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Prostaglandins and cellular reaction in uterine flushings. II. Effect of PG synthesis inhibition in IUD users

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Abstract

The effect of oral administration of indomethacin (100 mg/day), a potent inhibitor of prostaglandin (PG) biosynthesis, on the PG levels and cellular profile in the uterine flushings in response to the use of an IUD (Lippes Loop size C) was studied in sixty women. Indomethacin reduced the cell counts in both follicular and luteal phases of menstrual cycles before and after IUD insertion. The anti-inflammatory drug decreased PGE2 and PGF2alpha levels in both phases of the cycle before IUD insertion. After insertion, it inhibited only the formation of PGF2alpha and its 13,14-dihydro-15-keto metabolite in the luteal phase but not in the follicular phase. In long-term users, however, the drugs reduced the levels of all PGs studied in the luteal phase and only PGF2alpha and its metabolite in the follicular phase. The implications of these findings in the mechanisms of contraceptive action of IUDs and their side effects are discussed.

Introduction

The prostaglandins (PGs) have been implicated in the mechanisms of action of IUDs and their side-effects, particularly abnormal bleeding and pain [1]. Some PGs are chemotactic, causing leukocyte migration; these cells can also synthesize and release PGs [2,3]. This complex integrated biochemical relationship is presently ill-defined, and it is difficult to indicate if the observed cellular reaction with IUDs is due to local PG release. Moreover, it is of interest to understand whether either of these two

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events is totally dependent on the other or if both are induced by a common factor such as the presence of a foreign body *in utero*.

Many of the side-effects (especially pain, bleeding and expulsion) of IUDs can be induced by PGs through various mechanisms [1]. The ability of non-steroidal anti-inflammatory drugs (NSAIDs), known inhibitors of PG synthesis, to control these side-effects supports the concept of a key role for PGs in the pathogenesis of these problems [4,5]. Therapy with NSAID is effective in reducing IUD-related heavy menstrual blood loss, but a correlation between systemic intake of these drugs and inhibition of local uterine release of PGs has not yet been documented [6,7].

The present study was planned to evaluate the effect of oral intake of indomethacin, as NSAID, on PG levels and the cellular reaction in the uterine cavity in the presence of an inert IUD. The results would help in the understanding of the integrated relationship between the biochemical and biological responses in an attempt to explain the role of either event in the mechanisms of action and side-effects of IUDs.

Patients and methods

Sixty healthy fertile women between 20 and 37 years (mean 29.7 years) of age with regular menstrual cycles and normal blood loss were studied.

Group I: Control cycle and short-term users. This included thirty cases who selected IUDs (Lippes Loop size C) as their contraceptive method of choice.

Group II: Long-term IUD users. This group also included thirty subjects who had Lippes Loop size C inserted at least for two years.

Uterine washing was done using a Gravlee jet washer (as previously described [8]) around the sixth to the eighth day of the menstrual cycle and two weeks afterwards; it was repeated in the next cycle under indomethacin therapy (100 mg/day starting from the first day of the cycle) (Indocid (R) capsules 25 mg, Merck, Sharp and Dohme). This was done in both short-term users before and three months after IUD insertion, and long-term IUD users. The recovered fluid, containing a prostaglandin synthetase inhibitor to prevent *in vitro* PG formation, was filtered through millipore filters and the entangled cells were identified and counted. The cell-free fluid was used for estimation of PGE2, PGF2alpha and their 13,14-dihydro-15-keto metabolites by specific RIAs as stated elsewhere [8].

Results

Prostaglandin levels in the uterine wash fluid

(A) PGE2 (Figure 1): Before IUD insertion (short-term IUD users), the mean ± SE PGE2 levels in the follicular phase decreased in the indomethacin-treated cycle from 227.5 ± 60.2 pg/ml to 14.6 ± 1.3 pg/ml ($p < 0.05$), while in the luteal phase, it decreased from 144.5 ± 30.9 pg/ml to 64.9 ± 23.7 pg/ml ($p < 0.05$).

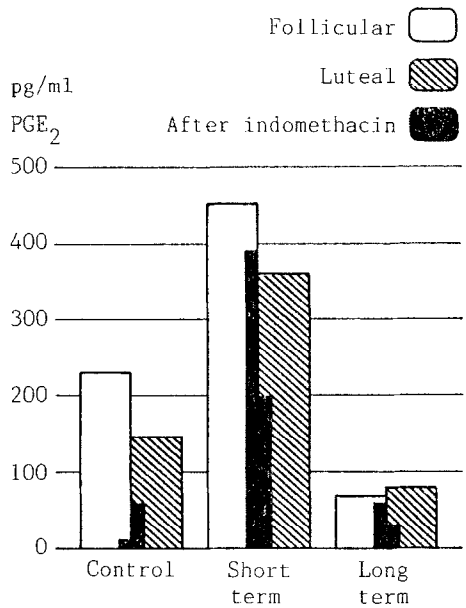

Figure 1 Effect of systemic administration of indomethacin on the PGE2 level (pg/ml) in the uterine flushing fluid during the follicular and luteal phases of control cycles and three months after IUD insertion as well as in long-term IUD users

After IUD insertion, the PGE2 level (mean ± SE) in the follicular phase was also reduced from a control level of 452.4 ± 102.9 pg/ml to a level of 392.0 ± 126.9 pg/ml in the indomethacin-administered cycle ($p > 0.05$). In the luteal phase, the level was 366.6 ± 107.5 pg/ml without indomethacin and decreased in the next cycle with indomethacin to 196.2 ± 118.5 pg/ml ($p > 0.05$).

In long-term IUD users, the PGE2 level (mean ± SE) was also reduced as a result of indomethacin intake from 70.4 ± 10.4 pg/ml to 61.2 ± 41.6 pg/ml in the follicular phase ($p > 0.05$), while in the luteal phase it decreased from 81.3 ± 14.1 pg/ml to 30.2 ± 9.0 pg/ml in the indomethacin-treated cycle ($p < 0.05$).

(B) PGE2 metabolite (Figure 2): Before IUD insertion (short-term IUD users), the PGE2 metabolite level (mean ± SD) in the uterine wash showed insignificant changes in both the follicular and luteal phases of the indomethacin-treated cycles, as compared with control cycles; follicular control level was 11.7 ± 1.7 pg/ml and indomethacin-treated level was 13.0 ± 4.0 pg/ml, while in the luteal phase, these values were 11.1 ± 2.0 pg/ml and 10.5 ± 2.3 pg/ml, respectively ($p > 0.05$).

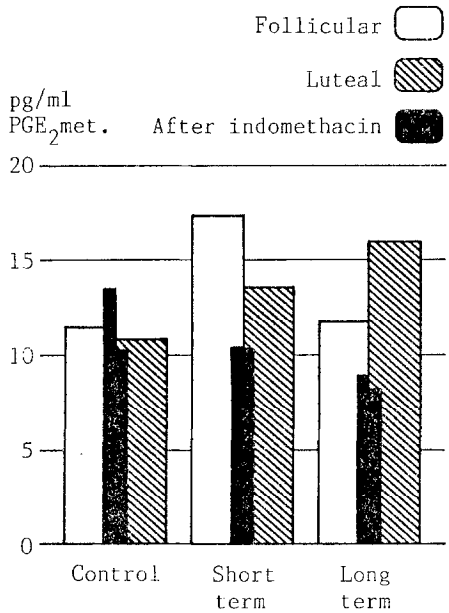

Figure 2 Effect of indomethacin on the levels of 13,14-dihydro-15-keto PGE2 (pg/ml) in uterine flushings from control cycles, in short-term and long-term IUD users

Also, after IUD insertion in short-term users, the level (mean ± SE) of 13,14-dihydro-15-keto PGE2 was insignificantly different in the follicular and luteal phases of control cycle from the corresponding levels of indomethacin-administered cycles (follicular level of 17.1 ± 3.9 pg/ml changed to 10.3 ± 5.1 pg/ml during indomethacin intake while the luteal level of 14.0 ± 2.2 pg/ml decreased in the indomethacin cycle to 10.9 ± 2.5 pg/ml) ($p > 0.05$). In long-term users, the follicular metabolite level (mean ± SE) of 12.0 ± 1.5 pg/ml decreased in the indomethacin-treated cycle to 8.9 ± 1.3 pg/ml ($p > 0.05$). However, in the luteal phase, the level decreased significantly from 16.4 ± 1.7 pg/ml to 8.3 ± 1.1 pg/ml ($p < 0.05$).

(C) PGF2alpha (Figure 3): In short-term IUD users before insertion, the level decreased from a control follicular mean ± SE value of 28.8 ± 2.6 pg/ml to 15.0 ± 6.0 pg/ml in the indomethacin-intake cycle ($p < 0.05$). The corresponding levels in the luteal phase of both cycles were 33.1 ± 1.7 and 14.4 ± 1.4 pg/ml respectively ($p < 0.05$).

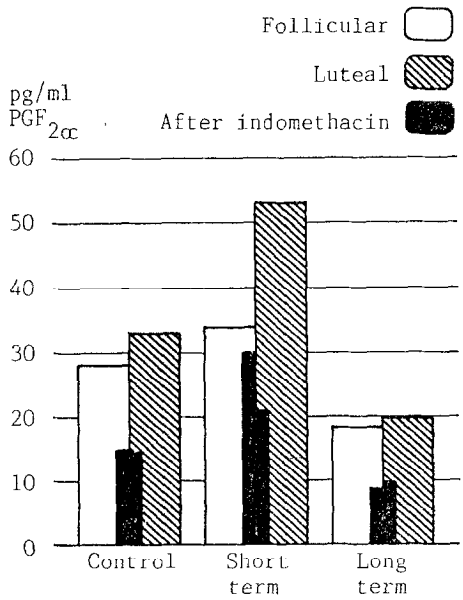

Figure 3 Effect of indomethacin on PGF2alpha levels (pg/ml) in uterine flushing fluid from control cycles and 3 months after IUD insertion as well as in long-term IUD users

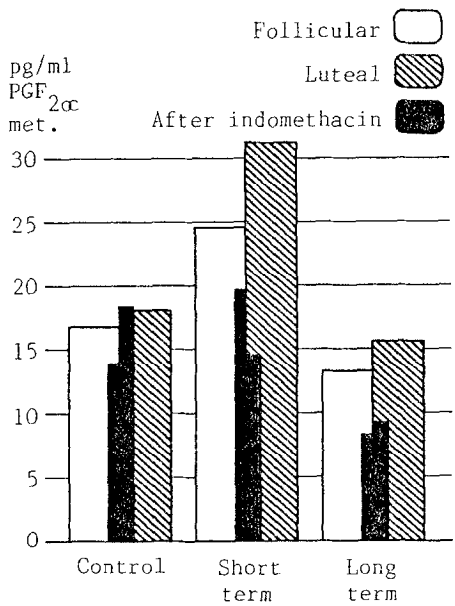

Figure 4 Effect of indomethacin intake on the levels (pg/ml) of 13,14-dihydro-15-keto PGF2alpha in uterine flushing fluid from control cycles, in short-term and long-term IUD users

After IUD insertion, the follicular PGF2alpha level (mean ± SE) was 34.2 ± 4.8 pg/ml, which insignificantly decreased to 30.2 ± 5.3 pg/ml in the indomethacin-treated cycle ($p > 0.05$). In the luteal phase, however, the indomethacin-induced decrease from 53.6 ± 4.9 pg/ml to 21.2 ± 6.9 pg/ml was significant ($p < 0.05$).

In long-term IUD users, the follicular level of 18.3 ± 2.3 pg/ml decreased in the indomethacin cycle to 9.0 ± 2.1 pg/ml, whereas in the luteal phase, it decreased from 20.0 ± 2.1 pg/ml to 9.9 ± 1.5 pg/ml respectively ($p < 0.05$).

(D) PGF2alpha metabolite (Figure 4): Before IUD insertion (short-term users), the decrease in 13,14-dihydro-15-keto PGF2alpha levels in the uterine wash because of indomethacin intake was insignificant in both the follicular and luteal phases ($p > 0.05$).

After IUD insertion, the decrease in metabolite level in the follicular phase of the indomethacin-administered cycle from an original level of 24.8 ± 3.3 pg/ml to 19.8 pg/ml was not significant ($p > 0.05$). In the luteal phase, however, the decrease from 31.3 ± 3.3 pg/ml to 14.5 ± 2.7 pg/ml during intake of indomethacin was significant ($p < 0.05$).

In long-term IUD users, there was a statistically significant decrease in PGF2alpha metabolite in the uterine wash fluid as a result of indomethacin administration from 13.3 ± 1.4 pg/ml to 8.3 ± 1.0 pg/ml in the follicular phase and from 15.8 ± 1.9 pg/ml to 9.2 ± 0.9 pg/ml in the luteal phase ($p < 0.05$).

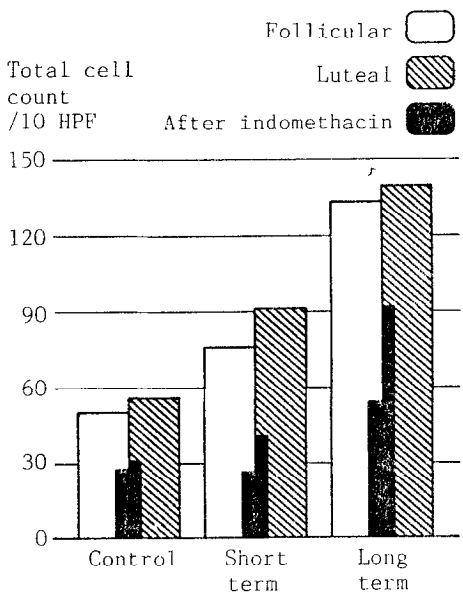

Figure 5 Effect of indomethacin on the total cell count/10 high-power fields (HPF) in uterine flushing fluid obtained in the follicular and luteal phases of control cycles, and after IUD insertion (short- and long-term). Note that the NSAID inhibited the cellular reaction in the uterine milieu at all phases of the cycle before and after IUD insertion, particularly among short-term IUD users

The cellular environment in the uterine wash fluid

(A) Control cycle of short-term users: Indomethacin produced a significant decrease in the cellular population. Some cells were damaged and broken in their cytoplasmic membrane with irregular morphology and fragmented nucleus. In the follicular phase, the total cell count (mean ± SE) decreased significantly from 50.0 ± 3.5 to 27.0 ± 4.9/10 HPF ($p < 0.05$) (Figure 5). Polymorphs constituted 46.7%, the macrophages 46.5% and the lymphocytes 6.7% of the total cell count. In the luteal phase, the cell population decreased significantly after oral intake of indomethacin, from 55.7 ± 2.9 to 31.5 ± 4.1/10 HPF ($p < 0.05$). The polymorphs constituted 45.75%, the macrophages 49.5%, and the lymphocytes 4.6% of the total cell population (Table 1).

Table 1 Effect of indomethacin (100 mg/day) on the cellular environment (count/10 HPF) in the uterine wash fluid in the control cycle of short-term IUD users

		Polymorphs		*Macrophages*		*Lymphocytes*		*Total count*	
		Before treatment	*After treatment*	*Before treatment*	*After treatment*	*Before treatment*	*After treatment*	*Before treatment*	*After treatment*
Follicular phase	Mean ± SE	26.2 ± 1.7	12.6* ± 3.3	18.6 ± 2.8	12.6* ± 1.7	5.1 ± 0.8	1.8* ± 0.8	50.0 ± 3.5	27.0* ± 4.9
	%	52.4	46.7	37.3	46.5	10.2	6.7	100.0	100.0
Luteal phase	Mean ± SE	29.7 ± 2.2	14.4* ± 2.2	22.1 ± 0.9	15.6* ± 2.8	3.8 ± 0.4	1.5* ± 0.3	55.7 ± 2.9	31.5* ± 4.1
	%	53.3	45.7	39.7	49.5	6.8	4.7	100.0	100.0

* = Significant at $p < 0.05$ compared to non-indomethacin cycle

(B) Short-term users after device insertion: There was a statistically significant decrease in the total cell population, as well as in the number of polymorphs, macrophages and lymphocytes, during indomethacin use, both in the follicular and luteal phases. Some cells showed irregular morphology with damaged and broken outer cytoplasmic membranes and fragmented nuclei. In the follicular phase, the total cell population (mean ± SE) decreased from 77.0 ± 3.4 to 26.5 ± 10/10 HPF ($p < 0.05$) (Figure 5). After indomethacin treatment, the polymorphs constituted 44.7%, the macrophages 45.2% and the lymphocytes 9.8% of the total cell population. In the luteal phase, the total cell population decreased from 91.3 ± 4.4 to 40.9 ± 4.9/10 HPF ($p < 0.05$). After indomethacin, the polymorphs constituted 40.7%, the macrophages 50.8% and the lymphocytes 8.4% of the total cell population (Table 2).

Table 2 Effect of indomethacin (100 mg/day) on the cellular environment (count/10 HPF) in the uterine wash fluid in short-term IUD users, after device insertion

		Polymorphs		*Macrophages*		*Lymphocytes*		*Total count*	
		Before treatment	*After treatment*	*Before treatment*	*After treatment*	*Before treatment*	*After treatment*	*Before treatment*	*After treatment*
Follicular phase	Mean ± SE	42.3 ± 1.6	11.8* ± 3.4	28.9 ± 1.5	12.0* ± 1.7	5.8 ± 0.7	2.6* ± 0.7	77.0 ± 3.4	26.5* ± 5.7
	%	54.9	44.7	37.5	45.2	7.5	9.8	100.00	100.00
Luteal phase	Mean ± SE	42.9 ± 6.6	16.6* ± 1.5	40.4 ± 3.4	2.8* ± 3.3	8.0 ± 0.7	3.4* ± 0.5	91.3 ± 4.4	40.9* ± 4.9
	%	46.9	40.7	44.2	50.8	8.7	8.4	100.0	100.0

* = Significant at $p < 0.05$ compared with non-indomethacin cycle

(C) Long-term IUD users: During the indomethacin cycle, there was a significant decrease in the number of total cell count. Some cells showed irregular morphology with damaged and broken cytoplasmic membranes and fragmented nuclei. In the follicular phase, the number of total cell count (mean ± SE) decreased during indomethacin treatment from 134.2 ± 10.8 to 54.0 ± 4.7/10 HPF ($p < 0.05$). The polymorphs constituted 41.8%, the macrophages 50.9% and the lymphocytes 7.2% of the total cell population after oral administration of indomethacin. In the luteal phase, the number of total cells decreased as a result of indomethacin treatment from 139.4 ± 12.2 to 91.7 ± 6.4/10 HPF ($p < 0.05$). The polymorphs constituted 65%, the macrophages 32.1% and lymphocytes 2.8% of the total cell population after indomethacin intake (Table 3).

Discussion

Vane demonstrated that aspirin-like substances exert their anti-inflammatory action through the inhibition of microsomal enzymes involved in the synthesis of PGs [4]. There is a good correlation between the anti-inflammatory activities of aspirin-like drugs and their anti-prostaglandin synthetase activities [9]. Tomlinson and associates found indomethacin to be 2000 times more potent than aspirin as an inhibitor of PG biosynthesis [10].

Table 3 Effect of indomethacin (100 mg/day) on the cellular environment (count/10 HPF) in the uterine wash fluid in long-term users

		Polymorphs		*Macrophages*		*Lymphocytes*		*Total count*	
		Before treatment	*After treatment*	*Before treatment*	*After treatment*	*Before treatment*	*After treatment*	*Before treatment*	*After treatment*
Follicular phase	Mean ± SE	70.3 ± 5.3	22.7* ± 2.4	53.2 ± 6.3	27.7* ± 2.0	10.6 ± 2.3	3.9* ± 0.4	134.2 ± 10.8	54.4* ± 4.7
	%	52.3	41.8	39.6	50.9	7.9	7.2	100.0	100.0
Luteal phase	Mean ± SE	87.9 ± 7.6	59.7* ± 6.1	44.2 ± 4.5	29.4* ± 2.6	7.2 ± 1.13	2.6* ± 0.4	139.4 ± 12.2	91.7* ± 6.4
	%	63.0	65.0	31.7	32.1	5.2	2.8	100.0	100.0

* = Significant at $p < 0.05$ compared with non-indomethacin cycle

In the present study, it is obvious that the NSAID, indomethacin, had a significant effect in decreasing PGE2 and PGF2alpha levels in the endometrial jet wash fluid obtained during the follicular and luteal phases of control cycles. However, in short term IUD users, it was observed that indomethacin had no effect on the PG levels in the follicular phase while in the luteal phase it decreased only PGF2alpha and its major metabolite significantly. On the other hand, in long-term users, this drug effectively reduced all PGs measured in the luteal phase and only PGF2alpha and its metabolite in the follicular phase. These data indicate that indomethacin differentially inhibited the generation of various PGs studied at different phases of the control cycle and after short- and long-term use of IUDs. Moreover, it appears that the short-term presence of an intrauterine foreign body could interfere with the local inhibitory effect of indomethacin on PG synthetase activity in the follicular phase.

Despite the lack of increased PG synthesis in long-term IUD users [8], the NSAID caused a more pronounced inhibition of the PG synthesis in this group. It can thus be concluded that the long-term presence of a foreign body in the uterine cavity imposes a dual effect on PG synthesis; first, there is a lack of increased synthesis like that seen among short-term users, and, second, it does not interfere with the inhibitory effect of indomethacin. In other words, the marked inhibition of PG synthesis by indomethacin in long-term users is another form of adaptation.

In the present study, it was clearly demonstrated that indomethacin induces a significant reduction in the cell population in the uterine wash fluid in control cycles, and short- and long-term IUD users. Also, it was observed that some of the

inflammatory cells were damaged and some other cells showed fragmented nuclei in the indomethacin-treated cycle. The mechanism of these biological responses, however, is not clear at present.

Administration of NSAIDs during menstruation has proved to be an effective means of reducing menstrual blood loss among IUD users [5,7,11,12]. Several hypotheses were suggested to explain the mechanism of this response, the most prominent of which is the reduction of PG synthesis [5]. The data of the present work support such a hypothesis. Moreover, the failure of indomethacin to inhibit PG production significantly among short-term IUD users in the follicular phase is in agreement with the failure of NSAIDs to control intermenstrual spotting related to the IUD [13]. If PGs are involved in abnormal bleeding induced by IUDs, it is likely that the F types are the ones particularly related to this side-effect, since NSAID reduced this PG level only in the luteal phase of short-term users prior to the onset of menstruation. In long-term users, the significant reduction in the same compound also in the follicular phase may indicate a possible clinical value of NSAIDs in women with intermenstrual bleeding problems.

Regarding the mechanism of action of IUDs, the present data provide an additional evidence against a key role for the measured prostanoids in the prevention of pregnancy. This evidence relates to the observation that despite the use of indomethacin in sixty cycles with an IUD *in situ*, no pregnancies were recorded. The marked local reduction in cell populations induced by indomethacin without occurrence of pregnancy may also contradict the concept of a key role for the cellular reaction in the contraceptive mechanism of IUDs. If the cellular response is an essential factor in the IUD action, then the use of NSAID is particularly likely to disturb the function of the device shortly after insertion, where the maximum inhibition of cellular reaction was observed. In long-term IUD users, indomethacin reduced the cellular reaction in the luteal phase to the level of uninhibited counts in short-term users.

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Resumé

On a étudié chez 60 femmes les conséquences de l'administration par voie orale (100 mg/jour) d'indométhacine, puissant inhibiteur de la biosynthèse de la prostaglandine (PG), sur les niveaux de PG et le profil cellulaire dans les flux d'elimination utérine en résponse à l'utilisation d'un DIU (stérilet de Lippe, taille C). L'indométhacine a réduit le nombre des cellules tant pendant la phase folliculinique que pendant la phase lutéinique des cycles menstruels avant et après l'insertion du dispositif. Cet anti-inflammatoire a fait baisser les niveaux de PGE_2 et de PGF_{2alpha} au cours des deux phases du cycle avant l'insertion du stérilet. Après l'insertion, il n'a inhibé que la formation de la PGF_{2alpha} et de son métabolite 13,14-dihydro-15-cétone pendant la phase lutéinique mais non pendant la phase folliculinique. Toutefois, chez les utilisatrices de longue date, ces substances ont fait baisser les niveaux de toutes les prostaglandines étudiées pendant la phase lutéinique et de la seule PGF_{2alpha} et son métabolite pendant la phase folliculinique. Cet article examine les liens entre ces constatations et les mécanismes de l'action contraceptive des DIU ainsi que leurs effets secondaires.

Resumen

En sesenta mujeres se estudió el efecto de la administración oral de indometacina (100 mg/día), un potente inhibidor de biosíntesis de prostaglandina (PG), en los niveles de PG y en el perfil celular en lavados uterinos, en respuesta al uso de un DIU (Asa de Lippes, tamaño C). La indometacina redujo el contaje celular en ambas fases, folicular y luteal, de los ciclos menstruales antes y después de la inserción del DIU. La droga antiinflamatoria disminuyó los niveles de PGE_2 y PGF_{2alpha} en ambas fases del ciclo antes de la inserción del DIU. Después de la inserción solamente inhibió la formación de PGF_{2alpha} y sus 13,14-dehidro-15-keto metabolites en la fase luteal pero no en la fase folicular. Sin embargo, en las usuarias a largo plazo las drogas redujeron los niveles de todas las PG estudiadas en la fase luteal y solamente la PGF_{2alpha} y sus metabolites en la fase folicular. Se discutieron las implicaciones de estos hallazgos en el mecanismo de la acción anticonceptiva de los DIU y sus efectos colaterales.