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Intranasally delivered microdoses of bromocriptine (BCR) effectively reduces serum prolactin levels in hyperprolactinaemic patients

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It is well known that hyperprolactinaemia in the human leads to infertility. The therapy of choice in India has been the administration of bromocriptine (BCR) as tablets. This mode of administration is generally accompanied by undesirable side-effects such as giddiness, nausea, vomiting and postural hypotension. We demonstrate here the efficacy of microdoses of BCR administered intranasally (IN) to hyperprolactinaemic patients ($n = 6$) in reducing significantly the elevated serum prolactin levels and maintain them within the normal range. The IN mode of BCR administration, in addition to reducing the effective dose of the drug by 4–20-fold, results in little or no side-effects otherwise associated with oral therapy.

HYPERPROLACTINAEMIA could be a result of pituitary stalk compression (hypothalamo-pituitary disconnection), the presence of a pituitary tumour, or unexplained idiopathic causes. One of the principal effects of high concentrations of prolactin secretion is the inhibition of gonadotropin pulsatality, probably due to an effect at the hypothalamic GnRH pulse generator[1]. Hyperprolactinaemia-associated reproductive dysfunctions in the human female are known to result in menstrual irregularities like oligomenorrhea or amenorrhea, spontaneous or expressive galactorrhea and inhibition of ovarian steroidogenesis. The effect of hyperprolactinaemia in man, on the other hand, is yet to be clearly understood; but it is known to cause decrease in libido, leading to infertility.

Bromocriptine (BCR), a potent dopamine agonist that inhibits prolactin secretion from the pituitary, is the drug of choice in effective reduction of prolactin (PRL) concentration in the management of hyperprolactinaemic patients. However, being an ergot alkaloid, oral BCR (O-BCR) therapy results in side-effects such as nausea, vomiting and postural hypotension[2] in a large percentage of patients; the less common side-effects are headache, fatigue, abdominal cramps and constipation. Attempts at developing new agonists that are long-acting as well as have less adverse effects have been on the anvil in the recent past[1]. Hitherto, in India, BCR is available only in tablet form and the administered dose ranges from 5–20 mg or more per day.

Earlier studies from our laboratory and others have clearly demonstrated that intranasal (IN) administration of microdoses of steroids/drugs are effective in acting at the hypothalamo-pituitary axis and as such serve as a potential alternate method to achieve blockage of hormone release[3–9]. In the present pilot study we have sought to determine (a) whether IN-BCR treatment (in microdoses compared to the relatively larger doses of O-BCR) can effectively reduce the PRL concentration and maintain it within normal range (< 25 µg/l) in hyperprolactinaemic patients and (b) if the marked reduction in therapeutic dose results in a significant decrease in side-effects and better patient compliance.

Five female and one male hyperprolactinaemic patients volunteered to enter the study, which was cleared by the Ethics Committee of M. S. Ramaiah Medical Teaching Hospital, Bangalore. A written, informed consent was obtained from all of the volunteer-patients. The details of their age, diagnosis and clinical manifestations are provided in Table 1. Since all the patients were on O-BCR, they underwent a washout period of two weeks, during which time they were asked to stop taking the BCR tablets and were taught the precise use of the nasal spray device. The nebulizer used was obtained from Pfeiffer GmbH and Co. KG, Radolfzell, Germany, and delivers ~ 100 µl solvent/spray with an efficiency of > 90% as determined using a labelled [^{3}H] steroid. The solvent (vehicle) used to dissolve crystalline BCR (kindly provided by Serum Institute of India, Pune) comprised of ethanol/propylene glycol/distilled water in a ratio of 3 : 3 : 4. This solvent has earlier been successfully used to deliver steroid hormones in human volunteers[5].

Following the washout period, the volunteers were subjected to a two-week pretreatment schedule when the vehicle alone was delivered by IN route. Resting levels of serum PRL were determined from three blood samples collected on day 14, 7 and 1 of this phase of

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Table 1. Comparative efficacy data of O-BCR (retrospective) and IN-BCR (current) in treatment of hyperprolactinaemia

Patient/ volunteer ID	Sex*	Age	Diagnosis	Serum PRL† (μg/l)		Clinical manifestations		O-BCR		IN- BCR		Reduction in dose of IN-BCR over O-BCR
				–T	V	Irregular cycles	Galactorrhea	Dose (mg/d)	Side effects‡	Dose (mg/d)	Side-effects$	
A	F	48	Prolactinoma (macroadenoma)	160	78	Menopausal		2.5	Yes (+++)	0 125 (2)	Nil	20-fold
B	F	25	Hyperprolactinaemia	156	103	Yes	Yes	10.0	Yes (+++)	2.0 (8)	Nil	5-fold
C	F	30	Hyperprolactinaemia+IDDM	137	84	Yes	Yes	7 5	Yes (++)	2.0 (8)	Nil	3.75-fold
D	F	28	Hyperprolactinaemia	108	81	Yes	No	5.0	No (–)	0.5 (2)	Nil	10-fold
E	F	26	Hyperprolactinaemia	67	60	Yes	Yes	5.0	Yes (+++)	0.5 (2)	Nil	10-fold
F	M	30	Acromegaly	96	82	—	—	5.0	Yes (++)	1.0 (4)	Nil	5-fold

*F, female; M, male.
†Serum PRL levels before initiation of O-BCR therapy (–T) and during IN-vehicle (V) spray period. Note that within 3–4 weeks of IN-BCR treatment, the PRL levels reached the normal range.
‡The degree of side effects (nausea, vomiting) is expressed as moderate (++) to severe (+++).
$Excepting in volunteer A, where the dose/100 μl, solvent spray was 125 μg, in the rest of the volunteers it was 250 μg/100 μl spray. The number of sprays received by each volunteer is provided in the parenthesis.

study. During the first four weeks of treatment phase, all the patients received IN-BCR in increasing, uniform split doses (ranging from 125 μg to 2000 μg/day) twice daily after standardized meals. Those patients who did not respond to the lower doses were treated with the next increment in the dose of IN-BCR and this dose was either continued till the end of the study or further increased based on weekly review of serum prolactin levels. This permitted us to determine the effective dose of IN-BCR required to bring down the PRL levels to within the normal range in each case, which, as discussed later, could differ from one volunteer to the other. From the fifth week onwards, each of the volunteers continued to receive the respective effective dose of IN-BCR till the end of the treatment phase (24 weeks). Serum prolactin was determined using the human prolactin radioimmunoassay kit kindly provided by the NIAMDD, Bethesda, MD, USA, according to the procedure described earlier[10]. The range of serum PRL in normal male and female determined either by this method or using a commercially available kit (Coat-A-Count, Diagnostic Products Corporation, CA, USA) were essentially similar (5–25 μg/l).

All the volunteers were subjected to routine ENT examinations before, during and after the IN-BCR treatment period for any local, drug-related allergic or other reactions. In addition to monitoring the female volunteers for changes in galactorrhea by periodic examination, they were critically interviewed by the collaborating clinician with regard to the status of side-effects with O-BCR vs. IN-BCR and their preference for intranasal vs. oral mode of BCR treatment. The details of menstrual cyclicity were also recorded during these consultations.

For the purpose of comparison both within a volunteer and among the volunteers, the changes observed in serum PRL levels during different phases of this study are represented as individual graphs in Figure 1 (*a–f*).

At the time of first diagnosis, all the patients had serum PRL level in the range of 50–160 μg/l. Upon O-BCR treatment, the PRL levels were reduced to within the normal range (< 25 μg/l). Following the washout period and the two-week vehicle treatment phase the serum PRL levels rose to above-normal range (40–100 μg/l) in all the volunteers (Figure 1). Administration of IN-BCR in increasing doses during the first four-week period of treatment phase brought about a significant reduction in the elevated PRL levels from an initial 40–100 μg/l to 15–30 μg/l ($p < 0.05$) within this period of treatment. The dose of IN-BCR required to bring about this decrease in PRL levels, however, varied for individual volunteers and this ranged from 125 μg/day (volunteer A) to 2000 μg/day (volunter B). The effective IN-BCR dose did not seem to depend on the resting PRL level but appeared to be a function of the responsivity of the individual. Thus, once this effective concentration of IN-BCR was arrived at for each of the volunteers, they continued to receive this effective dose till the end of the treatment period (24 weeks) to determine further the efficacy of IN-BCR to maintain PRL levels well within the normal range. In addition, replacement of IN-BCR spray with IN-vehicle spray (between weeks 20 and 22) in volunteer A resulted in a significant increase in the serum PRL levels (from 14 μg/l to 70 μg/l; $p < 0.05$) within two weeks. However,

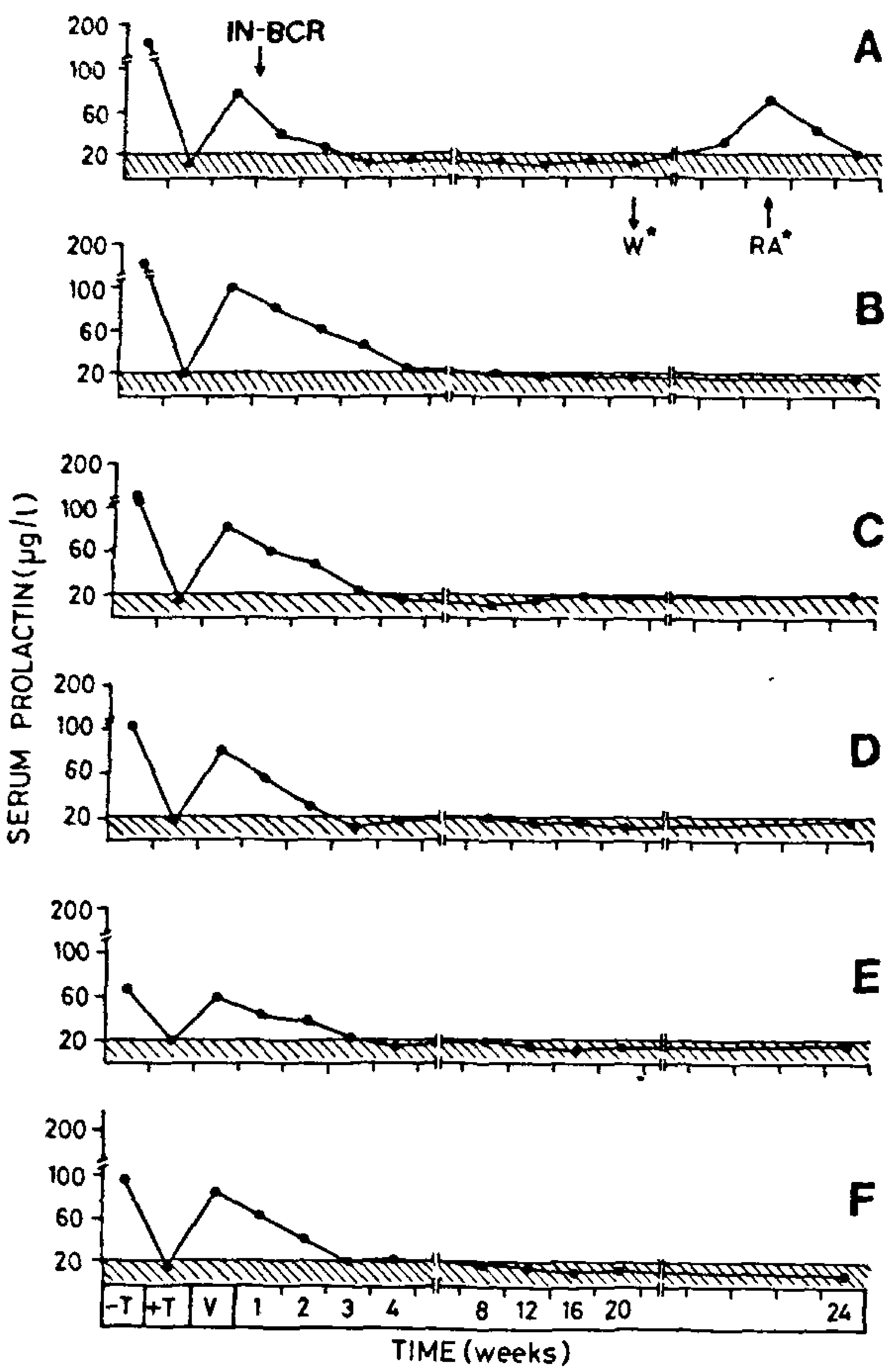

Figure 1. Longitudinal serum PRL profile in hyperprolactinaemic volunteers treated with IN-BCR. A–F, volunteer ID. Serum prolactin levels: *–T*, before start of O-BCR regimen; *+T*, during O-BCR therapy; V, during IN-vehicle therapy. ↓ indicates initiation of IN-BCR therapy; W and RA refer to time of withdrawal and readministration of IN-BCR, respectively, in volunteer A (*) only. Shaded area represents normal PRL levels (5–20 µg/l). Dose of IN-BCR. A – 125 µg/d; B,C – 2000 µg/d; D,E – 500 µg/d, F – 1000 µg/d.

readministration of IN-BCR in the same dose as before (125 µg/day) was able to suppress effectively the elevated PRL level to within the normal range during the next two-week period, thus reiterating the efficacy of this mode of drug administration (Figure 1 *a*).

Periodic clinical examination of the volunteers and their interview by the collaborating clinician/ENT specialist revealed that IN-BCR therapy, in addition to being well accepted, was preferred over the oral therapy. Although the volunteers complained of minor irritation at the time of administration of the spray (due perhaps to the alcoholic content of the solvent), thorough ENT examination before and during the treatment phase did not reveal any local, allergic reactions in any of the volunteers. Four out of the five female patients who were in the reproductive age group of 25–30 years started regular menses by weeks 6–8 of treatment phase and continued to have normal menstrual cycles till the end of the 24-week study period. These female volunteers, who had mild to moderate galactorrhea during the pretreatment phase, were observed to have decreased intensity in this state upon IN-BCR treatment, similar to that observed with O-BCR therapy. In the one menopausal female volunteer, who had hyperprolactinaemia due to pituitary macroadenoma (verified by CT scan), although IN-BCR spray brought down the serum PRL levels to within the normal range, it apparently had no effect on the tumour size. Serial CT scans, visual field tests and fundoscopy performed once every three months in this patient both during O-BCR (retrospective) and during IN-BCR (current) treatment to determine any change in the tumour size revealed no significant reduction in the tumour size by either mode of therapy. This volunteer (A) subsequently underwent surgical treatment for removal of the adenoma. The male volunteer (F) also showed a significant decrease in serum PRL levels following IN-BCR spray (Figure 1 *f*). None of the volunteers complained of any side-effects with IN-BCR therapy and this was unlike what most of them had earlier experienced with O-BCR treatment (Table 1).

For the past few decades BCR has been the most widely used dopamine agonist in the treatment of hyperprolactinaemia[1, 11–14]. The incidence of side-effects following O-BCR treatment, however, has been an unresolved issue, particularly when long-term management of these patients is contemplated. Attempts at developing alternate, long-term effective modes of BCR treatment have, to some extent, been successful[15, 16]. Thus, administration of 2.5 mg BCR by the vaginal route (V-BCR) has been shown to increase significantly the circulating levels of BCR (compared to O-BCR treatment) and has been observed to be equally effective in reducing PRL levels, suggesting thereby that V-BCR may result in a reduction in the overall dose required, thus improving compliance without compromising on therapeutic efficiency[17]. Likewise, treatment with long-acting O-BCR (Parlodel SRO) is shown to be an alternative for the treatment of hyperprolactinaemia, having better tolerance and being equally effective over its predecessor, Parlodel[16]. A pilot study carried out by us with a newer solvent formulation has indicated that it should be possible to replace the alcohol-based vehicle with an aqueous-based vehicle without loss in efficacy of the drug (unpublished observations).

In the present study, we have clearly demonstrated that in hyperprolactinaemic patients IN-BCR treatment can be (a) as effective as O-BCR (at doses 1/4th to 1/20th of O-BCR) in reducing as well as maintaining PRL levels within the normal range over prolonged periods of treatment, (b) well tolerated with little or no side-effects, thus increasing the compliance (acceptance and tolerance) of the patients and (c) highly effective in bringing about a significant reduction in clinical manifestations like galactorrhea and facilitating reinitiation of normal menses. The IN-BCR therapy as such appears to be a potential alternative to O-BCR in the management of hyperprolactinaemia.

A larger multicentric trial is envisaged to determine clearly the benefits of IN-BCR treatment in the management of hyperprolactinaemia. Such a study may also show if IN-BCR is beneficial in reducing the size of prolactinomas following prolonged period of treatment. Yet another use to which such kind of therapy can be adopted is in the management of long-term Parkinsonism, where patients are dependent on BCR in addition to L-DOPA throughout their life[1].

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Plasticity of Z-DNA as observed in the crystal structures of non-self-complementary hexanucleotides

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The crystal structures of two non-self-complementary hexadeoxyribonucleotides, d(CACGCG) · d(CGCGTG) and d(CGCACG) · d(CGTGCG), containing a single A : T base pair each have been solved. Both the sequences are left-handed Z-DNA. The conformation of d(CACGCG) · d(CGCGTG) is very similar to Z-DNA conformations reported earlier, while that of d(CGCACG) · d(CGTGCG) is substantially different. A shift in the position of the A : T base pair is probably responsible for inducing a change in the structure of the tightly wound Z-DNA helix. This is the first time that such large distortions have been observed in closely packed crystals of Z-DNA sequences.

SINCE the discovery, by solution spectroscopic studies[1] and single crystal X-ray diffraction[2], of left-handed Z-DNA in $d(CG)_n$ sequences, evidence has accumulated to support a possible significant biological role for this form of the genetic molecule. Among these is the discovery of regions of the eukaryotic chromosome which cross-react with antibodies raised against Z-DNA sequences[3] and the presence of Z helicogenic $(CG)_n$ sequences in the DNA of histidine D gene of *Salmonella*[4]. Repetitive (TG/CA)*n* sequences capable of adopting Z-DNA conformation have also been found to occur in human and rodent genomes[5]. Studies of the Z-DNA propensities of polynucleotides and short linear DNA fragments of different base sequences have indicated that large amounts (up to 50%) of A : T base pairs in alternating $(CG)_n$ sequences do not prevent Z-DNA formation[6]. A strict alternation of the sequence (i.e. pyrimidine–purine) has also been found neither to be necessary[7] nor sufficient[8,9] to induce Z-DNA.

High-resolution Z-DNA structures have been observed in crystallographic studies of a variety of sequences[10–12], many of them containing the features indicated above. Thus, d(CGATCG) crystallized as Z-DNA without an alternating purine–pyrimidine sequence[7]. A : T base pairs in Z-DNA have also been seen in the crystals of $d(m^5CGTAm^5CG)$[11], d(CGCATGCG)[10], d(CGTACGTACG)[14] and d(CACGTG)[15]. Experimental studies[13,15] and theoretical calculations [16] have indicated a lower stability for Z-DNA when A : T base pairs are present. The high-resolution crystal structures of Z-DNA fragments have provided some clues to rationalize this Z-DNA phobicity of A : T base pairs.

All the crystal structures studies on Z-DNA (in fact,