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Note

Predictive value of hGH-RIA kit for assaying carp growth hormone

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ABSTRACT

Growth hormone (GH) levels in serum and pituitary extracts of three major Indian carps viz, rohu, catla and mrigal were estimated using human GH RIA kit. The cross reactivity observed between human GH and carp GH clearly indicates that both share a relatively large number of common antigenic determinants. This was further confirmed by parallel shift in values of standard and diluted rohu pituitary extracts. Therefore, human GH RIA kit may be successfully employed to monitor carp serum GH levels. This may also help in screening fish growth promoters of commercial interest for potential aquaculture.

Growth rates in cultured fishes have been known to be elevated by neuroendocrine factors (Lin *et al.*, 1994), through administration of GH or recombinant GH preparation (Lin *et al.*, 1993) and by producing GH transgenic lines of fish. Oral administration of recombinant salmon growth hormone to rainbow trout *Oncorhynchus mystkis* has been shown to elevate serum GH (Moriyama *et al.*, 1993). Under the above conditions information on growth hormone levels can help in elucidating the mechanism by which growth is stimulated and also to facilitate rapid determination of optimal concentration and appropriate time of the day when such stimulating factor/GH preparation can be administered. The major handicap associated with such trials in Indian fishes is non availability of fish growth hormone RIA kit. Although growth hormone assays have been developed for fish (Kosugi *et al.*, 1995; Marchelidon *et al.*, 1996) commercial kits are not yet available. For the widely cultured Indian major carps, attempts are being made for production of GH transgenic fish, but no laboratory to our knowledge is working on developing GH assay. Human GH has been reported to have about 32 % structural homology with that of carp GH (Chao *et al.*, 1989) and commercial human GH RIA kit is also readily available in India. This prompted us to estimate the levels of GH in serum and pituitary extracts of the Indian major carps using this kit.

Fingerlings of Indian major carps *Catla catla, Labeo rohita* and *Cirrhinus mrigala* collected from a local farm

were acclimatised to laboratory conditions for a week before the actual experiments. They were fed once daily with a mixed diet containing wheat bran : wheat flour : gram flour at a ratio of 1:1:0.5 mixed with live tubifix worms at the rate of 2 % total body weight. The excess food and faecal matter were removed and water was changed daily. The fishes (16-43 g) were sorted out in different groups keeping the variation in body weight range to a minimum.

The blood was collected 15 min after administration of test compound or saline in 1.5 ml eppendorf tubes through direct cardiac puncture using 1 x 100 mm unheparinised fine glass capillaries. Four to five fingerlings were used for each blood sample. The serum from blood samples was separated at room temperature (26°C ± 1°C). The samples were centrifuged at 2000 rpm for 20 min. The clear pale-straw coloured serum was decanted in another eppendorf tube and stored at – 70°C till analysis. For each assay two serum samples were analysed. The whole pituitary was collected by vivisecting the cranium dorsally and pituitary extract was prepared by homogenising 20-40 mg wet weight of pituitary tissue in 0.05 M phosphate buffer in 0.15 M saline pH 7.2. One per cent n-butyl alcohol (v/v) was added prior to homogenisation to minimise foaming and to serve as a preservtive. The crude extracts were then allowed to stand at 4°C for a period of one hour prior to centrifugation. Each pituitary assay was based on minimum of three samples. The extract was centrifuged at 2500 rpm for 20 minutes at 4°C and clear supernatent was stored at – 70°C till analysis.

Measurement of serum GH was carried out by radioimmunoassay using hGH–RIA kit supplied by Bhabha Atomic Research Centre, Mumbai, India as per methodological description given with the kit.

A significant inhibition in the binding of labelled hGH with rabbit antiserum to hGH was observed by GH present in the serum and pituitary extracts of Indian major carps (Table 1). In the three species, serum GH levels were 5.45 ± 1.51, 9.20 ± 2.65 and 1.53 ± 0.52 ng/ml whereas pituitary GH levels were 9.72 ± 1.16, 14.06 ± 3.28, and 9.95 ± 0.04 ng/ml in *L. rohita*, *C. catla* and *C. mrigala* respectively.

Further owing to non-availability of purified carp GH, we have used natural source to validate our findings. The pure pituitary extract showed 30.66 % binding which is equivalent to 9.72 ± 1.16 ng/ml of carp GH concentration, whereas serum sample showed 62.56 % binding equivalent to 2.94 ± 0.33 ng/ml GH. Further pituitary extracts of *L. rohita* was diluted with that of its serum samples in the ratio of 1:1, 3:1 and then reversing the ratio to, 1:3. The radioimmunoassay results obtained are very startling as shown in Fig. 1. After dilution with carp serum, the GH levels were found to decrease proportionately

TABLE 1. *Base levels of GH in the serum and pituitary extracts of the three Indian major carps*

Species	Month	GH (ng/ml)	
		Serum	Pituitary extract
Labeo rohita	Oct.	11.10 ± 6.04	--
	Nov.	5.45 ± 1.51	9.72 ± 1.16
Catla catla	Oct.	10.95 ± 2.20	15.52 ± 5.45
	Nov.	9.20 ± 2.65	14.06 ± 3.28
Cirrhinus mrigala	Oct.	-	25.70 ± 2.28
	Nov.	1.53 ± 0.52	9.95 ± 0.04

- Not sampled.

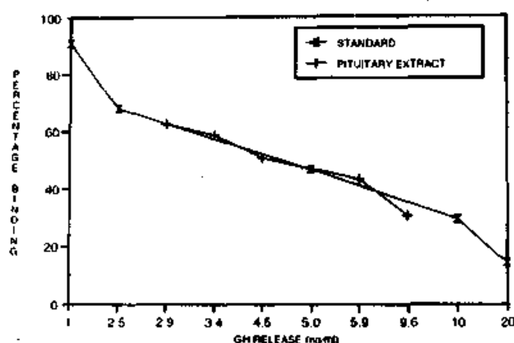

Fig. 1. The dose response curve using cocktails (3:1, 1:1, and 1:3) of pituitary extract and serum of rohu. The pituitary GH was 9.6 and serum GH, 2.9 ng/ml.

and there was an obvious parallel shift in the concentration of carp GH and standard values obtained with hGH–RIA kit.

The base level values of serum GH obtained on Indian major carps are comparable to the one already reported in goldfish (Marchant and Peter, 1986; Marchant *et al.*, 1989). The cross reactivity observed between human GH and carp GH clearly suggested that the human GH share a relatively large number of common antigenic determinants with carp GH. Nevertheless in view of the known structural differences between human and carp GH it may be pointed out that the measurement of carp growth hormone in our experiment represents a semi quantitative profile. Thus, the measurement of fish GH levels using hGH–RIA kit may reflect a near quantitative or qualitative estimate of carp GH.

The present study has thus indicated the feasibility of utilising human GH – RIA kit for screening of compounds of structural diversity with the objective of developing commercially feasible growth promoter for aquaculture.

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