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Isolation and Partial Purification of Hepatic Stimulatory Substance

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THE PURPOSE of this study was to purify and characterize hepatic stimulatory substance (HSS), a protein found in the liver that has been reported to stimulate hepatic DNA synthesis and hepatocyte replication and that was previously identified by LaBrecque et al[1-3] and Francavilla et al in the rat liver,[4,5] as well as by Starzl et al in the canine liver.[6,7]

HSS has been partially purified by successive steps, involving ethanol precipitation, ultrafiltration, and fast protein lipid chromatography (FPLC). The activity of this factor has been tested in 40% hepatectomized rats.

MATERIALS AND METHODS

Animals

Male Fischer (F344) rats (180 to 200 g) and weanling Fischer (F344) rats (60 to 90 g), were purchased from Hilltop Lab Animals, Scottsdale, PA, and were kept in temperature and light controlled rooms. They received food and water ad libitum.

Surgical Procedures

Forty percent partial hepatectomies (PH) were performed in rats according to Higgins and Anderson.[8] Control animals underwent a sham operation consisting of laparotomy and manual manipulation of the liver.

Preparation of Hepatic Extracts

In Table 1, the preparation of HSS from liver cytosol is described. However, liver homogenates can also be used as the source of HSS as described by LaBrecque and Pesch[1] and us.[6,7]

Sodium Dodecyl Sulfate (SDS) Polyacrylamide Gel Electrophoresis

SDS polyacrylamide slab gels with a 7.5% to 20% gradient were prepared and developed according to Laemmli.[9] Protein bands were visualized by Coomassie blue R 250 according to Weber and Osborn.[10]

Protein Determination

Protein concentration was determined by the method of Lowry et al[11] or by the method of McKnight[12] for the determination of submicrogram quantities.

Determination of the Activity of HSS and Its Fractions

The activity of various fractions was determined in vivo using 40% hepatectomized rats. Experiments were carried out according to LaBrecque and Pesch.[1] [^{3}H]-thymidine incorporation, labeling and mitotic indexes were determined as previously described.[13]

Statistical Analysis

Statistical analysis of groups was carried out by one-way analysis of variance using SPSS/PC statistical software (SPSS, Inc, Chicago) on an IBM-AT microcomputer.

RESULTS AND DISCUSSION

Table 2 describes the purification steps of HSS and the physico-chemical characteristics of active fractions named F_{150} on the basis of its elution from the FPLC columns with MaCl at 150 mmol/L.

HSS seems to be a protein with a molecular weight (MW) between 50 and 14 kd, which is

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Supported by Research grants from the Veterans Administration and Project Grant No. AM-29961 from the National Institutes of Health, Bethesda, MD, and by Grant No. 885/02 16544 from the Consiglio Nazionale delle Ricerche, Italy.

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0041-1345/88/2001-1271$3.00/0

Table 1. Preparation and Purification of HSS

Source
Weanling male F-344 Fischer rats
Purification steps
Remove liver between 7 and 8 am
Prepare cytosol as previously described[13]
Heat at 65°C for 15 minutes
Centrifuge 30,000 g, at 4°C for 20 minutes
Collect supernanant and add to 6 volumes of cold ethanol
Stir for two hours at 4°C
Centrifuge 37,000 g, at 4°C for 20 minutes
Resuspend the pellet (OH-F) in NHA-acetate 150 mmol/L, pH6.5 and homogenize
Centrifuge 10,000 g, at 4°C for ten minutes
Filter the freshly prepared OH-F through Amicon PM30 ultrafiltration membrane
Concentrate the PM30 filtrate using Amicon YC05 ultrafiltration membranes
Lyophilize the concentrated material and keep at −70°C (30 kd-F)
Redissolve 30 kg-F in 5 mmol/L phosphate buffer for chromatography (FPLC using mono Q HR 5/5 column)
Elute the active fraction (F_{150}) using a linear 0 to 200 mmol/L NaCl gradient at 150 mmol/L NaCl
Dialyze F_{150} in NH_4-acetate, lyophilize and keep at −70°C

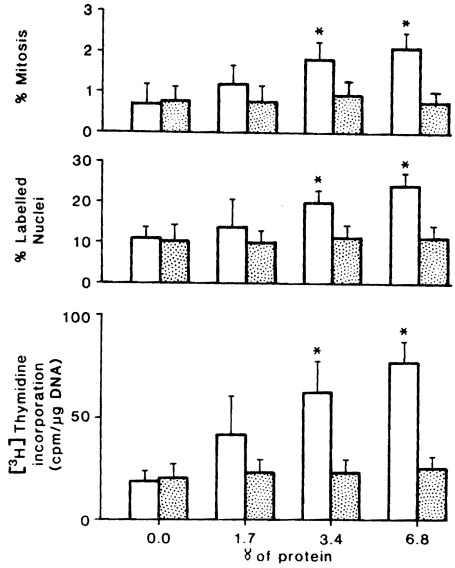

Fig 1. Dose response curve in 40% hepatectomized rats injected with F_{150}. DNA synthesis, percent of labeled nuclei, and percent of mitosis were determined as reported in Materials and Methods. The values shown are the averages of ten determinations for each level of F_{150} ± SD and the average of four determinations for each level of serum albumin in PBS ± SD. *, Significantly different from the control value PBS, $P < .05$.

resistant to neuraminidase, is destroyed by trypsin, and is resistant to heating at 95°C for ten minutes. The purification of F_{150} results in a 38,000-fold increase of activity over the original cytosol. As previously described,[14] each preparation of F_{150} obtained from FPLC chromatography was completely free of recognizable hormones such as insulin, glucagon, vasopressin, and EGF.[14] The activity of each fraction during the various purification steps was tested in 40% hepatectomized rats. All fractions demonstrated significantly more activity than PBS when injected into rats.

The activity of F_{150} was dose-dependent over a range of 1.76 μg to 6.8 μg per 100 g body weight of the recipient rat. Thymidine incorporation results were confirmed by labeling and mitotic index results, as shown in Fig 1.

Table 2. Steps of Purification of HSS and Chemical and Physico-Chemical Properties of Fraction F_{150} Obtained From Weanling Rat Liver

Material	Protein Injected in Each Rat (mg/2 mL)	DNA Synthesis (cpm/mgF DNA)*	Resistant to			Purificaiton (Fold)
			Heat	Trypsin	Neuro-aminidase	
Cytosol	75	43,350 ± 8,820†				—
65°C Supernatant	20	56,720 ± 10,240†				6
OH-F	10	66,350 ± 11,350†				15
30 kd	2.75	63,520 ± 13,220†				102
F_{150}	0.003	54,380 ± 10,200	100%	30%	100%	38,100

*[^{3}H]-thymidine incorporation in a 40% hepatectomized rat injected with PBS was 16,500 cpm/mg DNA. The numbers are the averages from no less than 20 different rats ± SD.

†Significantly different from controls, $P < .05$.

It has been clearly demonstrated that F_{150} is organ, but not species specific.[14] This fraction, as it presently exists, contains a few proteins, as indicated by SDS page with a MW ranging between 14 and 60 kd. Further studies are in process to obtain the final purification of HSS. Completely purified HSS would represent an important step in the knowledge of hepatocyte proliferation as well as in clinical therapy.

The use of growth factor therapy for acute liver failure as well as in acute rejection after liver transplantation in animals and in humans is, in fact, the main objective of this study. In fact, we have already shown that this type of therapy, using fractions obtained during the HSS purification, significantly improves the survival rate of rats intoxicated with the selective hepatotoxin D-Galactosamine.[13]

ACKNOWLEDGEMENT

We are grateful to John Prelich for technical assistance.

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