EFFECTS OF OCHRATOXIN A ON TISSUE GLYCOGEN LEVELS IN RATS

Shigetoshi SUZUKI and Tetsuo SATOH
Institute of Food Microbiology, Chiba University, Narashino, Chiba, Japan

Accepted December 14, 1972

Abstract—A single oral dose (15 mg/kg body wt.) of Ochratoxin A caused a depletion of hepatic glycogen level and an elevation in the cardiac glycogen level in both intact and adrenalectomized rats. When tested in adrenalectomized rats under pretreatment of hydrocortisone, Ochratoxin A showed similar effects on hepatic glycogen level as was seen in intact rats. These changes in hepatic glycogen levels of Ochratoxin A treated rats may be attributed to interference with endocrine balance.

Ochratoxin A (OCT A) was found to be a toxic fungal metabolite isolated from Aspergillus ochraceus Wilh. (1) and Penicillium viridicatum Westling (2). With regard to the effects of OCT A on carbohydrate metabolism, Theron et al. (3) reported simultaneous changes in hypertrophy of smooth-endoplasmic reticulum and diminution or loss of hepatic glycogen in electronmicroscopic studies, while, Purchase and Theron (4) demonstrated that the electronmicroscopic pattern of storage of liver glycogen observed in rat liver after OCT A administration was similar to glycogen storage disease type VIII and IX. Since no information has been reported on the biochemical aspects of OCT A toxicity in vivo and quantitative analysis of the tissue glycogen levels, the present study was undertaken to investigate the effects of OCT A on tissue glycogen levels by colorimetric assay in intact rats. Adrenalectomized rats (ADREX) were also used, since mobilization of tissue glycogen may be the result of an interference with endocrine balance.

MATERIALS AND METHODS

OCT A was isolated from a culture Aspergillus ochraceus Wilh. (IFM 4443) and purified by column chromatography as reported previously (5). All rats used in this study were male Wistar strain weighing 230-300 g, maintained on standard commercial diet and water, plus addition of 0.9 % NaCl solution in ADREX, ad libitum. Throughout the present study, OCT A was given orally as suspension in 0.1 % carboxymethylcellulose solution. Animals in the control group were administered carboxymethylcellulose alone and this vehicle was found to have no effect on tissue glycogen levels. Bilateral adrenalectomies were performed by dorsal approach under pentobarbital anesthesia 5 days before animals were sacrificed.

At the indicated time-intervals after OCT A administration, the animals were sacrificed by decapitation. Duplicate specimens of hepatic and cardiac tissues were quickly removed and frozen with dry ice until use. The glycogen levels in tissues were analyzed according to the anthrone method (6).
RESULTS

As illustrated in Table 1, OCT A treated intact rats (15 mg/kg) caused a significant decrease (P<0.01) in hepatic glycogen levels 4 hr later and the level gradually recovered to that of the control within 5 days. A remarkable reduction in hepatic glycogen levels (P<0.001) was also observed in the ADREX after 4 hr. This reduction continued until death of rats within 7 hr after administration. Conversely, the cardiac glycogen level was

Table 1. Time course on changes in liver glycogen levels in intact and adrenalectomized rats after Ochratoxin A treatment*.

| Time (hr) | Glycogen (mg/g wet wt.) |
|-----------|------------------------|
|           | LIVER                  |
|           | Treated                |
|           | Control                |
|           |                        |
| 2         | 111.58±2.79 (5)        |
| 4         | 30.71±1.44 (6)         |
| 5         | 65.74±3.04 (5)         |
| 6         | 34.75±2.95 (5)         |
| 16        | 64.81±4.12 (5)         |
| 24        | 84.07±6.59 (5)         |
| 48        | 76.33±6.78 (5)         |
| 72        | 100.52±4.89 (4)        |
| 120       | 45.08±6.16 (3)         |
| 168       | 54.60±1.08 (4)         |

| Time (hr) | Glycogen (mg/g wet wt.) |
|-----------|------------------------|
|           | ADREX                  |
|           | Treated                |
|           | Control                |
|           |                        |
| 2         | 116.79±3.80 (5)        |
| 4         | 73.08±5.07 (5)         |
| 5         | 104.69±4.57 (5)        |
| 6         | 70.19±5.92 (5)         |
| 16        | 118.67±7.94 (5)        |
| 24        | 116.60±5.79 (6)        |
| 48        | 90.30±7.03 (5)         |
| 72        | 81.09±5.22 (5)         |
| 120       | 54.60±1.08 (4)         |
| 168       | 22.79±3.31 (5)         |

* Values represent the means±standard error. Figures in parentheses indicate number of animals employed. P values calculated by F-test. N.S.: not significant. Ochratoxin A was administered p.o. (15 mg/kg).

Table 2. Time course on changes in heart glycogen levels in intact and adrenalectomized rats after Ochratoxin A treatment*.

| Time (hr) | Glycogen (mg/g wet wt.) |
|-----------|------------------------|
|           | HEART                  |
|           | Treated                |
|           | Control                |
|           |                        |
| 2         | 6.11±0.13 (4)          |
| 4         | 6.13±0.33 (9)          |
| 5         | 9.23±0.68 (5)          |
| 6         | 3.63±0.46 (5)          |
| 16        | 3.72±0.32 (8)          |
| 24        | 4.27±0.49 (5)          |
| 48        | 3.85±0.39 (5)          |
| 72        | 1.72±0.42 (5)          |
| 120       | 2.02±0.36 (4)          |
| 168       | 2.76±0.55 (4)          |

| Time (hr) | Glycogen (mg/g wet wt.) |
|-----------|------------------------|
|           | ADREX                  |
|           | Treated                |
|           | Control                |
|           |                        |
| 2         | 5.98±0.40 (4)          |
| 4         | 3.60±0.81 (7)          |
| 5         | 4.88±0.24 (5)          |
| 6         | 3.88±1.07 (6)          |
| 16        | 4.24±0.57 (8)          |
| 24        | 4.61±0.15 (4)          |
| 48        | 3.68±0.34 (5)          |
| 72        | 1.91±0.35 (5)          |
| 120       | 2.76±0.55 (4)          |
| 168       | 1.93±0.22 (5)          |

* Explanation as in Table 1.
increased slightly, but statistically significant ($P<0.05$) within 4–6 hr after administration of OCT A in intact rats. Cardiac glycogen levels of OCT A treated ADREX were increased significantly ($P<0.05$) after 4 hr and this elevation recovered to that of control 6 hr after dosing (Table 2).

On the other hand, pretreatment with hydrocortisone resulted in significant depletion in the hepatic glycogen level in OCT A treated ADREX showing a pattern similar to that seen in the intact group, and this elevation in cardiac glycogen level was recovered to the control level (Table 3). Table 4 shows the relationship between doses of OCT A and tissue glycogen levels in ADREX and intact rats 4 hr after OCT A treatment. A remarkable depletion in the hepatic glycogen level was apparent in the 15 mg/kg of OCT A in intact rats ($P<0.01$), with all doses in ADREX ($P<0.001$), whereas, the cardiac glycogen level was not affected in lower doses of OCT A in intact rats and ADREX.

In order to determine whether or not glycogen depletion in the liver and glycogen elevation in the heart were caused by OCT A per se or its metabolite, ochratoxin a (OCT a), rats were treated orally with OCT a in a dose of 9.5 mg/kg, which is comparable to

---

**Table 3. Effects of pretreatment with hydrocortisone on changes in tissue glycogen levels in ADREX by administration of Ochratoxin A.**

| Tissue | Hydrocortisone (25 mg/kg s.c.) | Glycogen (mg/g wet wt. tissue) | Significance |
|--------|-------------------------------|-------------------------------|-------------|
|        | None                          | OCT A treated | Control | |
| Liver  | Pretreated*                   | 39.62±0.85 (4) | 101.30±8.97 (5) | $P<0.01$ |
| Heart  | None                          | 5.62±0.85 (7)  | 2.50±0.41 (6)  | $P<0.05$ |
|        | Pretreated*                   | 5.04±0.20 (5)  | 4.31±0.68 (5)  | N.S. |

* Hydrocortisone was administered s.c. daily x 3.
Liver and heart were removed 4 hr after administration of OCT A.
OCT A was administered p.o. (15 mg/kg). Explanation as in Table 1.

**Table 4. Relationship between doses of Ochratoxin A and tissue glycogen levels in intact and adrenalectomized rats*.**

| Dose (mg/kg p.o.) | 0   | 5   | 10  | 15  |
|-------------------|-----|-----|-----|-----|
|                   |     |     |     |     |
| Intact            |     |     |     |     |
| Liver             | 88.19±8.96 (5) | 70.04±5.63 (5) | 66.36±6.77 (5) | 31.56±3.27 (7) |
| Heart             | 3.60±0.81 (7)  | 5.31±0.31 (4)  | 3.73±0.08 (5)  | 6.13±0.33 (9)  |
|                   | N.S. | N.S. | N.S. | N.S. |
|                   |     |     |     |     |
| ADREX             |     |     |     |     |
| Liver             | 40.95±2.37 (7) | 0.97±0.07 (5)  | 1.18±0.07 (5)  | 1.65±0.29 (9)  |
| Heart             | 2.50±0.41 (6)  | 2.75±0.41 (4)  | 3.20±0.33 (8)  | 5.62±0.18 (7)  |
|                   | P<0.001 |     |     |     |

* Liver and heart were removed 4 hr after OCT A administration.
Explanation as in Table 1.
the equivalent mole of OCT A in a dose of 15 mg/kg. No significant depletion in hepatic glycogen levels or elevation of cardiac glycogen levels was observed (Table 5).

DISCUSSION

It has been recognized that some fungal toxins reduce hepatic glycogen levels, however, reports have not covered cardiac glycogen levels. Schank and Wogan (7) reported that daily administration of Aflatoxin B1 (60 µg/kg p.o.) for five successive days caused significant decrease in hepatic glycogen in ducklings, but not in male rats in spite of administration of higher dose (600 µg/kg p.o.). Raj et al. (8) also observed a significant reduction in hepatic glycogen of chicks by treatment with Aflatoxin B1 after a single dose of 2.7 mg/kg i.p., but no change was seen with mice (9). Rubratoxin B, a toxin obtained from fungi Penicillium rubrum Stoll, was found to decrease hepatic glycogen level in mice (10). Hara (11) observed that hepatic glycogen level was depleted significantly in mice by treatment with Islanditoxin, a peptidic metabolite of Penicillium islandicum Sopp.. Reduction of the hepatic glycogen level in most studies described above was due to liver function damage.

The results in the present study demonstrate that a single oral dose (15 mg/kg body wt.) of OCT A caused a depletion of hepatic glycogen level and an elevation of cardiac glycogen level in both intact and ADREX rats. Changes in hepatic glycogen levels after OCT A treatment are opposite to those demonstrated by Purchase and Theron (4). An explanation of these discrepancies has yet to be clarified. Effects of OCT A on the hepatic glycogen levels in ADREX may to some extent be due to an inhibition in the glycogen biosynthesis stimulated by hydrocortisone, or an increase in glycogen breakdown. In addition, these changes in tissue glycogen levels could be attributed to the effects of OCT A per se, not OCT α. No changes in liver composition as regards lipid, or protein content are observed. In addition, the alteration in liver glycogen levels is not associated with marked histologic alteration in microscopic studies (12).

Acknowledgements: The authors wish to thank Prof. K. Miyaki and Dr. M. Yamazaki of this institute for their interest and encouragement in this work.
OCCHRATOXIN A AND GLYCOGEN

REFERENCES

1) Scott, de B.: Mycopathol. Mycol. Appl. 25, 213 (1965)
2) Walbeek, W., van, Scott, P.N., Narwig, J. and Lawrence, J.W.: Can. J. Microbiol. 15, 1281 (1969)
3) Theron, J.J., van der Merwe, K.J., Liebenberg, N., Joubert, H.J.B. and Nel, W.: J. Path. Bact. 91, 521 (1966)
4) Purchase, I.F.H. and Theron, J.J.: Food Cosmet. Toxicol. 6, 479 (1968)
5) Yamazaki, M., Maebayashi, Y. and Miyaki, K.: Appl. Microbiol. 20, 452 (1970)
6) Good, C.A., Kramer, H. and Somogyi, M.: J. biol. Chem. 100, 485 (1933)
7) Shank, R.C. and Wogan, G.N.: Toxicol. Appl. Pharmacol. 9, 468 (1966)
8) Raj, H.G., Shankaran, R. and Venkitasubramanian, T.A.: Indian J. Biochem. 7, 55 (1970)
9) Shankaran, P., Shankaran, R., Raj, H.G. and Venkitasubramanian, T.A.: Br. J. exp. Path. 51, 487 (1970)
10) Hayes, A.W. and Wilson, B.J.: Toxicol. Appl. Pharmacol. 17, 481 (1970)
11) Hara, T.: Tokyo Iyaku Zasshi 72, 136 (1964)
12) Kanisawa, M.: Private Communication