Effects of Certain Drugs on Serum Bilirubin Level and Hepatic UDP-Glucuronyl Transferase Activity (Sodium Salicylate, Sodium Hippurate, Sodium Dehydrocholate, Sodium Phenobarbiturate and Prednisolone)

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Bilirubin is excreted from the liver as a glucuronic acid ester. For the formation of bilirubin glucuronide, UDP-glucuronyl transferase (abbrevaited as UDPGT) is required. This enzyme is contained in the microsome of hepatic cells. In the following experiments the influence of drugs on hyperbilirubinemia in bile duct-ligated rat was studied. Drugs studied were as follows: sodium salicylate, sodium hippurate, sodium dehydrocholate, sodium phenobarbiturate and prednisolone. These drugs were reported to increase biliary excretion of BSP and bilirubin or expected to improve hyperbilirubinemia.

EXPERIMENTAL METHODS

(I) Influence of drugs on hyperbilirubinemic rats

Male Wistar rats weighing 180–240g were anesthesized with ether and placed on the table in a supine position. Common bile duct was exposed through median abdominal incision and the peripheral end of the common duct was ligated, followed by double ligation immediately below the inflow of hepatic duct. Abdominal incision was then closed. After 4 days of supply of solid ration and water, they were used as hyperbilirubinemic rats (rats with ligation of bile duct) in the experiment.

Following drugs were intravenously injected from the saphenous vein each 5 animals making up each group. Before and 30, 60, 90 and 120 minutes after injection, 0.6ml blood sample each was obtained from the tail vein.

a) Sodium dehydrocholate (10mg/100g b.w.)
b) Sodium phenobarbiturate (5mg/100g b.w.)
c) Sodium salicylate (6mg/100g b.w.)
d) Sodium hippurate (7mg/100g b.w.)
e) Prednisolone (prednisolone sodium hemisuccinate 1.5mg/100g b.w.)

Bilirubin was determined by the method of Ichida et al.[1]. Since ascorbic acid

used in the original method is unstable, hydroxylamine hydrochloride was used instead.

(II) Method of measurement of UDPGT

Double measurement of UDPGT was always carried out by the following two methods.

a) Method of Perona et al.[2] (4MU method)

This method uses 4-methyl umbellipherone (4MU) as a glucuronide receptor. After incubation for 15 to 30 minutes, measurement was carried out by Hitachi 203 fluorospectrophotometer. 4MU (4-methyl umbellipherone) of conjugated type was expressed with mμ mole/mg protein. Protein concentration of liver supernatant was 0.4–1.4mg by Daughaday's method[3].

b) Lueders' method[5] modified by Yamamoto[4] (p-nitrophenol method)

Mixture consisting of 0.6 μ mole p-nitrophenol, 1 μ mole uridine 2-phosphoglucuronic acid, 75 μ mole Tris buffer (pH 7.4), 10 μ mole $MgCl_2$, 0.4ml liver supernatant (protein content 3.3–6.2 mg) adjusted to total volume of 1.5ml, was incubated at 37°C for 30 minutes, followed by addition of 1ml of 6.7 % trichloracetic acid. After centrifugation, 0.2 N NaOH was added to 1ml supernatant for color development. With the use of Hitachi-Perkin-Elmo photoelectric colorimeter, amount of conjugated nitrophenol was measured at 400 mμ.

(III) Liver UDPGT activity of rat injected with drugs

In normal rats, test drugs were injected into the tail vein for 3 days. Liver was removed 30 minutes after the injection on the third day. Supernatant was prepared and UDPGT activity was measured by the 4MU method and the p-nitrophenol method described above. The dose for injection was 10mg/100g b.w. for sodium dehydrocholate, 5 mg/100g b.w. for sodium phenobarbiturate, 7 mg/100g b.w. for sodium hippurate, 6mg/100g b.w. for sodium salicylate and 1.5mg/100g b.w. for prednisolone. In the control group, 0.1ml/100g b.w. physiological saline was injected.

EXPERIMENTAL RESULTS

(I) Influence of drugs on hyperbilirubinemic rats

Table 1 summarized the effect of bile duct ligation on serum bilirubin concentration. In the control group, serum total bilirubin concentration was 5.76mg/dl, and the value after 120 minutes was 5.68mg/dl with scarcely any change. Direct type bilirubin was 4.38 mg/dl before and 4.22 mg/dl after experiment. In the group treated with sodium salicylate, total bilirubin concentration was 5.82 mg/dl before and 4.80 mg/dl after 120 minutes. Direct bilirubin fell from 4.52 mg/dl to 3.76 mg/dl after 120 minutes, while indirect bilirubin fell slightly from 1.30 mg/dl to 1.06 mg/dl.

Table 1. Influence of drugs on hyperbilirubinemia

Drugs examined	time	total bilirubin (mg/dl)		direct bilirubin (mg/dl)		indirect bilirubin (mg/dl)	
		mean	S.D.	mean	S.D.	mean	S.D.
Control	before	5.76	2.16	4.38	1.41	1.38	0.88
	30′	5.88	2.14	4.28	1.65	1.60	0.65
	60′	5.94	2.20	4.50	1.44	1.44	0.80
	90′	5.80	2.23	4.42	1.54	1.38	0.88
	120′	5.68	2.17	4.22	1.47	1.46	0.81
Sodium salicylate	before	5.82	1.92	4.52	1.49	1.30	0.60
	30′	5.54	2.05	4.44	1.71	1.10	0.57
	60′	5.38	2.01	4.32	1.69	1.06	0.67
	90′	5.20	1.85	4.24	1.66	0.96	0.50
	120′	4.80	1.71	3.76	1.44	1.06	0.60
Sodium hippurate	before	5.54	1.18	4.26	0.82	1.28	0.54
	30′	5.66	1.05	4.48	0.61	1.18	0.58
	60′	5.64	1.07	4.44	0.44	1.20	0.70
	90′	5.48	0.90	4.46	0.54	1.02	0.47
	120′	5.42	0.91	4.30	0.60	1.12	0.44
Sodium dehydro-cholate	before	5.60	0.56	4.06	0.71	1.54	0.30
	30′	5.70	0.47	4.04	0.72	1.66	0.47
	60′	5.56	0.46	3.98	0.43	1.58	0.33
	90′	5.60	0.30	3.86	0.42	1.74	0.52
	120′	5.46	0.45	3.80	0.70	1.64	0.40
Sodium phenobar-biturate	before	9.18	3.54	6.44	1.81	2.74	1.76
	30′	8.62	3.03	6.16	1.90	2.46	1.13
	60′	8.62	2.86	5.88	1.51	2.76	1.38
	90′	8.42	2.73	5.64	1.71	2.78	1.07
	120′	8.14	2.67	5.56	1.70	2.54	0.95
Predni-solone	before	6.68	1.03	5.38	0.84	1.30	0.56
	30′	6.43	1.25	4.97	1.34	1.47	0.33
	60′	6.35	1.34	4.70	1.11	1.65	0.53
	90′	6.28	1.09	4.48	0.68	1.80	0.71
	120′	6.02	1.14	4.23	0.90	1.78	0.69

Similar chage was also noted in the group given sodium phenobarbiturate. Total bilirubin was 9.18 mg/dl before and 8.14 mg/dl after 120 minutes. Direct type bilirubin was 6.44 mg/dl before and 5.56 mg/dl after 120 minutes. In the group treated with prednisolone, the result was somewhat less favorable. Total bilirubin was 6.68 mg/dl before and 6.02 mg/dl after 120 minutes. Direct type bilirubin fell from 5.38 mg/dl before to 4.23 mg/dl after 120 minutes. In the group treated with sodium hippurate and sodium dehydrocholate, however, decrease of neither serum total bilirubin nor direct type bilirubin was noted.

(II) Liver UDPGT activity in rats injected with drugs

Table 2 summarized the results of liver UDPGT activity determination in rats treated with drugs for 3 days. Average activity in the control group by 4MU method was 17.1 mμ mole/mg protein after 15 minutes, and 34.5 mμ mole/mg protein after 30 minutes. In the group treated with sodium dehydrocholate, 15 minutes value was 69.7 and 30 minutes value 234.5. In the group treated with

prednisolone, 15 minutes value was 22.7 and 30 minutes value 105.6.

In the group treated with sodium phenobarbiturate, 15 minutes value was 29.3 and 30 minutes value 72.4. In the group treated with sodium hippurate, 15 minutes value was 31.7 and 30 minutes value 72.3. Enzyme activity was increased in each of these groups. In the group treated with sodium salicylate, however, 15 minutes value was 25.6 and 30 minutes value 45.9, without significant increase of activity.

Average activity in the control group by p-nitrophenol method was 8.9 mμ mole/mg protein. The corresponding value was 13.4 in the group treated with sodium dehydrocholate, 14.2 in the group treated with prednisolone, and 17.9 in the group treated with sodium phenobarbiturate, suggesting a marked increase in enzyme activity. In the group treated with sodium hippurate, however, the value was 10.6, suggesting a slight tendency of increase. In the group treated with sodium salicylate, the value was 9.0, with no activation at all.

Table 2. Influence of drugs on hepatic UDPGT activity

Drugs examined	Conjugated 4MU mμ mole/mg protein		Conjugated p-nitrophenol mμ mole/mg protein
	15 minutes value	30 minutes value	
	mean ± S.D.	mean ± S.D.	mean ± S.D.
Control	17.1 ± 5.4	34.5 ± 7.9	8.9 ± 1.5
Sodium salicylate	25.6 ± 4.7	45.9 ± 8.8	9.0 ± 1.3
Sodium hippurate	31.7 ± 10.2	72.3 ± 27.1	10.6 ± 2.2
Sodium dehydrocholate	69.7 ± 30.5	234.5 ± 72.2	13.4 ± 2.1
Sodium phenobarbiturate	29.3 ± 11.8	72.4 ± 16.2	17.9 ± 3.6
Prednisolone	22.7 ± 14.6	105.6 ± 58.3	14.9 ± 0.9

DISCUSSION

Remmer et al.[6] suggested the non-specific stimulation of enzyme activity of liver microsomes by phenobarbital. Later, this drug was found to decrease serum bilirubin, especially non-conjugated bilirubin. The action of phenobarbital to alleviate jaundice was considered to be due to the activation of UDPGT activity of liver microsome. In liver disease in which the decrease of activity of this enzyme was either demonstrated or suggested, this drug was used with favorable results[7) 8) 9) 10) 11) 12)]. With the use of phenobarbital, histological findings also suggested intensification of enzyme activities in the microsome[13)], while increase of UDPGT activity was biochemically demonstrated[7) 14) 15) 16) 17) 18)].

In the experiment of the authors, phenobarbital was shown to decrease serum

bilirubin markedly in bile duct-ligated rats. Administration of this drug for 3 days was found to increase liver UDPGT activity in support of this concept. Phenobarbital is therefore without effect on the jaundice of rat with congenital absence of UDPGT enzyme[19]. The mechanism of action of this drug is thus mainly directed to UDPGT enzyme but other mechanism of alleviation of jaundice is also conceivable. Phenobarbital stimulates the excretion of ICG and BSP in bile and the intensifying action of BSP conjugating enzyme activity also participates. Facilitating action on pigment excretion in bile appears to be the most important factor[20][21][22][23]. However, explanation of the increased pigment excretion in bile with the increase of the volume of bile alone might be rather hasty in drawing conclusion. Application of this principle itself to bilirubin might also be dangerous. However, the increased conjugated type bilirubin might well be excreted in increased amount in response to the increase of the amount of bile. Phenobarbital is also reported to facilitate the ability of the liver to take up bilirubin[24]. The protein Y specifically binding bilirubin within the liver cell[25] should also be noted with reference to this. On the other hand, phenobarbital was also reported to decrease the activity of UDPGT inhibitors[26].

The action of phenobarbital to decrease bilirubin concentration in blood is not only due to the intensifying action of UDPGT activity, but also other factors. In the results of our authors in rats with ligation of bile duct, the decrease of bilirubin in blood due to phenobarbital is mainly the result of decrease of direct type bilirubin. This does not necessarily exclude other factors and whether or not liver UDPGT activity is decreased in obstructive jaundice is yet unknown. This drug, however, mainly intensifies the enzyme action, facilitating the transition of indirect bilirubin to direct form in the liver. Direct form is probably excreted via the renal glomerulus.

ACTH or corticosteroid has been reported to decrease serum bilirubin value in obstructive jaundice[27][28][29][30]. Various theories were advanced on the action of corticosteroids decreasing serum bilirubin. Choleretic action of corticosteroid was emphasized by some[31] but denied by others[32][33].

Increase of bilirubin excretion in urine by steroid has been denied[28][30]. Steroid does not delay destruction of red cells[30]. Inhibitory action on shunt bilirubin synthesis has been considered to be important but is not yet experimentally proved[34]. Consequently, explanation of the action of corticosteroid to decrease serum bilirubin is at present impossible.

In the experiment of the authors, prednisolone definitely decreased serum bilirubin of bile duct-ligated rats, facilitating liver UDPGT activity. Since adrenocortical steroid was reported to inhibit UDPGT activity as shown the use of o-aminophenol[35], it appears to be the only reliable factor at present, though it is rather risky to use the authors to explain the inhibiting action on serum bilirubin.

Sodium salicylate is reported to decrease serum bilirubin concentration through

inhibiting the combination between plasma albumin and bilirubin[36)37)]. However, since this drug facilitates the excretion of BSP into bile and also has a mild choleretic action[38)39)], inhibitory action on protein binding does not appear to the only factor in the decrease of serum bilirubin.

In the experiment of the authors using bile duct-ligated rats, sodium salicylate decreases serum bilirubin and did not influence the liver UDPGT activity, so that the main cause of the decrease of serum bilirubin by this drug appears to be the inhibition on the combination between plasma albumin and bilirubin.

Sodium dehydrocholate is a potent hydrocholagogue. This drug decreases bilirubin concentration in bile but total bilirubin excretion in bile is reported to increase[40)41)], to decrease or to be unchanged[42)]. These results depend on the dose of the drug, time of observation, state of liver function and on the difference in experimental animals. Effect of sodium dehydrocholate on serum bilirubin is variable, decrease[43)] and increase[41)44)45)] have been reported without constant tendency. Sodium dehydrocholate has an action of inhibiting combination between albumin and BSP[38)] or bilirubin[46)]. Besides choleretic action, this drug has diuretic action and the possibility of loss of direct type bilirubin out of the body by this action should be seriously considered. Action of facilitation of transition of indirect type bilirubin into brain tissue was also recognized[47)]. Under such condition, decrease of serum bilirubin by this drug may be expected. However, no decrease of serum bilirubin was noted in the bile duct-ligated rats in our hand. While no bilirubin excretion in bile appears to take place into bile, the absence of augmenting action on liver UDPGT activity in sodium dehydrocholate represents the greatest reason.

Sodium hippurate has a facilitating action on liver UDPGT activity and a detoxifying action, and is clinically used for toxic liver disease[48)]. Unlike salicylate, long term administration does not cause liver damage[49)]. This drug also has a mild choleretic action and inhibitory action on the combination between plasma albumin and BSP.

In the experiment using liver slices, it does not act on BSP conjugating enzyme, but it intensifies BSP conjugating enzyme in experiments with liver homogenate[38)]. Since this drug does not enter the bile[50)], it probably does not enter the liver cell, and this is probably responsible for the discrepancy between experiments using liver slices and those using homogenates.

Consequently, the facilitation of excretion of BSP into bile might be due to the action of this drug on liver cell membrane.

In the experiment of the authors, failure of decrease of serum bilirubin value in bile duct-ligated rats by sodium hippurate might be explained with this fact. In the present experiment, UDPGT activity of ether conjugation through OH is always utilized, unlike bilirubin conjugation by esterification through COOH.

CONCLUSION

Animal experiments were carried out on the effect of drugs on hyperbilirubinemia and liver UDP-glucuronyl transferase activity. Following results were obtained.

1) Sodium phenobarbiturate, sodium salicylate and prednisolone decreased serum bilirubin especially direct type bilirubin in bile duct-ligated rats. No such action was noted in sodium hippurate and sodium dehydrocholate.

2) Drugs were injected in normal rats for 3 days to determine liver UDPGT activity. Sodium phenobarbiturate, sodium dehydrocholate, sodium hippurate and prednisolone intensified UDPGT activity but sodium salicylate was devoid of such action.

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