Effect of growth hormone on human alveolar macrophage oxidative metabolism

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Abstract

Background – Growth hormone (GH) has diverse immunological actions and has been shown to augment oxidative metabolism in rat peritoneal and porcine alveolar macrophages and both human and animal neutrophils. A study was performed to determine the effects of GH on human alveolar macrophages in vitro.

Methods – Macrophages were harvested from 10 patients undergoing broncho-alveolar lavage and incubated with 0, 10 and 100 nmol/ml GH for four hours. Oxidative metabolism was assessed by means of a fluorescent assay using FMLP and *E coli* as stimulants. Fluorescence was measured using flow cytometry.

Results – No difference in basal or stimulated oxidative metabolism was found between the GH and control groups.

Conclusions – GH does not have a direct stimulatory action on human alveolar macrophages in vitro. However, this does not exclude an indirect effect in vivo. The results contrast with previous studies on animal alveolar macrophages.

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Human leucocytes have been shown to synthesise growth hormone (GH),¹ and GH and its growth promoting peptide insulin-like growth factor (IGF)-1 have diverse immunological actions.² Normal thymic and lymphoid cells have binding sites for GH and prolactin.³ GH primes human neutrophils via the prolactin (PRL) receptor.⁴ Porcine alveolar macrophages and rat alveolar and peritoneal macrophages show augmented superoxide response to GH.² The cytolytic potential of natural killer (NK) cells is also effected by GH and, indeed, NK cells from GH-deficient children have an intrinsic defect compared with healthy controls in that they fail to respond to interferon.⁵ A beneficial role for GH in malnourished patients with chronic obstructive pulmonary disease has also been suggested.⁶

This study was undertaken to determine the effect of GH on human alveolar macrophage oxidative metabolism and to see if the findings in animal studies could be demonstrated in humans.

Methods

Alveolar macrophages were harvested from 10 normal healthy male volunteers by means of bronchoalveolar lavage according to standard

guidelines. Lavage fluid was centrifuged at 250g for 10 minutes and the resulting pellet was resuspended in DMEM supplemented with 10% fetal calf serum and 5% penicillin and streptomycin and made up to a final concentration of 1×10^6 macrophages/ml (>90% viability by trypan blue exclusion). These were then incubated with two concentrations of GH (10 nmol/ml and 100 nmol/ml) or vehicle control for four hours. The concentrations and time points were derived from concentration-response and time course experiments. Alveolar macrophages were assessed for oxidative metabolism using the fluorogenic substrate DHR 123.⁷ Briefly, 100 μ l of 1×10^6 macrophages/ml were incubated under three conditions, alone, with 20 μ l of 1×10^{-6} M FMLP, or with 20 μ l opsonised *E coli* (1×10^9 bacteria/ml) for 10 minutes at 37°C. 20 μ l of substrate, which is fluorescent on interaction with reactive oxygen species, was then added. FACS lysing solution (1 ml) was added to fix the macrophages. Samples were centrifuged, washed, resuspended in PBS, and stored at 4°C in the dark until FACS analysis. Immunofluorescence analysis was conducted with the use of a FAC-Scan equipped with an argon laser providing excitation wavelength of λ_{ex} = 488 nm and collection of fluorescent signal at λ_{em} = 530 + 15 nm (green fluorescence) and λ_{em} = 595 + 30 nm (red fluorescence). The intensity of fluorescence was used as an indicator of oxidative metabolism.

Statistical analysis was by means of ANOVA.

Results

There was no significant difference between the control and the growth hormone groups at either concentration (fig 1).

Figure 1 Effect of growth hormone (GH) on oxidative metabolism. Values are mean (SE).

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Discussion

Our results contrast with previously described results in animal macrophages.² It is possible that the effects of GH on human macrophages are mediated in vivo by IGF-1 or somatomedin C, the synthesis of both of which are stimulated by GH. However, GH has been shown to prime human neutrophils directly and IGF-1 antibodies do not antagonise this effect. GH augments human granulopoiesis in vitro by inducing synthesis of somatomedin C.⁸ The fact that we demonstrated no increase in oxidative metabolism with GH stimulation rules out an effect mediated via macrophage-derived somatomedin C or IGF-1. It does not, of course, exclude a more distant paracrine mode of action in vivo.

While our method of measuring oxidative metabolism differs from the other studies on GH, it has been previously validated.⁷ It measures intracellular reactive oxygen metabolite release, but it is unlikely there would be a rise in extracellular free radicals without a rise in intracellular levels.

Functional heterogeneity of diverse macrophage and T cell properties has been demonstrated both for intra-species populations of macrophages⁹ and inter-species¹⁰ populations of T cells. We propose that our failure to demonstrate an augmenting effect of GH on human alveolar macrophage oxidative metabolism represents a further example of inter-

species functional heterogeneity. In conclusion, we have shown that growth hormone fails to stimulate oxidative metabolism directly in human alveolar macrophages. This does not rule out an indirect effect in vivo.

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