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Comments on *Inhibition of Growth of Androgen-independent DU-145 Prostate Cancer* In Vivo *by Luteinising Hormone-releasing Hormone Antagonist Cetrorelix and Bombesin Antagonists RC-3940-II and RC-3950-II*, Jungwirth *et al.*, *Eur J Cancer* 1997, 33(7), 1141–1148

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We read with great interest the paper by Jungwirth and associates in the July issue of the *European Journal of Cancer* [1]. The paper raises the question of the direct inhibitory effect of luteinising hormone-releasing hormone (LHRH) analogues (both antagonists and agonists) on the growth of androgen-independent prostate tumour cells *in vivo* and *in vitro*. Similar experiments performed in our laboratory have led to partially different results, and we believe that further studies are required before drawing definitive conclusions.

Jungwirth and associates showed that the LHRH antagonist Cetrorelix (100 μg/day/animal subcutaneous (s.c.), for 4 weeks) significantly inhibited the growth of the androgen-independent prostate tumour DU 145 in nude mice. In contrast, a similar treatment performed with the LHRH agonist [D-Trp^6]LHRH was completely ineffective. Moreover, their *in vitro* studies showed that Cetrorelix significantly decreased ^{3}H-thymidine incorporation into DU 145 cells, but only at very high concentrations.

The data obtained in our laboratory, using the same LHRH antagonist, only partially agree with those of Jungwirth and associates. We have found that the administration of Cetrorelix (via osmotic minipumps, 50–100 μg/day/animal s.c. for 14 days) to nude mice bearing xenografts of the DU 145 prostate cancer does not provide consistent results, significantly inhibiting tumour growth in only one experiment of the two performed. In contrast, the LHRH agonist Zoladex (via osmotic minipumps, 100 μg/day/animal s.c. for 14 days) was consistently effective in reducing DU 145 tumour growth *in vivo* [2]. In *in vitro* experiments, we have observed that Cetrorelix is a potent inhibitor of DU 145 cell proliferation, even at low concentrations (10^{-9}–10^{-6} M; Figure 1, inset). The LHRH agonist Zoladex also exerts an antiproliferative action on DU 145 cells (Figure 1), confirming our previously published data obtained with another LHRH agonist (Buserelin) [3]. Interestingly, when the antagonist and the agonist were given simultaneously, Cetrorelix did not antagonise the inhibitory action of Zoladex; no summation or potentiation of the inhibitory effect exerted separately by each compound was observed (Figure 1).

The reasons for these discrepancies are still unclear, but we would like to propose the following considerations:

(1) The length of the treatment might be crucial for the efficacy of Cetrorelix, when given *in vivo* to nude mice. From the studies of Jungwirth and associates it is clear that only after 21 days of treatment did the inhibitory effect of the analogue become significant. In our experiments, Cetrorelix was given for only 14 days.
(2) The experimental conditions adopted in the different laboratories (cell culture conditions, LHRH analogue used, length of treatment, etc.) might influence the ability of DU 145 cells to respond to LHRH analogues.
(3) We have previously demonstrated that a LHRH inhibitory loop (LHRH-like peptide, LHRH receptors) is expressed in DU 145 cells, and have suggested that LHRH agonists might inhibit prostate tumour cell

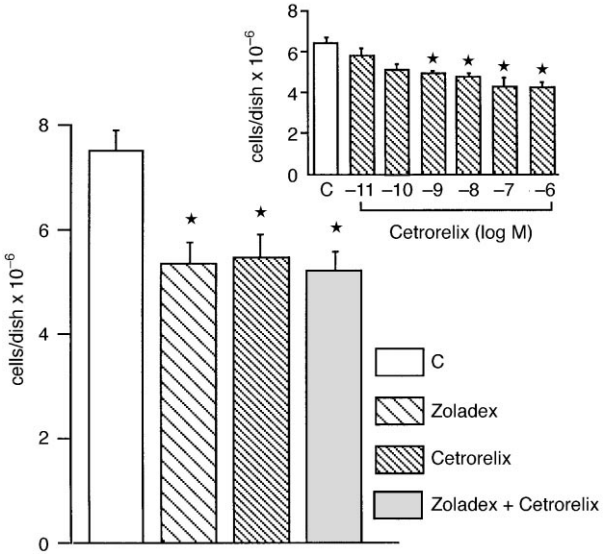

Figure 1. Effects of a 4-day treatment with the luteinising hormone-releasing hormone (LHRH) agonist Zoladex (10^{-6} M) or with the LHRH antagonist Cetrorelix (10^{-6} M), either alone or in combination, on the proliferation of DU 145 cells *in vitro*. Inset. Effects of a 4-day treatment with different doses of Cetrorelix on the proliferation of DU 145 cells. Results are expressed as the mean cell number per plate ± standard error. C, controls without drugs. *$P < 0.05$ versus controls (Dunnett's test).

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proliferation through the activation of LHRH receptors [3]. On the basis of these findings, we propose that the inhibition of prostate cancer cell proliferation induced by Cetrorelix may emphasise the presence, in this analogue, of agonistic properties which may appear when it acts directly on prostate tumour cells. This may indicate that Cetrorelix acts as a LHRH antagonist at the level of the pituitary, and as a LHRH agonist at the level of the prostate. If this is the case, a more accurate characterisation of the pharmacological profile of Cetrorelix activity on prostate tumour cells appears necessary to clarify its precise mechanism of action.

In conclusion, it is clear that Cetrorelix directly inhibits the proliferation of androgen-independent prostate cancer cells. However, the selection of the experimental protocols seems to be crucial for detecting its antitumour activity. Finally, and most importantly, the profile of the pharmacological activity of this compound at the level of the tumour needs further clarification before definitive conclusions can be drawn.

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3. Dondi D, Limonta P, Moretti RM, Montagnani Marelli M, Garattini E, Motta M. Antiproliferative effects of luteinizing hormone-releasing hormone (LHRH) agonists on the human androgen-independent prostate cancer cell line DU 145: evidence for an autocrine-inhibitory LHRH loop. *Cancer Res* 1994, **54**, 4091–4095.

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Response from A.V. Schally, A. Jungwirth and A.M. Comaru-Schally

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We welcome the comments of Limonta and associates [1] regarding our article [2], but we do not consider all of the viewpoints expressed by them to be either definitive or necessarily correct. Firstly, the demonstration of the inhibitory effect of the modern luteinising hormone-releasing hormone (LH-RH) antagonist Cetrorelix on the growth of human androgen-independent prostate cancers *in vivo* was made not only in the DU-145 line as reported in our paper [2], but also in the PC-3 line [3]. At least 2 weeks of administration of Cetrorelix are necessary to obtain growth inhibition and the inconsistent results of Limonta and associates *in vivo* [1] are due, as the authors indicated, to a short treatment period. Further, growth inhibition of DU-145 tumours by the LH-RH agonist Zoladex does not correspond to well-established events in clinical settings, where all hormonal therapies, including LH-RH agonists, aimed at androgen deprivation in patients with advanced prostatic cancer do not prevent an eventual relapse. The patients eventually die, apparently of androgen-independent prostate cancer [2–4].

The question of a direct effect of LH-RH analogues, both agonistic and antagonistic, on various tumours is interesting and important and has received much attention from various investigators, including Limonta, Dondi, Motta and associates [1, 4, 5]. However, most effects *in vitro* are obtained only at very high concentrations and may not be applicable under clinical conditions. Previously, the authors described stimulatory effects of an LH-RH antagonist on the growth of DU-145 cells cultured *in vitro* [5] and now their letter reports that Cetrorelix, a modern and well-characterised LH-RH antagonist, inhibits DU-145 proliferation.

The conjectures of an inhibitory LH-RH loop on prostate cancers and especially of agonistic effects of LH-RH antagonists, like Cetrorelix, on prostate cancer are difficult to accept. An LH-RH loop may exist on various tumours and it has also been postulated by others, but it is more likely to be of a stimulatory nature, since LH-RH is not an antiproliferative hormone, like somatostatin. Such a stimulatory loop could in turn be inhibited by LH-RH agonists, antagonists and antisera to LH-RH, resulting in a tumour suppressing effect.

Very extensive pharmacological investigations have already been carried out on Cetrorelix [4, 6–8], although additional studies would, of course, be welcome. The efficacy of Cetrorelix in the treatment of patients with advanced prostate cancer has already been demonstrated [4, 6–8]. The use of antagonists such as Cetrorelix appears to be particularly appropriate in order to induce immediate tumour inhibition in patients with metastatic invasion of the spinal cord or metastases to the brain and liver, in whom the flare-up in disease that is occasionally caused by the LH-RH agonists, must be avoided [6, 7]. Thus, many favourable characteristics of Cetrorelix have already been established and studies in progress should define others.

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