Effects of growth hormone and insulin-like growth factor-I on the iron-induced lipid peroxidation in the rat liver and porcine thyroid homogenates

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Abstract

OBJECTIVES: Growth hormone (GH) and its tissue mediator, insulin-like growth factor-I (IGF-I), are involved in oxidative processes, lipid peroxidation (LPO) included. Bivalent iron (Fe²⁺) is frequently used to experimentally induce oxidative damage to macromolecules (Fe²⁺ + H₂O₂ + H⁺ → Fe³⁺ + ⋅OH + H₂O). The aim of the study was to evaluate the effect of GH and/or IGF-I on the iron-induced LPO in the rat liver and porcine thyroid homogenates.

METHODS: Rat liver and porcine thyroid homogenates were incubated in presence of GH (100; 10; 1.0; 0.1; 0.01; 0.001; 0.0001 µg/ml) or IGF-I (1000; 100; 10; 1.0; 0.1; 0.01; 0.001; 0.0001 µg/ml) or GH (100 µg/ml) + IGF-I, or FeSO₄ + H₂O₂ plus GH and/or IGF-I. The level of LPO was expressed as concentrations of malondialdehyde + 4-hydroxyalkenals (MDA+4-HDA) per mg of protein.

RESULTS: GH and/or IGF-I did not change the basal level of oxidative damage to lipids. In the rat liver homogenates, GH did not affect the iron-induced LPO, whereas IGF-I – in the lowest two concentrations – enhanced the process. In porcine thyroid homogenates, GH – in its two lowest concentrations – prevented, whereas in other concentrations, it enhanced the iron-induced LPO. IGF-I, in all used concentrations, enhanced the iron-induced LPO.

CONCLUSION: GH and/or IGF-I may reveal prooxidative effects. This fact does not support their application in the treatment of disorders associated with increased oxidative damage.

INTRODUCTION

Reactive oxygen species (ROS), free radicals included, are formed in living organisms under physiological conditions. An overproduction of ROS, due to the action of either external or internal factors, may cause increased damage to macro-

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Abbreviations:
- ANOVA - one-way analysis of variance
- GH - growth hormone
- IGF-I - insulin-like growth factor-I
- LDL - low-density lipoprotein
- LPO - lipid peroxidation
- MDA+4-HDA - malondialdehyde + 4-hydroxyalkenals
- PUFAs - polyunsaturated fatty acids
- ROS - reactive oxygen species
MATERIAL AND METHODS

The procedures, used in the study, were approved by the Ethical Committee of the Polish Mother’s Memorial Hospital – Research Institute.

GH from porcine pituitary, the human recombinant IGF-I expressed in E. coli, and ferrous sulphate (FeSO₄) were purchased from Sigma-Aldrich (St. Louis, MO). The LPO-586 kit for LPO was purchased from Calbiochem (La Jolla, CA). Other chemicals were of analytical grade and came from commercial sources.

Rat livers were collected from 20 male Wistar rats (the average age of 2 months; weighing about 160 g each). Porcine thyroids were collected from 20 male animals (the average age of 12 months) at a slaughterhouse. Immediately after collection, tissues were frozen on solid CO₂ and stored at –70 °C until assay.

Liver fragments and thyroid tissues were homogenized in ice cold 50 mM Tris-HCl buffer (pH 7.4, 10% or 5%, w/v – in final incubation volume, respectively) and then, incubated for 30 min at 37 °C in presence of the examined substances.

In order to induce LPO, homogenates of rat livers or porcine thyroids were incubated in presence of FeSO₄ (15 µM or 40 µM, respectively) + H₂O₂ (0.1 mM or 0.5 mM, respectively) (Karbownik et al. 2000; Karbownik & Lewinski 2003a). Additionally, liver or thyroid homogenates were incubated in presence of:

- **Experiment I**: GH (100, 10, 1.0, 0.1, 0.01, 0.001, 0.0001 µg/ml) alone (to check its effect on the basal LPO), or FeSO₄ (15 µM or 40 µM, respectively) + H₂O₂ (0.1 mM or 0.5 mM, respectively) + GH (100, 10, 1.0, 0.1, 0.01, 0.001, 0.0001 µg/ml);
- **Experiment II**: IGF-I (1000, 100, 10, 1.0, 0.1, 0.01, 0.001, 0.0001 µg/ml) alone (to check its effect on the basal LPO), or FeSO₄ (15 µM or 40 µM, respectively) + H₂O₂ (0.1 mM or 0.5 mM, respectively) + IGF-I (1000, 100, 10, 1.0, 0.1, 0.01, 0.001, 0.0001 µg/ml);
- **Experiment III**: GH (100 µg/ml) + IGF-I (1000, 100, 10, 1.0, 0.1, 0.01, 0.001, 0.0001 µg/ml) (to check their joint effect on the basal LPO), or FeSO₄ (15 µM or 40 µM, respectively) + H₂O₂ (0.1 mM or 0.5 mM, respectively) + GH (100 µg/ml) + IGF-I (1000, 100, 10, 1.0, 0.1, 0.01, 0.001, 0.0001 µg/ml).

The reactions were stopped by cooling the samples on ice. Each experiment was run in duplicate and was repeated three times.

**Measurement of LPO products**

The concentrations of malondialdehyde + 4-hydroxy-alkenals (MDA+4-HDA), as an index of LPO, were measured in liver and thyroid homogenates.

The homogenates were centrifuged at 3000 × g for 10 min at 4 °C. The supernatant was mixed with 650 µl of a methanol:acetonitrile (1:3, v/v) solution, containing a chromogenic reagent, N-methyl-2-phenylindole, and vortexed. After addition of 150 µl of methanesulfonic acid (15.4 M), the incubation was carried out at 45 °C for 40 min. The reaction between MDA+4-HDA and N-methyl-2-phenylindole yielded a chromophore, which was spectrophotometrically measured at the absorbance of 586 nm, using a solution of 4-hydroxynonenal (10 mM) as standard.

The level of LPO was expressed as the amount of MDA+4-HDA (nmol) per mg of protein.
**Measurement of protein concentration**

Protein concentration was measured, using the Bradford's method (Bradford 1976), with bovine albumin as standard.

**Statistical analysis**

The data were statistically analyzed, using a one-way analysis of variance (ANOVA), followed by Student-Newman-Keuls’ test. Statistical significance was determined at the level of $p<0.05$. The results are presented as means ± SEM.

**RESULTS**

In all the experiments, FeSO$_4$ + H$_2$O$_2$, added into the incubation medium, increased the level of LPO (Figures 1–6).

The incubation of rat liver or porcine thyroid homogenates in presence of either GH (used in concentrations of 100, 10, 1.0, 0.1, 0.01, 0.001, 0.0001 µg/ml) or IGF-I (used in concentrations of 1000, 100, 10, 1.0, 0.1, 0.01, 0.001, 0.0001 µg/ml), or GH (100 µg/ml) plus IGF-I (1000, 100, 10, 1.0, 0.1, 0.01, 0.001, 0.0001 µg/ml) did not affect the basal LPO (Figures 1–6).

In rat liver homogenates, GH, used together with FeSO$_4$ + H$_2$O$_2$, did not cause any changes in the iron-induced LPO (Figure 1). In turn, IGF-I, whereas in concentrations of 1000, 100, 10, 1.0, 0.1, 0.01 µg/ml did not affect the Fenton reaction-induced LPO, in the lowest used concentrations (0.001 or 0.0001 µg/ml), it even enhanced FeSO$_4$ + H$_2$O$_2$-induced LPO in rat liver homogenates (Figure 2). The incubation of liver homogenates in presence of FeSO$_4$ (15 µM) + H$_2$O$_2$ (0.1 mM) + GH (100 µg/ml) + IGF-I (1000, 100, 10, 1.0, 0.1, 0.01, 0.001, 0.0001 µg/ml) failed to cause any significant changes in FeSO$_4$ + H$_2$O$_2$-induced LPO (Figure 3).

In porcine thyroid homogenates GH, used in concentrations of 100, 10, 1.0, 0.01 µg/ml, significantly enhanced FeSO$_4$ + H$_2$O$_2$-induced LPO, whereas in the lowest used concentrations, i.e. 0.001, 0.0001 µg/ml, it completely prevented FeSO$_4$ + H$_2$O$_2$-induced LPO (Figure 4). IGF-I, in all used concentrations, significantly enhanced FeSO$_4$ + H$_2$O$_2$-induced LPO. However, the stimulatory effects of the highest (1000, 100, 10, 1.0 µg/ml), as well as the lowest (0.001, 0.0001 µg/ml) IGF-I concentrations were significantly stronger than those of IGF-I, used in concentrations of 0.1 and 0.01 µg/ml (Figure 5). When the homogenates were incubated in presence of GH (100 µg/ml) plus IGF-I (used in concentrations of 1000, 100, 10, 1.0, 0.1, 0.01, 0.001, 0.0001 µg/ml), they significantly – in all used concentrations of IGF-I – increased FeSO$_4$ + H$_2$O$_2$-induced LPO (Figure 6).

**DISCUSSION**

Our study has been the first attempt to evaluate the effects of GH and IGF-I (used alone or together) on the basal and the iron-induced LPO in the liver and thyroid homogenates. The selection of the liver and the thyroid gland for such a study is justified by several factors. The liver is one of the target organs for GH and the main source of IGF-I, being the main mediator of GH tissue effects. The liver plays an important role in detoxifying processes; hepatocytes contain a variety of enzymes and proteins involved in the detoxification of potentially harmful substances. The thyroid gland is also involved in the regulation of the body’s energy balance and metabolism. The effects of GH and IGF-I on LPO in these tissues may provide insights into the mechanisms of action of these hormones and their role in the regulation of metabolic processes.

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**Fig. 1.** Concentrations of MDA+4-HDA in rat liver homogenates, incubated for 30 min in presence of FeSO$_4$ (15 µM) + H$_2$O$_2$ (0.1 mM), or GH (100, 10, 1.0, 0.1, 0.01, 0.001, 0.0001 µg/ml), or FeSO$_4$ (15 µM) + H$_2$O$_2$ (0.1 mM) + GH (100, 10, 1.0, 0.1, 0.01, 0.001, 0.0001 µg/ml). Bars represent means ± SEM of three independent experiments run in duplicates. *p<0.05 versus Controls.

**Fig. 2.** Concentrations of MDA+4-HDA in rat liver homogenates, incubated for 30 min in presence of FeSO$_4$ (15 µM) + H$_2$O$_2$ (0.1 mM), or IGF-I (1000, 100, 10, 1.0, 0.1, 0.01, 0.001, 0.0001 µg/ml), or FeSO$_4$ (15 µM) + H$_2$O$_2$ (0.1 mM) + IGF-I (1000, 100, 10, 1.0, 0.1, 0.01, 0.001, 0.0001 µg/ml). Bars represent means ± SEM of three independent experiments run in duplicates. *p<0.05 versus Controls; ♦p<0.05 versus Fe.
of antioxidant enzymatic systems, so they are able to reduce the amount of oxygen free radicals generated during cellular activity (Brown-Borg et al. 2002; Hayes & McLellan 1999; Kireev et al. 2007; Miyamoto et al. 2003). The thyroid gland is an organ in which ROS are involved and even are indispensable for physiological processes (Karbowska & Lewinski 2003b). H$_2$O$_2$ is an essential factor for biosynthesis of thyroid hormones.

And also, the presence of the antioxidative enzymes (Rhee 1999; Sadani & Nadkarni 1996; Sugawara et al. 1988) and antioxidative proteins (peroxiredoxins) (Kim et al. 2000) has been well documented in the thyroid gland. GH and IGF-I are important for optimizing thyroid growth and function (thyroid hormone secretion, metabolism and action) (Chen et al. 2004; Dumont et al. 2003; Fayet & Hovsepian 2004; Lewinski et al. 1993).
In our study, the observation of the “lack” of GH and/or IGF-I effects on the basal LPO in rat liver and porcine thyroid, was unexpected, but very desirable. This finding may indicate that GH and IGF-I are worth testing for their potential protective effects against oxidative damage. According to our knowledge, only in one study the effects of GH and IGF-I on the oxidative processes were evaluated under in vitro conditions. In this study, GH or IGF-I, applied in concentrations similar to those used in our study, however for a much longer time (24 hours), decreased the activity of antioxidative enzymes in hepatocytes derived from normal, wild-type mice (Brown-Borg et al. 2002). However, that decrease in antioxidative enzyme activities (if any) did not result in any increase of LPO in the present study, which could be due either to much shorter time of incubation (1 hour vs. 24 hours) or to direct effects of GH and/or IGF-I on LPO in vitro. In turn, in agreement with our results, in only one in vivo study did GH not change the basal level of oxidative damage to lipids (expressed as the level of malondialdehyde) in the liver of normal, wild-type mice (Chen et al. 2004). In contrast to our findings, in the liver of normal rats treated with GH (single administration) (Youn et al. 1998) or IGF-I (a 2-week administration) (Castilla-Cortazar et al. 1997), the level of the basal LPO was significantly reduced, compared with untreated healthy controls. This apparent disagreement between the above cited study and the present study may be explained by the fact, that in vitro effects can not be directly extrapolated to the in vivo conditions.

In the present study neither GH nor IGF-I were able to prevent the iron-induced LPO in rat liver homogenates. The observation of the “lack” of protective effects of GH and IGF-I may support the concept that the liver, as the main target organ for GH-action and the main source of IGF-I, is less susceptible to exogenous administration of these two factors, even when they are used in relatively high concentration. On the other hand, IGF-I, when used in the lowest concentrations (0.001 and 0.0001 µg/ml), increased the iron-induced LPO in liver tissue. These unexpected differences between effects caused by high and low concentrations of IGF-I are difficult to explain. One can suppose that IGF-I, which is always “a factor in place” in the liver, may strongly influence oxidative processes in this tissue by enhancing oxidative damage induced by any pathophysiological factor. However this occurs, when IGF-I is in concentrations close to physiological, slightly exceeding upper normal ranges. It is not excluded that this prooxidative effect of IGF-I results, at least to a certain extent, from the mentioned above inhibitory action on antioxidative enzyme activities in the liver (Brown-Borg et al. 2002). In turn, the “lack” of prooxidative effects of IGF-I in high concentrations may result from lower penetration of the factor to cellular membranes due to the mechanical “blockade” but such an explanation is only hypothetical.

In the porcine thyroid, both GH and IGF-I significantly affected the iron-induced LPO. GH, in the lowest used concentrations (0.001 and 0.0001 µg/ml), was able to protect against oxidative damage, caused by the Fenton reaction, thus revealing antioxidative effects; those effects of GH are the only protective effect against the iron-induced LPO, observed in our study. This finding suggests that GH being in concentrations closed to physiological contributes to oxidative balance. It is worth mentioning that also other authors observed protective effects of GH against LPO. For example, GH decreased LPO in rats with thioacetamide-induced liver cirrhosis (Chen et al. 2004), in rat liver homogenates after thermal injury (Youn et al. 1998), and in the intestine homogenates in septic rats (Huang et al. 2002; Jung et al. 2003).

In other – higher – used concentrations, we observed prooxidative effect of GH, relying on enhanced oxidative damage to lipids, already caused by iron. Thus, GH, when in excess, contributes to oxidative damage in the thyroid gland, which may result in different pathologies, such as goiter in patients with acromegaly (Kasagi et al. 1999) and an increased risk of cancer in acromegalics (Jenkins et al. 2006; Matyja et al. 2006; Orme et al.1998), although thyroid cancer specifically has not been described with relation to acromegaly. Potential prooxidative properties of GH excess in the thyroid gland were additionally confirmed with even stronger prooxidative effects of IGF-I in the tissue, as observed in the present study.

Consequently to our observations, prooxidative effects of GH and IGF-I have been confirmed in several in vivo and in vitro studies in animals and in humans. GH and IGF-I excess is associated with increased oxidative stress and early decline in antioxidative enzymes activity in transgenic mice overexpressing GH (Anderson et al. 2006; Hauck & Bartke 2001). Also patients with active acromegaly have significantly higher the basal LPO, expressed as a plasma level of malondialdehyde (Yarman et al. 2003).

The question arises if GH or IGF-I could be used as an external protective factor, potentially comparable to melatonin (Reiter et al. 2008a; 2008b) with regard to their antioxidative action, in the case of disorders other than GH deficiency, associated with increased oxidative stress. Our in vitro results do not support such a use of either of the two substances. Also other studies do not favor such a recommendation. GH treatment, administered in patients with critical illnesses, associated with increased oxidative stress (Karbownik-Lewinska et al. 2007), appeared to double the mortality and worsen the morbidity (Takala et al. 1999). Concerning IGF-I, there is currently unambiguous evidence for an increased risk of cancer, a condition always associated with elevated oxidative stress, in patients with high IGF-I concentrations, even when IGF-I concentrations remain within high-normal ranges (Hankinson et al. 1998; Kurek et al. 2000).
In conclusion, GH and/or IGF-I may directly contribute to oxidative balance in the liver and the thyroid under physiological conditions but, in the case of induced oxidative stress, they may reveal prooxidative effects, which fact does not support their application in the treatment of disorders, other than GH deficiency, associated with increased oxidative damage.

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REFERENCES


