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Effects of Salicylate and Other Enhancers on Rectal Absorption of Erythropoietin in Rats

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Abstract—To develop a new treatment for patients with anaemia in which erythropoietin (EPO) can be given without injection, the effects of promoters of the rectal absorption of EPO were studied. Recombinant human (rHu) EPO (5000 units) in a dosing solution or in a rectal suppository was placed in the rectum of healthy rats and changes in serum EPO levels were monitored by an enzyme-linked immunosorbent assay. Without a promoter, rHuEPO was not absorbed. Sodium glycocholate, sodium caprate, and sodium salicylate in the solution of rHuEPO increased the absorption of rHuEPO. Sodium salicylate or sodium caprate in the suppository with rHuEPO also increased its absorption. The bioavailability of rHuEPO in a suppository containing 5% sodium salicylate compared with that by an intravenous injection was 1·2%. rHuEPO given in rectal suppositories containing sodium salicylate and inserted once a day for 6 consecutive days increased erythropoiesis in peripheral blood.

Erythropoietin (EPO) is the primary humoral regulator of erythropoiesis. It is a heavily glycosylated polypeptide of 34–39 kDa with a half of its molecular weight being contributed by sugar moieties (Jacobs et al 1985). EPO has been produced by recombinant DNA technology for use as a therapeutic agent (Jacobs et al 1985; Lin et al 1985; Powell et al 1986; Goto et al 1988). It is an accepted treatment for anaemia of patients undergoing haemodialysis (Winearls et al 1986; Eschbach et al 1987; Urabe et al 1988). Currently, polypeptide hormones such as EPO are administered by injection because of their poor membrane permeability. For patients undergoing haemodialysis, the standard EPO treatment is an intravenous injection two or three times a week; for patients undergoing peritoneum dialysis, subcutaneous injections are experimentally given once a week. Injections are painful and sometimes difficult to administer compared with other dosage forms. A more convenient route could make EPO more acceptable as an agent for the treatment of anaemia caused by the reduced production of EPO. Rectal administration of EPO might be more acceptable to patients than injections. To make the rectum permeable to polypeptide hormones such as EPO, administration with an absorption promoter is necessary (Moore et al 1986; Murakami et al 1988). In this work we chose three promoters with different actions in increasing absorption by the rectum, and examined their effects on the absorption of EPO by the rectum of rats.

Materials and Methods

Purified recombinant human EPO (rHuEPO)

rHuEPO from the culture supernatant of baby hamster kidney cells that had been engineered to produce rHuEPO was purified to homogeneity as described previously (Goto et al 1989).

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Animals

Wistar male rats, 9–11 weeks old (Charles River Japan, Atsugi, Kanagawa, Japan), 350–400 g, were kept for at least one week before experimentation in a room with a controlled environment (23±2°C, 50±10% relative humdity, 12 h light-dark cycle)

Assay of serum EPO concentrations

The serum concentrations of EPO were measured by a sandwich type enzyme-linked immunosorbent assay (ELISA) using two monoclonal antibodies that recognized different epitopes on the peptide chain (Goto et al 1989).

Rectal administration of EPO solution

Dosing solutions of rHuEPO (25000 units mL^{-1}) in 10 mM sodium phosphate buffer (pH 7·4) containing 2, 5, or 10% absorption promoter (sodium glycocholate, sodium caprate or sodium salicylate) or no promoter were prepared. A portion of dosing solution (200 μL, containing 5000 units of rHuEPO) was placed into the rectum of a rat as follows. Rats were starved for 16 h before dosing and anaesthetized with diethyl ether. To seal the rat anus, a silicone rubber septum connected with a smooth-tip syringe needle was glued to the anus with a quick-acting adhesive (Alon-Alpha for surgical use, Sankyo, Tokyo, Japan). After the injection, the needle was removed from the septum. A small amount of blood (about 0·4 mL) was collected from the tail vein before and at specified times after injection, and serum was obtained by centrifugation (Tomy MRX-150, 10000 rev min^{-1} for 10 min) and analysed for EPO.

Administration of EPO suppository

A rectal suppository containing rHuEPO was prepared as follows: a promoter (sodium caprate or sodium salicylate) was added to Witepsol H-15 (Dynamit Nobel Chemicals, Troisdorf-Oberlat, Germany), which had been melted at 40°C, and mixed to disperse the promoter homogeneously. The promoter concentrations tested were 2, 5, and 10%. Lyophilized rHuEPO ($1·23 \times 10^6$ units mL^{-1}) in 10 mM sodium phosphate buffer (pH 7·4) was added to give a

Table 1. Serum concentrations of EPO after rectal administration of rHuEPO solutions containing various absorption promoters.

Promoters	Conc. (%)	No. of rats	Serum EPO concn (m unit mL^{-1}) 0 min	15 min	30 min	60 min	120 min	AUC_{0-2} (m unit h mL^{-1})
None	—	3	ND	ND	ND	ND	ND	0
Sodium glycocholate	2	4	ND	175±36	170±45	158±43	112±30	282±69
	5	4	ND	158±38	129±20	114±22	78±19	212±26
	10	3	ND	230±21	294±13	158±34	71±20	321±26
Sodium caprate	2	4	ND	205±50	190±32	120±11	75±15	250±30
	5	3	ND	128±17	238±26	278±46	219±61	439±72
	10	3	ND	34±11	98±11	282±18	274±44	394±23
Sodium salicylate	2	2	ND	81±16	200±27	218±49	132±38	324±62
	5	3	ND	369±289	431±266	366±140	292±28	674±288
	10	3	ND	233±46	458±82	390±34	304±67	675±84

Values are means s.e. ND; not detected (detection limits, 20 m unit mL^{-1}).

concentration of 5000 units/100 mg of suppository base. One hundred milligrams of the solution was pipetted into an Eppendorf tube with a capacity of 0·5 mL and cooled at 4°C to make a cone-shaped suppository. For a single administration, an EPO suppository was inserted into the anus of a rat that had been starved for 16 h and anaesthetized with diethyl ether. For short-term examination (less than 4 h), the anus was immediately sealed with a quick-acting adhesive after the insertion of the suppository. For a long-term examination, the anus was immediately sealed with a silicone rubber septum after the insertion of the suppository, and the septum was held in place with an elastic adhesive bandage for 4 h. Before and after the insertion, blood was collected from the tail vein of the rat and the concentration of serum EPO was determined. Results were compared with those of an intravenous injection prepared by the dilution of rHuEPO to 12500 units mL^{-1} in 10 mM sodium phosphate buffer (pH 7·4) containing 0·1% rat serum albumin; 0·4 mL of the solution was injected into the tail vein of rats. At specified times, about 0·4 mL of blood was collected from the tail vein and assayed for the serum concentration of EPO.

In-vivo biological activity of EPO suppositories

A suppository containing 5000 units of rHuEPO and 5% sodium salicylate was inserted into the rectum of rats. The anus was immediately sealed with a silicone rubber septum and the septum was fixed with an elastic adhesive bandage for 2 h. Administration was repeated in the same way once daily for 6 consecutive days. Results were compared with those of a subcutaneous injection prepared by the dilution of rHuEPO to 250 units mL^{-1} in 10 mM sodium phosphate buffer (pH 7·4) containing 0·1% rat serum albumin; 0·2 mL of the solution was injected into rats once a day for 6 consecutive days. At specified times, about 0·4 mL of blood was collected from the tail vein of the rat with EDTA disodium as the anticoagulant and was used for haematological examination.

Haematological examinations

Red blood cell counts, the haematocrit, and haemoglobin were measured with an automated microcell counter (Sysmex E-4000, Toa Medical Electronics, Tokyo, Japan). Reticulocytes were counted by the method of Brecher. Statistical analysis was by analysis of variance.

Results

The EPO solution without an absorption promoter, did not change the serum EPO concentration (Table 1). The concentration of serum EPO had increased 15 min after administration of the EPO solution containing any one of the promoters (sodium glycocholate, sodium caprate and sodium salicylate) and reached a maximum at about 60 min or earlier, and then gradually decreased. The area under the curve for the

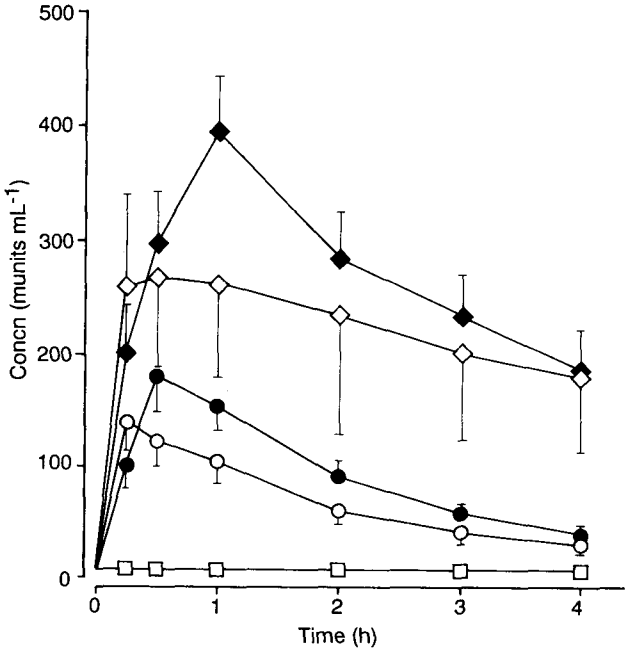

FIG. 1. Serum concentrations of EPO after rectal administration of a suppository. An EPO suppository (5000 units) containing no promoter (□), 2% (○) or 5% (●) sodium caprate, or 2% (◇) or 5% (◆) sodium salicylate was inserted into the rectum of rats. Values are means±s.e. of 3–6 rats.

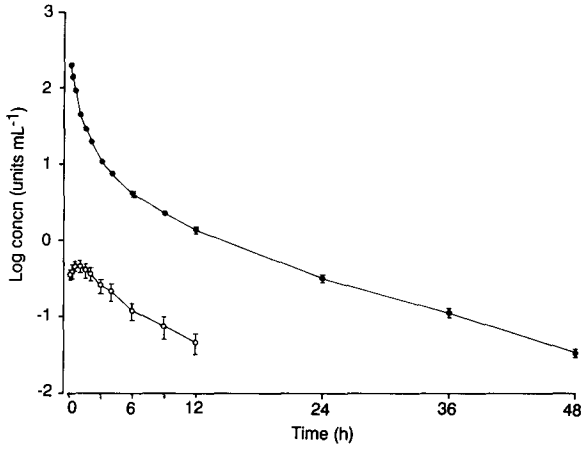

FIG. 2. Serum concentrations of EPO after an intravenous injection or insertion of a suppository. A suppository containing 5000 units of rHuEPO and 5% of sodium salicylate was inserted into the rectum of rats (○) or a solution containing 5000 units of rHuEPO was injected intravenously into rats (●). Values are means ± s.e. of 5 rats.

EPO concentration from 0 to 2 h (AUC_{0-2}) was calculated by moment analysis. The AUC_{0-2} was greatest with sodium salicylate and smallest with sodium glycocholate.

The two more effective promoters, sodium salicylate and sodium caprate, were tested in suppositories. An EPO suppository without an absorption promoter did not change the serum EPO concentration (Fig. 1). The promoters increased the absorption of EPO from the rectum. The largest increase was with 5% sodium salicylate; at 4 h after administration, the serum concentration of EPO was about 200 m units mL^{-1} with either 2 or 5% sodium salicylate.

We also compared the serum EPO level after rectal administration with an EPO suppository containing 5% sodium salicylate to that after intravenous injection of EPO (Fig. 2). The concentration of serum EPO at 24 h after the administration of the rectal suppository was below the detection limit for EPO (20 munits mL^{-1} serum) by ELISA. The serum EPO concentration could still be measured at 48 h after administration by intravenous injection. AUC_{0-12} after rectal administration and that after intravenous injection were calculated to be $2{\cdot}15 \pm 0{\cdot}48$ and 180 ± 9 units h mL^{-1}, respectively. The values of $AUC_{0-\infty}$ by these two routes were estimated to be $2{\cdot}34 \pm 0{\cdot}55$ and 194 ± 11 units h mL^{-1}, respectively. The bioavailability of EPO in the suppository compared with that by intravenous injection was thus 1·2%.

When a suppository was inserted into the rectum of rats daily for 6 days, the red blood cell count, the haematocrit, the haemoglobin, and the reticulocyte count were significantly ($P < 0{\cdot}05$) higher than those of the untreated controls (Fig. 3). The differences from the values reached with subcutaneous injections with 50 units of EPO (1% the amount

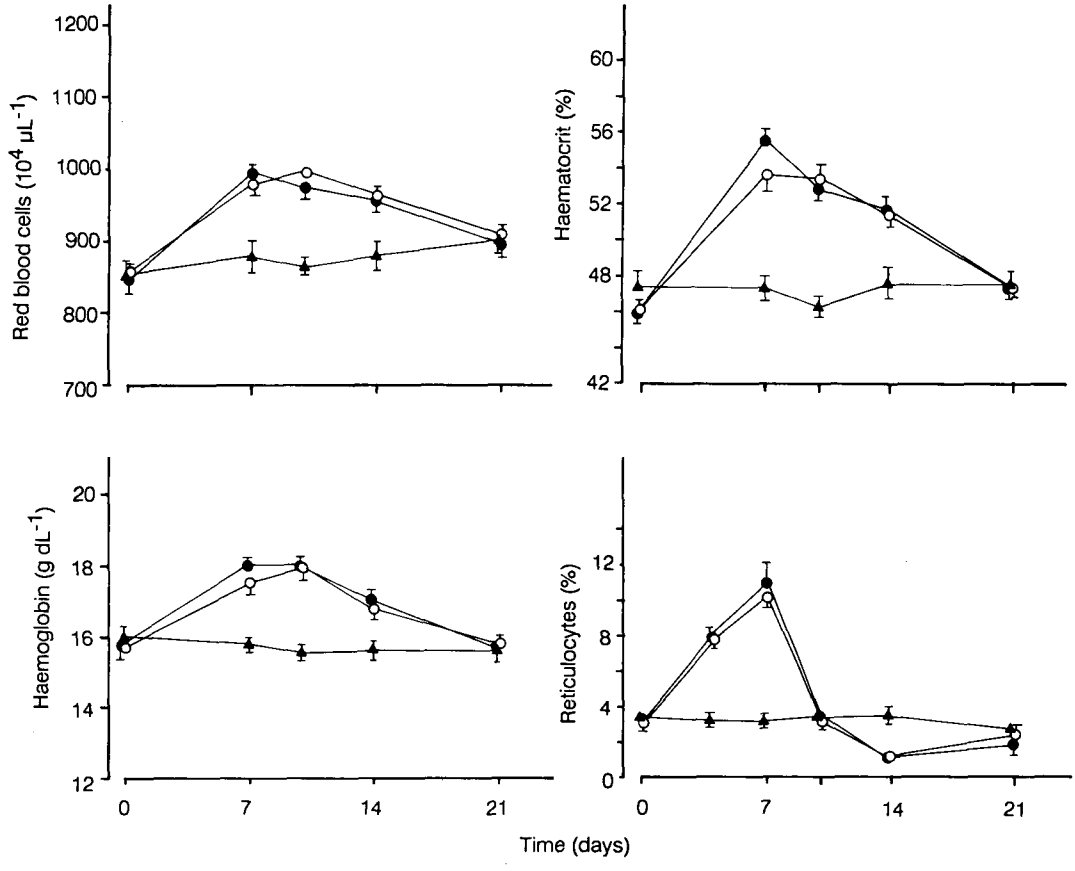

FIG. 3. Erythropoiesis of rats treated with repeated rectal administration of EPO. Rats were given EPO once a day for 6 consecutive days. Symbols: ● rectal suppository containing 5000 units of rHuEPO and 5% sodium salicylate, ○ subcutaneous injection of 50 units of rHuEPO, ▲ controls. Values are means ± s.e. of 5 rats.

contained in each suppository) were insignificant. Thus, EPO suppositories inserted into the rectum of rats had an in-vivo effect, increasing erythropoiesis in the peripheral blood.

Discussion

Polypeptide hormones such as EPO administered without injection may pass through membranes and express their hormonal activities. Two transport routes, paracellular and transcellular, have been proposed for membrane permeation. Bile salts such as sodium glycocholate seem to bind with calcium ions (Murakami et al 1984) and sodium caprate changes the pore size in the tight junctions of membranes (Tomita et al 1988). These promoters probably increase the permeability of membranes to hydrophilic macromolecules via the paracellular route. Sodium salicylate seems to increase transport through both routes (Nishihata et al 1986).

In this study, we chose sodium glycocholate, sodium caprate, and sodium salicylate which have different actions in increasing absorption by the rectum, and examined their effects on the rectal absorption of EPO in rats. These promoters significantly increased the absorption of EPO by the rectum (Table 1, Fig. 1). However, it is not clear which mechanisms are involved in the increased absorption of EPO in the rectum caused by these promoters, though probably both are involved. Moreover, consideration must also be given to absorption enhancement associated with mucosal damage (van Hoogdalem et al 1990). Rectal suppositories may be useful for the administration of EPO (Fig. 3). The bioavailability of EPO in suppository form was about 1% compared with intravenous injection (Fig. 2), but bioavailability might be increased by use of a different combination of absorption promoter and suppository base.

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