efficacy in the treatment of endometriosis remains poorly investigated. Due to the differences in the mechanisms of phytochemicals and progestins, some synergistic effects may be expected when they are used in combination. Therefore, this study aims to investigate the effect of a combination of DNG and DIM on *in vitro* and *ex vivo* models, as well as its potential clinical use based on a series of cases of patients with endometriosis treated with single or combination therapy.

2. Material and methods

2.1. Cell culture

Ishikawa cells, a human aromatase expressing endometrial adenocarcinoma cell line [31], were cultured in Dulbecco's modified Eagle's medium (DMEM; GIBCO), supplemented with 5% (v/v) heat-inactivated fetal bovine serum (FBS; SIGMA), and maintained under standard conditions (37 °C, 5% CO_2, humidified atmosphere).

2.2. Chemicals

DIM and DNG were obtained from Naari Pte. Ltd., Singapore.

2.3. Ex vivo model

Twelve patients (age between 34 and 51 years) were recruited at the Sophien- and Hufeland-Hospital, Weimar, Germany. Endometriotic tissue was collected from five patients under visual observation and washed in physiological saline solution to discard chocolate fluid and excess of red blood cells. Normal endometrium was obtained at hysterectomy from 7 normally cycling women without endometriotic lesions who were not on hormonal treatment at the time of surgery. No significant difference in age was observed between the two groups.

Explant samples of ~200 mg were divided into small pieces of ~40 mg. Explants were cultured in DMEM medium (GIBCO), supplemented with 5% FBS and 1% penicillin/streptomycin (PAA Laboratories) in presence of DNG (10^{-7} M), DIM (10^{-7} M) or their combination. Treatments were administered in triplicates.

2.4. Proliferation test

Cell proliferation was assessed by using the colorimetric Cell Proliferation ELISA BrdU assay (Roche) following the manufacturer's instructions. Briefly, cells were labelled with BrdU and then fixed to a 96-well plate. BrdU incorporated in the DNA was detected by a monoclonal antibody conjugated with peroxidase and quantified after enzymatic substrate reaction at 370 nm (reference wavelength: 492 nm). For experiments on Ishikawa cells, 10,000 cells were seeded in 96-well plates and allowed to grow for up to 72 h in presence of different concentrations of DNG and DIM. Results are expressed as relative to the vehicle control.

2.5. Viability test of tissue explants

CellTiter96™ AQ non-radioactive cell proliferation kit (Promega) was used to determine cell viability in primary endometrial tissues as recommended by the manufacturer. Endometrial tissue explants (~40 mg) were seeded in 96-well plates in DMEM medium containing 5% (v/v) FBS and 1% penicillin/streptomycin in triplicates. The next morning, supernatants were collected for measurement of basal expression of estrogen and progesterone. Subsequently, medium was exchanged by DMEM (5% FBS) supplemented with different concentrations of DNG, DIM or their combination. After 72 h of incubation, supernatants were collected for final measurement of hormones. Finally, 100 µl DMEM (phenol- and serum-free) and 20 µl MTS solution was added to each well containing tissue and further incubated for 2 h. After removing the remaining tissue, cell viability was quantified in the supernatants assessed as absorbance at 490 nm. Values are expressed as relative to vehicle control.

2.6. Estradiol and progesterone detection

17β-Estradiol and progesterone levels were detected in tissue culture supernatants by electrochemiluminescence immunoassay "ECLIA" on a Roche-Cobas analyzer (Cobas - Roche) according to manufacturer's instructions. Results are expressed as ratio of final concentration (72 h) vs. initial concentration (0 h), and expressed as relative to vehicle treated controls.

2.7. Clinical cases

A single center, clinical observation based on case reports has been performed to evaluate the effect of DNG-DIM combination versus DNG alone on bleeding pattern and on endometriosis-associated pelvic pain (EAPP). After signing informed consent and laparoscopic confirmation of endometriosis, 10 women were enrolled and randomly divided in two groups: 1) DNG alone or 2) DNG and DIM. Drugs were administered orally over 3 months at a dose of 2 mg DNG once per day (DNG, Visanne®, Bayer/Germany) and 100 mg DIM three times per day (BioResponse –DIM®, Springfield Nutraceuticals BVZ/The Netherlands). The patients returned 3 times (once per month) to the study site for assessment of EAPP by using a Visual Analogue Scale (VAS). Between visits, patients were asked to complete a diary with regard to bleeding pattern and use of additional analgesic medication. Two patients were excluded from the study due to inconstant recording of bleeding patterns or long-time treatment interruption. The reason for interruption was not the occurrence of adverse effects. Eight patients (between 26 and 41 years) finished the study (Fig. 2).

The observation plan and consent form including data protection statement were approved by the Ethics Committee of the Thuringian Medical Association (Ethik-Kommission der Landesärztekammer Thüringen) under registration number 35291/2014/116, study number 1299dim14ct on 09/10/14.

2.8. Statistics

Results are shown as mean ± SEM. Data were analyzed using Student's *t*-test or ANOVA as indicated in the figure legends.

3. Results

3.1. In vitro model

Effects of DNG and DIM on cell proliferation was analyzed in Ishikawa cells by BrdU incorporation. For regular proliferation medium supplementation with 5% FBS was necessary, as well as daily renewal of media containing the active compounds (data not shown). A significant dose-dependent anti-proliferative effect was observed in cells treated with DNG (10^{-9}–10^{-5} M) after 72 h of incubation. At high concentrations (10^{-6} and 10^{-5} M) proliferation was significantly reduced after only 48 h of incubation (Fig. 3A). DIM treatment reduced proliferation of Ishikawa cells dose-dependently when applied at 10^{-7}–10^{-5} M for 72 h. Earlier effects are observed at 24 and 48 h when using 10^{-5} M and 10^{-6} M, respectively (Fig. 3A). Based on these observations we have chosen 10^{-7} M as the concentrations of both compounds for further experiments.

3.2. Ex vivo model

To investigate the effect of combined administration of DNG and DIM, viability of endometrial tissue from patients with and without endometriosis was assessed after 72 h of treatment. In the endometriotic tissue, a 9% decrease of cell viability was observed after incubation with 10^{-7} M DNG alone, but at the same concentration, DIM reduced cell viability significantly by 25%. This effect was significantly enhanced by combination of DNG and DIM, which resulted in a decrease of approx. 40% of the control cell viability. 17β-estradiol secretion was not affected by DNG alone but treatment with DIM and the combination therapy (DNG-DIM) resulted in a significant reduction of approximately 27% (Fig. 3B).

In tissue obtained from patients after hysterectomy without history of endometriosis, only a slight decrease of cell viability was observed when treated with DNG alone (9% decrease), but no differences were observed after incubation with DIM alone or in combination. Likewise, estradiol release remained almost constant in all experiments (Fig. 3B). Progesterone was undetectable in the supernatants of tissue cultures.

3.3. Clinical cases

To compare the effects of single or combined therapy on EAPP and bleeding patterns, reports of patients with endometriosis treated with DNG or DNG-DIM combination were statistically evaluated. Patients in the DNG group started at a slightly lower median intensity of EAPP than the patients in the DNG-DIM group (62.8 ± 8.1 vs. 69.2 ± 12.9 mm, respectively). In both groups EAPP improved significantly compared to the reported value at the first visit. At the fourth and final medical visit, the median intensity of EAPP was still higher in the DNG group (20.8 ± 14.8 mm) than in the DNG-DIM group (5.5 ± 5.5 mm), but not significantly (Fig. 4A).

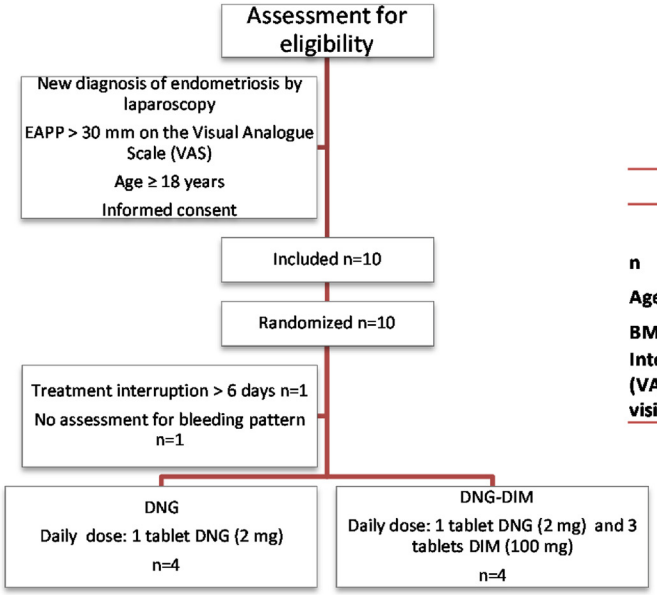
Analysis of patient bleeding patterns for 84 days showed a significant improvement in the number of days with no bleeding in the DNG-DIM group compared to the DNG group (64.5 ± 8.0 days vs. 47.0 ± 2.5, respectively). Spotting and light bleeding also was less frequent in the DNG-DIM group compared to the DNG group (9.8 ± 4.0 vs.16.2 ± 5.4 and 5.0 ± 2.1 vs.15.75 ± 7.0), but without statistical significance (Fig. 4B). Other bleeding parameters did not differ between the two groups.

The number of episodes was slightly higher in the DNG-DIM group than in the DNG group (3.7 ± 1.1 vs. 2.7 ± 0.5), but the duration of bleeding episodes was significantly shorter in the DNG-DIM group (5.5 ± 1.2 days) than in the DNG group (17.3 ± 4.9 days) (Fig. 4C).

In general, both treatments were well tolerated. In a scale of "very good, good, moderate, bad" the tolerability was mostly classified as "very good" or "good". In the DNG group, the tolerability was assessed as "moderate" once at visit 2 and once at visit 4. In the DNG-DIM group, tolerability was assessed as "moderate" twice at visit 2. In no case, tolerability was rated as "bad".

4. Discussion

Several theories have been proposed to explain the pathogenesis of endometriosis including changes in immune, hormonal, genetic and epigenetic factors [32]. Since progesterone plays an important role in the regulation of endometrial growth, synthetic progestins have been



	DNG		DNG-DIM		
	mean	SD	mean	SD	p
n	4		4		
Age [years]	30.75	3.20	35.50	6.86	0.26
BMI [kg/m2]	28.65	9.91	21.21	1.47	0.19
Intensity of EAPP (VAS) [mm] at visit 1	62.75	16.15	69.25	25.79	0.68

Fig. 2. Composition and description of study groups.

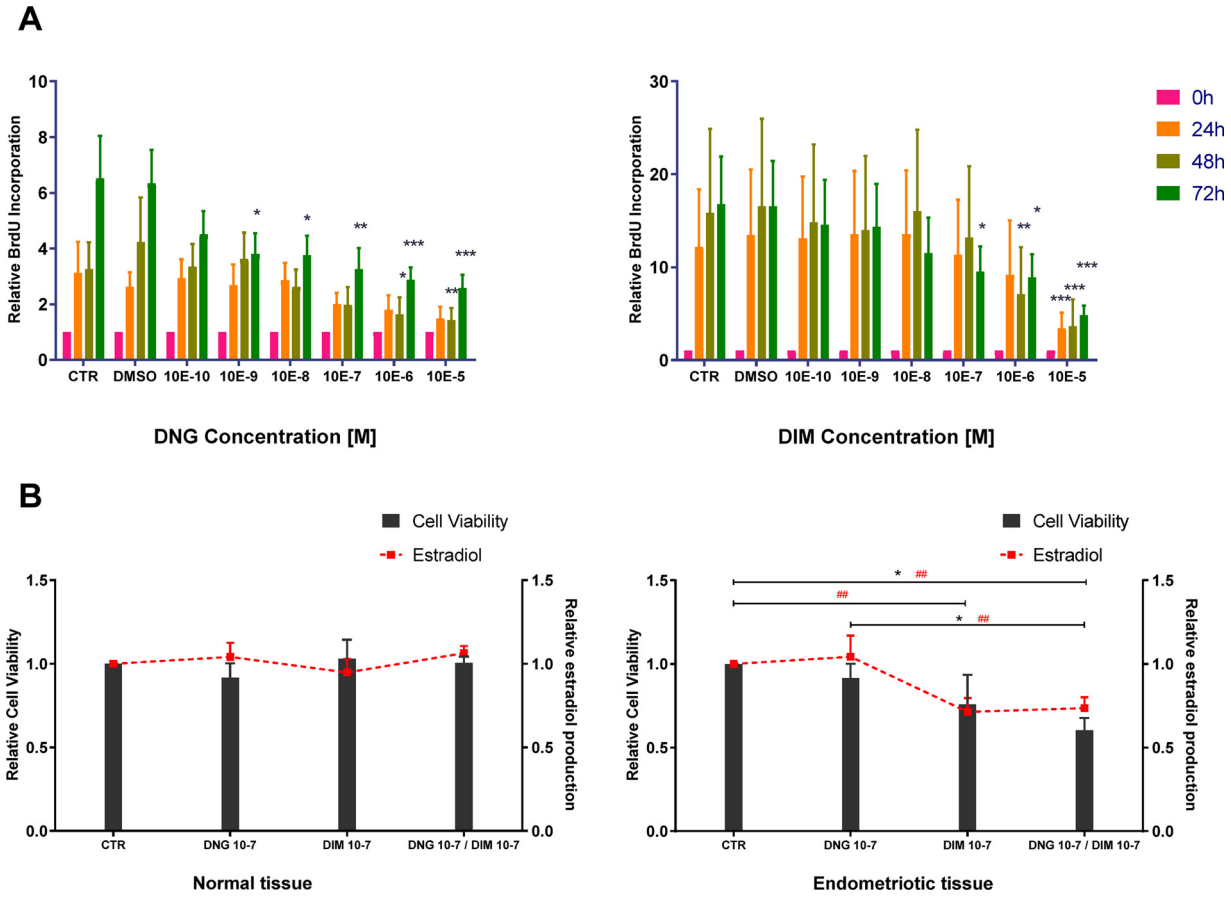


Fig. 3. A) Anti-proliferative activities of DNG (n = 7) and DIM (n = 4) in Ishikawa cells as assessed by BrdU assay. B) Cell viability and 17β-estradiol secretion in primary tissues from normal endometrium and endometriotic tissue. Error bars indicate standard error of the mean. Student's *t*-test * $p < 0.05$; ** (or $^{\#\#}$ for estradiol) $p < 0.01$; ***, $^{\#\#\#}$ $p < 0.001$.

developed to solve the problem.

DNG belongs to the most used progestins for the treatment of endometriosis due to its unique pharmacokinetic profile, which is characterized by progesterone receptor selectivity, anti-androgenic properties, moderate anti-gonadotrophic action, and inhibition of bone resorption [33]. This 19-norprogestin does not bind to sex hormone-binding globulin (SHBG) and albumin, which results in a higher percentage of unbound biologically active compounds (~10% vs. 0.5–4% for other progestins), short serum half-life of 6.5 h, small distribution volume (one-compartment situation), and high oral bioavailability by

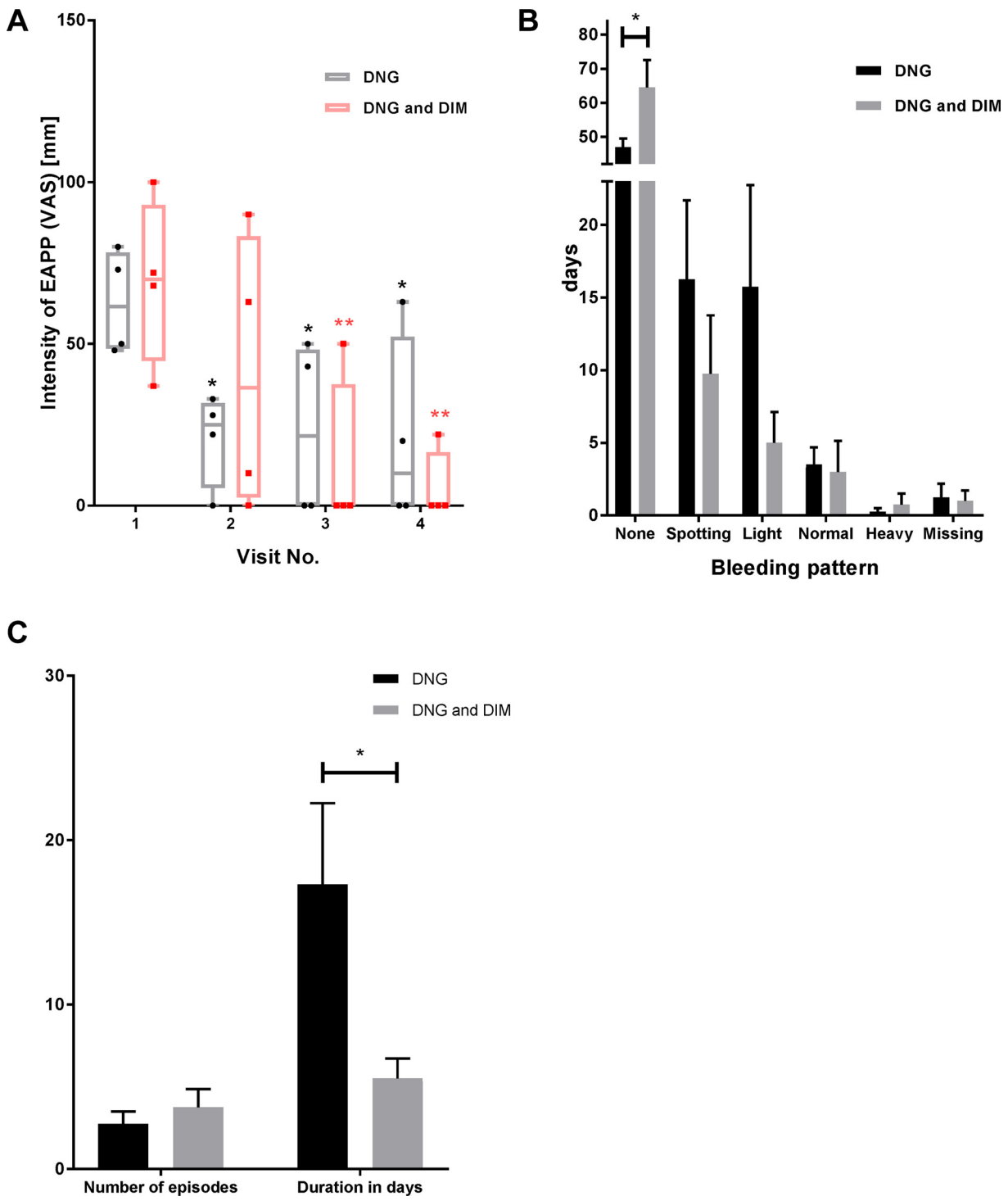


Fig. 4. Comparison of clinical parameters in patients upon DNG or DNG-DIM combination therapy. A) Intensity of EAPP at each medical visit. Dots show each individual value, box plots indicate 25 and 75 percentile and median, error bars show full range of values. B) Mean number of days per patient with the respective bleeding pattern and C) mean number of bleeding episodes and bleeding days/episode within a period of a total of 84 days. Error bars indicate standard error of the mean. Two-way ANOVA, * $p < 0.05$.

90.5% [34].

Recent studies demonstrate that the hypo-estrogenic environment created by DNG inhibits endometriotic lesion growth (Reviewed in [35]). The first hint for the use of DNG for the treatment of endometriosis came from experimental models showing reduction of the volume of autogenous transplanted endometrium including angiogenesis in rats and rabbits. The proposed mechanism is an increase in the natural killer activity in peritoneal fluid cells and decreased apoptosis and interleukin-1β production in peritoneal macrophages [36–40]. DNG also inhibits aromatase and cyclooxycenase-2 expression and prostaglandin E2 production in human endometriotic stromal cells and increases the progesterone receptor isoform B:A ratio in patients with ovarian endometriosis which improves progesterone resistance in endometriotic tissue [41,42].

Due to its efficacy, DNG has been approved for treatment of endometriosis in Europe and Canada, but side effects are often reported.

Accumulating evidence has demonstrated that lifestyle and diet may influence the risk of endometriosis, such as fruit and vegetables consumption [43]. Furthermore, dietary consumption of cruciferous vegetables *(Brassicas)* was proven to reduce estrogen-dependent malignancies such us breast, endometrial and cervical cancer [29]. *Brassicas* are rich in IC3 and its biologically active dimer DIM which can diminish the effect of estrogen on tissue growth. The involved mechanisms include suppression of genes driven by the estrogen receptor, and the control of enzymes such us CYP1A1 resulting in metabolites that are antiproliferative and proapoptotic [29]. Since different cellular mechanisms control DNG and DIM effects, it may be hypothesized that their combination further improves the treatment of endometriosis.

In this study, *in vitro, ex vivo* models as well as a series of cases were used to establish effects of DNG and its combination with DIM for the treatment of endometriosis.

The *in vitro* study on Ishikawa cells confirmed the anti-proliferative activities of DIM and DNG. Based on the dose-dependent effects, DNG has a higher efficacy *in vitro* starting at 10^{-9} M. Previous reports demonstrated 10^{-7} M as an effective DNG dose for reducing prostaglandin E2 production, aromatase expression and proliferation in endometrial and stromal cells [41,44]. Likewise, treatment with DNG at 10^{-5} and 10^{-7} M diminishes inflammation-induced proliferation as assessed in primary endometriotic stromal cell cells treated with TNF-α [14]. A concentration of 10^{-7} M DNG is similar to the blood levels resulting from administration of 1 mg DNG twice daily [41], and was therefore selected for further experiments.

The effects of DIM on Ishiwaka cells have been reported only in one study demonstrating a significant reduction of proliferation upon treatment with DIM at 10^{-5} M [30]. Our results demonstrate that continuous treatment with DIM at 10^{-7} M is sufficient for significant reduction of Ishikawa cell proliferation. This concentration is 100-fold lower than the serum concentration of *Brassica* vegetable eaters consuming 200 g of Brussels sprouts daily [30], which allows the translation of these *in vitro* results to the *in vivo* situation.

An *ex vivo* model was used to investigate the combination treatment of DNG and DIM on primary tissue explants from endometriosis patients and controls. In endometrial tissue explants obtained upon hysterectomy from patients without endometriosis no effects of DNG, DIM or their combination were found on cell viability and estradiol secretion. In contrast, cell viability was reduced in endometriotic explants treated with 10^{-7} M DNG and 10^{-7} M DIM alone. This effect was significantly enhanced when DNG and DIM were applied in combination.

DIM but not DNG was effective in reducing estradiol secretion highlighting the differences in the action mechanisms of these compounds and the potential of DIM to specifically reverse estrogen effects [29]. Combination of DIM and DNG induced the strongest reduction in cell viability and estradiol secretion suggesting a potentiating effect of the DNG-DIM combination. These effects were observed only in tissue from endometriosis lesions and not from normal endometrium demonstrating a pathology-specific effect similar to that on prostate cells, where DIM selectively induces apoptosis in cancerous, but not in normal cells [45]. A pharmacodynamics trial reported that monotherapy with DNG at doses > 2 mg results in declining E2 concentrations, but still within the physiologic range, in healthy volunteers [46]. Our results suggest that a DNG-DIM combination may specifically reduce estradiol levels in endometriotic lesions, but minimizes a general hypoestrogenism, the main drawback of the current hormonal treatment. Extremely low levels of E2 may result in bone mineral loss, vasomotor symptoms and atrophy of secondary sexual characteristics, while elevated E2 levels induce endometriotic lesions to growth [47]. Therefore, endometriosis treatment should be designed to fit within the “estradiol therapeutic window”, in which estradiol is reduced to levels at which the growth of endometriotic lesions is not stimulated but bone loss remains minimal [47].

To investigate the potential use of DNG-DIM combination for the treatment of women with endometriosis, a clinical observation was carried out in a small group of volunteers. Health outcome (EAPP and bleeding patterns) was reported by patients after being randomized in two groups: DNG and DNG-DIM therapy. The chosen dosage of DNG (2 mg/day) corresponds to that of Visanne®, which is approved in many countries for the treatment of endometriosis [48], and matches the optimized concentration of our *in vitro* and *ex vivo* studies [41]. Likewise, the total dosage of DIM of 300 mg/day corresponds with reported clinical studies [28]. The application interval (three times daily) of an absorption-enhanced DIM preparation (100 mg DIM/ hard gelatin capsule) is based on the short half-life of 4.5 h [49].

In the group of patients treated only with DNG, endometriosis associated pain (EAPP) was significantly reduced already at the first visit, whilst the DNG-DIM combination group reported significant differences starting at the second visit. However, 75% of the women in the combination group did not complain pain after three cycles, whilst 50% of the women in the DNG group had pain until the end of the study. This reduction in pelvic pain occurred within the first 8 weeks of treatment and is an improvement compared to previously reported effects of DNG alone requiring 24–52 weeks of treatment [50]. Despite the small size of the groups, there is a clear trend in the positive effect of DNG-DIM combination compared to DNG alone. This was further demonstrated in the bleeding patterns where a significant reduction in the number and the duration of bleeding/spotting episodes was observed in the DNG-DIM group compared to the DNG group. No additional side effects were reported in the DNG-DIM group and the tolerability was more favorable than in the DNG group. Further clinical studies should assess potential side effects of the combination therapy and confirm the improvement of DNG therapy by additional application of DIM.

5. Conclusion

Combinatory DNG-DIM therapy is more effective than DNG alone in reducing viability and estradiol secretion of endometriotic but not normal endometrial tissue *ex vivo*. A series of clinical cases indicates that application of DNG-DIM combination may ameliorate bleeding patterns in women with endometriosis.

Declaration of interest

I confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. I further confirm that the order of authors listed in the manuscript has been approved by all of them.

Acknowledgment/disclosure

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