Effects of bile acids and endotoxin on the function and morphology of cultured hamster Kupffer cells

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Summary. The mechanisms of hepatic reticuloendothelial cell dysfunction in obstructive jaundice were investigated using cultured hamster Kupffer cells. The introduction of free bile acids, cholic acid (CA) at concentrations over 2 mM and chenodeoxycholic acid (CDCA) over 1 mM inhibited colloidal carbon pinocytosis. CA and CDCA at concentrations over 0.5 mM inhibited IgG-coated sheep red blood cell phagocytosis. With the application of conjugated bile acid and endotoxin at concentrations over 50 lag/ml, endocytic function was inhibited. With bile acids, a dose-dependent increase in the concentration of β-glucuronidase occurred in the culture medium, and with endotoxin a time-dependent increase in β-glucuronidase was noted. Bile acids produced alterations in cell organelles before destruction of the cell membrane. The presence of endotoxin led to the appearance of large vacuoles in the cytoplasm. These observations suggest that bile acids and endotoxin inhibit Kupffer cells by different mechanisms. We tentatively conclude that bile acids rather than endotoxin influence Kupffer cells in vivo.

Key words: Kupffer cell – Bile acid – Endotoxin – Bile duct obstruction

Materials and methods

Animals. Adult female Golden hamsters (Seiwa Co., Nakatsu, Oita, Japan), weighing 100–150 g, were used. These animals were fed a standard pellet diet and water ad libitum.

Chemicals. Cholic acid (CA), taurocholic acid (TCA), glycocholic acid (GCA), taurochenodeoxycholic acid (TCDCA) and glycochenodeoxycholic acid (GCDCA) were obtained from Sigma Chemical Co. (St. Louis, Mo., USA). According to the suppliers, these chemicals have a purity of 98%. Chenodeoxycholic acid (CDCA), with a purity of 95% (TLC), was obtained from Calbiochem Co. Lipopolysaccharide JW (E. Coli 0127: B8) was obtained from Difco Labo. (Detroit, Michigan, USA).

Isolation and culture of Kupffer cells. Nonparenchymal liver cells were isolated by a procedure based on a method described by Knook and Sleyster (1976) and Munthe-Kaas et al. (1975). Preoperative biliary drainage in patients with severe obstructive jaundice may help to minimise complications such as sepsis, endotoxemia, disseminated intravascular coagulation (DIC) (Wardle and Wright 1970; Oka et al. 1983), acute respiratory distress syndrome (ARDS), renal failure (Bailey 1976; Wilkinson et al. 1976), hepatic failure (Wilkinson et al. 1974), and multiple organ failure (MOF) (Keller et al. 1985). In obstructive jaundice, the deposition of bile in tissues occurs, the entry of bacteria and endotoxin from the intestine increases, and functions of the hepatic reticuloendothelial system are disturbed (Wilkinson et al. 1976; Bailey 1976).

The exact mechanism involved in dysfunction of the hepatic reticuloendothelial system in patients with obstructive jaundice remains obscure. In the present study, we attempted to determine how the concentration of bile acids (Adler et al. 1977; Eklund et al. 1980) and endotoxin (Wilkinson et al. 1976; Bailey 1976), which increase in the sinusoids in obstructive jaundice, affect the function and morphology of the cultured hamster Kupffer cells (KCs).
were cultivated in a plastic dish with a diameter of 35 mm (Falcon). After 24 h the medium was changed and after 48 h the cells were used for the experiments.

Treatment with bile acids and endotoxin. After 48 h culture, bile acids (CA, TCA, GCA, CDCA, TCDCA, or GCDCA) or endotoxin were incubated with the cells for 1, 6, 12 and 24 h. The final concentration of bile acids and endotoxin ranged from 0.5 to 5 mM and from 2 to 100 μg/ml, respectively. After incubation the medium was used for β-glucuronidase and lactate dehydrogenase assays.

The viability was 95.2% with 4 mM CA, 94.3% with 4 mM TCA, 33.3% with 2 mM CDCA, 0% with 3 mM CDCA, 97.1% with 2 mM TCDCA and 0% with 3 mM TCDCA for 1 h incubation. It was 96.3 and 85.0% after incubation with endotoxin 100 μg/ml for 1 h and 24 h, respectively.

Measurement of colloidal carbon pinocytosis and erythropagocytosis. This procedure was based on the method of Kirn et al. (1980). After treatment with bile acids or endotoxin, the cells were washed with Hanks' solution and the medium was changed. After 1 h, colloidal carbon (Pelikan Commercial Ink, Gunther Wagner, Hannover, FRG) was added to each dish (400 μg/10^6 Kupffer cells). After 1 h incubation at 37°C, the cells were washed three times with PBS and then incubated with 1% sodium lauryl sulfate. The carbon content was determined by measuring the optical density (O.D.) at 750 nm. Under these conditions, one unit of O.D. corresponded to 70 μg colloidal carbon/ml. As Fc portions of rabbit Ig bind well to Fc receptors on hamster Kupffer cells, sheep erythrocytes opsonized with sheep erythrocyte antibodies produced in rabbits (Ishizu Pharmaceutical Co., Osaka, Japan) were added to each dish (final concentration, 40 erythrocytes/Kupffer cell) for 1 h at 37°C. The cells were then treated with 0.85% NH₄Cl for 10 min to lyse the erythrocytes which were not internalized, washed three times with PBS and incubated with 1% sodium lauryl sulfate. The number of erythrocytes internalized was determined by measuring the hemoglobin content of the lysate at 410 nm. Under these conditions, one unit of O.D. corresponded to 2.0 × 10^6 SRBC/ml of lysate.

Assay of β-glucuronidase and lactate dehydrogenase (LDH). After treatment with bile acids or endotoxin, the medium was used for the assay of β-glucuronidase and LDH. β-glucuronidase was assayed spectrophotometrically with 4-nitrophenyl-D-glucuron (Boehringer Mannheim GmbH, FRG) as the substrate, using the method of Kato et al. (1960). LDH was assayed a standard method (Babson and Philips 1965).
ing cytoplasm formed ruffles (Fig. 1 C). By trans-
eter and the nucleus protruded in the center of
cells appeared spindle-shaped or star-like; they
with this isolation method the yield of NPC was
microvilli were prominent and the extend-
microscopy adherent KCs were 20-30 μm in diam-
contained dense bodies and the nucleus was not
blue exclusion test. The purity by uptake of latex
beads after 48 h culture was 91.2 ± 3.4% (Fig. 1 A).

By phase contrast microscopy cultured Kupffer

**Results**

**Characterization of nonparenchymal cells (NPC)**

With this isolation method the yield of NPC was
7.2 ± 2.6 × 10^7 cells/hamster. The viability after iso-
lated was 95.4 ± 3.8%, determined by the trypan
blue exclusion test. The purity by uptake of latex
beads after 48 h culture was 91.2 ± 3.4% (Fig. 1 A).
By phase contrast microscopy cultured Kupffer
cells appeared spindle-shaped or star-like; they
contained dense bodies and the nucleus was not
sharply delineated (Fig. 1 B). By scanning electron
microscopy adherent KCs were 20-30 μm in diam-
eter and the nucleus protruded in the center of
the cell; microvilli were prominent and the extend-
cytoplasm formed ruffles (Fig. 1 C). By trans-
mission electron microscopy normally cultured

**Effect of bile acids**

Free cholic acid at concentrations over 2 mM and
free chenodeoxycholic acid at concentrations over
1 mM inhibited significantly colloidal carbon pino-
cytosis by KCs (Table 1). Free CA and free CDCA
at concentrations over 0.5 mM inhibited signifi-
cantly IgG-coated SRBC phagocytosis by KCs
(Table 1).

Since in vivo, bile acids which contact KCs are
mostly conjugated, changes in endocytic functions
of KCs were studied using conjugated bile acids.
The endocytic function of KCs was progressively
inhibited with increasing concentrations of bile
acids.

There was no significant difference in SRBC
phagocytosis between CA and TCA, except with
2 or 3 mM, between CA and GCA, between
CDCA and GCDCA, except with 1 mM and be-
tween CDCA and TCDCA (Table 2). There was
no significant difference in colloidal carbon pino-
cytosis between CA and TCA, between CA and
GCA, and between CDCA and TCDCA. There
was a significant difference between CDCA and
GCDCA (Table 3).

Table 4 shows the activity of β-glucuronidase
released into the medium following treatment with
bile acids. The activity increased significantly at
concentrations of over 1 mM CDCA, 2 mM
GCDCA, 2 mM TCDCA, and 2 mM CA. The in-
crease occurring with CDCA was much greater
than that with CA. LDH activity secreted into the
medium from KCs after incubation with 4 mM
CA, 4 mM TCA, 4 mM CDCA and 4 mM
TCDCA was 0, 3.7, 7.4 and 0 U, respectively.
There were no significant differences from the con-
trol value (17.4 U).

In phase contrast micrographs the KCs treated
with 5 mM CA became smaller rounded, and the
cytoplasmic volume diminished (Fig. 2 A). By scan-
ing electron microscopy KCs were contracted
and filopodia were revealed more clearly (Fig. 2 B).
KCs treated with 1 mM CDCA showed similar
morphologic changes.

After treatment with CA or CDCA below con-
centrations of 5 mM and 1 mM, respectively, the
cells became rounded and the number of microvilli
diminished as the concentration of bile acids in-
creased. Mitochondria, endoplasmic reticulum and
the nuclear envelope were vacuolized and lys-
osomes became scanty (Fig. 2 C, D).

| Table 1. Endocytosis by cultured Kupffer cells treated with free bile acids for 1 h |
|---|---|---|
| Concentration of bile acids (mM) | C.C. pinocytosis (μg/10^6 Kc) | SRBC phagocytosis (SRBC/Kc) |
| CA | 0 | 76.5 ± 2.6 (100) | 8.68 ± 0.54 (100) |
| 0.5 | 75.1 ± 6.3 (98.1) | 7.14 ± 0.34 (82.3) |
| 1.0 | 77.1 ± 3.2 (100.8) | 7.35 ± 0.54 (84.7) |
| 2.0 | 60.1 ± 10.2* (78.5) | 5.88 ± 0.41* (67.7) |
| 5.0 | 42.6 ± 8.1* (55.7) | 5.33 ± 1.00* (61.3) |
| CDCA | 0 | 69.0 ± 8.9 (100) | 9.24 ± 0.91 (100) |
| 0.5 | 66.8 ± 14.0 (96.8) | 6.86 ± 0.84* (74.2) |
| 1.0 | 43.0 ± 16.8* (62.4) | 5.46 ± 0.50* (59.1) |
| 2.0 | 23.4 ± 8.7* (33.5) | 0.98 ± 0.22* (10.6) |
| 5.0 | 1.4 ± 0.8* (2.1) | 0.98 ± 0.32* (10.6) |

The data are expressed as mean ±SE of triplicates from four independent experiments.

* P < 0.05, compared with endocytosis without bile acids.

Values in parentheses show the percentage of endocytosis without bile acids.

C.C.: colloidal carbon, SRBC: sheep red blood cell, CA: cholic acid, CDCA: chenodeoxycholic acid.

Morphology. Phase contrast microscopy was performed with a Nikon KIAPHOT-TMD. The cells adherent to a cover slip (Lux) placed in the bottom of the dish at seeding were fixed with 2.5% glutaraldehyde in 0.1 M cacodylate buffer for 1 h, postfixed in 1% OsO4 for 1 h and then dehydrated in a graded series of ethanol. The cover slips for scanning electron microscopy were immersed in isomyl acetate and critical-point drying was carried out using Hitachi HCP-2. Fragments of dried samples were sputter-coated with gold. The samples were observed in, and photographed with, a "JEOL, JSM-840" electron microscope at 5 KV. The cover slips for transmission electron microscopy were embedded in Epon. Thin sections doubly stained with uranyl acetate and lead citrate were examined at 80 KV in a JEOL JEM-100-CX electron microscope.

Statistical analysis. The values are expressed as mean ± SE. The unpaired Student's t-test was performed to determine the significance of differences between the means. P values less than 0.05 were considered to be statistically significant.
Table 2. IgG-coated SRBC phagocytosis by cultured KCs treated with free and conjugated bile acids for 1 h

| Bile acids | Control  | Concentration of bile acids (mM) | 1   | 2   | 3   | 4   |
|------------|----------|---------------------------------|-----|-----|-----|-----|
| CA         | 3.89 ± 0.89 (100) | 2.84 ± 0.51 (73.0) | 2.38 ± 0.32 (61.3) | 1.79 ± 0.23 (45.9) | 1.54 ± 0.81 (39.6) |
| TCA        | 3.85 ± 0.63 (99.1) | 3.57 ± 0.40* (91.9) | 2.49 ± 0.20* (64.0) | 2.00 ± 0.24 (51.4) |
| GCA        | 2.52 ± 0.51 (64.9) | 2.31 ± 0.59 (59.5)   |     |     |     |     |
| CDCA       | 4.52 ± 0.27 (100) | 0.88 ± 0.63 (19.4)   | 0.42 ± 0.31 (9.3)  | 0.39 ± 0.23 (8.5)  | 0.42 ± 0.20 (9.3)  |
| TCDCA      | 2.07 ± 0.14 (45.7) | 0.21 ± 0.12 (4.7)    | 0.21 ± 0.12 (4.7)  | 0.04 ± 0.02 (0.8)  |
| GCDCA      | 2.52 ± 0.28* (55.8) | 0.11 ± 0.10 (2.3)    | 0.39 ± 0.32 (8.5)  | 0.21 ± 0.10 (4.7)  |

The data are expressed as mean ± SE of triplicates from two independent experiments.
Values show SRBC phagocytosis (SRBC/KC).
Values in parentheses show the percentage of control.

* P < 0.05, compared with phagocytosis of KCs treated with free bile acids.

CA: cholic acid, TCA: taurocholic acid, GCA: glycocholic acid, CDCA: chenodeoxycholic acid, TCDCA: taurochenodeoxycholic acid, GCDCA: glycochenodeoxycholic acid.

Table 3. Colloidal carbon pinocytosis by cultured KCs treated with free and conjugated bile acids for 1 h

| Bile acids | Control  | Concentration of bile acids (mM) | 1   | 2   | 3   | 4   |
|------------|----------|---------------------------------|-----|-----|-----|-----|
| CA         | 48.2 ± 9.1 (100) | 41.1 ± 6.6 (85.3) | 40.6 ± 5.5 (84.3) | 38.8 ± 10.8 (80.6) | 37.8 ± 6.0 (78.5) |
| TCA        | 39.1 ± 0.5 (81.2) | 37.0 ± 3.0 (77.0) | 36.0 ± 1.0 (74.9) | 35.0 ± 5.8 (72.8) |
| GCA        | 45.4 ± 9.3 (94.2) | 38.3 ± 5.3 (79.6) | 35.5 ± 2.3 (73.8) | 30.7 ± 4.5 (63.9) |
| CDCA       | 58.0 ± 10.8 (100) | 23.4 ± 4.5 (40.4) | 18.6 ± 5.3 (32.2) | 18.4 ± 5.0 (31.7) | 15.6 ± 2.8 (27.0) |
| TCDCA      | 30.5 ± 8.3 (52.6) | 23.2 ± 8.1 (40.0) | 21.2 ± 1.5 (36.5) | 20.7 ± 3.5 (35.7) |
| GCDCA      | 59.0 ± 12.3* (101.7) | 46.6 ± 4.3* (80.4) | 38.3 ± 3.3* (66.1) | 35.3 ± 11.6* (60.9) |

The data are expressed as mean ± SE of triplicates from two independent experiments.
Values show colloidal carbon pinocytosis (µg/10⁶ KCs).
Values in parentheses show the percentage of control.

* P < 0.05, compared with pinocytosis of KCs treated with free bile acids.

CA: cholic acid, TCA: taurocholic acid, GCA: glycocholic acid, CDCA: chenodeoxycholic acid, TCDCA: taurochenodeoxycholic acid, GCDCA: glycochenodeoxycholic acid.

Effect of endotoxin

SRBC phagocytosis was inhibited significantly at concentrations over 100 µg/ml endotoxin after 1 h incubation and at concentrations over 50 µg/ml after 6, 12, and 24 h incubation (Fig. 3A).

Colloidal carbon pinocytosis was activated significantly by endotoxin at concentrations over 10 µg/ml after 1 h incubation, but was inhibited significantly at concentrations over 10 µg/ml after 24 h incubation. There was little change in pinocytosis after 6 and 12 h incubation (Fig. 3B).
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Table 4. $\beta$-glucuronidase in medium from cultured KCs treated with bile acids for 1 h

| Bile acids | Concentration of bile acids (mM) | 0     | 1       | 2        | 3       | 4       |
|------------|----------------------------------|-------|---------|----------|---------|---------|
| CA         | 25.5±4.5 (100)                   | 30.0±12.0 (118) | 43.5±5.2* (171) | 91.5±13.5* (359) | 120.0±19.5* (471) |
| CDCA       | 19.2±1.2 (100)                   | 103.2±21.6* (538) | 148.8±8.4* (775) | 146.4±6.0* (762) | 132.0±3.6* (688) |
| TCDCA      | 19.2±1.2 (100)                   | 18.0±2.4 (94)    | 127.2±10.8* (663) | 122.4±8.4* (638) | 116.4±8.4* (606) |
| GCDCNA     | 19.2±1.2 (100)                   | 24.0±2.4 (125)   | 153.6±9.6* (800)  | 140.4±7.2* (731)  | 141.6±20.4* (738) |

Values show U/dl·h. The data are expressed as mean ±SE of triplicates from two independent experiments. Values in parentheses show the percentage of activity without bile acids.

* $P<0.05$, compared with the activity without bile acids.

CA: cholic acid, CDCA: chenodeoxycholic acid, TCDCA: taurochenodeoxycholic acid, GCDCNA: glycochenodeoxycholic acid.

Fig. 2A. Phase contrast micrograph showing KCs cultured for 2 days and treated with 5 mM CA for 1 h. The cytoplasm was reduced in volume and the KCs were round. Note the space between each KC. ×500. (B) Scanning electron micrograph showing KCs cultured for 2 days and treated with 5 mM CA for 1 h. The KCs are contracted, the ruffles have disappeared and filopodia are clearly evident. ×5000. (C) Transmission electron micrograph showing KC cultured for 2 days and treated with 5 mM CA for 1 h. KC are rounded and the number of microvilli is reduced. Mitochondria, endoplasmic reticulum and the nuclear envelope are vacuolized and the lysosomes decreased in number. ×5000. (D) Transmission electron micrograph showing KC cultured for 2 days and treated with 1 mM CDCA for 1 h. The KC shows almost the same changes as those treated with 5 mM CA. ×8000.
The activity of \( \beta \)-glucuronidase in the medium changed little after 1, 6 and 12 h incubation with endotoxin at concentrations less than 100 \( \mu \)g/ml but increased significantly after 24 h incubation at concentrations over 10 \( \mu \)g/ml (Fig. 3C).

Colloidal carbon pinocytosis and \( \beta \)-glucuronidase secretion after incubation with lower concentrations of endotoxin showed the same tendency as with the higher concentrations (Table 5).

LDH activity secreted by KCs into the medium after incubation with 50 and 100 \( \mu \)g/ml endotoxin was 39.7 and 9.9 U, respectively. There were no significant differences from the control value (36.0 U).

KCs incubated with endotoxin became slightly enlarged and rounded, as viewed with phase contrast microscopy (Fig. 4A, B). By scanning electron microscopy the KCs were enlarged, the cell surface became granular, and the microvilli were reduced and appeared irregular (Fig. 4C). By transmission electron microscopy the KCs were seen to contain large vacuoles, suggesting the presence of autophagic vacuoles in the cytoplasm. The plasma membrane, mitochondria, endoplasmic reticulum and Golgi apparatus changed little (Fig. 4D).

**Discussion**

Dysfunction of the hepatic reticuloendothelial system in clinical obstructive jaundice has been studied in vivo (Drivas et al. 1976; Holman and Rikkers 1982). As it is difficult to elucidate factors influencing Kupffer cells in vivo, cultured Kupffer cells were used in the present study. Rats are not a suitable species because chenodeoxycholic acid in jaundiced rats changes to \( \beta \)-muricholic acid and is detoxified (Greim et al. 1972; Danielsson 1973). Hamsters resemble humans with regard to biliary anatomy and the composition of bile acids (Anderson et al. 1972; Kuroki and Hoshita 1983), and were used in the present study.

This work revealed that primary bile acids (cholic acid and chenodeoxycholic acid) at concentrations over 0.5 mM altered the cultured KCs both functionally and morphologically. The concentrations of bile acids used was higher than that in the serum in obstructive jaundice (Williams et al. 1975; Van Berge et al. 1976). However, the concentration of bile acids in bile canaliculi in obstructive jaundice has been measured to be 10–20 mM (Klaasen 1971; Erlinger and Dhumeaux 1974). Bile acids in the bile duct enter the sinusoid through leaky tight junctions and capillaries and bile acids in hepatocytes may leak into the sinusoid (Forker 1969; Sternlieb and Quintana 1985). Therefore, the concentration of bile acids in sinusoids may rise to the concentrations used in this study (Scharschmidt et al. 1981).

Functional disturbances of cultured KCs by primary bile acids were assumed to be related to various mechanisms. One might be an alteration in the Fc receptor necessary to phagocyte IgG-coated SRBC (Munthe-Kaas 1976; Kaplan et al. 1975). The present study demonstrated that IgG-coated SRBC phagocytosis was inhibited by bile acids even at a concentration of 0.5 mM. However, at this concentration colloidal carbon pinocytosis, which does not require special receptors, was not inhibited, suggesting that receptor alteration may not be a major influence. The detergent effect of bile acids has been considered to be an important cause of cellular dysfunction (Fodorowski et al. 1978; Kakis and Yousef 1978). In our study, bile acids at concentrations over 0.5 mM CDCA or 0.5 mM CA interfered with the endocytic function of KCs. Although at these concentrations the
Table 5. Colloidal carbon pinocytosis (A) and β-glucuronidase secretion (B) after incubation with lower endotoxin concentrations for 1 h and 24 h

| Incubation time | Concentration of endotoxin (µg/ml) | 0 (control) | 2 | 4 | 6 | 8 | 10 |
|-----------------|-----------------------------------|-------------|---|---|---|---|----|
|                 |                                   | 0 (control) | 2 | 4 | 6 | 8 | 10 |
| A 1 h           |                                   | 67.5±11.5   | 117.5±11.5* | 107.5±5.5* | 124.0±13.0* | 115.5±5.5* | 126.0±14.0* |
|                 |                                   | 107.2±17.2  | 173.9±17.2  | 158.6±7.9  | 183.3±19.2  | 170.9±7.9  | 186.2±20.7  |
| 24 h            |                                   | 48.5±6.5    | 32.5±4.0    | 29.5±6.5   | 25.5±2.5*   | 29.0±4.0*   | 28.5±3.0*   |
|                 |                                   | 13.8±13.8   | 67.6±8.3    | 61.4±13.8  | 52.4±4.8    | 60.0±8.3    | 58.6±6.2    |
| B 1 h           |                                   | 186.0±12.0  | 200.0±1.6   | 166.0±10.2 | 200.6±4.4   | 180.1±14.2  | 186.3±12.4  |
|                 |                                   | 6.5±10.7    | 107.5±1.1   | 89.2±5.4   | 107.5±2.2   | 96.8±7.5    | 100±6.5     |
| 24 h            |                                   | 194.1±16.2  | 218.3±10.4  | 252.3±16.2*| 252.4±10.4* | 250.0±14.4* | 254.4±18.2* |
|                 |                                   | 6.2±11.3    | 9.2±10.4    | 129.9±8.2  | 129.9±5.2   | 128.9±7.2   | 130.9±9.3   |

The data are expressed as (A) µg/10^6 KCs and (B) U/dl-h
The data are expressed as mean ± SE of triplicates from one experiments
* P<0.05, compared with control
Values in parentheses show the percentage of control

Fig. 4A. Phase contrast micrograph showing KCs cultured for 2 days and treated with endotoxin 100 µg/ml for 1 h. The KCs are slightly enlarged and rounded. ×250. (B) Phase contrast micrograph showing KCs cultured for 2 days and treated with endotoxin 100 µg/ml for 24 h. There are no conspicuous changes, compared with the findings after 1 h incubation. ×500. (C) Scanning electron micrograph showing KC cultured for 2 days and treated with endotoxin 100 µg/ml for 24 h. The KC is enlarged, the cell surface is granular, the microvilli are reduced in number and appear irregular. (D) Transmission electron micrograph showing KC cultured for 2 days and treated with endotoxin 100 µg/ml for 24 h. The KC is expanded with large vacuoles originated from lysosomes. Cytoplasmic membrane, mitochondria, endoplasmic reticulum and nuclear envelope are intact. ×8000
membrane of KCs did not seem to be altered, the cellular organelles showed definite changes. Therefore, plasma membrane damage may not be the primary event. The pseudopodia and microvilli decreased in number and were shortened with increased concentrations of bile acids, hence, the microfilaments may also play an important role in KC function. In our study, the lysosomes decreased in number and \(\beta\)-glucuronidase activity in the medium increased as the concentration of bile acids increased. Dysfunction may follow the decrease in lysosomal fusion degradation, which is the last step of endocytosis after attachment to the cell membrane, internalization and intracellular transport (Praaning-van Dalen et al. 1982), secondary to damage and decrease of lysosomes.

In our experiments, endocytic function and \(\beta\)-glucuronidase release by KCs were activated after incubation with endotoxin for 1 h, but were inhibited after incubation for 24 h. Tanner et al. (1983) reported that N-acetyl-\(\beta\)-glucosaminidase (NAG) produced by resident macrophages was unchanged following endotoxin exposure for 2 h but increased when stilboestrol and Corynebacterium parvum were given to the donor rats before the macrophages were isolated. Bhatnagar et al. (1981) reported that chemiluminescence and \(O_2^−\) production by cultured rat KCs were activated by incubation with zymosan for less than 40 min. Their data paralleled ours with regard to incubation with endotoxin for 1 h. However, we did not demonstrate that cultured KCs are activated by endotoxin, because the species used, incubation time, activator and parameters differed.

At endotoxin concentrations over 50 \(\mu\)g/ml, SRBC phagocytosis by KCs was inhibited; but colloidal carbon pinocytosis was not inhibited with similar concentrations of endotoxin and incubation for less than 12 h. It was reported that the concentration of endotoxin in vivo did not exceed 50 \(\mu\)g/ml (Al-Tuwaijri et al. 1981; Kuratsune et al. 1983). Therefore, the concentration of endotoxin which inhibited the endocytic function of KCs in this study was much higher than the concentration measured in vivo. The mechanism by which endotoxin caused damage to cultured KCs differed from that caused by primary bile acids. The KCs exposed to endotoxin had many cytoplasmic vacuoles considered to have originated from lysosomes and cell swelling in vivo (Frenzel et al. 1977; McCuskey et al. 1983) and in vitro (Maier and Ulevitch 1981; Morland and Kaplan 1977). The present study confirmed that the same changes occurred with 100 \(\mu\)g/ml endotoxin. Therefore, KC dysfunction after the application of endotoxin was considered to be caused by excessive endocytosis (Mathison and Ulevitch 1979; Ruiter et al. 1981).

In our study, KCs treated with bile acids for 1 h at concentrations over 2 mM CA or 1 mM CDCA produced significant increases in the concentration of \(\beta\)-glucuronidase in the culture medium. KCs treated with bile acids for 24 h produced similar changes (data not shown). It was therefore assumed that \(\beta\)-glucuronidase secretion by KCs treated with bile acids was dose-dependent. When endotoxin carried to the liver is taken up and detoxified by KCs (Mathison and Ulevitch 1979; Praaning-Van Dalen et al. 1981), \(\beta\)-glucuronidase is secreted by these cells. In our study, the secretion of \(\beta\)-glucuronidase did not increase with 100 \(\mu\)g/ml of endotoxin after less than 12 h incubation, but did increase with 10 \(\mu\)g/ml endotoxin after 24 h incubation. Therefore, the activation of KCs seemed to depend on the incubation time. There was also a difference between bile acids and endotoxin with regard to their effects on the secretion of \(\beta\)-glucuronidase from KCs.

We tentatively conclude that primary bile acids rather than endotoxin influence KC function in obstructive jaundice.

The present study demonstrated that primary bile acids and endotoxin altered the function and morphology of the KCs in vitro. However, in vivo, other factors have to be considered and the interaction of these factors requires attention. It was reported that the absorption of endotoxin from the intestine increased as the bile acids in intestine decreased and that the reverse was also true (Bailey 1976; Cahill 1983). It was also reported that bile acid itself has a detergent effect on endotoxin (Shands and Chun 1980; Evans et al. 1982).

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