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Effect on the Canine Eck Fistula Liver of Intraportal TGF-β Alone or with Hepatic Growth Factors

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Transforming growth factor-β canceled the hepatocyte proliferation caused by transforming growth factor-α when the two substances were mixed and administered through a disconnected central portal vein branch after creation of an Eck fistula. In contrast, transforming growth factor-β had no antidotal action on the stimulatory effects of insulin or full test doses of insulinlike factor-2, hepatocyte growth factor, epidermal growth factor or triiodothymanine. A minor antidotal effect on hepatic stimulatory substance activity could be detected, but only with hepatic stimulatory substance was given in doses smaller than those known to cause maximum stimulatory response. These results suggest a highly specific pharmacological and physiological interaction between transforming growth factor-α and transforming growth factor-β in the modulation of liver growth control. (HEPATOLOGY '992;16:1267-1270.)

The Eck fistula (portacaval shunt) in dogs is a useful tool in the identification and study of hepatic growth-modulating substances, of which some of the most potent have little or no effect on hepatocytes in culture (1). This operation is followed by tripling of hepatocyte proliferation, atrophy of hepatocytes within 4 days to about half their original volume and establishment of a new stable state after 4 days (2). Growth stimulating substances, when infused into the tied-off central portal vein after this operation, augment further the heightened proliferation of the Eck fistula liver and prevent liver atrophy after creation of the fistula (1, 3-7). These effects have been termed hepatotrophic. In contrast, transforming growth factor-β (TGF-β), a known inhibitor of hepatocyte proliferation in vitro (8, 9), has the opposite effect in that it suppresses hepatocyte proliferation after creation of the Eck fistula and further reduces the size of the atrophied hepatocytes (1). The striking TGF-β effect is easily overridden with insulin (1).

In this study, we examined further the interaction of TGF-β with the five substances that were the most potently hepatotrophic in the Eck fistula model (1): insulin, insulinlike growth factor-2 (IGF-2), TGF-α, hepatocyte growth factor (HGF) and hepatic stimulatory substance (HSS). In addition, the interaction of TGF-β was tested with other molecules: epidermal growth factor (EGF), which is highly stimulatory in vitro (10) but not in vivo; and triiodothymanine (T_3), which causes feeble stimulation only in the in vivo system (1, 11). The results revealed a strong, although not absolute, specificity of the TGF-α interactions with TGF-β.

MATERIALS AND METHODS

The Experimental Model. Details of the experimental model have been described (1, 3, 4). In experiments testing a single drug, the left portal vein was used for infusion (Fig. 1A) and the right portal vein was ligated. For double infusions, catheters were placed in both main portal branches and connected to separate infusion pumps (Fig. 1B). The dogs were active after surgery and appeared clinically well. They were fed sugar water *ad libitum* on the day of surgery and a regular diet thereafter.

The pathological endpoints were the same as those previously described (1-7). Four days after creation of the portacaval shunt, 0.2 mCi/kg of intravenous [³H]thymidine was given (specific activity = 80 to 90 Ci/mmol; Du Pont–New England Nuclear, Boston, MA). Two hours later, while dogs were under sodium phenobarbital anesthesia, specimens were taken from the left and right lobes of the liver and fixed in 10% normal buffered formalin. At autopsy, after each dog was killed with an overdose of anesthesia, the patency of the anastomosis and the correct position of the catheter tip were confirmed. The liver tissue was processed and stained with hematoxylin and eosin.

Autoradiography was carried out with Kodak NTB2 liquid emulsion (Eastman Kodak Co., Rochester, NY) with an exposure time of at least 30 days. The number of replicating hepatocytes as an index of hepatocyte regeneration was determined by counting the number of [³H]thymidine-labeled nuclei per 1,000 hepatocytes. The sizes of individual hepatocytes (index of hypertrophy or atrophy) were determined by tracing the outlines of at least 500 midzonal liver cells projected onto standard-thickness paper, cutting out the individual silhouettes and weighing them. This method has been shown to be accurate in determining hepatocyte size and has been validated by planimetry and studies of unicellular organisms, the sizes of which have been determined directly (12).

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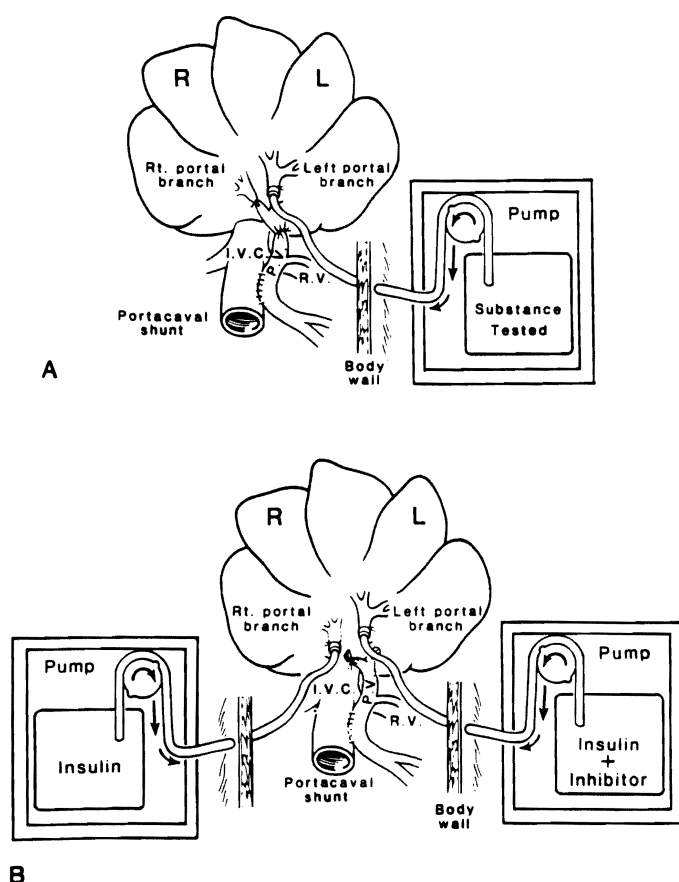31/1/40940

FIG. 1. Eck fistula model for postoperative infusion into the (A) left portal vein and (B) the left and right portal vein.

In control experiments (data not shown), we established that the vehicle used for the test substances is inert. Each animal served as its own control because the cell replication and cell sizes in the directly infused liver lobes could be compared with these parameters in the contralateral lobes that were not infused or infused with different solutions. In addition, we used as fresh controls animals not subjected to surgery and animals with Eck fistula only. Animals from the group of Eck fistula controls were killed on days 1 to 4 after surgery, enabling us to note the pace of change and the difference in changes of different liver lobes (Table 1).

Growth Stimulators Tested. The hormones we used to stimulate growth were semisynthetic human insulin (E.R. Squibb & Sons, Inc., Princeton, NJ) and T_3 (Sigma Chemical Co., St. Louis, MO). Growth stimulating factors used were HSS produced in our laboratory (13); recombinant HGF (also called hematopoietin [14]), generously donated by Dr. George Michalopoulos of the University of Pittsburgh; IGF-2 and EGF (Collaborative Research, Inc., Bedford, MA); and TGF-α, (Peninsula Laboratories, Inc., Belmont, CA).

Growth Factor Inhibitors Tested. TGF-β (Collaborative Research, Inc., Bedford, MA) was infused alone (Fig. 1A) or mixed with selected growth stimulating factors (Fig. 1B) and infused into one of the branches of the portal vein.

Statistical Analysis. Data are reported as mean ± S.D. Student's one-tailed *t* test was used to determine the significance of differences. A p value less than 0.05 was considered significant.

RESULTS

Controls. The day-to-day changes in the liver after creation of Eck fistula were the same as those reported previously (1-4) with rapid atrophy that was equal in the right and left lobes. We saw a nearly threefold increase in cell mitosis (Table 1).

TGF-β Infusion. At a TGF-β dose of 5 ng/kg/day, th normal proliferative response of 1.5 to 4.5 labelet hepatocytes/1,000 hepatocytes was cut to about 2.: labeled hepatocytes/1,000 hepatocytes in the TGF-β-infused left liver lobes; in the uninfused right lobes we found a full proliferative effect. In addition, the hepa tocytes were significantly more atrophied in the lefl lobes than in the uninfused right lobes (Table 1).

TGF-β Plus Growth Stimulators. As previously reported (1), a strong hepatotrophic response was evident in the lobes infused only with insulin, IGF-2, HSS, HGF or TGF-α. Modest responses to T_3 and EGF were seen (Table 2). TGF-β at a dose 10 times greater than that shown to be antihepatotrophic in the control experiments documented in Table 1 caused no reduction in these responses except for those induced by TGF-α (Table 2).

TGF-β completely eliminated the TGF-α actvity. In addition, it suppressed the normally occurring proliferative response characteristic of Eck fistula and aggravated hepatocyte atrophy (Table 2).

Dose-response Relationship of HSS vs. that of TGF-β. Although 50 ng/kg/day HSS still had its full hepatotrophic effect after mixing with 50 ng/kg/day TGF-β, smaller HSS doses of 25 and 10 ng were partly or completely inactivated after mixing with the fixed large dose of TGF-β (Table 3).

DISCUSSION

TGF-β, a 25,000-Da homodimeric peptide, is known to be a powerful *in vitro* inhibitor of various cell types (15, 16), including hepatocytes stimulated with EGF and TGF-α (9). During the liver regeneration that reaches its peak 24 hr after partial hepatectomy in rats, the tissue TGF-β level increases significantly, but not until after this peak of regeneration is reached. Thereafter the TGF-β concentration rises four to eight times by 72 hr after hepatectomy and then recedes to the control level by 96 hr. Such findings have prompted speculation that TGF-β modulates liver regeneration by counteracting the growth stimulation of TGF-α, EGF and other regeneration-promoting factors (17).

In contrast to the classic "burst" of liver regeneration after partial hepatectomy, the heightened cell renewal after creation of the Eck fistula builds to a new steady state within 4 days after surgery and remains constant thereafter. In this investigation it was demonstrated that 5 ng/kg/day TGF-β prevented the increase in hepatocyte proliferation invariably seen in the Eck fistula liver. It also worsened the characteristic hepatocyte atrophy.

In subsequent experiments, 50 ng/kg/day TGF-β was infused to simulate the high concentrations of endogenous TGF-β seen during regeneration after hepa-

TABLE 1. Hepatocyte size and autoradiographic labeling

| Group | No. of dogs | Days after PCS | TGF-β dose (ng/kg/day) | No. of labeled hepatocytes per 1,000 hepatocytes[a] | | Cell size units[a] | |
				Left lobe	Right lobe	Left lobe	Right lobe
1	2	No surgery	—	1.55 ± 0.18	1.55 ± 0.08	0.161 ± 0.002	0.160 ± 0.003
2	3	1	—	1.43 ± 0.43	1.36 ± 0.4	0.192 ± 0.02	0.192 ± 0.023
3	3	2	—	6.33 ± 0.82[b]	6.26 ± 0.64[b]	0.134 ± 0.008[b]	0.134 ± 0.009[b]
4	3	3	—	5.03 ± 0.27[b]	4.96 ± 0.3[b]	0.128 ± 0.009[b]	0.130 ± 0.008[b]
5	4	4	—	4.4 ± 0.3[b]	4.2 ± 0.3[b]	0.095 ± 0.01[b]	0.096 ± 0.015[b]
6	3	4	5	2.4 ± 0.5[c, d]	4.6 ± 0.8	0.064 ± 0.02[c, d]	0.103 ± 0.01
7	2	4	50	2.1 ± 0.05[c, d]	4.9 ± 0.7	0.071 ± 0.006[c, d]	0.106 ± 0.004

PCS = portacaval shunt.

Group 1: dogs that did not undergo surgery; groups 2 to 5: dogs 1 to 4 days after PCS; groups 6 and 7: dogs with PCS and 4-day continuous infusion of TGF-β into the left branch of the portal vein.

[a] Data expressed as mean ± S.D.

[b] $p < 0.001$ vs. groups 1 and 2.

[c] $p < 0.01$ vs. own right lobe.

[d] $p < 0.05$ vs. left lobe of group 5.

TABLE 2. Hepatocyte size and autoradiographic labeling after continuous infusion of growth stimulating factor into the right portal vein branch and infusion of growth stimulating factor plus TGF-β (50 ng/kg/day) into the left portal infusion of branch

| Group | No. of subjects | Stimulating substances | Dose (ng/kg/day) | No. of labeled hepatocytes per 1,000 hepatocytes[a] | | Cell size units[a] | |
				Left lobe	Right lobe (no inhibitor)	Left lobe	Right lobe (no inhibitor)
1	3	Insulin	0.16	15.0 ± 0.8	14.9 ± 0.4	0.187 ± 0.018	0.165 ± 0.01
2	1	IGF-2	50	11.0	10.3	0.148	0.1477
3	2	HSS	50	13.8 ± 0.2	13.3 ± 0.15	0.142 ± 0.006	0.143 ± 0.004
4	1	HGF	50	11.3	11.2	0.164	0.163
5	2	T_3	100	7.4 ± 0.65	7.3 ± 0.8	0.126 ± 0.016	0.127 ± 0.013
6	2	EGF	150	5.15 ± 0.2	5.05 ± 0.05	0.119 ± 0.001	0.120 ± 0.001
7	2	TGF-α	50	3.4 ± 0.25[b]	12.6 ± 0.5	0.082 ± 0.006[b]	0.159 ± 0.002

[a] Data expressed as mean ± S.D.

[b] $p < 0.001$.

TABLE 3. Hepatocyte size and autoradiographic labeling after continuous infusion of HSS into the right portal vein branch and HSS plus TGF-β (50 ng/kg/day) into the left portal vein branch

| Group | No. of subjects | HSS (ng/kg/day) | No. of labeled hepatocytes per 1,000 hepatocytes[a] | | Cell size units | |
			Left lobe	Right lobe (no inhibitor)	Left lobe	Right lobe (no inhibitor)
1	1	10	2.3	7.5	0.093	0.141
2	1	25	5.8	9.2	0.104	0.153
3	2	50	13.0 ± 0.2	13.3 ± 0.15	0.142 ± 0.006	0.143 ± 0.004

[a] Data expressed as mean ± S.D.

tectomy. These high concentrations are considered the result of the development of resistance to TGF-β (18). In the 4 days after creation of the Eck fistula, the larger dose of TGF-β (50 ng/kg/day) abrogated the stimulation usually caused by TGF-α. Because the reactivity to TGF-α alone was fully retained in the contralateral lobes, it appeared that TGF-β was inactivated almost completely with one pass through the liver. Under the same conditions of testing, all of the other potent hepatotrophic factors that were examined (insulin,

IGF-2, HGF, HSS, T_3 and EGF) were unaffected by mixing and coadministration with TGF-β.

These data indicate that growth-stimulating factors of hepatocytes can be divided into two groups: those resistant to modulation by TGF-β and those whose proliferation-inducing qualities are dramatically reduced or even canceled by TGF-β. However, it should also be emphasized that the foregoing classification of hepatic stimulatory factors according to their reaction to coadministration with TGF-β depends in part on dose

selection. This was demonstrated when HSS (which is usually unaffected by TGF-β) was given in reduced quantities (10 or 25 ng/kg/day) that by themselves evoked minimum proliferative responses. Under these circumstances, the minor HSS-induced response could be eliminated with TGF-β.

Further discussion of these findings does not seem warranted. However, we do emphasize the seemingly high degree of specificity of the TGF-β/TGF-α interaction. Even EGF, which ostensibly has the same binding sites as TGF-α (19), was not affected by TGF-β. This pharmacological specificity is consonant with a critical role of the balance of these growth factors in the control of cell proliferation, as has been proposed by others (20, 21).

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