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# SHORT COMMUNICATIONS

## Interaction of synthetic compounds with UTP-D-glucose 1-phosphate uridylyl transferase of *Echinococcus multilocularis* metacestodes

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Many compounds have been screened for their activity against *Echinococcus multilocularis* metacestodes, the causative agent of alveolar hydatid disease [1, 2], but studies involving rational approaches are few. Parasite enzymes are potential targets for selective chemotherapy, so to try to select such enzymes, one approach has been to examine the activity of particular enzymes in parasites and in infected host livers.

Glycogen represents an energy reserve for the metacestode and its accumulation in cells of the parasite has been shown by electron microscopy [3]. For this reason there has been a great deal of interest in determining the regulatory mechanisms of this pathway. UTP-D-glucose 1-phosphate uridylyl transferase or UDP-glucose pyrophosphorylase (EC 2.7.7.9) is a crucial enzyme in glycogen biosynthesis. Sarciron *et al.* [4] have shown that the host enzyme has kinetic and physical parameters different to those of the parasite. This enzyme was retained as a potential target for chemotherapy.

Toward this end, the synthesis of inhibitors was envisaged using as the starting point the observation that alkaline phosphatase like UDP-glucose pyrophosphorylase recognizes a phosphorylated substrate and can be inhibited by isatin. Modifications of the basic structure of isatin were therefore undertaken as previously described [5].

The present paper deals with the effects of two ring-opened derivatives of isatin **1**: ethyl oxanilate **2** and parachloroethyl oxanilate **3** (Fig. 1) against the activity of UDP-glucose pyrophosphorylase in cestodes *in vivo* and the repercussion upon glucose and glycogen content both in the metacestodes and livers of infected animals.

### *Materials and methods*

*Enzymatic activities. Animals and treatment.* Three-month-old Mongolian Gerbils *(M. unguiculatus)* were infected by intraperitoneal inoculation with 50 mg of *Echinococcus multilocularis* metacestodes (germinal layer and protoscolices). In this experimental model, development of the parasite is restricted to the peritoneal cavity with no hepatic invasion during the time course of the experiment. The treatment was started three months after infection. Animals were divided into three groups of five. The first and second groups were treated per os once daily with synthetic compounds **2** and **3** respectively at 50 mg·kg$^{-1}$ body weight for 18 days. The third group served as controls. Autopsy was carried out the eighteenth-day after the start of treatment. The livers or the metacestodes of the animals of each group were pooled.

The enzymatic activity of UDP-glucose pyrophosphorylase was measured in the cytosol.

After cellular fractionation, all subsequent operations were carried out at +4°.

All tissues were transferred to Tris–HCl 20 mM, pH 8 in 0.25 M sucrose and homogenized for 10 min using a scissor homogenizer, a grinder (5 min) and sonicated for 10 sec (power 15 W). The homogenate was then centrifuged at 10,000 *g* for 30 min. The resulting mitochondrion-free supernatant was centrifuged at 100,000 *g* for 60 min in a 52 TI rotor (Beckmann) and the cytosol and the microsomal fraction were separated.

*Assay.* Protein concentrations were determined by the method of Bensadoun and Weinstein [6] using bovine serum albumin as a standard.

Glucose concentration was determined by a gluco-kit (Biomérieux 61303 with orthotoluidine). Glycogen was measured spectrophotometrically according to the procedure of Kepler and Decker [7] using amyloglucosidase. UDP-glucose dehydrogenase type VI was obtained from Sigma Chemical Co. (St Louis, MO).

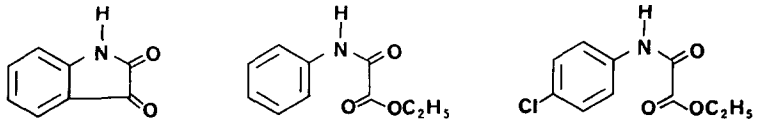
### *Results and discussion*

The data in Table 1 show that in the presence of oxamates **2** and **3** inhibition of UDP-glucose pyrophosphorylase of the cestode *Echinococcus multilocularis* was observed. Results in Table 1 also show that the compound 3 is a better inhibitor of UDP-glucose pyrophosphorylase of the cestode than compound **2**. A possible explanation for this difference of activity may be that the parachlorinated compound **3** is more lipophilic than the unsubstituted compound **2** and may therefore cross membrane barriers more readily.

In view of these results further experimentation was carried out only with compound **3** and its effect on the levels of glucose and glycogen was determined on livers of infected hosts and on metacestodes.

Oxamate **3** increases the levels of glucose (Table 2) and glycogen (Table 3) in metacestodes. Glucose uptake is increased thereby bringing about a concomitant increase in glycogen. On the other hand in the livers of infected animals treated by this oxamate, glucose uptake is slightly decreased and there is no detectable glycogen. One reason for this latter observation may be that liver glycogen is degraded to glucose which is then taken up preferentially by metacestodes to increase its own glycogen reserve.

As regards the key enzyme in glycogen synthesis: the UDP-glucose pyrophosphorylase activity is less inhibited




**1** **2** **3**

Fig. 1. Structural formulae of **1** isatin (2,3 indolinedione); **2** ethyloxanilate and **3** parachloroethyloxanilate. (For review of synthesis see Ref. 5.)

Table 1. Effects of the oxamates **2** and **3** on UDP-glucose pyrophosphorylase specific activities in the *Echinococcus multilocularis* metacestodes and in the liver of infected Mongolian gerbil

| | Livers activities | Metacestodes activities |
|---|---|---|
| Controls | 198.67 ± 6.60 | 240 ± 29.44 |
| Oxamate **2** | 97.13 ± 5.39 ↘ (51%) | 144.75 ± 7.37 ↘ (39.7%) |
| Oxamate **3** | 70.58 ± 1.51 ↘ (64.5%) | 51.44 ± 1.97 ↘ (78.6%) |

UDP-glucose pyrophosphorylase activity is expressed as nmol·min$^{-1}$/mg proteins. Each value is the mean of three determinations ± SE.

Table 2. Repercussion of UDP-glucose pyrophosphorylase inactivation by oxamate **3** on glucose content in the *Echinococcus multilocularis* metacestodes and in the liver of infected Mongolian gerbil

| | Livers content | Metacestodes content |
|---|---|---|
| Controls | 2.77 ± 0.01 | 4.43 ± 0.11 |
| Oxamate **3** | 2.73 ± 0.01 ↘ (1%) | 6.0 ± 0.01 ↗ (135%) |

Glucose concentration is expressed as mmol/mg proteins. The results are means ± SE.

by oxamate **3** in liver than in metacestodes. The increased susceptibility of the metacestode enzyme could probably be due to the previously described differences in the kinetic and physical parameters of this enzyme compared to those from the liver of infected animals [4].

Table 3. Repercussion of UDP-glucose pyrophosphorylase inactivation by oxamate **3** on glycogen content in the *Echinococcus multilocularis* metacestodes and in the liver of infected Mongolian gerbil

| | Livers content | Metacestodes content |
|---|---|---|
| Controls | 0.014 ± 0.001 | 0.086 ± 0.001 |
| Oxamate **3** | 0 ↘ (100%) | 0.236 ± 0.003 ↗ (270%) |

Glycogen concentration is expressed as μmol/mg proteins. The results are means ± SE.

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## Trifluoperazine does not inhibit the acute metabolic effects of insulin in rat adipocytes

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Calcium has been implicated in mediating some of the biochemical responses of insulin [1]. The effect of calcium in certain cases was attributed to its binding to calmodulin, a ubiquitous calcium binding protein [2]. Calmodulin has been demonstrated to activate a variety of enzymes, including several protein kinases [3–5], plasma membrane $Ca^{2+}$–$Mg^{2+}$ ATPase [6] and cyclic nucleotide phosphodiesterase [2, 7]. A role for calmodulin in insulin action has been obtained directly from studies with calcium and calmodulin [8, 9], and also by inference from studies with "inhibitors" of calmodulin [10, 11]. The phenothiazines, specifically trifluoperazine, have been shown to inhibit calcium–calmodulin-activated processes [12]. There is not, however, a general consensus as to the role of calmodulin in the mechanism of insulin action.

Insulin is the major anabolic hormone regulating intermediary metabolism, yet the biochemical events coupling the insulin–receptor interaction with the modulation of intracellular processes remain to be fully elucidated [13]. Autophosphorylation of the insulin receptor is enhanced in response to insulin [14, 15] and in addition, "second messengers" are released into the intracellular milieu [16, 17]. Trifluoperazine can inhibit insulin receptor phosphorylation, but without altering the effect of insulin to stimulate glucose transport [18]. Since the effect of insulin to regulate glucose transport is dissociable from its