MATHEMATICAL-STATISTICAL EVALUATION OF HYPOPHYSEAL AND OVARIAN RESPONSE TO Gn-RH AND TO D-Leu^{6}-des Gly^{10}-LH-RH-EA STIMULATION IN PATIENTS WITH AMENORRHEA

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SUMMARY

In a group of 30 women with secondary amenorrhea the administration of D-Leu^{6}-des Gly^{10}-LH-RH-EA Analogue 25 mcg i.m. leads to a more intense and prolonged gonadotrophin response than synthetic Gn-RH 100 mcg i.v.

The ovarian response to Gn-Rh is remarkable and lasts for approximately 24 hours; the stimulation with Analogue leads to a response which is slightly more intense than the one induced by Gn-RH, but probably of longer duration.

Since many years the administration of synthetic Gn-RH (Gn-RH test) is successfully employed to study the functionality of the hypothalamic- hypophyseal- ovarian axis ([1-8]). This decapeptide has also been used as a therapeutic agent to obtain ovulation and pregnancy in women with amenorrhea and/or sterility ([9-15]). Its short half-life and rapid metabolism ([16]) led to the research for more potent and long acting LH - RH Analogues ([17 - 20]). Such Analogues were obtained by modifying some aminoacids of the natural decapeptide sequence. Several new Analogues of LH-RH have been shown to be more active in releasing LH and FSH than the synthetic peptide.

Many Authors ([21-25]) carried out studies testing the hypophyseal response to stimulation with Analogues; some of them ([26-30]) also tested the ovarian response, but only few ([31-32]) took into consideration the FSH-LH and E_2 responses to both synthetic Gn-RH and analogue.

The aim of our study is to test the action of one Analogue: the D-Leu^{6}-des Gly^{10}-LH-RH-EA, toward natural Gn-RH: the comparison is made through a mathematical and statistical analysis of both hypophyseal and ovarian response in a group of women with amenorrhea.

MATERIAL AND METHODS

30 women, 16 to 31 year old (average 20 years), with secondary amenorrhea were examined. None of the patients showed organic alteration of the genital tract, signs of virilization, galactorrhea and/or hyperprolactinemia. All subjects went through the same diagnostic schedule of stimulation and blood sample collection.

Diagnostic schedule:

— Day 1 (BASE): Blood samples withdrawn at the minutes 0, 240, 360, 480 (corresponding to the hours 9 a.m., 1 p.m., 3 p.m., 5 p.m.).

— Day 2 (Gn-RH): Blood samples withdrawn at the minute 0 (9 a.m.), followed by first stimulation with Gn-RH (Relisorm, Serono) 100 mcg i.v.; then, samples withdrawn after 15, 30, 45, 60 minutes. At 120′ sample collection followed by second stimulation with Gn-RH 100 mcg;

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then, samples collected every 15 minutes (135′, 150′, 165′, 180′) and eventually at 240′ (1 p.m.), 360′ (3 p.m.), 480′ (5 p.m.).

— Day 3 (ANALOGUE): Sample collection at the time 0′, followed by stimulation with Analogue 25 mcg i.m. (D-Leu^6-des Gly^{10}-LH-RH EA, Biodata, Serono); then, sample collection after 60, 120, 180, 240, 360, 480 minutes.

The blood samples, collected in heparined testtubes, were centrifuged on the same day; plasma was separated and kept at −20 °C until the hormonal testing time. We made a radioimmunological assay of LH, FSH and 17-β-E_2 in every plasma sample. Biodata kits were used for FSH and LH, and Nordiclab kits for 17-β-E_2.

Statistical analysis:

Mathematical-statistical analysis was made with Apple II plus computer. We compared, for every single hormone in each of the 30 patients, at the corresponding times:

a) The mean values at the minutes 0, 240, 360, 480 on day 1 (BASE) to those obtained on day 2 (Gn-RH).

b) The mean values of day 1 (BASE) to those of day 3 (ANALOGUE).

c) The mean values of day 2 (Gn-RH) to those of day 3 (ANALOGUE).

We calculated the average ($\bar{x}$) and the standard deviation (S.D.) for each withdrawing time.

In order to assess the significance of the differences between the hormonal values, we used Student's t test for paired data. The integral of FSH, LH and 17-β-E_2 values was calculated by Simpson's method to assess the global hormonal dismission in each day. Identical statistical analysis was then made on integrals: average, standard deviation, Student's t test.

Table 1. — *Comparison between LH values on day 1 (BASE) and 2 (after Gn-RH).*

Time (min.)	Day of collection	Average	S.D.	Student's t test	P%
0′	1) BASE	13.35	8.39	1.49	85.82
	2) Gn-RH	10.56	7.74		
240′	1) BASE	10.91	8.59	−6.05	99.99
	2) Gn-RH	69.13	55.11		
360′	1) BASE	11.19	8.68	−4.57	99.98
	2) Gn-RH	33.93	28.96		
480′	1) BASE	10.43	7.78	−2.73	99.97
	2) Gn-RH	21.94	24.00		

Table 2. — *Comparison between LH values on day 1 (BASE) and 3 (after ANALOGUE).*

Time (min.)	Day of collection	Average	S.D.	Student's t test	P%
0′	1) BASE	13.35	8.39	−0.37	27.28
	3) ANALOGUE	14.35	15.54		
240′	1) BASE	10.91	8.59	−9.18	99.99
	3) ANALOGUE	116.36	63.69		
360′	1) BASE	11.19	8.68	−7.42	99.99
	3) ANALOGUE	101.10	65.90		
480′	1) BASE	10.43	7.78	−6.18	99.99
	3) ANALOGUE	82.83	63.46		

RESULTS

A) Hypophyseal response

LH hormone

Statistical analysis of LH assays are shown on tables 1, 2, 3.

Tab. 1 shows that the differences between time 0′ values are not significant while the differences in all the following times (240′, 360′, 480′) are significant.

All average differences between LH values shown on table 2 are significant, except for initial values (time 0′).

The comparison beween LH values after the two stimulations (tab. 3) shows that all differences are significant, except for time 0′ values.

Tab. 4 shows that mean values increase after Gn-RH and grow further after Analogue: differences between averages are significant.

FSH hormone

The following tables (5, 6, 7) show statistical analysis on FSH assays.

The difference between average values (tab. 5) is not significant at the time 0′, but is highly significant at all the successive times.

Table 3. — *Comparison between LH values on day 2 (after Gn-RH) and 3 (after ANALOGUE).*

Time (min.)	Day of collection	Average	S.D.	Student's t test	P%
0′	2) Gn-RH	10.56	7.74	−1.65	89.51
	3) ANA-LOGUE	14.35	15.54		
240′	2) Gn-RH	69.13	55.11	−5.97	99.99
	3) ANA-LOGUE	116.36	63.69		
360′	2) Gn-RH	33.93	28.96	−6.46	99.99
	3) ANA-LOGUE	101.10	65.90		
480′	2) Gn-RH	21.94	24.00	−5.80	99.99
	3) ANA-LOGUE	82.83	63.46		

Table 4. — *Comparison between integral LH values on three days: 1 (BASE), 2 (after stimulation with Gn-RH) and 3 (after stimulation with ANALOGUE).*

Time (min.)	Day of collection	Average	S.D.	Student's t test	P%
	1) BASE	6299.45	7177.54	−6.12	99.99
	2) Gn-RH	26507.40	17694.20		
$\int_0^{480}$	1) BASE	6299.45	7177.54	−7.42	99.99
	3) ANA-LOGUE	36943.16	22420.84		
	2) Gn-RH	26507.40	17694.20	−5.68	99.99
	3) ANA-LOGUE	36943.16	22420.84		

Table 5. — *Comparison between FSH values on day 1 (BASE) and 2 (after Gn-RH).*

Time (min.)	Day of collection	Average	S.D.	Student's t test	P%
0′	1) BASE	7.77	3.24	1.47	85.18
	2) Gn-RH	7.01	3.03		
240′	1) BASE	7.39	3.59	−6.29	99.99
	2) Gn-RH	23.19	14.35		
360′	1) BASE	7.36	2.61	−6.23	99.99
	2) Gn-RH	16.03	8.09		
480′	1) BASE	7.43	3.10	−4.30	99.96
	2) Gn-RH	13.60	7.57		

On tab. 6 the differences between data are all significant, except for time 0′ values.

On tab. 7, differences are not significant at the times 0′ and 240′, while they are significant at all the other times.

On tab. 8, all differences between data are significant.

Table 6. — *Comparison between FSH values on day 1 (BASE) and 3 (after ANALOGUE).*

Time (min.)	Day of collection	Average	S.D.	Student's t test	P%
0′	1) BASE	7.77	3.24	0.95	34.82
	3) ANA-LOGUE	9.43	9.78		
240′	1) BASE	7.39	3.59	−9.21	99.99
	3) ANA-LOGUE	26.20	11.68		
360′	1) BASE	7.36	2.61	−8.52	99.99
	3) ANA-LOGUE	27.36	13.37		
480′	1) BASE	7.43	3.10	−8.13	99.99
	3) ANA-LOGUE	24.63	12.12		

Table 7. — *Comparison between FSH values on day 2 (after Gn-RH) and 3 (after ANALOGUE).*

Time (min.)	Day of collection	Average	S.D.	Student's t test	P%
0′	2) Gn-RH	7.01	3.03	−1.34	81.38
	3) ANA-LOGUE	9.43	9.78		
240′	2) Gn-RH	23.19	14.35	−1.60	88.37
	3) ANA-LOGUE	26.20	11.68		
360′	2) Gn-RH	16.03	8.09	−6.71	99.99
	3) ANA-LOGUE	27.36	13.37		
480′	2) Gn-RH	13.60	7.57	−6.07	99.99
	3) ANA-LOGUE	24.63	12.12		

Table 8. — *Comparison between integral values of FSH on the 3 days: 1 (BASE), 2 (after Gn-RH) and 3 (after ANALOGUE).*

	Day of collection	Average	S.D.	Student's t test	P%
	1) BASE	3296.16	1274.04	−7.26	99.99
	2) Gn-RH	7896.33	3821.08		
$\int_0^{480}$	1) BASE	3296.16	1274.04	−7.70	99.99
	3) ANALOGUE	9178.83	4462.67		
	2) Gn-RH	7896.33	3821.08	−2.60	98.63
	3) ANALOGUE	9178.83	4462.67		

COMMENT

From the results of the previous tables we suggest that:

a) Since no significant difference is seen between data at the time 0′, both kinds of stimulation are carried out under basal conditions: thus the hormonal differences in the following times (minute 240, 360, 480) are not invalidated by environment, psychological or dietetic factors, and the second stimulation (with Analogue) is not influenced by the previous one (with Gn-RH).

b) The gonadotrophin response to Gn-RH stimulation tends to decrease at the minute 480 and is exhausted by the end of the day: this is corroborated by the initial values of day 3 which are not different from those of the two previous days.

The response to Analogue grows progressively and remains intense even after 8 hours.

c) Both gonadotrophins give good response to Gn-RH, but their response is much higher after Analogue.

B) OVARIAN RESPONSE

The results of statistical 17-β-E_2 assays are shown on tab. 9, 10, 11. Integral values are reported on tab. 12.

Table 9. — *Comparison between 17-β-E_2 values on day 1 (BASE) and 2 (after Gn-RH).*

Time (min.)	Day of collection	Average	S.D.	Student's t test	P%
0′	1) BASE	61.01	56.06	1.80	92.20
	2) Gn-RH	46.31	40.80		
240′	1) BASE	50.43	40.26	−4.63	99.94
	2) Gn-RH	90.03	65.47		
360′	1) BASE	54.60	50.28	−5.59	99.99
	2) Gn-RH	146.70	106.69		
480′	1) BASE	62.01	64.98	−3.97	99.93
	2) Gn-RH	173.20	159.06		

Table 10. — *Comparison between 17-β-E_2 values on day 1 (BASE) and 3 (after ANALOGUE).*

Time (min.)	Day of collection	Average	S.D.	Student's t test	P%
0′	1) BASE	61.01	56.06	−1.95	94.26
	3) ANALOGUE	86.86	79.90		
240′	1) BASE	54.43	40.26	−3.30	99.71
	3) ANALOGUE	86.36	48.53		
360′	1) BASE	54.60	50.28	−5.84	99.99
	3) ANALOGUE	155.66	84.75		
480′	1) BASE	62.01	64.98	−5.46	99.99
	3) ANALOGUE	223.70	146.40		

Table 11. — *Comparison between 17-β-E_2 values on day 2 (after Gn-RH) and 3 (after ANALOGUE).*

Time (min.)	Day of collection	Average	S.D.	Student's t test	P%
0′	2) Gn-RH	46.31	40.80	−3.41	99.78
	3) ANALOGUE	86.86	76.90		
240′	2) Gn-RH	90.03	65.47	0.34	24.82
	3) ANALOGUE	86.36	48.53		
360′	2) Gn-RH	146.70	106.69	−0.47	35.37
	3) ANALOGUE	155.66	84.75		
480′	2) Gn-RH	173.20	159.06	−1.36	82.07
	3) ANALOGUE	223.70	146.40		

Table 12. — *Comparison between integral values of 17-β-E_2 in the 3 days: 1 (BASE), 2 (after Gn-RH) and 3 (after ANALOGUE).*

	Day of collection	Average	S.D.	Student's t test	P%
	1) BASE	24481.20	19788.61	−4.99	99.99
	2) Gn-RH	44189.81	29668.84		
$\int_0^{480}$	1) BASE	24481.20	19788.61	−5.08	99.99
	3) ANALOGUE	47950.50	22408.90		
	2) Gn-RH	44189.81	29668.84	−0.82	41.90
	3) ANALOGUE	47950.50	22408.90		

Tab. 9 shows that there is no difference between basal values (time 0′) in the 2 successive days while all differences in the following times are significant (minute 240, 360, 480). Estrogenic values grow progressively and reach their highest mean value at the minute 480.

The comparison on tab. 10 shows that all differences between mean values are significant at the times 240, 360, 480. Although the comparison between initial values (time 0′) is not significant, it has a high P (= 94.26%). In fact, day 3 mean value at the time 0′ is higher than that of day 1: this is probably due to the previous stimulation with Gn-RH (day 2). The estrogenic response to this stimulation evidently continues for more than 24 hours.

Hormonal data after the 2 stimulations are different (tab. 11) only at the time 0′; in the successive times, despite the higher values after ANALOGUE, differences are not significant.

Tab. 12 confirms that the value difference between BASE and after Gn-RH, and between BASE and after ANALOGUE are significant, while differences after Gn-RH and after ANALOGUE are not significant: the estrogenic response to both stimulations is thus similar, at least for the times we examined.

COMMENT

The 17-β-E_2 assays suggest that:

a) There is a good ovarian response to either Gn-RH or Analogue stimulation.

b) The response after stimulation with Gn-RH is tardive and prolonged, as it begins at the minute 240 and continues for at least 24 hours. This is shown by the significance of the differences at the time 0′ on table 11.

c) After stimulation with Analogue, the response remains intense and prolonged: compared to basal values the difference is significant (tab. 10), while it is not when compared to the values after Gn-RH. However, mean values are higher after Analogue.

d) The persistence of the Analogue effect cannot be established owing to the short hospitalization time of only 8 hours after drug administration. Since at that moment the hormonal response is intense and progressively growing, we assume that it persists for a long time.

CONCLUSIONS

Our data suggest that the hypophyseal response induced by D-Leu^6-des Gly^{10}-LH-RH-EA Analogue administration is much higher and more prolonged than after Gn-RH. On the contrary, the ovarian response to Analogue is only a little higher than to Gn-RH; however, we can presume that it is more prolonged with Analogue. In accordance with many Authors ([21-32]) we think that the much longer and intense effect of the long-acting Analogue can be very useful in clinical field.

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