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INCREASING THE PLASMA HALF-LIFE OF TRICHOSANTHIN BY COUPLING TO DEXTRAN

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Abstract—Trichosanthin (TCS) is a plant protein which has a wide spectrum of pharmacological activities. It was demonstrated recently that this compound suppressed the replication of human immunodeficiency virus (HIV-1) *in vitro*. The mechanism of action is believed to be inhibition of protein synthesis. Trichosanthin is a low molecular weight protein which is expected to be easily filtered and eliminated through the kidney. To minimize renal loss, the molecular size of trichosanthin can be increased by coupling to dextran. The larger complex will not undergo glomerular filtration and therefore renal loss can be prevented. This study investigates the kidney's role in trichosanthin elimination and the beneficial effect afforded by coupling to dextran in prolonging plasma half-life. For this purpose, a radioimmunoassay has been developed to determine the concentration of TCS in plasma and urine. The sensitivity of this assay is in the nanogram range. Trichosanthin was coupled to dextran T40 by a dialdehyde method and successful coupling was confirmed by gel filtration chromatography. The complex retained specific binding to trichosanthin antibodies with decreased affinity which can be partially reversed after incubation with dextranase; an enzyme that digested dextran. The pharmacokinetics of intravenously administered trichosanthin (0.75 mg/kg) was compared between two groups of rats with normal and impaired renal function (bilateral renal arterial ligation). Rats with ligation showed a decrease in plasma clearance from 4780 ± 570 to 220 ± 20 μL/min and an increase in the mean residence time from 9 ± 1 to 145 ± 16 min. Despite the several-fold difference in these parameters, recovery of trichosanthin from normal rat urine was only 0.38 ± 0.05%. This value can be increased by using higher injection doses. The data indicate that the kidney is an important organ for the elimination of trichosanthin. When the dextran–trichosanthin complex was injected into normal rats trichosanthin activity was not detected in the urine. All the pharmacokinetic parameters suggest that the dextran–trichosanthin complex stayed longer in the body and maintained a much higher plasma concentration than trichosanthin.

Trichosanthin (TCS) is a basic plant protein extracted from the root tubers of *Trichosanthes kirilowii* of the Cucurbitaceae family. Its purified form is also known as GLQ223 [1–3]. The molecular weight is about 26,000 daltons and consists of 247 amino acids. It is a single polypeptide chain for which the primary, secondary and the 3-dimensional structure [4–6] have been established and the gene encoding TCS has been isolated and cloned [7].

Clinical trials in China showed that TCS administered in single doses from 5 to 12.5 mg induced midtrimester abortion [8]. Trichosanthin is also known to inhibit the growth of choriocarcinoma *in vitro* [9]. Recently McGrath *et al.* [10] demonstrated that this compound inhibits replication of human immunodeficiency virus (HIV-1) in primary monocyte/macrophages and T-lymphoblastoid cells *in vitro*. The mechanism of action is believed to be related to inhibition of protein synthesis [11, 12]. Trichosanthin is a Type I ribosome-inactivating protein. It inhibits protein synthesis by inactivating eukaryotic ribosomes through catalytic cleavage of a single adenine base from a highly specific site on the 28S RNA of the 60S ribosomal subunit [13–16].

The TCS molecule is small enough to undergo glomerular filtration, and therefore significant renal clearance of this compound is anticipated. This will lead to a reduction of its half-life in the body. Should glomerular filtration be an important determinant for TCS elimination, coupling of TCS to dextran could effectively increase its molecular size and therefore enable it to escape glomerular filtration. The idea of preventing glomerular filtration of one molecule by coupling it to another macromolecule is not new. For example, in the search for a better blood substitute, coupling of dextran to hemoglobin prevented its renal loss and prolonged its half-life in the circulation. Biological activity was mostly retained after coupling and the complex was found to be non-immunogenic in homologous species [17–20].

This study describes the coupling of TCS to dextran T40 and also examines whether the complex has an improved half-life in the circulation. A sensitive radioimmunoassay was also developed to detect changes in plasma and urine TCS concentration.

MATERIALS AND METHODS

Preparation of antigen and antisera. Trichosanthin was purified from *Trichosanthes kirilowii* of the Cucurbitaceae family according to a previously described method [1]. Polyclonal antiserum against

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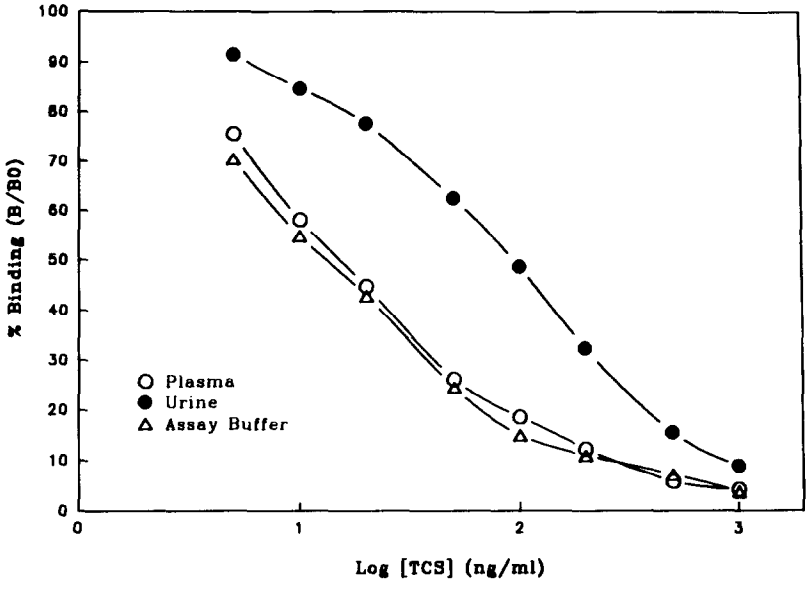

Fig. 1. Typical standard curves for TCS assay performed in buffer, plasma and urine.

denatured TCS was raised in 3-month-old (2.5 kg) albino rabbits. Crystalline trichosanthin (100 μg in 0.25 mL normal saline containing 0.5% sodium dodecyl sulphate) was denatured by heat treatment at 80° for 10 min and then diluted by normal saline to a final concentration of 200 μg/mL. An equal volume of Freund's complete adjuvant was added to emulsify the antigen. At the beginning, 1 mL of this emulsion was injected intradermally at the back of a rabbit. Five booster injections containing the same quantity of antigen but emulsified with Freund's incomplete adjuvant were given at equal time intervals for the following 15 weeks. During this time, the activities of the antiserum were monitored by immunoblotting [21] using the ProtoBlot™ system (Promega, WI, U.S.A.). Two weeks after the last injection the rabbit with the highest antibody titre was killed and the antiserum stored at −20°.

Iodination of TCS. Trichosanthin was iodinated by using the chloramine-T method. The reaction mixture contained 500 μCi of $Na^{125}I$ (in 5 μL 500 mM phosphate buffer, pH 7.5), 10 μg chloramine-T (in 10 μL PBS, pH 7.6) and 5 μg TCS (in 10 μL PBS, pH 7.6). Metabisulphite (50 μg in 100 μL PBS, pH 7.6) was added after 10 sec to stop the reaction. The unreacted iodide was removed by gel filtration on a Sephadex G-75 column (1 × 14 cm). Non-specific binding sites were saturated by rinsing the column with 1 mL of 2% bovine serum albumin (BSA) followed by 30 mL of PBS (pH 7.6). The iodination mixture was eluted with PBS (pH 7.6), and 1.5 mL fractions of the eluate were collected into test tubes containing 0.2 mL 1% BSA and 0.1% sodium azide in the elution buffer. The four tubes containing the highest count of [^{125}I]TCS were used and stored at 4°.

Radioimmunoassay procedure. Radioimmunoassay was performed by using a double-antibody polyethylene glycol (PEG) method under equilibrium conditions. The assay buffer contained 0.01 M PBS, 1% BSA and 0.1% sodium azide, which was used to dilute TCS standards, antisera, normal rabbit serum and labelled antigen. A typical reaction mixture contains 100 μL each of [^{125}I]TCS (about 20,000 cpm), 2% normal rabbit serum, standard (5–1000 ng/mL) or sample, antisera (final concentration 1:25,000) and assay buffer. Non-specific binding is accessed by replacing the aliquots of antisera and standard with same volumes of assay buffer. The final volume is 500 μL in all cases and all tubes were made in duplicates. The assay tubes were then incubated overnight at room temperature.

To separate the free antigen from the antibody–antigen complex, 200 μL of goat anti-rabbit γ-globulin (1:40 in assay buffer with 10% PEG 6000) was added and the mixture incubated for 15 min at 4°. It was then centrifuged for 4 min at 9000 rpm at 0°. The supernatant was removed, washed once with ice-cold PBS and the radioactivity in the pellet determined with a Kontron gamma counting system. Results were automatically calculated using a computer program, based on log–logit transformation. Under these conditions, the specific binding was about 33% while the non-specific binding was less than 3%.

Coupling of trichosanthin to dextran T40. Dextran–trichosanthin (DX–TCS) conjugate was prepared by the dialdehyde method as described previously [17]. In this method, 10 mL of dextran T40 (100 mg/mL) was first oxidized by 1 mL of sodium periodate (120 mg/mL) and then left overnight in the dark at 4°. Afterwards, freshly prepared sodium bisulphite (300 mg/mL) was added until the mixture turned brown and then colourless. The activated dextran was then dialysed against distilled water. Coupling of TCS to dextran was done at different TCS/dextran molar ratios of 1:100, 1:50, 1:25, 1:10, 1:5 and 1:2.5, respectively. The pH of the mixture was adjusted to

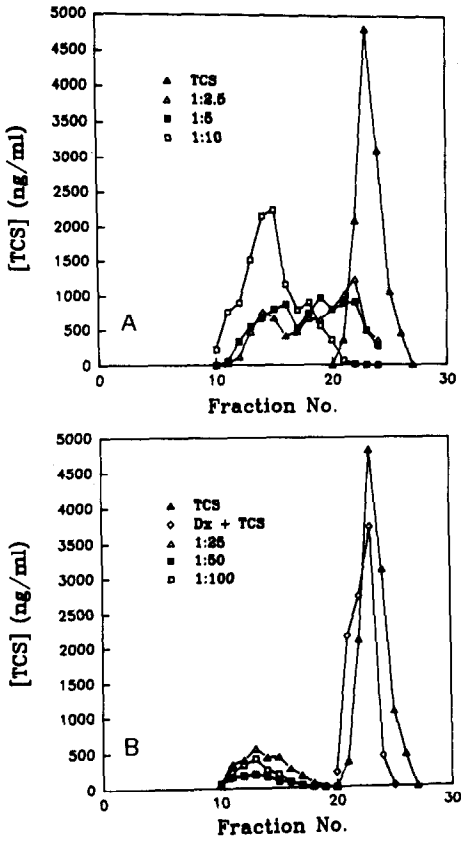

Fig. 2. Elution profile of different reaction mixtures at different dextran and TCS ratios on Sephadex G200 column.

9.5 by adding 0.3 M sodium carbonate and the mixture was left overnight at 4°. Successful coupling was confirmed by Sephadex G-200 column (1.8 × 55 cm) chromatography eluted with PBS, pH 7.6. Four-millilitre fractions were collected and the TCS concentration of each fraction was determined by radioimmunoassay as described above. An alternative way to assess coupling is by ultrafiltration through a membrane with molecular cut off of 30,000 (Centricon 30). Trichosanthin is small enough to pass through the membrane and appears in the filtrate while DX–TCS will be excluded. Plasma protein binding to TCS can also be evaluated similarly. If there is significant protein binding to TCS its filtration will be impaired.

Binding of DX–TCS to TCS antibodies. To examine whether DX–TCS retains its specific binding capacity to TCS antibodies, competitive binding studies were performed as described above. Iodinated TCS competes with DX–TCS for binding to the TCS antibodies. A binding study of DX–TCS with the dextran moiety digested by incubating the complex with dextranase at pH 7.6 and 36° for 20 hr was performed also. The respective effects of dextran T40, dextranase and both on the TCS radioimmunoassay were examined by adding these substances to the assay.

Pharmacokinetic study. The pharmacokinetics of a single i.v. bolus injection of TCS or DX–TCS were compared in Sprague–Dawley rats weighing between 350 and 400 g. After an overnight fast, the rats were anesthetized with 4.5% pentobarbital (60 mg/kg). The carotid artery and jugular vein were cannulated for blood sampling and drug injection, respectively. Trichosanthin (0.75 mg/kg) was injected into normal (group 1, N = 6) and renal arterial ligated (group 2, N = 8) rats, while DX–TCS was injected into normal rats (group 3, N = 6). Renal function was assessed by tritiated inulin. Plasma samples (0.3 mL) were taken at 2, 5, 15, 25, 35, 45, 90, 135 and 180 min and urine samples were collected at 45, 90, 135 and 180 min after drug administration. Plasma and urine concentrations of TCS or DX–TCS were determined by the radioimmunoassay described above. Determination of plasma DX–TCS was done

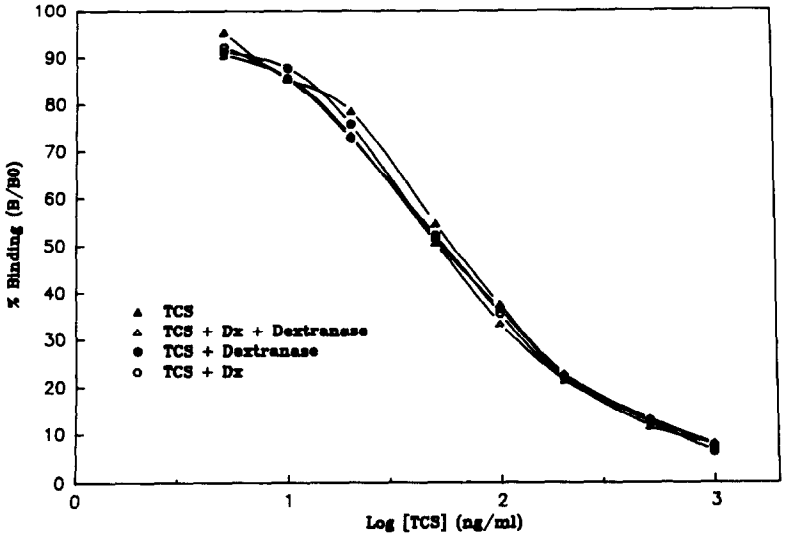

Fig. 3. The effect of dextran and/or dextranase on the TCS competitive binding curve.

under disequlibrium condition using DX–TCS as standards to increase the sensitivity of the assay.

Data analysis. Pharmacokinetic parameters were calculated by the BITRI computer program for compartmental analysis and the PKCALC computer program for non-compartmental analysis. Statistical analysis of group differences were performed by Tamhane's multiple comparison with unequal variances [22] at significant level: $P < 0.05$.

RESULTS

Radioimmunoassay for TCS

Typical standard curves obtained from the radioimmunoassay are shown in Fig. 1. For the curve produced from the standard assay buffer addition of rat plasma makes no statistical difference, while addition of urine shifted the curve to the right. The slopes of the three curves were almost identical. The intra-assay and inter-assay variances were 7.5 and 9.7%, respectively. The sensitivity of the assay was found to be 2.5 ng/mL in plasma and 5.0 ng/mL in urine.

Coupling of TCS to dextran

After chemical coupling of TCS to dextran, TCS activity was eluted at a much faster rate (Fig. 2) on the Sephadex column. This indicated successful coupling and elution of the DX–TCS complex which had a larger molecular size and moved faster in the column. There was clear separation between TCS and DX–TCS when the molar ratio of the reaction was greater than 1:25 (Fig. 2B) whereas overlapping representing unreacted TCS occurred below this ratio (Fig. 2A). The lowest molar ratio (1:25) producing complete coupling will be used as a standard reaction condition to prepare the complex for all subsequent experiments. In addition, ultrafiltration study through a Centricon 30 (molecular cut off 30,000) showed that TCS was able to pass through the filter while no detectable DX–TCS activity was found in the filtrate, supporting that coupling of TCS to dextran was complete. In a similar experiment, it was also found that incubation of TCS with plasma did not affect its filtration (filtration of TCS in PBS and plasma yielded filtrate concentration of 269 ± 54 and 284 ± 62 ng/mL respectively). This demonstrated that plasma protein binding to TCS was minimal.

Binding of DX–TCS to TCS antibodies

The radioimmunoassay for TCS was not affected by the presence of dextran T40, dextranase or both (Fig. 3). When this assay was applied on DX–TCS, the binding curve shifted to the right suggesting a decrease in affinity between DX–TCS and the TCS antibodies. The shift can be partially reversed when the dextran moiety of the complex was digested by dextranase (Fig. 4).

Pharmacokinetics

In order to investigate the role of the kidney in eliminating TCS, renal function of group 2 rats was impaired by bilateral ligation of the renal arteries. Since the kidney is the only organ that removes inulin, plasmal inulin concentration should decrease with time in normal rats with normal renal function. In contrast, a constant plasma inulin concentration indicates complete loss of renal function. In the present experiment, loss of renal function in the ligated rats can be demonstrated by the constant inulin concentration (Fig. 5). When TCS was injected into normal rats, there was a rapid decrease in plasma concentration. In contrast, injection of the same dose of DX–TCS resulted in a much higher plasma TCS concentration throughout the experiment (Fig. 6). The plasma DX–TCS concentration in normal rats was qualitatively similar to plasma TCS concentration in ligated rats. Comparing with TCS injection (into normal and ligated rats), DX–TCS injection also resulted in having the longest mean residence time and lowest rate of plasma clearance (Table 1). All these parameters suggested that the modified form of TCS remained much longer in the circulation. The total amount of TCS that could be recovered from normal rat (group 1) urine within the experimental period of 180 min was $0.38 \pm 0.05\%$ of the amount administered. The percentage recovery was much higher ($3.70 \pm 0.70\%$) when the dose was doubled and further increased to $20.67 \pm 3.54\%$ when the dose was 3 mg/kg. Almost all the urine TCS was recovered in the first 45 min ($86 \pm 5\%$). No DX–TCS activity was detected in any rats infused with DX–TCS (group 3).

Most of the pharmokinetic parameters calculated from compartmental or non-compartmental analysis were very similar. In compartmental analysis, TCS injection (group 1) was consistent with a three-compartment model while DX–TCS injection (group 3) or TCS injection into ligated rats (group 2) were consistent with a two-compartment model. All pharmacokinetic parameters (Table 1) were significantly different between the three groups of rats ($P < 0.05$).

DISCUSSION

A sensitive radioimmunoassay method for detecting TCS in the nanogram range is described in this study. The assay performed in rat plasma was not different from those in standard assay buffer. However, there was a right shift of the standard curve when the assay was performed in urine, suggesting the presence of inhibitory substances in urine that affect antibody–antigen reaction.

Trichosanthin has a molecular weight of about 26,000 daltons which is small enough to pass through the glomerulus and be lost in urine. This means that TCS is expected to be cleared rapidly by the kidney. Pharmacokinetic studies with ^{125}I-labelled TCS in mice demonstrated that the compound was rapidly taken up by the kidney and excreted. Blood elimination half-life was about 10 min [23]. There are numerous examples that the plasma half-life of such a protein can be prolonged by coupling it to a macromolecule like dextran [17, 24, 25]. The mechanism of increased plasma persistence may be due to decreased glomerular filtration or protection from degradation afforded by dextran. The biological activity of the protein is usually retained and the antigenicity sometimes reduced due to masking of antigenic determinants [26, 27]. Appropriate drug

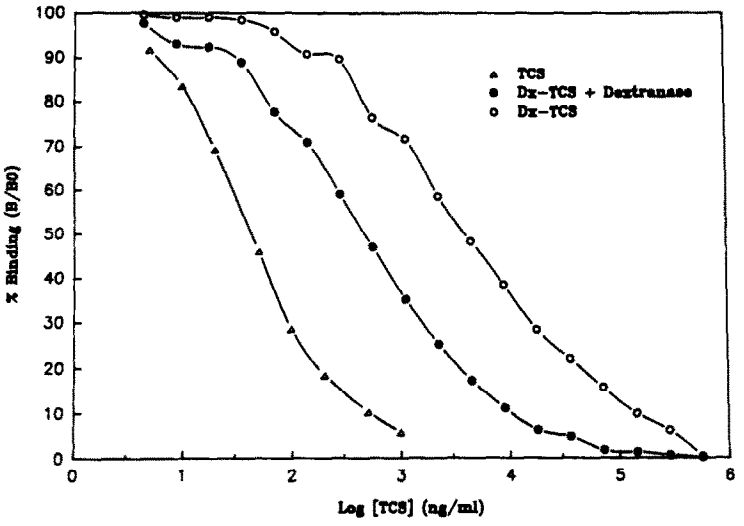

Fig. 4. The effect of dextranase on the DX–TCS competitive binding curve. Dextranase was incubated with DX–TCS for 24 hr.

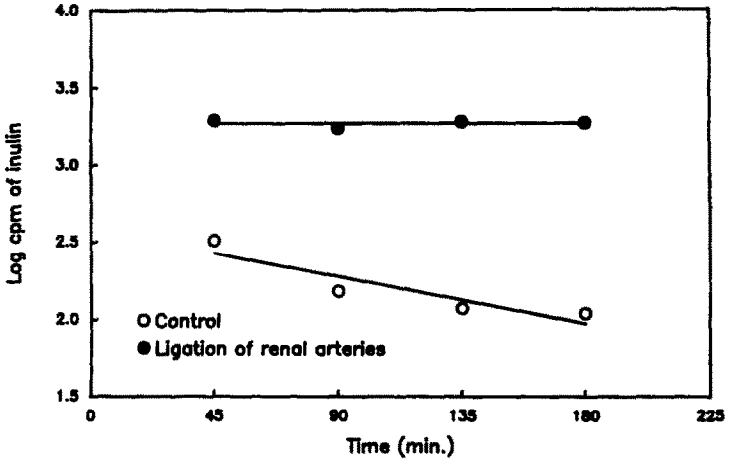

Fig. 5. Plasma inulin concentration in normal and ligated rats. Same amount of inulin was administered at time zero. Values are expressed as means ± SEM, N = 6.

modification of this type can be valuable in improving drug half-life in circulation and cutting out side effects. This study describes the coupling of TCS to dextran by a dialdehyde method. Some antibody binding behaviour and pharmacokinetics of the covalent complex were also investigated.

The binding curve of DX–TCS to TCS antibodies shifted parallel to the right of the TCS binding curve. This suggests specific binding of DX–TCS to the TCS antibodies but with decreased affinity relative to TCS. This is likely to be due to steric hindrance of dextran on TCS so that some antigenic determinants on TCS are not accessible by the antibodies. This point is supported by observing an increase in DX–TCS binding affinity towards that of TCS after the dextran moiety of DX–TCS was degraded by dextranase. Free dextran or dextranase do not seem to affect the binding.

Injection of DX–TCS produced the highest plasma TCS concentration at any time during the experiment compared to injection of the same dose of TCS into normal and renal arterial ligated rats. No DX–TCS activity was detected in urine. The pharmacokinetic parameters such as plasma clearance (Cl_p), mean residence time (MRT) and area under curve (AUC_0^∞) all support that DX–TCS remained in the circulation much longer than TCS. If glomerular filtration of TCS is the only parameter affected, one will anticipate that the pharmacokinetics of DX–TCS in normal rats will be the same as TCS in renal arterial ligated rats. The present experimental result indicated that DX–TCS remained longer in the

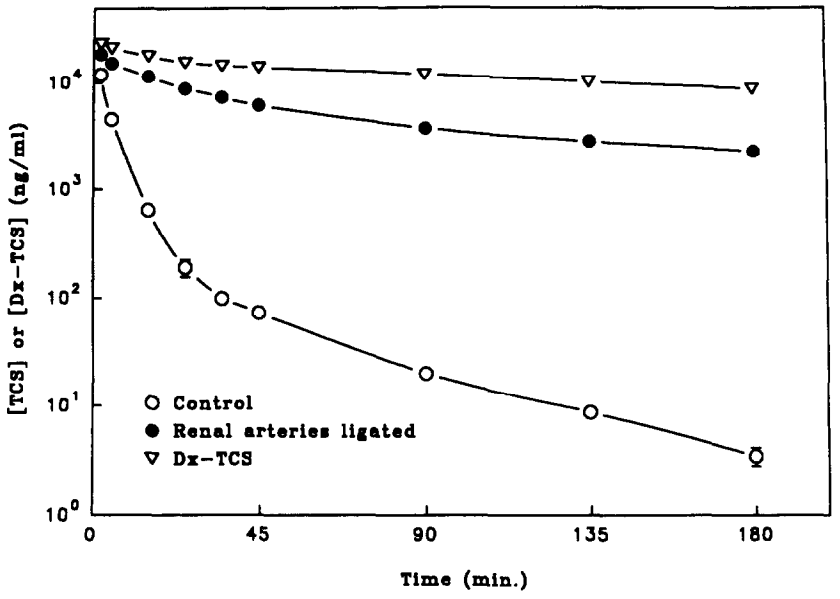

Fig. 6. Plasma TCS or DX-TCS concentration in normal and ligated rats. Same amount of TCS or DX-TCS was administered at time zero. Values are expressed as means ± SEM.

Table 1. A summary of some pharmacokinetic parameters in normal rats infused with TCS or DX-TCS and ligated rats infused with TCS

Non-compartmental analysis	Normal (N = 6)	Ligated (N = 8)	DX-TCS (N = 6)
V_z (mL/kg)	507 ± 36	97 ± 6	45 ± 1
V_{ss} (mL/kg)	127 ± 9	84 ± 4	45 ± 1
AUC_0^∞ (μg·min/mL)	59 ± 6	1285 ± 102	5095 ± 1634
Cl_p (μL/min)	4780 ± 570	220 ± 20	60 ± 40
MRT (min)	9 ± 1	145 ± 16	308 ± 41

Values are expressed as means ± SEM.
* $P < 0.05$.
Abbreviations: V_z, apparent distribution volume; V_{ss}, steady state distribution volume; AUC_0^∞, area under curve; Cl_p, plasma clearance; MRT, mean residence time.

plasma compartment than TCS in ligated rats. This suggests that coupling of TCS to dextran not only reduces its rate of renal clearance, but also its rate of non-renal degradation. The covalent complex may be more resistant to enzymatic degradation due to protection afforded by dextran.

In compartmental analysis, it appears that a major distribution compartment for TCS is lost after coupling to dextran. This compartment is likely to be the kidney into which the complex DX-TCS cannot enter due to the large molecular size. The same compartment is also lost when TCS is infused into ligated rats which do not have a functional kidney. Furthermore, the apparent (V_z) and steady state (V_{ss}) volume of distributions are all reduced after coupling, suggesting that the larger DX-TCS complex has a smaller distribution volume.

The mean residence time of TCS was roughly 10 min in normal and 145 min in ligated rats. It meant that more than 60% of the administered TCS was removed within the experimental period of 180 min. Since all pharmacokinetic parameters suggested that the renal component assumed a more significant role in eliminating TCS, a large percentage of the amount excreted should be through the kidney and recoverable from urine. However, only 0.38 ± 0.05% of the administered TCS were recovered from urine within the 180 min of experiment. It led to the speculation that after glomerular filtration, an ample amount of TCS was somehow retained by the kidney. It can either precipitate in the renal tubule or be reabsorbed by tubular cells. The amount of TCS cleared by the kidney therefore cannot be fully recovered in urine. Renal tubular TCS reabsorption is more likely for the following reasons: (1) Kidney is the major site for metabolism of small molecular weight proteins which undergo glomerular filtration (e.g. ribonuclease, retinol binding protein, insulin) [28–30]. In 1 hr, the proximal tubular cells are capable of

reabsorbing up to 0.2 mg/kg body weight of small molecular weight proteins [31]. This quantity is in the same order of magnitude as the amount of TCS administered meaning that the kidney is capable of reabsorbing most of the TCS. (2) Majority of the urine TCS (86 ± 5%) was recovered in the first 45 min after injection. In this period, plasma TCS concentration and therefore the filter load was highest. This exceeded the capacity of the kidney to reabsorb TCS and a relatively large amount of TCS was recovered from the urine. Afterwards, plasma concentration decreased drastically. The kidney was able to reabsorb most of the filtered TCS and therefore little was excreted in the urine. (3) For the same reasoning, increasing the injection dose to 1.5 and 3 mg/kg increased the recovery to 3.7 and 20%, respectively. This means that recovery can actually be increased by using a higher injection dose to exceed the renal reabsorptive capacity of TCS so that it can be split into the urine.

In conclusion, covalent coupling of TCS to dextran prolongs the survival of TCS in the circulation. The complex retained specific binding capacity to TCS antibodies but with decreased affinity. This may improve the plasma half-life and reduce the antigenicity of TCS. The biological activity of the complex remains to be explored.

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REFERENCES

1. Yeung HW, Wong DM, Ng TB and Li WW, Purification of three isolectins from root tubers of *Trichosanthes kirilowii* (Tian hua fen). *Int J Pept Protein Res* **27**: 325–333, 1986.
2. Wang Y, Qian RQ, Gu ZW, Jin SW, Zhang LQ, Xia ZX, Tian GY and Ni CZ, Scientific evaluation of Tian Hua Fen: history, chemistry and application. *Pure Appl Chem* **58**: 789–798, 1986.
3. Yeung HW, Poon SP, Ng TB and Li WW, Isolation and characterization of an immunosuppressive protein from *Trichosanthes kirilowii* root tubers. *Immunopharmacol Immunotoxicol* **9**: 25–46, 1987.
4. Pan KZ, Zhang YM, Lin YJ, Wu CW, Zheung A, Chen YZ, Dong C, Ma XQ, Wang YP, Wu S, Zhang MG, Chen SZ, Xia ZX, Tian GY, Ni CZ, Fan, ZC, Ma YL and Sun XX, The backbone structure of Trichosanthin. In: *Advances in Chinese Medicinal Materials Research* (Eds. Chang HM, Yeung HW, Tso WW and Koo A), pp. 297–303. World Scientific Publication, Singapore, 1985.
5. Wang Y, A new biologically active plant protein. In: *Advances in Chinese Medicinal Materials Research* (Eds. Chang HM, Yeung HW, Tso WW and Koo A), pp. 289–295. World Scientific Publication, Singapore, 1985.
6. Kubota S, Yeung HW and Yang JT, Conformation of abortifacient proteins: trichosanthin, α-momorcharin and β-momorcharin. *Biochim Biophys Acta* **871**: 101–106, 1986.
7. Chow TP, Feldman RA, Lovett M and Piatak M, Isolation and DNA sequence of a gene encoding α-Trichosanthin, a type I ribosome-inactivating protein. *J Biol Chem* **265**: 8665–8669, 1990.
8. Cheng KF, Midtrimester abortion induced by *Radix trichosanthis*: morphologic observations in placenta and fetus. *Obest Gynecol* **59**: 494–498, 1982.
9. Tsao SW, Yan KT and Yeung HW, Selective killing of choriocarcinoma cells *in vitro* by trichosanthin, a plant protein purified from root tubers of the Chinese medicinal herb *Trichosanthes kirilowii*. *Toxicon* **24**: 831–840, 1986.
10. McGrath MS, Hwang KM, Caldwell SE, Gaston I, Luk KC, Wu P, Ng VL, Crowe S, Daniels J, Marsh J, Deinhart T, Lekas PV, Vennari JC, Yeung HW and Lifson JD, GLQ223: an inhibitor of human immunodeficiency virus replication in acutely and chronically infected cells of lymphocyte and mononuclear phagocyte lineage. *Proc Natl Acad Sci USA* **86**: 2844–2848, 1989.
11. Yeung HW, Li WW, Feng Z, Barbieri L and Stripe F, Trichosanthin, α-momorcharin and β-momorcharin: identity of abortifacient and ribosome-inactivating proteins. *Int J Pept Protein Res* **31**: 265–268, 1988.
12. Zhang X and Wang J, Homology of tirchosanthin and ricin A chain. *Nature* **321**: 477–478, 1986.
13. Endo Y, Mitsui K, Motizuki M and Tsurugi K, The mechanism of action of ricin and related toxic lectins on eukaryotic ribosomes. *J Biol Chem* **262**: 5908–5912, 1988.
14. Stripe F, Bailey S, Miller SP and Bodley JW, Modification of ribosomal RNA by ribosome-inactivating proteins from plants. *Nucleic Acid Res* **16**: 1349–1367, 1988.
15. Endo Y, Isurugi K, Yutsudo T, Takeda Y, Dgasawara T and Igarashi K, Site of action of a vero toxin (VT2) from *Escherichia coli* 0157: H7 and of shiga toxin on eukaryotic ribosomes. *Eur J Biochem* **171**: 45–50, 1988.
16. Brigotli M, Rambeeli F, Zamboni M, Montaneno L and Sperti S, Effect of α-sarcin and ribosome-inactivating proteins on the interaction of elongation factors with ribosomes. *Biochem J* **257**: 723–727, 1989.
17. Tam SC, Blumenstein J and Wong JTF, Soluble dextran-hemoglobin complex as a potential blood substitute. *Proc Natl Acad Sci USA:* **73**: 2128–2131, 1976.
18. Blumenstein J, Tam SC, Chang JE and Wong JTF, Experimental transfusion of dextran-hemoglobin. In: *Blood Substitutes and Plasma Expanders* (Eds. Jamieson GA and Greenwalt TJ), pp. 205–212. Alan R. Liss Inc., New York, 1978.
19. Tam SC, Blumenstein J and Wong JTF, Blood replacement in dogs by dextra–hemoglobin. *Can J Biochem* **56**: 981–984, 1978.
20. Cunnington PG, Jenkins SN, Tam SC and Wong JTF, Oxygen binding and immunological properties of complexes between dextran and animal hemoglobin. *Biochem J* **193**: 261–266, 1981.
21. Sambrook J, Fritsch EF and Maniatis T, Screening expression libraries with antibodies and oligonucleotides. In: *Molecular Cloning. A Laboratory Manual*, 2nd edn, Chapter 12. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.
22. Tamhane AC, Multiple comparisons in model I one-way ANOVA with unequal variances. *Commun Statistics* **A6**: 15–32, 1977.
23. Byers V, Levin A, Waites L, Starrett B, Mayer R, Clegg J, Price M, Robin R, Delaney M and Baldwin R, A phase I/II study of trichosanthin treatment of HIV disease. *AIDS* **4**: 1189–1196.
24. Marshall JJ, Humphreys JD and Abramson SL, Attachment of carbohydrate to enzymes increases their circulatory lifetimes. *FEBS Lett* **83**: 249–252, 1977.
25. Melton RG, Wiblin CN, Foster RL and Sherwood RF, Covalent linkage of carboxypeptidase G_2 to soluble dextran—I. Properties of conjugates and effects on plasma persistence in mice. *Biochem Pharmacol* **36**: 105–112, 1987.
26. King TP, Kochoumian L, Ishizaka K, Lichtenstein LM and Norman PS, Immunochemical studies of dextran

coupled ragweed pollen allergen, antigen E. *Arch Biochem Biophysics* **169**: 464–473, 1975.
27. Seppala I and Makela O, Antigenicity of dextran–protein conjugates in mice: effect of molecular weight of the carbohydrate and comparison of two modes of coupling. *J Immunol* **143**: 1259–1264, 1989.
28. Bernard A, Amor AO, Viau C and Lauwerys R, The renal uptake of proteins: a non-selective process in conscious rats. *Kidney Int* **34**: 175–185, 1988.
29. Mogenson CE and Sølling K, Studies on renal tubular protein reabsorption: partial and near complete inhibition by certain amino acids. *Scan J Clin Lab Invest* **37**: 477–486, 1977.
30. Cojocel C, Franzen-Sieveking M, Beckmann G and Baumann K, Inhibition of renal accumulation of lysozyme (basic low molecular weight protein) by basic protein and other basic substances. *Pflügers Arch* **390**: 211–215, 1981.
31. Waldmann TA, Strober W and Mogielnicki RP, The renal handling of low molecular weight protein. *J Clin Invest* **51**: 2162–2174, 1972.