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REVIEW

Does dienogest influence the inflammatory response of endometriotic cells? A systematic review

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Abstract
Objective and design A systematic review of all literature was done to assess the ability of the progestin dienogest (DNG) to influence the inflammatory response of endometriotic cells.
Main outcome measures In vitro and in vivo studies report an influence of DNG on the inflammatory response in eutopic or ectopic endometrial tissue (animal or human).
Results After strict inclusion criteria were satisfied, 15 studies were identified that reported a DNG influence on the inflammatory response in endometrial tissue. These studies identified a modulation of prostaglandin (PG) production and metabolism (PGE2, PGE2 synthase, cyclooxygenase-2 and microsomal PGE synthase-1), pro-inflammatory cytokine and chemokine production [interleukin (IL)-1β, IL-6, IL-8, tumor necrosis factor-α, monocyte chemoattractant protein-1 and stromal cell-derived factor-1], growth factor biosynthesis (vascular endothelial growth factor and nerve growth factor) and signaling kinases, responsible for the control of inflammation. Evidence supports a progesterone receptor-mediated inhibition of the inflammatory response in PR-expressing epithelial cells. It also indicated that DNG inhibited the inflammatory response in stromal cells, however, whether this was via a PR-mediated mechanism is not clear.
Conclusions DNG has a significant effect on the inflammatory microenvironment of endometriotic lesions that may contribute to its clinical efficacy. A better understanding of the specific anti-inflammatory activity of DNG and whether this contributes to its clinical efficacy can help develop treatments that focus on the inhibition of inflammation while minimizing hormonal modulation.

Keywords Dienogest · Inflammation · Endometriosis · Cytokines · Chemokines · Prostaglandin · Growth factors · Progestin · Progestogen

Introduction

Endometriosis is characterized by the growth of endometrial epithelial and stromal cells outside the uterine cavity and affects up to 15 % of reproductive age women [1]. It is associated with chronic pelvic pain, infertility [2] and a reduced quality of life [3]. The growth of endometriotic lesions is estrogen dependent and produces an increase in pro-inflammatory cytokine [4, 5], chemokine [6, 7] and growth factor [8] concentrations in the local environment. Current treatments for endometriosis target the estrogen dependence of the disease predominantly with progestogens and gonadotropin releasing hormone analogs (GnRHa) that create hypo-estrogenic and hyper-progestogenic environment via the inhibition of ovarian follicle development and the subsequent reduction in estrogen

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production and serum concentrations [9–11]. The long-term induction of a hypo-estrogenic state, however, will create significant side effects, as well as negatively impacting fertility options and are thus less than optimal for the treatment of reproductive age women. Improved treatment options are required.

Dienogest (DNG) is an orally active progestogen, highly selective for the progesterone receptor (PR) that exists in two major isoforms, PR-A and PR-B [12]. DNG is indicated for the long-term treatment of endometriotic pain [13, 14] and is associated with an increase in the patients' quality of life [15, 16] that is maintained even after the discontinuation of therapy [17]. DNG treatment with 2 mg day^{-1}, however, reduces estrogen concentrations only to levels that remain within the normal range of women in the early follicular phase of the menstrual cycle and are still higher than those mediated by GnRHa buserelin and leuproreline, but with a comparable improvement in clinical symptoms [10]. This suggests that it may not be required to eliminate the estrogen production completely for effective treatment, or that another, non-hormonal activity may contribute to DNG effectiveness.

In addition to estrogen dependence, the inflammatory microenvironment of endometriotic lesions can significantly contribute to both disease progression [18] and symptomology [19], and therefore, may also represent a viable target for the treatment of endometriosis that has not yet been fully exploited [20]. Progesterone receptor (PR) activation, the primary target for DNG also regulates inflammation in endometrium. Progesterone inhibits inflammation in endometrial myometrial cells through regulation of the nuclear factor (NF)κB, the transcription factor responsible for the pro-inflammatory cytokine production in endometrial tissue [21]. Furthermore, a reciprocal relationship between PR-B expression and NFκB exists in endometrial tissue [22]. Therefore, given the minimal decrease of systemic estrogen and the importance of inflammation to endometriotic disease progression, it is possible that an anti-inflammatory mechanism may also contribute to the effectiveness of DNG treatment in endometriotic women. If present, such a mechanism of action may be a valid therapeutic target for endometriosis treatment that could be optimized to limit hormonal modulation.

Objectives

As a preliminary investigation, we therefore examined the literature to determine whether there was sufficient evidence to support a DNG influence on the inflammatory response of endometriotic cells, and secondly, to determine whether the mechanism is mediated by PRs activation.

Materials and methods

A systematic review of the in vitro and in vivo effects of DNG on endometrial and endometriotic cells was performed in accordance with Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. All aspects of the study were decided before the literature search and no post hoc changes were made. The relevant articles were identified through a search of the Medline, PubMed and EMBASE databases with the keywords 'dienogest' in combination with 'endometriosis' and 'inflammation' by one author (G.G.) and identified references were reviewed by a second author (B.D.M.). All searches were made in June 2015, and the databases were searched from January 1, 1987 through May 31, 2015. We assessed all potentially relevant articles and examined the reference lists for additional publications. Only English language articles were considered. Both published articles and accepted manuscripts were included. We did not consider abstracts and case reports.

The inclusion criteria were the examination of the inflammatory response of endometrial/endometriotic tissue after exposure to DNG. Primary outcomes were a change in the production, or concentration of pro-inflammatory molecules, including prostaglandins (PG), cytokines, chemokines and growth factors and intracellular signaling kinases that mediate inflammation. Institutional Review Board approval was not obtained for this systematic review, as it was determined as unnecessary since all information were extracted from previously published studies.

Results

Study selection

The predefined search terms generated 90 references in PubMed. Of these 90 references, 68 were excluded because they did not meet the eligibility criteria and were concerned mainly with clinical efficacy, safety and tolerability of DNG in endometriosis patients, leaving 22 remaining publications [23–44]. Six studies were excluded, as the outcomes were not considered linked to inflammation, but rather progesterone metabolism/activity and cell cycle interference [25, 27, 28, 30–32]. One additional study was excluded as it was performed only on cells from breast and endometrial cancers [24]. The final sample consisted of 15 references [23, 26, 29, 33–44] (Fig. 1). A total of 11 studies [26, 29, 33–41] were identified that examined inflammatory effects on endometrial or endometriotic cells after DNG treatment in vitro and three studies [23, 42, 43] that

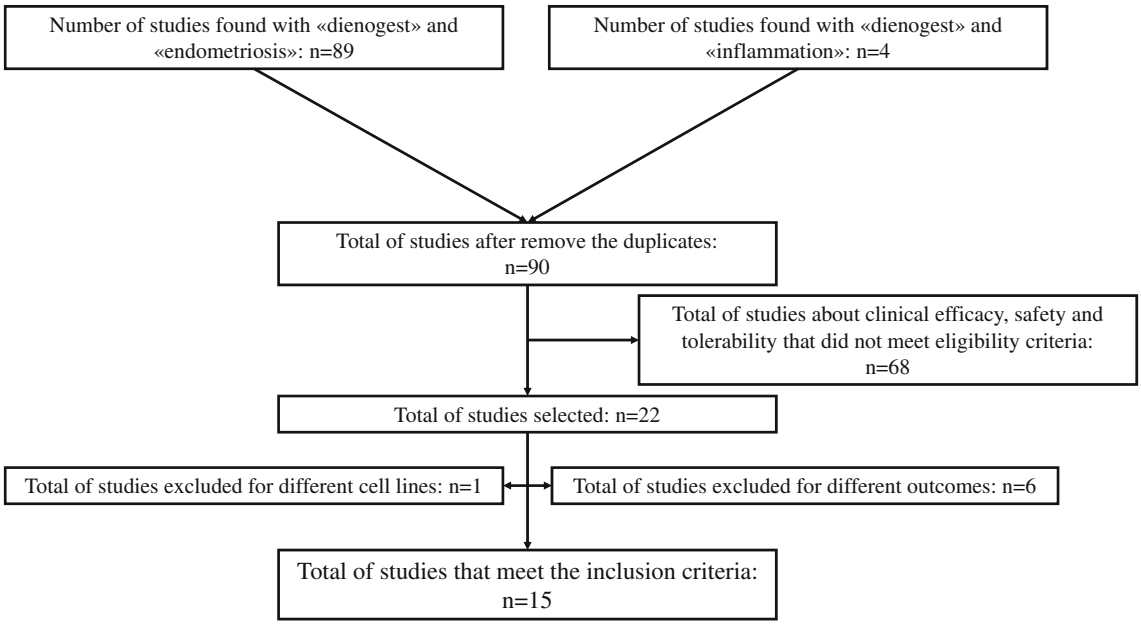

Fig. 1 Flowchart of the systematic review process used during this study

examined DNG effect on inflammation in animals (mice). The final study is an in vivo study [44] that evaluated the impact of DNG on endometrial tissue of human subjects.

Study characteristics and risk of bias

When assessing in vitro studies the use of different cell culture models and conditions is a potential source of bias. In this review, all epithelial cell studies were performed on immortalized cell lines, except one [35]. Stromal cell cultures were isolated from either the eutopic or the ectopic tissue of endometriotic women. Furthermore, both monolayer cell cultures [37, 38] and spheroid cell cultures [33, 35] have been included, which may also introduce a degree of bias. Both cell type and the conditions for each study have been clearly stated throughout the manuscript. DNG concentrations are also a source of potential bias and are clearly reported where appropriate, and range between 10^{-5} and 10^{-9} M. One final source of variation was the expression of PRs, known to vary in endometriosis patients [45], cell types and primary cell preparations (Tables 1, 2).

DNG influence on the inflammatory response

Prostaglandins and converting enzymes

In an in vitro model of immortalized epithelial cells transfected with either PRA or PRB, the treatment with DNG significantly inhibited CYP19A1, prostaglandin E2 Synthase (PGES) and microsomal (m)PGES-1 mRNA and prostaglandin E2 (PGE2) protein concentrations in PRA+/B+ epithelial cells, an effect that could be attenuated by the specific anti-progesterone RU486 [33]. DNG also inhibited CYP19A1 in immortalized epithelial cells from a peritoneal lesion (12Z) [29]. In a series of immortalized epithelial cell lines derived from ovarian endometriomas that utilized either the endogenous (PRA−/PRB−; EMosis-CC/TERT1) or the overexpression PR isoforms (PRA; EMosis-CC/TERT1/PRA, PRB; EMosis-CC/TERT1/PRA−/PRB+) there was an inhibition of the cyclooxygenase (COX)-2, mPGES and CYP19A1 mRNA in both the PRA+ and PRB+ cells, but not in the endogenous PRA−/B− cells when treated with DNG (10^{-7} M) [33]. Epithelial cells of endometrioma tissues collected in women after treatment with DNG also expressed a lower proportion of aromatase positive cells compared with controls [44].

Other studies indicate a similar influence of DNG on stromal cells. In primary stromal cells isolated from endometriomas and grown in a spheroid cell culture, DNG treatment (10^{-7} M) significantly reduced the expression of both COX2 and CYP19A1 mRNA and PGE2 protein expression [34]. Aromatase inhibition was also confirmed in stromal cell spheroid cultures from ovarian endometriomas [35]. Neither the PR subtype ratio nor the influence of specific PR antagonists was investigated.

Inflammatory cytokines and chemokines

Using the immortalized epithelial ectopic and eutopic cell culture models transfected with either PRA or PRB described previously [33], DNG treatment (10^{-7} M) mediated an inhibition of interleukin (IL)-6, IL-8 and

Table 1 The influence of DNG on the inflammatory reaction of endometrial epithelial cells in vitro and the potential mechanism of action

Effect of DNG	Expression levels evaluated			PRs dependent activity evaluated		References
	mRNA	Proteins	Others	Yes/no	Way	
Inhibition of PGE2		X		Yes	Limited to the cells that express PRA or B	Ichioka et al. [33]
	X	X		Yes	Reversed by RU-486 Not present in cells with low levels of PRs	Shimizu et al. [26]
Reduction of COX-2	X			Yes	Limited to the cells that express PRA or B	Ichioka et al. [33]
	X			No		Shimizu et al. [26]
Reduction of PGE2 synthase	X			No		Shimizu et al. [26]
Reduction of mPGES-1	X			Yes	Limited to the cells that express PRA or B	Ichioka et al. [33]
	X			No		Shimizu et al. [26]
IL-6 downregulation	X			Yes	Limited to the cells that express PRA or B	Mita et al. [36]
IL-8 downregulation		X (LPS/HMGB1 activated)		Yes	Limited to the cells that express PRB	Mita et al. [36]
	X			Yes	Limited to the cells that express PRA or B	Ichioka et al. [33]
Reduction of MCP-1		X (LPS/HMGB1 activated)		Yes	Limited to the cells that express PRB	Mita et al. [36]
	X			Yes	Limited to the cells that express PRA or B	Ichioka et al. [33]
Reduction of aromatase	X			Yes	Not present in cells with low levels of PRs	Shimizu et al. [26]
	X			Yes	Limited to the cells that express PRA or B	Ichioka et al. [33]
	X			No		Beranič and Rižner [29]
NF-κB inactivation			Binding activity to DNA (EMSA)	No		Shimizu et al. [26]
			NF-κB gene activity (reporter gene assays)	Yes	Limited to the cells that express PRB	Mita et al. [36]
VEGF downregulation	X			Yes	Limited to the cells that express PRA or B	Ichioka et al. [33]
NGF downregulation	X	X	Bioactivity (assaying neurite outgrowth of rat pheochromocytoma cells-12 cells)	Yes	PRA or B both mediate the effect that is reduced in PR negative cells	Mita et al. [40]
	X			Yes	Limited to the cells that express PRA or B	Ichioka et al. [33]

LPS/HMGB1 lipopolysaccharide and high-mobility group box 1, *PR* progesterone receptor, *NF-κB* nuclear factor-κB, *PG* prostaglandin, *COX* cyclo-oxygenase, *mPGES* microsomal PGE synthase, *IL* interleukin, *MCP-1* monocyte chemoattractant protein-1, *VEGF* vascular endothelial growth factor, *NGF* nerve growth factor, *EMSA* electrophoretic mobility shift assay

monocyte chemotactic protein (MCP)-1 in both the PRA+ and PRB+ cells, but not in the PRA/B− cells [33]. DNG (10^{-7} to 10^{-9} M) mediated these anti-inflammatory effects through the inhibition of the toll-like receptor 4 (TLR4) mRNA expression and a subsequent inhibition of TLR4 attenuated IL-8 production [36]. In primary endometriotic

Table 2 The influence of DNG on the inflammatory reaction of endometrial stromal cells and the potential mechanism of action

Effect of DNG	Expression levels evaluated			PRs dependent activity evaluated		References
	mRNA	Proteins	Others	Yes/no	Mechanism of action	
Inhibition of PGE2		X		No		Yamanaka et al. [34]
Reduction of COX-2	X	X		No		Yamanaka et al. [34]
IL-8 downregulation		X (TNF-α and E2 activated)		Yes	The effect was not attenuated by the coadministration of RU486	Horie et al. [37]
Reduction of SDF-1	X (E2 activated)	X (E2 activated)		No		Okada et al. [38]
Reduction of aromatase	X	X		No		Yamanaka et al. [34]
NF-κB inactivation			NF-kB activation (EMSA) (TNFα and E2 activated)	No		Horie et al. [37]
		X	NF-kB DNA-binding activity (ELISA)	No		Yamanaka et al. [34]
VEGF downregulation	X (E2 activated)	X (E2 activated)		No		Okada et al. [38]

E2 estradiol, *PR* progesterone receptor, *NF-κB* nuclear factor-κB, *PG* prostaglandin, *COX* cyclo-oxygenase, *IL* interleukin, *SDF-1* stromal cell-derived factor-1, *VEGF* vascular endothelial growth factor, *EMSA* electrophoretic mobility shift assay, *TNFα* tumor necrosis factor α, *ELISA* enzyme-linked immunosorbent assay

stromal cells, DNG (10^{-7} M) reduced tumor necrosis factor (TNF) α-induced (0.1 ng/mL) IL-8 expression, which could not be reversed by coadministration of RU486 [37]. DNG also attenuated estradiol (E2)-induced stromal cell-derived factor (SDF)-1 mRNA and protein production in endometrial stromal cells from women with fibroids but without endometriosis [38]. DNG (10^{-7} M) also reduced TNFα production and secretion by peritoneal macrophages [39], and in an autotransplanted model of Sprague–Dawley rats DNG reduced IL-1β expression in isolated peritoneal macrophages [23].

Growth factors

DNG treatment reduced the mRNA expression of vascular endothelial growth factor (VEGF) and nerve growth factor (NGF) in PRA+ and PRB+, but not PRA−/B− immortalized endometrial epithelial cells [33]. DNG (10^{-7} to 10^{-9} M) also inhibited NGF mRNA and protein expression and reduced neurite outgrowth of rat pheochromocytoma cells, which are positive for PR expression [40]. In endometrial stromal cells from non-endometriotic women, VEGF mRNA and protein production was also inhibited by DNG treatment (10^{-7} to 10^{-9} M) [38]. In S-180 mouse tumor cells, topical DNG inhibited embryonic angiogenesis in a dose-dependent manner and its oral administration (1 mg kg^{-1} day^{-1}) significantly suppressed angiogenesis [42]. In a rat endometrial autograft model, oral DNG (1 mg/kg body weight/day) reduced the size of the microvascular network, decreased microvessel density and influenced the structure of the newly formed microvessels compared to controls [43].

Cellular-based modulators of the inflammatory response

DNG (10^{-7} M) inhibited NFκB-binding activity in immortalized endometriotic epithelial cells [26] and in lipopolysaccharide and high-mobility group box 1 (LPS/HMGB1) stimulated endometrial epithelial cells overexpressing PR–B [36]. In endometriotic stromal cells, treatment with DNG (10^{-7} M) attenuated TNFα stimulated NFκB activation in both monolayer [37] and spheroid cultures [34]. Lastly, it has also been shown that there was a lower expression of HLA-DR expression in peritoneal macrophages from women with endometriosis and that incubation with DNG (10^{-7} M) could restore this to similar levels as controls [39], suggesting DNG may influence immune cells.

Discussion

DNG is used for the long-term treatment of endometriosis by creating a hypo-estrogenic environment that inhibits endometriotic lesion growth. However, it is possible that DNG has additional mechanisms of actions that contribute

to lesion reduction. Given the ability of progesterone to influence inflammation, we postulated that DNG might also influence the inflammatory reaction that supports endometriotic lesion progression. Through this systematic review, we identified a total of 15 studies that reported an influence of DNG on the production of PGs (PGE2, COX-2 and mPGES-1) [26, 33–35], pro-inflammatory cytokines (IL-1β, IL-6 and TNF-α) [33, 36, 37], chemokines (IL-8, MCP-1 and SDF-1) [33, 36, 38] and growth factors (VEGF and NGF) [33, 38, 40] in endometriotic related cells. Although the number of studies that met the strict inclusion criteria for this manuscript is small, we believe that they provide sufficient preliminary evidence to indicate a local, anti-inflammatory role for DNG on endometriotic cells that deserves further attention.

Inflammation is a significant factor in the progression of endometriosis and the inflammatory mediators found to be regulated by DNG all contribute to endometriotic lesion progression. TNF-α stimulates the production of downstream cytokines in both epithelial and stromal cells [46, 47], leading to an increase in peritoneal fluid levels of numerous pro-inflammatory mediators including IL-6, MCP-1 [48, 49] and IL-8 [6] in endometriotic women. The response to inflammation and, therefore, the ability of DNG to influence it may also be lesion dependent as SDF-1 is significantly increased in the peritoneal fluid of women with deep infiltrating endometriosis (DIE) [50] and MCP-1, IL-6 and TNFα production is enhanced in recto-vaginal septum lesions compared to lesions from other locations [51]. Inflammation also influences the surrounding microenvironment as VEGF and NGF also stimulate angiogenesis and nerve growth. The ability of DNG to attenuate the production of all these cytokines and growth factors, therefore, will not only influence the viability of the endometriotic cells themselves, but also the supporting endothelial and stromal cells and extend its influence beyond hormonal modulation.

The mechanism by which DNG mediates this anti-inflammatory effect in endometriotic cells is difficult to infer from the data available. The majority of studies that we identified reported a DNG influence on inflammation via in vitro experiments. These in vitro models provide the advantage of being isolated from the influence of reduced follicular estrogens, thereby allowing the direct effects of DNG to be unmasked, confirming a local effect. How this local effect is mediated, however, is not clear. Endometriotic lesions produce their own estrogenic supply and are considered progesterone resistant and the modified PR activity is one of the primary mechanisms that allow endometriotic lesions to remain at ectopic locations through a dysregulation of decidualization [20, 52]. In epithelial cells, a DNG-mediated effect on inflammation occurred only when PR protein was present through an exogenous transfection, supporting a PR-based mechanism. In the stromal cells, such a mechanism could not be confirmed from the data available as the presence of PR was rarely examined, although in all studies a DNG effect on inflammation was observed without the need for exogenous PR expression. A progesterone resistance would suggest that local DNG effects occur through non-PR-mediated mechanisms, although PR expression and progesterone resistance in endometriotic lesions remain controversial [45, 53]. The exact mechanism of local DNG effects and whether these effects can be replicated in vivo, therefore, remains an open question.

Another consideration is whether anti-inflammatory DNG effects can also be mediated by its systematic influence on circulating estrogen concentrations. Estrogen can both stimulate and suppress the immune response, dependent on the cell type and estrogen concentrations [54]. In dendritic cells, estrogen stimulates inflammation [55, 56] whereas it suppresses the inflammatory response to influenza [57]. In endometriosis, both ERα and β have been observed in endometriotic cells [58] and estrogen treatment creates an inflammatory response in human endometrial stromal cells [59]. Therefore, the reduction of circulating estrogen by DNG could have a significant anti-inflammatory activity on both the immune and endometriotic cells in the peritoneal cavity.

Furthermore, there is also an interdependent relationship between estrogen and progesterone. For example, in breast cancer the PR is capable of influencing the cellular response to estrogen [60], whereas the physiological effects of progesterone may be amplified in the presence of estrogens due to the increased expression of PR [61]. Therefore, the imbalance of PR A and B expression in endometriotic lesions [62] could have a significant influence to how these cells respond to DNG through altered estrogen concentrations.

The potential of an estrogen-regulated inflammatory response by DNG treatment, whether through local or sytemic effects may also have significant implications for other estrogen-driven diseases that have an inflammatory component. Autoimmune diseases such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), autoimmune thyroid diseases (ATD) and multiple sclerosis (MS) all have a higher incidence in the female population [63]. Obese postmenopausal women are also at a higher risk of breast cancer due to the production of estrogen [64]. There may be, therefore, the possibility of using an anti-estrogen, anti-inflammatory drug, such as DNG in other estrogen-driven immune conditions. The therapeutic potential of targeting both inflammation and estrogen has already been proposed for MS [65].

The strongest evidence of a physiological effect of DNG on the inflammatory endometriotic environment comes

from the stromal cell studies; first, because these studies were performed in primary cell cultures and second, because transfection of PR was not required to detect an influence of DNG on the inflammatory response of these cells (Fig. 2). In normal functioning, endometrium stromal cells are the major target for progesterone [66] and are expected to have a significantly stronger PR expression; however, as the PR expression was not assessed in the majority of these studies it was difficult to determine if the local effects were mediated directly through the nuclear receptors or through another mechanism. Some of the results presented support a possibility for non-PR-mediated mechanisms; DNG-induced anti-proliferative effects in a rat model of endometriosis were observed even in the presence of a PR antagonist [23] and a DNG-mediated increase in IL-8 mRNA expression [37] and cell proliferation [41] was not attenuated by RU486 (Table 2). These preliminary data suggest that a non-PR mechanism should be investigated.

In contrast to the stromal cells, the epithelial cell studies were performed predominantly after the presence of PR proteins was determined (Fig. 2). Importantly, only a limited effect of DNG was observed in PR negative cells under basal conditions. If cells were transfected with PR to increase expression, however, a significant effect of DNG treatment on the mRNA production of inflammatory mediators was observed. These data suggest that in these immortalized epithelial cells the local DNG effects are mediated directly through PR activation and therefore that the PR status of epithelial cells in vivo will be important as to whether DNG has a physiological influence. When evaluated, the contribution of PR-A and PR-B isoforms has so far revealed similar biological activity [26, 33, 36, 40] (Tables 1, 2). Previous studies suggest that the inflammatory response of stromal cells is significantly stronger than the response of epithelial cells [47] and in fact, recent studies suggest that the stromal cell PR expression is sufficient for a progesterone-mediated inhibition of epithelial cell proliferation [12], as PR activation of stromal cells blocks the production of mitogenic mediators that influence epithelial cell viability [67].

Limitations

The major limitation of this systematic review is the small number of studies that met the strict inclusion criteria. However, we believe that this small number is reflective of the novelty and timeliness of this question, but was still sufficient to draw robust conclusions on the influence of DNG on inflammation. It was also not clear in some studies whether PR was present in the cell models examined, making it difficult to make conclusions on the mechanism of action.

Conclusions

This systematic review identifies sufficient evidence to support a local effect on endometriotic inflammation by DNG and identifies two important questions that remain

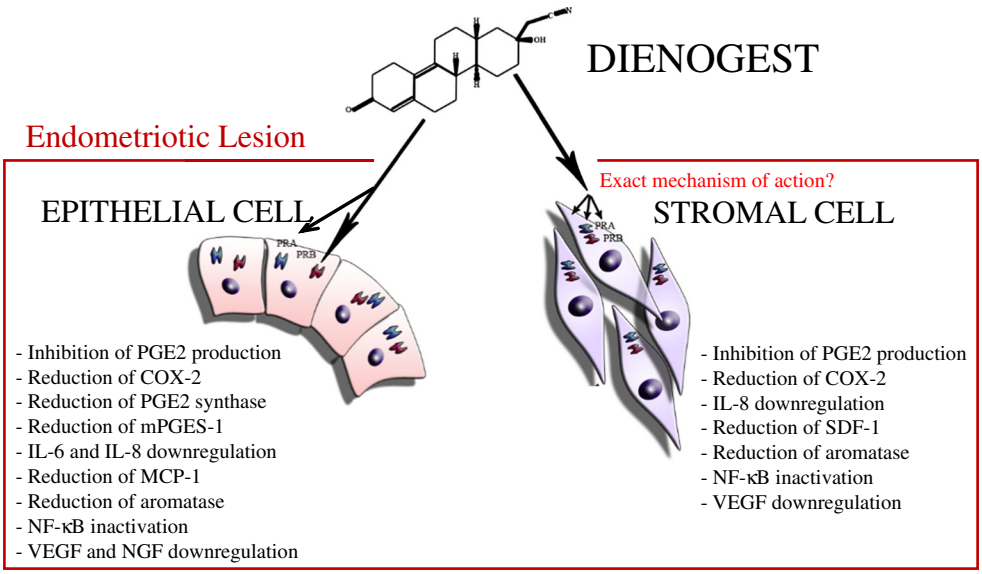

Fig. 2 In epithelial cells, DNG treatment was associated with a decrease in many different pro-inflammatory markers. This modulation, however, was only observed in cells positive for PR either through endogenous expression or PR transfection. DNG also modulates the inflammatory response of endometriotic stromal cells, however, the evidence was not clear on the mechanism of action and it is possible that this occurred through a non-PR-mediated mechanism

unanswered and should be addressed in future studies. In epithelial cells, it is likely the DNG effect is mediated through PR; however, the use of immortalized cell lines transfected with PR means that PR expression in endometriotic epithelial cells in vivo needs to be clarified. In primary stromal cells, a direct effect of DNG on inflammation is also present, although whether this occurs directly through PR modulation also needs confirmation.

In summary, the symptomatology and the progression of endometriosis are intricately linked to the hyper-inflammatory microenvironment stimulated by the growth of the ectopic endometrium. A better understanding of the local effects of DNG and other progestogens on local endometriotic tissue could facilitate the development of novel therapeutics that emphasize non-hormonal functions of this class of drugs and greatly improve their clinical profile.

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Compliance with ethical standards

Conflict of interest The authors of this review declare that they have no conflict of interest.

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