Urol Res (1990) 18:295–298

Urological Research

Transforming growth factor β-like activity in human hydrocele fluid

P. K. Saha, S. Kanda, H. Morimitsu, N. Nishimura, H. Kanetake, and Y. Saito

Department of Urology, Nagasaki University School of Medicine, Nagasaki, Japan

Accepted: August 1, 1989

Summary. In this study transforming growth factorβ (TGF-β)-like activity in human hydrocele fluid was investigated. Inhibition of DNA synthesis of adult rat hepatocytes in primary culture and stimulation of colony formation of normal rat kidney (NRK) fibroblasts, clone 49F in soft agar were observed in all acidified hydrocele fluids and these activites were neutralized by the specific antibody raised against human native TGF-β. In samples obtained from recurrent cases of hydrocele, TGFβ-like activity was observed in its active form (without acidification). These results suggest that human hydrocele fluid contains TGFβ-like activity and that the active form of TGF-β in recurrent hydrocele fluid may be responsible for the recurrence of the disease even after repeated aspiration.

Key words: Transforming growth factor – Hydrocele

TGF-β is a well-known bifunctional regulator of cell growth or cell differentiation [21]. It stimulates the anchorage-independent growth of mouse AKR-2B cells or NRK-49F cells [9] and inhibits the DNA synthesis of rat hepatocytes in primary culture [1, 11], mink lung cells [10] and vascular endothelium [14]. TGF-β was initially isolated from a culture medium conditioned by virally transformed cells [2]. Recently it has been found in many normal and malignant tissues [17]. TGF-β activity has been demonstrated in the malignant effusions of various cancerous patients [20], urine from normal, pregnant and tumor-bearing humans [23] and recently in synovial effusions [3]. TGF-β is usually secreted from the producer cells in a biologically inactive form, which can be activated by transient acidification [15, 24], urea treatment [8] or alkali treatment [6].

While hydrocele is a very common disorder, and recurrence after repeated aspiration is also commonly seen, the mechanism of recurrence is still not clear. We studied human hydrocele fluid in order to determine whether or not it contained TGFβ-like activity using the following four criteria: (i) activation with acidification (ii) inhibition of DNA synthesis of cultured rat hepatocytes (iii) stimulation of colony formatin of NRK-49F cells in soft agar and (iv) neutralization of the activity by a specific antibody to TGF-β. Our results indicate that TGFβ-like activity is present in all cases of hydrocele fluid irrespective of age. In addition, our results also demonstrate that only the fluid of recurrent cases contains the active form of TGF-β.

Materials and methods

Williams medium E was obtained from Flow Laboratories, U. K. Dulbecco's modified Eagle's medium was from Nissui Pharmaceuticals, Tokyo, Japan. Newborn calf serum was from GIBCO Oriental, Tokyo, Japan. Insulin and bovine serum albumin were purchased from Sigma Chemical Co. Aprotinin was from Mochida Pharmaceuticals, Tokyo, Japan. Dexamethasone and collagenase were from Wako Pure Chemical industries, Osaka, Japan. 5-[^{125}I]-iododeoxyuridine (2,200 ci/m mol) was from New England Nuclear Boston, MA, USA. Anti-TGF-β antibody was obtained from R&D systems, USA. Epidermal growth factor (EGF) was purified in our laboratory from the submaxillary glands of adult male mice using the method of Savage and Cohen [19]. Male Wistar rats were obtained from Otsubo Experimental Animals, Nagasaki, Japan.

Collection and acid treatment of hydrocele fluid

Fluids were collected from different patients by needle aspiration into disposable plastic syringes. These fluids were routinely centrifuged at 3,000 rpm for 10 min to remove the floating cells and stored at -20°C until used. Acid treatment of the fluid was carried out by the addition of acetic acid (final concentration 1 M) for 6 h at room temperature.

Isolation of rat hepatocytes

Parenchymal hepatocytes were isolated from adult male Wistar rats weighing 150–250 g by two-step collagenase perfusion as previously reported by Tanaka et al [22].

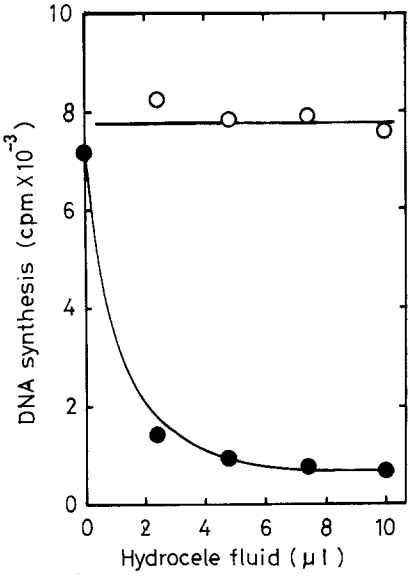

Fig. 1. Effect of human hydrocele fluid on DNA synthesis of adult rat hepatocytes in primary culture. Experimental conditions were as described in *Materials and methods.* Values are means of duplicate dishes. ○ = Untreated hydrocele fluid; ● = acid-treated hydrocele fluid

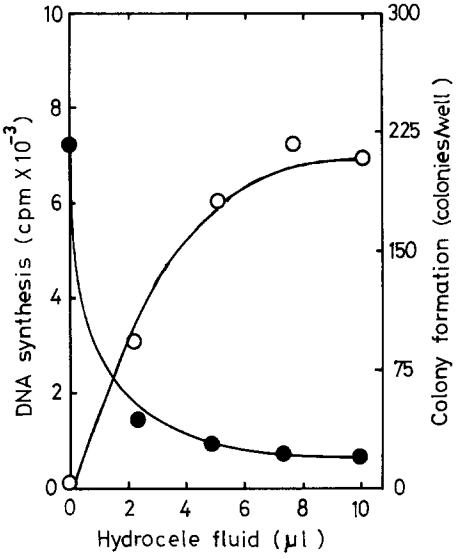

Fig. 2. Dose-dependent effects of acid-treated hydrocele fluid on DNA synthesis of adult rat hepatocytes in primary culture and colony formation of NRK-49F cells in soft agar. ○ = Colony formation of NRK-49F cells in soft agar; ● = DNA synthesis of hepatocytes

Assay of DNA synthesis

Isolated hepatocytes were suspended in Williams medium E supplemented with 5% newborn calf serum, 10^{-9} M insulin and 10^{-9} M dexamethasone and were incubated into 24-well multi-well plates coated with type 1 collagen at a density of 6.25×10^4 cells/cm^2. After 24 h, the medium was changed to a serum and hormone free medium containing 5 U/ml aprotinin, 10^{-7} M insulin and 10 ng/ml EGF. Untreated hydrocele fluid or neutralized activated fluid was then added to the cells. Incorporation of 5-[^{125}I]-iododeoxyuridine into hepatocytes was measured using the procedure described by Nakamura et al. [13].

Soft agar colony formation assay of NRK-49F cells

NRK-49F fibroblasts, obtained from the Japanese Cancer Research Resources Bank, were maintained in Dulbecco's modified Eagle's medium containing 10% fetal calf serum. Colony formation in soft agar was assayed in the presence of EGF as previously described [4, 11].

Neutralization of TGF β-like activity

Acidified hydrocele fluid was neutralized with sodium hydroxide and diluted with the culture medium containing 0.5% bovine serum albumin. Anti-human TGF-β IgG was then added and the fluid incubated for 2 h at 37°C. Finally, the fluid was added to the hepatocytes and DNA synthesis was assayed.

Results

Figure 1 shows the typical pattern of the inhibition of DNA synthesis of cultured rat hepatocytes by human hydrocele fluid. The acid-treated hydrocele fluid causes dose-dependent inhibition of DNA synthesis of rat hepatocytes stimulated by insulin and EGF, but untreated fluid does not show any activity (Fig. 1). This suggests that the inhibitory activity for DNA synthesis of rat hepatocytes is activated by acid treatment. It has been reported that not only TGF-β but also PDGI-α [12], interleukin-1β [13] and interleukin-6 [13] can suppress the DNA synthesis of adult rat hepatocytes. Of the four, only TGF-β stimulated colony formation of NRK-49F cells in soft agar. To confirm that this inhibitory effect on the growth of hepatocytes was due to the TGFβ-like activity, the acid activated hydrocele fluid was examined for its capacity to promote soft agar colony formation of NRK-49F cells (Fig. 2). Each of the fluids examined was found to promote soft agar colony formation by indicator cells in the presence of EGF.

Further, we studied the effect of anti-human TGF-β IgG on the inhibitory activity of hydrocele fluid on DNA synthesis of cultured rat hepatocytes. Table 1 shows that 40 μg/ml of specific antibody completely neutralized the TGFβ-like activity of hydrocele fluid. These results indicated that human hydrocele fluid possessed typical TGFβ-like activity. But the significance of the TGFβ-like activity in human hydrocele fluid was unclear. Next we screened the activity in hydrocele fluids using cultured rat hepatocytes. Table 2 shows a summary of the screening. All fluids obtained from recurrent cases were found to contain both the active and latent forms of TGFβ-like activity, while nonrecurrent fluids contained only the latent form of TGFβ-like activity. In recurrent cases (Table 2), we used the recurrent samples. Several samples collected from each case during the course of recurrence

Table 1. Neutralization of TGFβ-like activity by anti-TGF-β antibody

Addition	DNA synthesis (CPM)
None	1,157 ± 144
Insulin + EGF	12,014 ± 827
Insulin + EGF + Anti-TGF-β antibody	12,859 ± 722
Insulin + EGF + Acid treated hydrocele fluid	1,781 ± 81
Insulin + EGF + Acid treated hydrocele fluid +Anti-TGF-β antibody	11,807 ± 1,224

Hydrocele fluid examined here was obtained from case 5 of Table 2. Hepatocyte culture and assay of DNA synthesis were as described in *Materials and methods.* Values are means ± SD for duplicate experiments

Table 2. TGFβ-like activity in human hydrocele fluids obtained from different patients

Case No.	Age (years)	Untreated fluid (unit/ml)	Acid-treated fluid unit/ml
1	57	54	108
2	5	66	132
3	63	45	144
4	71	67	100
5	85	0	3,000
6	75	0	374
7	83	0	186
8	64	0	1,715
9	63	0	190
10	60	0	426
11	3	0	556
12	6	0	376
13	1 month	0	162

TGFβ-like activity resulting in half the maximal inhibiton of the DNA synthesis of rat hepatocytes was arbitrarily defined as one unit. Cases 1–4 are recurrent patients and cases 5–13 are nonrecurrent patients

were assayed separately, with similar results obtained for each respective case (data not shown). No significant differences were observed between adult and pediatric cases.

Discussion

TGF-β has two froms, an active form and a latent form. Most tissues secrete TGF-β in its latent form [15]. Although, the in vivo mechanism of activation of latent TGF-β is still unclear, it is possible that some enzymes such as endoglycosidase F and/or sialidase may play a role [7]. In our experiments, when we assayed untreated hydrocele fluid, only samples obtained from recurrent cases showed activity, while in nonrecurrent cases no activity was seen. However, acid-treated samples of both the recurrent and nonrecurrent cases demonstrated high activity (Table 2).

Hydrocele may be defined as a distinct increase in the amount of fluid in the tunica vaginalis. The tunica vaginalis is one of the serous cavities derived from the coelom and there is a constant interchange of fluid within all serous lined cavities, with accumulation of fluid occurring when an imbalance develops either of secretion or absorption in this interchange [5]. Inflammation or tumor of the testis or epididymis and surgical operations that destroy local veins or lymphatics may cause impairment of the secretion or absorption process and thus lead to the accumulation of fluid in the tunica vaginalis. In our series of patients only two adult cases (cases 1 and 5) had a past history of local inflammation, while in the other cases no secondary disease was found.

It has already been reported that normal pleural and peritoneal effusions contain TGFβ-like activity [18] and that the epithelium of the normal urinary tract secretes TGF-β [16]. In congenital hydrocele, TGF-β is thought to originate from the peritoneal fluid and in adult hydrocele, TGF-β is thought to be produced by the lining epithelium of the tunica vaginalis.

The presence of active TGF-β in recurrent hydrocele fluid suggests possible regulatory effects of TGF-β on secretion and absorption of fluid by the tunica vaginalis, as TGF-β is well-known for its multiple regulatory effects on other tissues and cells [21].

We conclude that in cases in which the fluid of the hydrocele testis contains an active form of TGF-β, the possibility of recurrence should be considered. Further study is required to understand the exact role of TGF-β in the development and /or maintainance as well as the frequent reapperance of fluid in the tunica vaginalis of hydrocele patients, even after repeated aspiration.

Acknowledgements. We thank Dr. Akihito Jodai for the supply of some hydrocele samples. We also thank M. Yoshimoto, T. Shimogama, Y. Harada and N. Yamaguchi for their outstanding technical assistance.

References

1. Carr BI, Hyashi I, Branum EL, Moses HL (1986) Inhibition of DNA synthesis in rat hepatocytes by platelet derived type-β transforming growth factor. Cancer Res 46:2330

2. DeLarco JE, Todaro GJ (1978) Growth factors from murine sarcoma virus-transformed cells. Proc Natl Acad Sci USA 75:4001
3. Fava R, Olsen N, Oja K, Moses H, Pincus T (1989) Active and latent forms of transforming growth factor β activity in synovial effusions. J Exp Med 169:291
4. Hirai R, Yamaoka K, Mitsui H (1983) Isolation and partial purification of a new class of transforming growth factors from an avian sarcoma virus transformed cell line. Cancer Res 43:5742
5. Huggins CB, Entz FH (1931) Absorption from normal tunica vaginalis testis, hydrocele and spermatocele. J Urol 25:447
6. Lyons MR, Oja JK, Moses HL (1988) Proteolytic activation of latent transfroming growth factor-β from fibroblast-conditioned medium. J Cell Biol 106:1659
7. Miyazono K, Heldin CH (1989) Role of carbohydrate structures in TGF-β1 latency. Nature 338:158
8. Miyazono K, Hellman U, Wernstedt C, Heldin CH (1988) Latent high molecular weight complex of transforming growth factor β1 purification from human platelets and structural characterization. J Biol Chem 263:6407
9. Moses HL, Tucker RF, Leof EB, Coffey RJ, Halper TrJ, Shipley GD (1985) Type-β transforming growth factor is a growth stimulator and a growth inhibitor. Cancer cells 3/ Growth factor and Transformation. Cold Spring Harbor Lab., New York, p 65
10. Moses HL, Coffey RJ, Leof EB, Lyons RM, Keski-Oja J (1987) Transforming growth factor β regulation of cell proliferation. J Cell Physiol [Suppl] 5:1
11. Nakamura T, Tomita Y, Hirai R, Yamaoka K, Kaji K, Ichihara A (1985) Inhibitory effect of transforming growth factor-β on DNA synthesis of adult rat hepatocytes in primary culture. Biochem Biophys Res Commun 133:1042
12. Nakamura T, Teramoto H, Tomita Y, Ichihara A (1986) Two types of growth inhibitor in rat platelets for primary cultured rat hepatocytes. Biochem Biophys Res Commun 134:755
13. Nakamura T, Arakaki R, Ichihara A (1988) Interleukin-1β is a potent growth inhibitor of adult rat hepatocytes in primary culture. Exp Cell Res 179:488
14. Owens GK, Geisterfer AAT, Young YWH, Komoriya A (1988) Transforming growth factor-β induced growth inhibition and cellular hypertrophy in cultured vascular smooth muscle cells. J Cell Biol 107:771
15. Pircher R, Lawrence DA, Jullien P (1984) Latent β-transforming growth factor in nontransformed and kirsten sarcoma virus-transformed normal rat kidney cells, clone 49F. Cancer Res 44:5538
16. Ranganathan G, Lyons R, Jiang NS, Moses H (1987) Transforming growth factor type β in normal human urine. Biochem Biophys Res Commun 148:1503
17. Roberts AB, Frolik CA, Anzano MA, Sporn MB (1983) Transforming growth factors from neoplastic and nonneoplastic tissues. Fed Proc 42:2621
18. Sairenji M, Suzuki K, Murakami K, Motohashi H, Okamoto T, Umeda M (1987) Transforming growth factor activity in pleural and peritoneal effusions from cancer and non-cancer patients. Jpn J Cancer Res 78:814
19. Savage CR, Cohen S (1972) Epidermal growth factor and a new derivative. Rapid isolation procedures and biological and chemical characterization. J Biol Chem 247:7609
20. Seo MK, Lynch KE, Podolsky DK (1988) Multiplicity of transforming growth factors in human malignant effusions. Cancer Res 48:1792
21. Sporn MB, Roberts AB, Wakefield LM, Assoian RK (1986) Transforming growth factor-β: biological function and chemical structure. Science 233:532
22. Tanaka K, Sato M, Tomita Y, Ichihara A (1978) Biochemical studies on liver functions in primary culture hepatocytes of adult rats. Hormonal effects on cell viability and protein synthesis. J Biochem [Tokyo] 84:937
23. Twarzik DR, Sherwin SA, Ranchalis J, Todaro GJ (1982) Transforming growth factors in urine of normal, pregnant and tumor-bearing humans. JNCI 69:793
24. Wakefield LM, Smith DM, Masui T, Harris CC, Sporn MB (1987) Distribution and modulation of the cellular receptors for transforming growth factor-beta. J Cell Biol 105:965

Y. Saito, MD
Department of Urology
Nagasaki University School of Medicine
Nagasaki-shi
852 Japan