Enhancement of Fibrinolysis and Thrombolysis by Polysorbate 80 (Tween 80®)

We wish to report some observations on urokinase (UK) which indicate that Polysorbate 80 (Tween 80®) can enhance fibrinolysis in vitro and thrombolysis in vivo. The enhancing effect in vitro is in part caused by a decrease in adsorption of UK to glass in the presence of Polysorbate. Whether a similar mechanism is involved in vivo is as yet unknown.

Experimental. UK preparations: Leo Pharmaceuticals, Copenhagen: Batch No. 161071, 4200 Ploug U/mg (equivalent to approximately 5250 C.T.A. units, as defined by the Committee on Thrombolytic Agents, Advisory to the National Heart Institute, National Institutes of Health). Batch No. 63012, 25,000 Ploug U/mg (approximately 31,250 C.T.A. units). Batch No. 63097, 7500 Ploug U/mg (approximately 9400 C.T.A. units). Sterling-Winthrop: Batch No. 64-489, 85,000 C.T.A. U/vial. Saline barbital buffer (SBB): 0.05 *M* barbital in 0.10 *M* NaCl at pH 7.75. Gelatine-buffer: SBB with 0.25% gelatine (granular, for bacteriological use, Fisher Scientific). Polysorbate-buffer: SBB with 0.005% Polysorbate 80 (Tween 80®, Nutritional Biochemical Corporation). Glass powder, 200 mesh (Fisher Scientific). Assays of fibrinolytic activity were performed on normal unheated fibrin plates prepared from plasminogen-rich bovine fibrinogen (0.1%)[1] with bovine thrombin (Leo Pharmaceuticals).

In vitro experiments. UK solutions containing Polysorbate in concentrations above 0.02%, when dropped on the fibrin plates, spread over the whole surface of the fibrin and could not be assayed. Polysorbate in a concentration of 0.005% was nearly as effective in preventing adsorption of UK to glass as a 0.25% gelatine solution (Table). After adsorption to glass in the presence of Polysorbate, additional fibrinolytic activity could be extracted from the glass with fresh Polysorbate solution, yielding an increase in total activity and suggesting a potentiating effect on UK. Gelatine behaved similarly (Table). When adsorption had taken place in SBB alone a small fraction only of the adsorbed UK could be recovered from the glass powder by treatment with Polysorbate. Fibrin plates containing 0.25% gelatine in the fibrin clot gave only a negligible increase in activity over the normal plates. In contrast, fibrin plates prepared with 0.005% Polysorbate gave a twofold increase in activity though the drops tended to spread making the reading of assays difficult.

In vivo experiments. Because Polysorbate is used in media for tissue cultures and can be injected into animals in reasonable concentrations without untoward signs of harm, it was thought of interest to see whether the thrombolytic properties of UK would be enhanced in the presence of Polysorbate.

Thrombi were produced in white New Zealand female rabbits (3–3.5 kg) in a 2 cm long segment of the marginal ear vein isolated between 2 bulldog clamps. Blood in the segment was withdrawn and replaced with 0.05 ml of 3% sodium morrhuate solution (27 gauge needle). After 15 sec both clamps were removed. The segment was observed at intervals by transillumination with a powerful pencil flashlight. During the next 15–20 min the lumen gradually became occluded by a thrombus and the flow of blood stopped. After the thrombus had been observed for an appropriate length of time the venous segment was removed under Nembutal® anesthesia. Paraffin sections were prepared and stained with hematoxylin and eosin. The lowest concentration of sodium morrhuate regularly producing complete occlusion was found to be 3%. None of the thrombi thus produced showed signs of lysis within 72 h. This concentration was, therefore, used in the comparative experiments. In all animal experiments sterile physiological saline was used as diluent for UK or Polysorbate.

2 different series of experiments were run. In the first series each of the thrombosed rabbits received i.v. every $^1/_2$ h in the contralateral ear 1 ml of UK solution (2500 C.T.A. U/ml). In 3 rabbits receiving the UK solution alone clot lysis occurred after 10, $10^1/_2$ and 12 h respectively. In 3 other rabbits receiving the UK in 5% Polysorbate solution clot lysis occurred after $6^1/_2$, $6^1/_2$ and 7 h. In control series in which the thrombosed animals received saline or Polysorbate solution alone no lysis occurred within 72 h.

Since thrombolysis depends upon the ability of UK to come into contact with the thrombus, in another series of experiments the animals were primed with UK so that thrombus formation took place in the presence of fibrinolytic activity in the circulating blood. In these animals 1 ml of the UK solution was injected i.v. 5 min before the venous segment was clamped and thrombus formation induced. Thereafter, 1 ml UK solution was given as before every $^1/_2$ h until the experiment was terminated. When the test solution contained UK alone (2500 C.T.A. U/ml), in 8 animals lysis was observed after the following periods of time in h: 7, 7, $6^1/_2$, $7^1/_2$, 7, 8, 10 and $9^1/_2$ (average: $7^{48}/_{60}$). In another similar group of 8 rabbits the test solution contained UK in 5% Polysorbate. Clot

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Effects of Polysorbate 80 and gelatine on adsorption of urokinase to glass

Glass mg/ml	SBB		Polysorbate-SBB		SBB		Gelatine-SBB	
	Supernatant	Eluate[a]	Supernatant	Eluate[a]	Supernatant	Eluate[b]	Supernatant	Eluate[b]
0	543	–	564	–	550	–	600	–
5	227	227	556	146	63	334	625	16
10	16	258	465	125	0	280	644	134
25	0	186	440	133	0	209	650	182

[a] Eluates with Polysorbate, 0.005%. [b] Eluates with gelatine, 0.25%. The urokinase (No. 161071) solutions containing 2.5 C.T.A. U/ml were shaken slowly for 30 min at room temperature with the amounts of glass powder mentioned. After centrifugation (15 min at approximately 1500 *g*) the supernatant was replaced with an equal volume of the solution mentioned[a, b] and the suspension was shaken for 15 min. The fibrinolytic activity was assayed by placing drops of 0.030 ml on the fibrin plates and recorded as the diameter products of the lysed zones (average of triplicate).

lysis occurred after the following periods in h: $2^3/_4$, $3^1/_2$, 4, $4^1/_2$, 4, 5, $3^1/_2$ and 4 (average: $3^{55}/_{60}$).

In a third series, separate solutions of UK (2500 U/ml) and 5% Polysorbate were given simultaneously in separate veins. In 9 animals lysis occurred after the following hours: $7^1/_2$, $7^1/_2$, 6, $4^1/_2$, 4, 5, $7^1/_2$, 7 and 8 (average: $6^{19}/_{60}$). This approaches the period of time required for UK alone.

Discussion. Many organic compounds are known to enhance lysis of fibrin clots[2]. Quaternary detergents enhance the fibrinolytic activity of trypsin and in small concentrations also that of streptokinase-activated human plasmin (chiefly consisting of SK-activator)[3]. Some non-ionic wetting agents also enhanced fibrinolysis by SK-activator, presumably due to a stabilizing influence[4]. Loss in activity caused by adsorption to glass can often be prevented by gelatine[5,6] or by Tween 80®[7]. Our results show that gelatine or Polysorbate 80 prevents or decreases the adsorption of UK to glass, but a potentiating effect appears also to exist, since the total yield of activity was increased. Triton X, another surface-active agent, releases plasminogen activator from lysosomes[8]. Intravenously applied, an inhibition of fibrinolysis was reported[9]. Sodium morrhuate, an anionic detergent, destroys fibrinolytically active cells of the vessel wall[10]. Our results indicate that Polysorbate enhances the effect of UK on thrombolysis in vivo. Whether this enhancement is due to a stabilization of the active compound or to a prevention of its removal by adsorption is as yet unknown. Interestingly, a compound enhancing thrombolysis in a simulated circulation system has been isolated from red blood cells[11], suggesting a possible role in the normal resolution of thrombi[12].

Zusammenfassung. Polysorbate 80 (Tween 80®), eine polyhydroxyle, nicht-ionisierbare, oberflächenaktive Substanz, vermindert die Adsorption von Urokinase an Glas, erhöht deren fibrinolytische Aktivität in vitro und steigert ihre thrombolytische Wirkung in experimentell hervorgerufenen Thrombosen im Kaninchen.

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Inhibition par les sels de tetrazolium de l'activité monoamine-oxydasique d'une suspension mitochondriale de cerveau de mouton

Les sels de tetrazolium sont d'un emploi fréquent en microscopie optique et électronique pour la détection de diverses deshydrogénases; c'est pourquoi il nous a paru intéressant d'étudier l'action éventuelle de ces sels sur l'activité de l'une d'entre elles: la monoamine-oxydase d'origine cérébrale.

Matériel et méthodes. (1) Source enzymatique. Nous avons choisi comme préparation enzymatique une suspension de mitochondries de cerveau de mouton préparée d'après Somogyi et al.[1]. Les cerveaux d'agneaux de 6–7 mois recueillis à l'abattoir immédiatement après l'abattage dans une solution de saccharose 0,25 *M* maintenue à environ 4 °C, sont ensuite broyés et homogénéisés au broyeur de Potter dans une solution de saccharose 0,25 *M* tamponnée à pH 7,4 (EDTA: 0,001 *M* – tampon *Tris* HCl 0,01 *M*). Le surnageant obtenu après une première centrifugation de 10 min à 1150 *g* est centrifugé 15 min à 12650 *g*. Le culot ainsi obtenu est repris par une solution de saccharose 0,25 *M* non tamponnée et centrifugé 10 min à 16500 *g*. Le dernier culot est finalement repris par une solution de saccharose 0,25 *M* non tamponnée de facon à obtenir environ 0,5 ml de suspension pour 1 g de tissu cérébral frais.

Toutes les opérations sont conduites en chambre froide entre 0 et 4 °C et les suspensions obtenues sont conservées congelées à – 20 °C.

Les préparations mitochondriales ont été contrôlées par une étude au microscope électronique. Les culots de la dernière centrifugation sont fixés à l'acide osmique à 2% en milieu tamponné selon Palade[2] pendant 1 h puis, après deshydratation à l'alcool éthylique, inclus dans l'araldite selon la méthode de Glauert et Glauert[3]. Les coupes ultra fines pratiquées grâce à un ultra-microtome Porter Blum ont été examinées sans coloration prealable avec un Superscope JEM. Les images obtenues montrent des plages de mitochondries semblant gonflées, d'aspect ovoïde et dont le grand axe mesure 1–1,5 μ. La pureté des préparations est supérieure à 95%.

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