

Studies on the goiter inhibiting action of iodolactones

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Abstract

The thyroid gland synthesizes 6-delta-iodolactone, a compound shown to inhibit goiter growth in vivo and cell proliferation in culture. The present studies were performed to characterize this effect further with the aim of exploring the possible therapeutic action of iodolactones. *Prevention assay*: rats were treated simultaneously with a goitrogen, methylmercaptoimidazole, and either 6-delta-iodo-lactone or 14-iodo-omega-lactone, a synthetic derivative, given either i.p. or p. o. Both compounds caused a significant decrease in thyroid weight irrespective of the route of administration, but oral administration was less effective. A dose-response relationship was observed, the minimal effective dose (i.p.) being 3 µg/day. *Involution assay*: goiter was first induced with methylmercaptoimidazole and then the iodolactones were injected. Both compounds caused a significant involution, which was dose-related. Acute (10 days) administration of the iodolactones did not produce significant changes in several serum parameters (total T3 and T4, cholesterol, total protein, urea and acetylcholinesterase). These results give further support to the potential therapeutic application of iodolactones.

Key words: Iodolactone; Goiter; Antigoitrogenic; Thyroid; Autoregulation

1. Introduction

The thyroid gland is capable of synthesizing two types of iodinated compounds: (a) iodothyronines and their precursors and (b) iodinated lipids (Chazenbalk et al., 1985). Among the latter, it has been demonstrated that the glands of rats (Boeynaems and Hubbard, 1980), pigs (Dugrillon et al., 1990) and humans (Dugrillon et al., submitted for publication) produce 6-iodo-delta-lactone, an iodinated derivative of arachidonic acid. Another iodocompound, iodoheptadecanal, has been also shown to be produced by the horse thyroid (Pereira et al., 1990).

In order to clarify the possible role of 6-iodo-delta-

lactone in thyroid physiology, we analyzed its action on gland function and growth. Previous results have shown that it inhibits several functional parameters, such as iodide uptake (Chazenbalk et al., 1988), formation of iodoprotein and H₂O₂ (Krawiec et al., 1988), and uptake of deoxyglucose and α-aminoisobutyric acid (Krawiec et al., 1991).

Moreover, it was demonstrated that 6-iodo-delta-lactone prevents goiter growth in rats (Pisarev et al., 1988) and that, in vitro, it inhibits thyroid cell proliferation in pigs (Dugrillon et al., 1990), humans (Dugrillon et al., submitted) and FRTL-5 cells (Pisarev et al., 1992). These results opened the possibility that this compound could be of potential use for the treatment of goiter and allied diseases.

These studies were performed in order to explore this point further. Another iodoarachidonate, 14-iodo-omega-lactone, a by-product in the synthesis procedure, was also assayed in these experiments. In this

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regard sporadic goiter and benign thyroid growth have been treated with iodide and/or iodothyronines. The data from different studies show that the results of such treatments are far from optimal, since reduced size of the abnormal gland was observed with great variability (see Gharib et al., 1987, for a study and review of this topic). This therapeutic failure can be explained in part by the lack of a clear explanation of the pathophysiology of these diseases (see Pisarev and Kleiman de Pisarev, 1980, for a review).

The aims of our studies were: (a) to define further the goiter preventing effect of iodolactones; (b) to determine whether these iodocompounds are active when administered p.o.; (c) to analyze if they can cause the involution of preformed goiter, and (d) to determine their possible side effects.

2. Materials and methods

2.1. Antigoitrogenic activity

Femal Wistar rats, 120–140 g body weight, were used in these studies.

2.2. Involution of preformed goiter

The rats were distributed into two groups: (a) solvent-treated and (b) injected with methylmercaptoimidazole, 5 mg/day for 10 days, i.p., to induce goiter. The treatment with the goitrogen was then discontinued and the rats were distributed into the following subgroups: (b1) without further treatment; (b2) 5 μ g/day of 14-iodo-omega-lactone; (b3) 5 μ g/day of 6-iodo-delta-lactone, both i.p. The animals were killed 7 and 14 days after this last treatment. The ratio thyroid weight mg/body weight (g) \times 100 was determined and

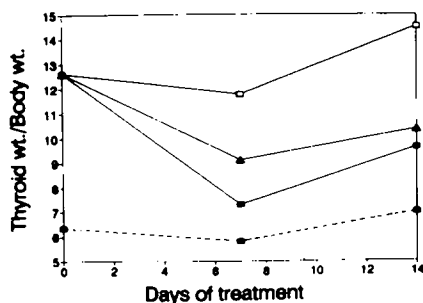


Fig. 1. The effect of 6-iodo-delta-lactone or of 14-iodo-omega-lactone on the weight of previously induced goiter. The rats were treated with methylmercaptoimidazole for 10 days and were then treated as indicated: □ — □ no further treatment; ▲ — ▲ 14-iodolactone; ■ — ■ 6-iodolactone; ■ - - - ■ solvent throughout the study. Each value is the average for four rats. Similar results were obtained in two additional experiments.

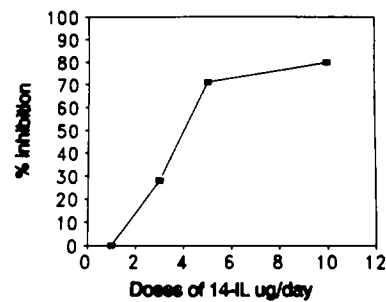


Fig. 2. Dose-response relationship of the goiter-inhibiting action of 14-iodo-omega-lactone. Each value is the average for four rats. The percent inhibition was calculated as described in the text.

the percent inhibition of goiter weight was calculated as follows:

$$\% \text{ variation} = \frac{1 - (\text{Exptal} - \text{control})}{(\text{MMI} - \text{control})} \times 100$$

where MMI = methylmercaptoimidazole.

2.3. Influence of route of iodolactone administration (Goiter prevention assay)

The rats were treated *simultaneously* from the start with 5 mg/day methylmercaptoimidazole, i.p., with the addition of 5 or 10 μ g/day of either iodolactone, administered either p.o. or i.p. The former was performed by using a stainless steel catheter attached to a syringe. Control rats were treated with the solvent. After 9 days the animals were killed, the ratio thyroid weight/body weight was determined, and the percent inhibition of goiter weight was calculated as above.

2.4. Possible side effects of iodolactone administration

The rats were treated with 10 μ g/day, i.p., of each iodolactone in parallel to the above described experiments. These animals were not treated with methylmercaptoimidazole. At the end of each experiment, blood was obtained, serum was separated by centrifugation at 4°C and the following assays were performed according to standard techniques: triiodothyronine (T3) and thyroxine (T4) were determined by radioimmunoassay, using DPC kits; urea was measured with a Bioclin kit; total protein was measured by the Biuret method, cholesterol with the Bioclin kit and acetylcholinesterase with the Boehringer Mannheim kit. The iodolactones were synthesized as already described (Monteagudo et al., 1992) and their purity was checked by reverse-phase high-pressure liquid chromatography (Boeynaems and Hubbard, 1980) and by ascending thin layer chromatography, using chloroform:methanol (97.5:2.5%, v/v). The spots were detected with I₂ vapour. Pure methylmercaptoimidazole was generously provided by Gador Laboratories of Argentina.

Table 1
Influence of route of administration on the goiter preventing effect of iodolactones

Treatment	Thyroid wt./Body wt.	% inhibition
Controls (12)	6.3 ± 0.15	
Methylmercaptoimidazole 5 mg/day (14)	13.8 ± 0.16 ^a	
Methylmercaptoimidazole + 14-iodolactone 5 µg/day i.p. (6)	9.6 ± 0.58 ^b	56
Methylmercaptoimidazole + 14-iodolactone 10 µg/day i.p. (4)	8.5 ± 1.15 ^b	71
Methylmercaptoimidazole + 14-iodolactone 5 µg/day oral (8)	11.6 ± 0.38 ^b	29
Methylmercaptoimidazole + 14-iodolactone 10 µg/day oral (10)	9.9 ± 0.28 ^b	52
Methylmercaptoimidazole + 6-iodolactone 5 µg/day i.p. (6)	10.1 ± 0.30 ^b	49
Methylmercaptoimidazole + 6-iodolactone 10 µg/day i.p. (4)	6.6 ± 1.60 ^b	94
Methylmercaptoimidazole + 6-iodolactone 5 µg/day oral (5)	10.6 ± 0.35 ^b	26
Methylmercaptoimidazole + 6-iodolactone 10 µg/day oral (4)	9.5 ± 2.90 ^b	42

The rats were treated simultaneously with methylmercaptoimidazole and the iodocompounds for 10 days. Each value is the average for the number of rats indicated in parentheses ± S.E.M. ^a $P < 0.01$ vs. controls; ^b $P < 0.01$ vs. methylmercaptoimidazole.

The statistical significance of the differences among the experimental groups was determined with Dunnett's *t*-test.

3. Results

3.1. Involution of preformed goiter

As shown in Fig. 1, administration of methylmercaptoimidazole, 5 mg/day for 10 days, caused a significant 100% increase in thyroid weight when compared to that of the control rats. At this point methylmercaptoimidazole was discontinued and the rats were distributed into the following groups: (a) pretreated with methylmercaptoimidazole, without further treatment; pretreated with the goitrogen and then treated with (b) 14-iodo-omega-lactone, 5 µg/day, or (c) 6-iodo-delta-lactone, 5 µg/day. The animals were killed after 7 and 14 days of treatment. Administration of 6-iodo-delta-lactone led to 75% and 63% inhibition after 7 and 14

Table 2
Serum thyroxine and triiodothyronine after administration of iodolactones

Treatment	Triiodothyronine (nmol)	Thyroxine (nmol)
Controls	1.75 ± 0.15 (9)	75.9 ± 6.4 (14)
6-Iodolactone 10 µg/day	1.78 ± 0.29 (8)	77.2 ± 6.4 (14)
14-Iodolactone 10 µg/day	1.43 ± 0.11 (14)	57.9 ± 2.6 (14)

The rats were treated for 10 days as indicated. Each value is the average ± S.E.M. for the number of animals indicated in parentheses, obtained in different experiments. Differences between means were not statistically significant.

days, respectively, while 14-iodo-omega-lactone caused 44% and a 48% inhibition at the corresponding periods.

3.2. Prevention of goiter growth

3.2.1. Dose-response relationship

When 14-iodo-omega-lactone was given simultaneously, via the i.p. route, with 5 mg/day of methylmercaptoimidazole, for 10 days, a progressive decrease in goiter growth was observed with increasing doses of this iodocompound: no inhibition with 1 µg/day, a 28% decrease with 3 µg/day, 71% with 5 µg/day, and 80% with 10 µg/day (Fig. 2).

3.2.2. Influence of route of administration

The prevention of goiter formation was compared in rats treated with the goitrogen, plus 6-iodo-delta-lactone or 14-iodo-omega-lactone given either p.o. or i.p. Table 1 depicts the results. The rats treated with 5 mg/day methylmercaptoimidazole had a 119% increase in their thyroid weight/body weight ratio. When

Table 3
Influence of administration of iodolactones on serum parameters

Treatment	Cholesterol (nmol/l)	Acetylcholinesterase (U/l)	Urea (nmol/l)	Protein (mg/l)
Control	1.60 ± 0.07	1622 ± 129	6.6 ± 0.02	61 ± 1
14-Iodolactone	1.65 ± 0.05	1556 ± 83	7.5 ± 0.02	64 ± 1
6-Iodolactone	1.76 ± 0.07	1572 ± 156	6.8 ± 0.03	65 ± 2

The rats were treated as described in Table 2. Each value is the average ± S.E.M. for four to eight rats. Differences among the means were not statistically significant.

groups of animals were treated simultaneously with 6-iodo-delta-lactone, the following inhibitions were observed: for 5 $\mu\text{g}/\text{day}$: i.p. 49% and p.o. 26%; for 10 $\mu\text{g}/\text{day}$: i.p. 94% and p.o. 42%. In the case of 14-iodo-omega-lactone the following inhibitions were observed: 5 $\mu\text{g}/\text{day}$: i.p. 56%, p.o. 29%; 10 $\mu\text{g}/\text{day}$: i.p. 71% and p.o. 52%.

3.2.3. Possible side effects of iodolactone administration

The effects of the injection of both iodolactones for 10 days on several serum parameters were examined. None of the parameters examined, such as total T4 and T3 (Table 2), urea, cholesterol, total protein and acetylcholinesterase activity (Table 3), were significantly different from those of the control rats.

4. Discussion

Previous results demonstrated that iodolactones can prevent goiter growth in rats (Pisarev et al., 1988) and inhibit thyrotropin (TSH) and forskolin-stimulated growth of FRTL-5 cells (Pisarev et al., 1992), epidermal growth factor-stimulated growth of pig-cells (Dugrillon et al., 1990) and phorbol ester-stimulated growth of human cells (Dugrillon et al., submitted for publication). In *in vivo* studies we observed a parallelism between goiter size and cyclic AMP and DNA content (Pisarev et al., 1988).

Taken together, these results show that iodolactones can impair both cyclic AMP-dependent and independent (tyrosine kinase and protein kinase C) growth. Previous studies have demonstrated that the goiter inhibiting action of iodolactones is not due to an inhibition of pituitary TSH secretion in rats since serum TSH, which was increased by goitrogen administration, remained the same in rats treated with methylmercaptoimidazole and iodolactones (which caused a significant inhibition of goiter size). This action of iodolactones cannot be ascribed to the iodide that would originate from their dehalogenation since injection of an equivalent amount of iodide failed to cause a significant change of thyroid size (Pisarev et al., 1988). Moreover, the direct inhibition of thyroid growth caused by these iodocompounds was confirmed in *in vitro* cell culture studies with FRTL-5 cells (Pisarev et al., 1992) and with pig thyroid cells (Dugrillon et al., 1990). The present data confirm and extend the previous observations concerning the inhibition of goiter growth and show, for the first time, that these iodocompounds can *prevent* the growth of methylmercaptoimidazole-induced goiter (when administered simultaneously) and also cause the *involution* of preformed goiter (when given after the goitrogen). Both effects were dose-related. Although the goiter induced by methylmercaptoimidazole in animals is different from that which

occurs in humans, these observations provide further support for the idea that these compounds are of potential use for this type of pathology.

A second point concerns the route of administration. We provide evidence that these iodocompounds are effective even when given p.o., an observation that further supports their therapeutic use. However, the results obtained would suggest that this route causes a decrease in the antigoitrogenic potency, indicating that a higher dose would be necessary in order to attain the same degree of pharmacologic effect obtained after i.p. administration. This would also suggest that, when administered p.o., the iodolactones are partially metabolized in the liver, with a decrease in their bioactivity.

Another of the objectives of the present studies was to determine whether the administration of iodolactones causes side effects which would preclude their therapeutic use. As a first step toward this goal we measured cholesterol and acetylcholinesterase, as an index of liver impairment, total protein, which indicates a deleterious effect on liver and/or general body metabolism, and urea, as a reflection of kidney function. Under the present experimental conditions no significant changes were observed in the serum parameters examined. This would suggest that no dramatic side effects are caused by the short-term administration of iodolactones. However, further studies are necessary, with longer times of treatment and measurement of other parameters, in order to exclude toxic side effects. It should be remarked that since 6-iodo-delta-lactone has already been demonstrated to be a naturally occurring compound (Boeynaems and Hubbard, 1980; Dugrillon et al., 1990 and submitted for publication) it is not surprising that it does not show dramatic toxic side effects on other systems.

Moreover, we have shown that these iodocompounds inhibit the thyroid membrane transport of several molecules, without having an effect on the same parameters in liver and kidney (Krawiec et al., 1991), suggesting a selective action on the thyroid gland, where these compounds are synthesized.

In summary, the present results provide further support to the idea that these iodolactones are potential useful therapeutic agents for the treatment of abnormal thyroid growth since they *prevent* goiter growth (when administered simultaneously with a goitrogen) and cause the *involution* of preformed goiter (when given after the goitrogen). These compounds are active p.o. and no significant side effects were detected after their administration.

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