Stimulation of Rat Liver Growth by a 1,3-Dithiole Derivative, KZ-1026

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Abstract—The oral administration of 2-(1,3-dithiol-2-ylidene)-1-phenyl-1,3-butanedione (KZ-1026) to normal rats at doses of 50, 100 and 200 mg/kg/day for 3 days accelerated liver enlargement in association with a dose-dependent increase in the total amounts of protein, RNA and DNA in the liver. The liver weight at 24 hr after the third dose of 200 mg/kg reached 174% of the control. With respect to the effect on liver enlargement, KZ-1026 differed from phenobarbital, since KZ-1026, unlike phenobarbital, increased hepatic DNA content without significant effects on P-450 and aminopyrine-N-demethylase. Incorporation of [3H]thymidine into liver DNA was stimulated by a single dose of KZ-1026 (200 mg/kg), and it peaked at 24 hr post dose (18 times the control), followed by an increase in the number of liver nuclei. Liver growth was also accompanied by an increasing hepatic reserve cell mass, expressed by the capacity of eliminating exogenous galactose from the blood stream. Pretreatment with KZ-1026 (200 mg/kg/day) for 3 days significantly improved the survival rate of subtotally hepatectomized rats from 39% to 78%. These findings indicate that KZ-1026 accelerates hepatocyte proliferation, resulting in an enhancement of liver functional mass in normal rats.

KZ-1026 (Fig. 1) synthesized in our chemistry laboratory was found to be protective against CCl4-induced acute liver injury in mice and also stimulative to the liver growth in rats (Y. Hirayama et al., unpublished data).

Liver enlargement is frequently observed in laboratory animals exposed to drugs or other xenobiotic compounds (1–5). These agents can be divided into two types: one type increases cell size (hypertrophy) and the other increases the number of cells (hyperplasia). In the normal liver, the cell proliferation is severely regulated, and thus a majority of hepatocytes remain quiescent (G0 or G1 arrest). Some of the growth factors and growth inhibitory factors (6–15) appear to be involved in this regulation, while synthetic agents that cause hyperplasia are also of interest and useful in the study of hepatocyte proliferation and its regulation. Since KZ-1026 appeared to be one of these drugs, we have examined the effect of KZ-1026 on biochemical parameters in the liver and on the number of hepatocytes in normal rats. In addition, the effect of KZ-1026 on the galactose elimination capacity plasma parameters in normal rats and on survival rates in subtotally (85%) hepatectomized rats were
investigated, in order to clarify whether the liver enlargement is accompanied by the enhancement of liver function.

Materials and Methods

Animals: Male Sprague-Dawley rats (Charles River Japan, Inc., Atsugi, Japan) weighing 180–200 g (6-week aged) were used in all experiments except the subtotal hepatectomy experiment which employed rats weighing about 300 g (8-weeks aged). The animals were kept in an air-conditioned room (23±2°C, 55±15% humidity) lighted between 6:00 a.m. and 6:00 p.m. and given laboratory chow (CA-1, Clea Japan Inc., Tokyo, Japan) and water ad libitum. All animals were acclimatized to the housing conditions for a week prior to use in the experiments.

Chemicals: KZ-1026 (Lot No. YT-292-1, synthesized at Banyu Pharmaceutical Co., Ltd., Tokyo, Japan) was suspended in a 0.5% carboxymethylcellulose sodium (CMC, Wako Pure Chemical Industries, Ltd., Osaka, Japan) solution with an ultrasonic homogenizer (Model US-300, Nihon Seiki Kaisha, Ltd., Tokyo, Japan) and given orally to rats in a volume of 5 ml/kg. The control group was given the same volume of vehicle only. Phenobarbital sodium was purchased from Sankyo Pharmaceutical Co., Ltd. (Tokyo, Japan) and [3H]thymidine (54 Ci/mmol), from Amersham Japan Co., Ltd. (Tokyo, Japan). All other chemicals used were of reagent grade.

Determination of biochemical parameters in plasma and liver of normal rats: KZ-1026 was given orally at doses of 50, 100 and 200 mg/kg/day for 3 days; and 24 hr after the last dose, the rats were killed by bleeding from the abdominal aorta with a heparinized syringe under ether anesthesia. Livers were excised and weighed, and a piece of each liver was used for the measurement of DNA, RNA and protein content. DNA and RNA were fractionated as follows: The tissue was homogenized with 4 volumes of 0.9% NaCl. A 1-ml aliquot of the homogenate was mixed with 1 ml of 10% trichloroacetic acid (TCA) and kept on ice for 20 min. The pellet of precipitated proteins was collected by centrifugation (3,000 rpm, 10 min), resuspended in 2 ml of ice-cold 5% TCA, left for 15 min and recentrifuged. The pellet was then resuspended in 2 ml ice-cold 5% perchloric acid (PCA) and warmed at 70°C for 15 min. The suspension was cooled and centrifuged at 3,000 rpm for 10 min. The pellet was re-washed in ice-cold 5% PCA and recentrifuged. The supernatants were combined and used for determination of DNA and RNA by means of the diphenylamine reaction (16) and orcinol reaction methods (17), respectively. Protein content was determined by the method of Lowry et al. (18). The following plasma parameters were measured with a Centrifichem Autoanalyzer (Encore, Baker Inst., PA, U.S.A.): total protein (TP), albumin (Alb), triglycerides (TG), total cholesterol (T.Chol), alkaline phosphatase (ALP), transaminases (GOT: glutamic oxaloacetic transaminase and GPT: glutamic pyruvic transaminase), amylase (Amy), glucose (Glc) and total bilirubin (T.Bil).

Comparison with phenobarbital: KZ-1026 (100 mg/kg) suspended and phenobarbital sodium (100 mg/kg) dissolved in 0.5% CMC were orally administered once a day for 6 days. On day 7, the rats were sacrificed, and the liver and the blood were collected. The protein, DNA and RNA contents in the liver and TP, Alb, T.Chol and PL in the plasma were measured as described above. Part of the liver was homogenized in 3 volumes of cold 1.15% KCl (pH 7.4) and centrifuged at 10,000×g for 30 min. The resultant pellet was suspended in 50 mM phosphate buffer (pH 7.6) containing 1 mM EDTA and used for the measurement of P-450 content. Microsomal protein and aminopyrine-N-demethylase (AND) activity were measured by the method of Omura and Sato (19) and Brodie and Axelrod (20), respectively.

Incorporation of [3H]thymidine into hepatic DNA: Rats were killed by bleeding from the carotid artery under ether anesthesia 6, 12, 24, 36 and 60 hr after a single dose of KZ-1026 at 200 mg/kg, p.o. [3H]Thymidine (300 µCi/kg) was injected intraperitoneally 2 hr before bleeding. The liver was excised, weighed and homogenized with 4 volumes of 0.9% NaCl, and the DNA fraction was extracted according to the method of Schneider (21). The radioactivity in the extracted DNA
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was measured in a liquid scintillation counter with external quenching correction.

**Determination of the number of hepatic nuclei:** Rats were sacrificed at 24, 72 and 168 hr after a single dose of KZ-1026 at 200 mg/kg, p.o. The liver was excised and weighed. Part of the left lateral lobe was resected, weighed and homogenized in 10 volumes of a solution containing 10 mM Tris-HCl (pH 8.0), 10 mM Mg-acetate, 1 mM EGTA and 0.2% Triton X-100, and then centrifuged (1,000 rpm, 5 min). The pellet was washed with the above solution first, then washed with phosphate-buffered saline (137 mM NaCl, 2.7 mM KCl, 8.1 mM Na2HPO4 and 1.5 mM KH2PO4), and finally suspended in phosphate-buffered saline. The number of nuclei in the suspension was measured using 2 types of blood cell counters (Coulter Counter Model ZB, Coulter Electronics Inst., FL, U.S.A. and EKDS blood cell counter, Kayagaki Irika Co., Tokyo, Japan). Part of the liver obtained at 72 hr after administration of KZ-1026 was used for counting the number of binucleates histologically. The liver was fixed in 10% buffered formalin, and the sections prepared from paraffin blocks were stained with hematoxylin and eosin. The ratio of binucleates in the liver was estimated by counting 900 to 1,100 cells per liver.

**Determination of galactose elimination:** Normal rats were given KZ-1026 orally at a dose of 200 mg/kg/day for 3 days. At 24, 48 and 72 hr after the last dose, a 35% glucose-saline solution (0.7 g/kg) was injected intravenously through the tail vein, and the blood was collected at 10-min intervals for 60 min after the galactose injection. The galactose concentration in the blood was measured with a Galactose-test Kit (Galactose-UV-Test, Boehringer Mannheim Yasunouchi Co., Ltd., Tokyo, Japan), and the t1/2 value was estimated. After the blood was collected, the rats were sacrificed under ether anesthesia, and the liver was excised and weighed.

**Subtotal hepatectomy:** KZ-1026 was given orally at a dose of 200 mg/kg/day for 3 days prior to the hepatectomy. Under ether anesthesia, rats were hepatectomized between 8:00 a.m. and 11:30 a.m. using the method of Gaub and Inversen (22) and then fasted for 2 days. The survival rate was estimated from the number of animals still alive at 48 hr after the operation.

**Statistical analysis:** All data were analyzed by Student’s t-test with the exception that the survival rate was analyzed by Fisher’s exact test. A “P” value lower than 0.05 was regarded as statistically significance.

**Results**

**Effect of KZ-1026 on biochemical parameters in plasma and liver of normal rats:** KZ-1026 increased liver weight dose dependently, and the liver weight paralleled the content of liver protein, RNA and DNA (Fig. 2). At 200 mg/kg of KZ-1026 for 3 days, liver weight, protein, RNA and DNA increased 68%, 52%, 78% and 47%, respectively. The rate of DNA increase was relatively smaller than the other parameters.

![Fig. 2. Effect of KZ-1026 on liver weight (A), protein (B), RNA (C) and DNA (D) content in normal rats. KZ-1026 was administered orally for 3 days, and rats were killed 24 hr after the last administration. Numbers in the histograms are percent increase over the control values. Each value is the mean±S.E. of 5 rats. Significantly different from the control value, *P<0.05, **P<0.01 and ***P<0.001.](image-url)
Table 1. Effect of KZ-1026 on several parameters in the plasma of normal rats

| Dose of KZ-1026 mg/kg | TP (g/dl) | Alb (g/dl) | Amy (U/l) | T.Chol (mg/d) | PL (mg/dl) |
|-----------------------|-----------|------------|-----------|--------------|------------|
| Control               | 4.6±0.07  | 3.1±0.06   | 911±32.6  | 57±1.9       | 119±5.3    |
| 50                    | 4.7±0.11  | 3.3±0.07   | 975±55.3  | 65±3.9       | 132±4.0    |
| 100                   | 4.8±0.16  | 3.2±0.10   | 1196±15.7 | 68±4.0*      | 131±7.7    |
| 200                   | 5.0±0.05*** | 3.3±0.07  | 1408±26.1*** | 105±3.5*** | 208±10.5*** |

KZ-1026 was administered orally once a day for 3 days. Each value is the mean±S.E. of 5 rats. Significantly different from the control value, *P<0.05 and ***P<0.001.

Table 2. Effects of KZ-1026 and phenobarbital sodium (PB) on some biochemical parameters in the liver of normal rats

| Drugs      | Dose (mg/kg) | BW (g)   | LW (g)   | RNA (mg/liver) | Protein (mg/liver) | DNA (mg/liver) | AND (nmole/mg protein) | P-450 (nmole/mg protein) |
|------------|--------------|----------|----------|----------------|-------------------|----------------|------------------------|--------------------------|
| Control    | —            | 246.0±4.39 | 10.9±0.38 | 86.1±5.60      | 2232±138.8        | 32.9±1.70      | 14.2±1.05              | 1.54±0.108               |
| KZ-1026    | 100          | 240.1±5.06 | 13.2±0.60* | 113.4±4.38**  | 2788±88.1**       | 43.4±0.97***   | 12.3±1.02              | 1.44±0.056               |
| PB         | 100          | 238.7±4.13 | 12.5±0.39* | 99.3±3.75     | 2710±106.1*       | 36.1±1.41      | 21.3±1.48**            | 2.88±0.165**             |

Drugs were administered orally once a day for 6 days. Each value is the mean±S.E. of 7 rats. Significantly different from the control value, *P<0.05, **P<0.01 and ***P<0.001.
than that of the other parameters, suggesting that the liver enlargement due to KZ-1026 was mainly attributable to hyperplasia but in part to hypertrophy. At 200 mg/kg, plasma TP, Amy, T.Chol and PL significantly increased to 9%, 55%, 84% and 75%, respectively (Table 1). However, the other plasma parameters such as TG, Glc, T.Bil, ALP and transaminases (GOT, GPT) did not change at any doses of KZ-1026, and no apparent hepatic or pancreatic injury was observed histologically (data not shown). These findings suggest an enhancement of protein and lipid metabolism in the liver.

Comparison of KZ-1026 with phenobarbital (PB): As shown in Table 2, KZ-1026 or PB given for 6 days at 100 mg/kg, p.o., stimulated the liver growth by 21.3% or 14.9%, respectively, associated with the increase in the RNA and protein contents in the liver. However, KZ-1026 and PB had different effects on the DNA and P-450 contents and AND activity. The DNA content was increased by KZ-1026 but not by PB, while P-450 and AND were remarkably increased by PB but not by KZ-1026. Different responses were also observed in some plasma parameters (Table 3). KZ-1026 induced significant increases in TP, T.Chol and PL in the plasma, while PB was ineffective.

Effect of KZ-1026 on hepatic DNA synthesis and the number of hepatic nuclei: Whether KZ-1026 enhances hepatocyte proliferation was investigated by determining the rate of DNA synthesis and the number of nuclei in the liver. The incorporation of [3H]-thymidine into hepatic DNA remarkably increased 24 hr after the single dose of KZ-1026 at 200 mg/kg (about 18 times higher than the control level), and then it returned to the control level 36 hr after the dose. Following the stimulation of DNA synthesis, a gradual increase in the DNA content was observed at 24 hr (18%), 36 hr (35%) and 60 hr (43%) (Fig. 3). The time-courses of liver weight, DNA content and hepatic nuclei after the

| Table 3. Effects of KZ-1026 and phenobarbital sodium (PB) on some biochemical parameters in the plasma of normal rats |
|---|---|---|---|---|---|
| Drugs | Dose (mg/kg) | TP (g/dl) | Alb (g/dl) | T.Chol (mg/dl) | PL (mg/dl) |
| Control | — | 5.2±0.05 | 3.3±0.03 | 56±2.6 | 124±8.1 |
| KZ-1026 | 100 | 5.6±0.06** | 3.6±0.06** | 76±1.7** | 150±6.0* |
| PB | 100 | 5.2±0.05 | 3.3±0.05 | 53±2.3 | 123±2.8 |

Drugs were administered orally once a day for 6 days. Each value is the mean±S.E. of 7 rats. Significantly different from the control value, *P<0.05 and **P<0.01.

Fig. 3. Effect of KZ-1026 on liver DNA content and [3H]-thymidine incorporation into hepatic DNA at various intervals after a single administration of KZ-1026 (200 mg/kg, p.o.). Each point indicates the mean±S.E. of 5 rats. When the error bar is absent, S.E. lies within the area of the point itself.
single dose of KZ-1026 (200 mg/kg, p.o.) are shown in Fig. 4A, B and C, respectively. The liver weight increased at 72 hr (33%) and 168 hr (15%) (Fig. 4A). In association with the increase in liver weight, the DNA content also increased at 24 hr (27%), 72 hr (37%) and 168 hr (22%) (Fig. 4B). The number of hepatic nuclei paralleled the DNA content, i.e., the increase of the hepatic nuclei was 20% at 24 hr, 42% at 72 hr and 28% at 168 hr (Fig. 4C). It was also found that KZ-1026 significantly decreased the population of binucleates (60% of the control), which was counted histologically 72 hr post-dose (Fig. 4D). All of these results indicate that KZ-1026 apparently augments DNA synthesis and the number of nuclei in the liver; that is, KZ-1026 enhances hepatocyte proliferation.

**Effect of KZ-1026 on galactose elimination capacity in normal rats:** Table 4 shows the relationship between liver enlargement and the t1/2 of galactose in the blood. After the administration of KZ-1026 at 200 mg/kg/day for 3 days, the liver weight increased by 51.4%, 57.0% and 16.3% of the control at 24, 48 and 96 hr after the last dose, respectively, while the t1/2 value decreased to 81.5%, 65.1% and 74.2% of the control values at 24, 48 and 96 hr after the last dose, respectively. These results suggest that the effect of KZ-1026 on the liver function develops later and persists longer than the effect on liver weight.

**Effect of KZ-1026 on the survival rate in subtotally hepatectomized rats:** As shown in Table 5, there was no appreciable difference between the KZ-1026 group and the control group in the percentage of liver resection, although pretreatment with KZ-1026 (200 mg/kg, p.o.) had increased the liver weight by

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**Fig. 4.** Effect of KZ-1026 on liver weight (A), DNA content (B), number of nuclei (C) and % of binucleate hepatocytes (D) after a single administration of KZ-1026 (200 mg/kg, p.o.). Percent of binucleate hepatocytes (D) shown is the value 72 hr after administration. Each point indicates the mean±S.E. of 5 rats. When the error bar is absent, S.E. lies within the area of the point itself. Significantly different from the control value, *P<0.05 and **P<0.01.

**Table 4.** Effect of KZ-1026 on the galactose tolerance test in normal rats

| Time after last dose (hr) | Galactose elimination time (t½, min) Control | KZ-1026 | Liver weight (g) Control | KZ-1026 |
|--------------------------|------------------------------------------|---------|--------------------------|---------|
|                          | (g)                                      |         |                          |         |
| 24                       | 26.0±0.49                                | 21.2±0.90** | 7.4±0.27                 | 11.2±0.22*** |
| 48                       | 29.5±1.18                                | 19.2±0.66**** | 7.9±0.33                 | 12.4±0.48*** |
| 96                       | 32.5±0.93                                | 24.1±1.82** | 8.6±0.30                 | 10.0±0.50*   |

KZ-1026 (200 mg/kg) was administered orally to rats for 3 days. Each value is the mean±S.E. of 5 rats. Significantly different from the control value, *P<0.05, **P<0.01 and ***P<0.001.
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Table 5. Effect of KZ-1026 on the survival rate of subtotally hepatectomized rats

| Group       | At time of operationa | 48 hr after operationb |
|-------------|-----------------------|------------------------|
|             | liver weight (g)      | % excused              | N   | survival rate (%) |
| Control     | 14.1 ± 0.33           | 84.1 ± 0.54            | 18  | 38.9              |
| KZ-1026     | 21.7 ± 0.83***        | 86.0 ± 0.71            | 18  | 77.8*             |

*aEach value is the mean ± S.E. of 8 rats. Significantly different from the control value, ***P < 0.001 (Student's t-test). bSignificantly different from the control value, *P < 0.05 (Fisher's exact test).

54% above the control value at the operation. Following pretreatment with KZ-1026, the survival rate at 48 hr after the operation was significantly improved from 38.9% to 77.8%.

Discussion

KZ-1026 administered orally at doses of 50, 100 and 200 mg/kg/day for 3 days induced a dose-dependent liver enlargement accompanied by liver DNA, RNA and protein content. The mode of action of KZ-1026 on liver enlargement differed from that of phenobarbital, a typical drug-metabolizing enzyme inducer, which also elicits a liver enlargement due to hypertrophy (Tables 2 and 3) (3, 4, 23–25). The single administration study suggested that KZ-1026 stimulates liver DNA synthesis. Moreover, the number of hepatic nuclei and DNA content increased coincidentally with the increase in liver weight. Although there is a high incidence of binucleation in the liver parenchyma (26), KZ-1026 rather decreased the incidence of binucleates as compared to the control. Therefore, the increase in hepatic nuclei induced by KZ-1026 will reflect an increase in the number of cells. In addition to these data, we observed KZ-1026 increases [3H]thymidine incorporation into DNA in an adult rat hepatocyte primary culture (27). These results suggest that the liver enlargement induced by KZ-1026 is mainly due to hepatocyte proliferation (hyperplasia). Although we have not tested the effect of KZ-1026 on liver non-parenchymal cells, it is of interest because these cells are reported to play an important role in liver functions (28). The increased liver weight regressed partially when the administration of KZ-1026 ceased (Fig. 4 and Table 4), although the increased DNA content persisted. In addition, the rate of DNA increase was relatively smaller than that of the liver weight. These observations may suggest that liver enlargement induced by KZ-1026 partly includes hypertrophy.

α-Hexachlorocyclohexane (α-HCH) is reported to cause liver hyperplasia in normal rats (29, 30). KZ-1026 seems to cause it in a similar manner, but more effectively than α-HCH (31, 32). However, it still remains to be elucidated whether the effect of KZ-1026 is essentially the same as that of α-HCH.

We also observed that KZ-1026 at a dose of 200 mg/kg/day for 3 days increased not only plasma TP, which is mostly synthesized in the liver, but also plasma T.Chol, PL and Amy levels (Table 1). It is well-known that bile duct injury causes an increase in plasma T.Chol and PL levels together with T.Bil level and that pancreatic injury causes an increase in plasma Amy level. However, no apparent injury was observed histologically in the liver or pancreas, and plasma T.Bil was not changed. Moreover, it is reported that the plasma Amy in normal rats is produced mainly by the liver (33, 34), and liver Amy is a secretory protein in normal rats (35).

From these results, we presumed that KZ-1026 enhances protein and lipid metabolisms accompanied by hepatocyte proliferation. Therefore, we also investigated whether KZ-1026 practically enhances essential liver functions.

As reported, the galactose elimination capacity reflects closely the functional reserve cell mass in the liver (36, 37). We demonstrated here that KZ-1026 significantly facilitated the galactose elimination rate in the normal rat, suggesting that KZ-1026 enhances the functional reserve cell mass. In addition, KZ-1026 also increased the survival rate of the acute hepatic failure model (subtotal hepatectomy). The increase in survival rate in subtotal hepatectomy is also reported with
20% glucose or testosterone. It is likely that 20% glucose or testosterone improves hypoglycemia or massive steatosis induced by hepatic resection respectively, resulting in facilitation of liver functions (22, 38, 39). It remains unclear how KZ-1026 improves the survival rate. We cannot rule out the possibility that KZ-1026 improves the liver functions after subtotal hepatectomy in the same manner as 20% glucose or testosterone. However, the most plausible interpretation may be as follows: pretreatment of rats with KZ-1026 increased the liver mass by 54%; thus, the subsequent subtotal (86%) hepatectomy resulted in a remnant liver of 21% the initial mass. In the control rats (without pretreatment with KZ-1026), the subtotal (84%) hepatectomy should have resulted in a remnant liver of 16% the initial mass. Thus, the 5% difference (21%–16%) in the remnant liver masses between KZ-1026 treated and untreated rats might account for the higher survival rate in the treated group.

These findings suggest that KZ-1026 enhances not only hepatocyte proliferation but also liver function, followed by hepatocyte differentiation. Interestingly, Tomonage et al. recently reported as follows: the expression of c-myc and c-Ha-ras which are stimulated in the regenerating liver are enhanced by KZ-1026 in the normal rat liver; KZ-1026 also enhances the expression of albumin mRNA, an index of hepatocyte differentiation, in the regenerating rat liver (40). This report supports the stimulative effect of KZ-1026 on both hepatocyte proliferation and differentiation. It has been reported that the hepatocyte proliferation is closely related to parasympathetic nerve and humoral factors (6). It is likely that KZ-1026, at least in part, affects hepatocytes directly since KZ-1026 is effective in a hepatocyte primary culture system. However, the possibility that the effect of KZ-1026 will be related to factors described above can not be ruled out since the in vivo effect of KZ-1026 on liver DNA synthesis is much stronger than the in vitro effect. Recently, Diaz-Gil et al. identified an albumin-bilirubin complex as a rat liver growth factor (14). Since KZ-1026 tends to increase albumin but not T.Bil in plasma, the stimulation of proliferation produced by KZ-1026 is not likely to be related to this factor. With respect with these matters, further investigations are necessary.

Although the detailed mechanism of action of KZ-1026 on stimulation of hepatocyte proliferation is still unclear, KZ-1026 should be a useful tool for studying the control mechanism for liver growth.

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References
1 Goldberg, L.: Liver enlargement produced by drugs: its significance. Proc. Eur. Soc. Study Drug Toxicity 7, 171–184 (1966)
2 Platt, D.S. and Cockrill, B.L.: Biochemical changes in rat liver in response to treatment with drugs and other agents-III. Effects of centrally acting drugs. Biochem. Pharmacol. 18, 459–473 (1969)
3 Grisham, J.M.: Effects of drugs of hepatic cell proliferation. In Drugs and the Cell Cycle, Edited by Zimmerman, A.M., Padilla, G.H. and Cameron, I.L., p. 95–136, Academic Press, New York and London (1973)
4 Schulte-Hermann, R.: Induction of Liver growth by xenobiotic compounds and other stimuli. CRC Crit. Rev. Toxicol. 3, 97–158 (1974)
5 Conney, A.H.: Pharmacological implications of microsomal enzyme induction. Pharmacol. Rev. 19, 317–366 (1967)
6 Alison, M.R.: Regulation of hepatic growth. Physiol. Rev. 66, 499–541 (1986)
7 Nakamura, T., Nawa, K. and Ichihara, A.: Partial purification and characterization of hepatocyte growth factor from serum of hepatectomized rats. Biochem. Biophys. Res. Commun. 122, 1450–1459 (1984)
8 Michalopoulos, G., Houck, K.A., Dolan, M.L. and Luetteke, N.C.: Control of hepatocyte replication by two serum factors. Cancer Res. 44, 4414–4419 (1984)
9 Goldberg, M.: Purification and partial characterization of a liver cell proliferation factor called hepatopoietin. J. Cell. Biochem. 27, 291–302 (1985)
10 Nakamura, T., Nawa, T., Ichihara, A., Kaie, N. and Nishino, T.: Purification and subunit of hepatocyte growth factor from rat platelets. FEBS Lett. 224, 311–316 (1987)
11 LaBrecque, D.R., Steele, G., Fogerty, S., Wilson, M. and Barton, J.: Purification and physical-chemical characterization of hepatic stimulator substance. Hepatology 7, 100–106 (1987)
12 Francavilla, A., DiLeo, A., Polimeni, L., Gavaler, J., Pellicci, R., Todo, S., Kam, I., Prelich, J., Makowka, L. and Starzl, T.E.: The effect of hepatic stimulatory substance, isolated from regenerating hepatic cytosol, and 50,000 and 300,000 subfractions in enhancing survival in experimental acute hepatic failure in rats treated with D-galactosamine. Hepatology 6, 1346–1351 (1986)

13 Gohda, E., Tsubouchi, H., Nakayama, H., Hiroto, H., Sakiyama, O., Takahashi, K., Miyazaki, H., Hashimoto, S. and Daikuhara, Y.: Purification and partial characterization of hepatocyte growth factor from plasma of a patient with fulminant hepatic failure. J. Clin. Invest. 81, 414–419 (1988)

14 Diaz-Gil, J.J., Sanchez, G., Trilla, C. and Escartin, P.: Identification of biliprotein as a liver growth factor. Hepatology 8, 484–486 (1988)

15 Nakamura, T., Teramoto, H., Tomita, Y. and Ichida, A.: Two types of growth inhibitor in rat platelets for primary cultured rat hepatocytes. Biochem Biophys. Res. Commun. 134, 755–763 (1986)

16 Burton, K.: A study of the condition and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem. J. 62, 315–323 (1956)

17 Schneider, W.C.: Determination of nucleic acids in tissues by pentose analysis. Methods Enzymol. 13, 680–684 (1957)

18 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J.: Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275 (1951)

19 Omura, T. and Sato, R.: The carbon monoxide binding pigment of liver microsomes. J. Biol. Chem. 239, 2370–2378 (1964)

20 Brodie, B.B. and Axelrod, J.: The fate of aminopyrine (Pyramidon) in man and methods for the estimation of aminopyrine and its metabolites in biological material. J. Pharmacol. Exp. Ther. 99, 171–184 (1950)

21 Schneider, W.C.: Phosphorus compounds in animal tissues. I. Extraction and estimation of deoxyxypenton nucleic acid and of pentose nucleic acid. J. Biol. Chem. 161, 293–303 (1945)

22 Gaub, J. and Inversen, J.: Rat liver regeneration after 90% partial hepatectomy. Hepatology 4, 902–904 (1984)

23 Kato, R., Loed, L. and Gelboin, H.V.: Microsome-specific stimulation by phenobarbital of amino acid incorporation in vivo. Biochem. Pharmacol. 14, 1164–1166 (1965)

24 Cajone, F. and Barnelli-Zazzeri, A.: The effect of phenobarbital on protein metabolism of liver. Results with isolated hepatocytes. Biochem. Pharmacol. 33, 725–729 (1984)

25 Tanaka, H., Watanabe, M. and Asahi, K.: Studies on the enlargement of the liver induced by continued administration of 1,3-diphenyl-5-(2-dimethylaminopropionamido)pyrazole (AP-14). Pharmacometr. 6, 291–312 (1972) (in Japanese)

26 Wheatley, D.N.: Binucleation in mammalian liver: Studies on the control of cytokinesis in vivo. Exp. Cell Res. 74, 465–469 (1972)

27 Katagiri, K., Suzuki, T., Monden, Y., Jinno, S., Hirayama, Y., Yamaki, T. and Yano, M.: KZ-1026, a hepatocyte growth accelerator, stimulates DNA synthesis of adult rat hepatocytes in primary culture. Tissue Culture Res. Commun. 7, 1–8 (1989)

28 Guguen-Guillouzo, C. and Guillouzo, A.: Modulation of functional activities in cultured rat hepatocytes. Mol. Cell. Biochem. 54, 35–56 (1983)

29 Schulte-Harmann, R., Koransky, W., Leberl, C. and Noack, G.: Hyperplasia and hypertrophy of rat liver induced by α-hexachlorocyclohexane and butylhydroxytoluene. Retention of the hyperplasia during involution of the enlarged organ. Virchows Arch. [B] 9, 125–134 (1971)

30 Schulte-Hermann, R., Leberl, C., Landgraf, H. and Koransky, W.: Liver growth and mixed-function oxidase activity: Dose-dependent stimulatory and inhibitory effects of α-hexachlorocyclohexane. Naunyn Schmiedebergs Arch. Pharmacol. 285, 365–366 (1974)

31 Parzefall, W., Münster, J. and Schulte-Hermann, R.: A comparative study on the effects of α-hexachlorocyclohexane and its metabolite β-pentachlorocyclohexane on growth and mono-oxygenase activities in rat liver. Biochem. Pharmacol. 29, 2169–2178 (1980)

32 Schulte-Hermann, R. and Schmitz, E.: Feedback inhibition of hepatic DNA synthesis. Cell Tissue Kinet. 13, 371–380 (1980)

33 Arnold, M. and Rutter W.J.: Liver amylase: II. Synthesis by the perfused liver and secretion into the perfusion medium. J. Biol. Chem. 238, 2760–2765 (1963)

34 Messer, M. and Dean, R.T.: Immunobiochemical relationship between α-amylases of rat liver, serum, pancreas and parotid gland. Biochem. J. 151, 17–22 (1975)

35 Hammerton, K. and Messer, M.: The subcellular and submicrosomal distribution of rat liver α-amylase activity. Biochim. Biophys. Acta 321, 597–602 (1973)
36. Takezaki, E., Nakanishi, T., Kawamoto, H., Kikukawa, M., Matsuura, T., Takeno, H., Olime, S., Kawakami, H. and Kajiyama, G.: Experimental study on galactose tolerance test as a test for functional reserve cell mass of the liver - comparison with ICG Rmax. Acta Hepatol. Japon. 24, 1235-1241 (1983) (Abs. in English)

37. Takezaki, E., Nakanishi, T., Watanabe, Y., Kawamoto, H., Kikukawa, M., Matsuura, T., Takeno, H., Kawakami, H. and Kajiyama, G.: Experimental study on galactose tolerance test as a test for functional reserve cell mass of the liver — galactose metabolism in regenerating rat liver. Acta Hepatol. Japon. 25, 1091-1096 (1984) (Abs. in English)

38. Weinbren, K. and Dowling, F.: Hypoglycaemia and the delayed proliferative response after subtotal hepatectomy. Br. J. Exp. Pathol. 53, 78-84 (1972)

39. Vic, P., Saint-Aubert, B., Astre, C., Bories, P., Bonardet, A., Descomps, B., Humeau, C. and Joyeux, H.: Complete liver regeneration in one-stage 90% hepatectomized rats treated with testosterone. Hepatology 2, 247-248 (1982)

40. Tomonaga, T., Ito, Y., Hayashi, H., Tabata, Y., Soeda, K., Imazeki, H., Yoshida, M., Odaka, M., Isono, Y., Hira, M., Shimada, T., Sakurai, S., Katagiri, K., Hirayama, Y., Yamaki, T. and Yano, M.: Effect of KZ-1026 on the hepatocyte proliferation and liver regeneration in rats. Abstract of the 88th Congress of Japanese Surgical Society, Niigata, p. 233 (1988) (in Japanese)