

# Delivery of glucocorticoid conjugate in rat gastrointestinal tract and its treatment for ulcerative colitis<sup>1</sup>

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ZHOU Si-Yuan, MEI Qi-Bing<sup>2</sup>, LIU Li, GUO Xin, QIU Bo-Sheng, ZHAO De-Hua, CHO Chi-Hin<sup>3</sup>  
(*Department of Pharmacology, Fourth Military Medical University, Xi-an 710032; <sup>3</sup>Department of Pharmacology, Faculty of Medicine, The University of Hong Kong, Hong Kong, China*)

**KEY WORDS** dextran; colitis; dexamethasone; trinitrobenzenesulfonic acid

## ABSTRACT

**AIM:** To evaluate colonic delivery and therapeutic effect of the newly synthesized dexamethasone (DX)-dextran (500 000) conjugate (DXD50) in the rat. **METHODS:** The amount of dexamethasone was measured in the contents from different parts of rat gastrointestinal tract and in plasma after ig conjugate. Therapeutic effect of conjugate and DX was tested in trinitrobenzenesulfonic acid-induced colitis in rat. Repair of colitis was assessed by measuring colonic ulceration area, colon weight, and colonic myeloperoxidase (MPO) activity. Systemic immunosuppression of DX was evaluated with weight of thymus and spleen and lymphocyte count in peripheral blood from rat with ulcerative colitis. **RESULTS:** Dexamethasone released from conjugate was mainly distributed in contents of cecum and colon. When DXD50 and DX  $0.25 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  were used ig to treat ulcerative colitis in rat, the ulcerative area of colon was reduced by 55.6 % and 33.3 %, respectively whereas colon weight was reduced by 17.9 % and 2.6 %, respectively. The conjugate had no effect on lymphocyte count in peripheral blood, spleen weight, and thymus weight of rat which could be reduced markedly by the same dose of DX ( $P < 0.05$  vs control). **CONCLUSION:** DXD50, which could specifically deliver DX to large intestine, is a promising agent in the treatment of human inflammatory bowel disease.

## INTRODUCTION

Ulcerative colitis is a common disease of the gastrointestinal (GI) tract. Although the administration of glucocorticoid has generally been proved effective, it is normally limited to treat disease in its acute form and can cause systemic side effects, such as adrenosuppression, immunosuppression, etc<sup>(1)</sup> on long term usage. In theory, selective delivery of drug to the colon could lower the required dose, reduce systemic side effects, and improve the curative effects. A variety of dosage forms have been investigated for their ability to efficiently deliver drugs to the lower gastrointestinal tract, including conjugate, bioerodible polymer coatings, and sustained-release dosage forms. Of these, conjugate represents a specific means to deliver a drug to the inflamed regions of the colon<sup>(2,3)</sup>. We synthesized a series of glucocorticoid conjugates with different molecular weight dextran, and observed their hydrolytic character *in vitro*<sup>(4)</sup>. The objectives of the present study were (1) to evaluate the pharmacokinetics of a newly synthesized colon-specific conjugate, dexamethasone (DX)-dextran (500 000, molecular weight of dextran = 500 000), DXD50; (2) to observe the efficacy of DXD50 in treatment for experimental ulcerative colitis in rat.

## MATERIALS AND METHODS

**Materials** DX was purchased from Tianjin Pharmaceutical Corporation, batch number 98-8-2. DXD50 was synthesized as described previously<sup>(4)</sup>. DXD50 is a white powder and DXD50 100 mg contained DX 4.5 mg. In addition, HPLC analysis showed that the DXD50 contained less than 0.3 % (w/w) non-covalently bound drug. 2,4,6-Trinitrobenzenesulfonic acid was purchased from Sigma Chemical Co. All other chemicals and solvents were either of HPLC grade or analytical reagent grade. Sprague-Dawley rats ( $200 \text{ g} \pm 20 \text{ g}$ ) were purchased from Experimental Animal Research Cen-

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<sup>2</sup> Correspondence to Prof MEI Qi-Bing.

Phn/Fax 86-29-337-4552. E-mail pharm@fmmu.edu.cn

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ter, Fourth Military Medical University. The animals were allowed water and laboratory chow *ad libitum*. A 12-h light-dark cycle was used. Main apparatus used was the high-performance liquid chromatography (HPLC, purchased from Beckman Cooperation).

**Delivery of conjugate in rat GI tract** The rats were randomized into 8 groups, 6 rats for each. As the dark circle ended at approximately 7:00, DXD50 5  $\mu\text{mol}/\text{kg}$  was administered *ig* to rat between 9:00 and 10:00. The rats were anesthetized by ether at appropriate time points (1, 2.5, 3.5, 4.5, 6, 9, 12, and 15 h after dosing) and 3–4 mL of blood was withdrawn from abdomino-aorta. (1) The blood was heparinized, and the plasma was separated by centrifugation (7 min, 4  $^{\circ}\text{C}$ , 2500  $\times g$ ). Plasma 1.0 mL and 200  $\mu\text{L}$  of prednisolone (internal standard) 1 mg/L were added into the tube. The mixture was extracted twice by vortexing with 2.0 mL of a mixture of methyl *tert*-butyl ether; pentane = 6:4 for 30 s. The sample was centrifuged for 3 min at 1000  $\times g$ . Each time, 1.5 mL of the organic phase was removed into tubes and evaporated in the ventilation cabinet. The residue was dissolved in 50  $\mu\text{L}$  of methanol and then 20  $\mu\text{L}$  was analyzed by HPLC<sup>(5)</sup>. The mobile phase consisted of 35 % of acetonitrile and 65 % of trisodium citrate 50 mmol/L adjusted to pH = 4.1 with phosphoric acid, and C<sub>18</sub> column (250 mm  $\times$  4.6 mm) was used. The flow rate was 1 mL/min, and the wave length of detection was 242 nm. (2) Immediately after collection of the blood, the contents of stomach, proximal small intestine (PSI), distal small intestine (DSI), cecum, and colon were removed. After the removal, the contents were diluted to 100 g/L with chilled (4  $^{\circ}\text{C}$ ) phosphate buffer (pH = 6.8, 0.1 mol/L). Diluted contents of stomach, PSI, DSI, cecum, and colon 0.7 mL was added into five different tubes, respectively. Into each of these tubes, 200  $\mu\text{L}$  of saturated aqueous sodium chloride and 200  $\mu\text{L}$  of prednisolonehemisuccinate (internal standard) 6 mg/L was added. The extraction of the samples was done by referring to the above step.

**Induction of ulcerative colitis** The rats were randomized into 6 groups (6 rats for each group) and then gently anesthetized by ether. A rubber catheter (OD, 2 mm) was inserted rectally into the colon such that the tip was 8 cm proximal to the anus. 2,4,6-Trinitrobenzenesulfonic acid dissolved in 38 % ethanol (v/v) was instilled into the lumen of the colon through the rubber catheter at the dose of 125 mg/kg<sup>(6)</sup>.

**Administration of DXD50 and DX to colitis rats** DXD50 0.05 – 1.25  $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  and DX 0.25  $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  were administered *ig* 6 h after the induction of ulcerative colitis for 7 d.

**Preparation of the samples and collection of data** The rats were decapitated after measuring the lymphocyte count in peripheral blood of rats. (1) Thymus and spleen were removed and weighed. (2) The colon was taken from the region which was 8 cm proximal to the anus. Along its mesenteric border, the colon was opened and gently rinsed of its contents with an iced NaCl solution 154 mmol/L. The colon was then placed flat, mucosal surface upwards, on a plate chilled at 4  $^{\circ}\text{C}$ . A transparent film was placed 5 mm above the mucosal surface, and the area of ulceration and total surface area were traced by a marker-pencil. The colon tissue was weighed after being dried on a filter paper. (3) The colonic myeloperoxidase activity was determined as previously reported<sup>(7)</sup>.

**Statistical method** All data were analyzed with SPLM software package (from Department of Statistics, Fourth Military Medical University, China). The statistical analysis of the data was performed according to One Factor Analysis of Variance.

## RESULTS

**Pharmacokinetics of DXD50** No DX could be determined in the contents of the stomach, proximal and distal small intestine within 15 h after DXD50 was administered *ig*. However, the active drug could be determined in the contents of cecum and colon at 4.5 h after dosing (Tab 1). Large amounts were released in the contents of the colon. The absorption rate of DX was low with the oral administration of DXD50 (Tab 2). The pharmacokinetic parameters of conjugate were: AUC = 430  $\mu\text{g} \cdot \text{h}^{-1} \cdot \text{L}^{-1}$ ,  $K_a = 0.36$ ,  $K_e = 0.31$ ,  $T_{peak} = 7.2$  h.

**Efficacy of DXD50** When DXD50 and DX (0.25  $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ ) were used *ig* to treat ulcerative colitis in rat, the ulcerative area of colon was reduced by 55.6 % and 33.3 %, respectively; colon weight was reduced by 17.9 % and 2.6 %, respectively; and the colonic MPO activity was reduced by 59.2 % and 56.6 %, respectively (Tab 3). This implied that DXD50 was able to facilitate the repair of damaged colonic mucosal better than the same dose of DX.

**Tab 1. Amount of DX released in contents of different parts of rat gastrointestinal tract after ig DXD50. Dose of conjugate was 5  $\mu\text{mol}/\text{kg}$ .  $n=6$ .  $\bar{x} \pm s$ .**

Time/h	Stomach	PSI	DX/ $\mu\text{g} \cdot \text{g}^{-1}$		Colon
			DSI	Cecum	
1	—	—	—	—	—
2.5	—	—	—	—	—
3.5	—	—	—	—	—
4.5	—	—	—	0.21 $\pm$ 0.07	0.1 $\pm$ 0.4
6	—	—	—	0.9 $\pm$ 0.3	2.0 $\pm$ 0.4
9	—	—	—	0.61 $\pm$ 0.25	1.22 $\pm$ 0.27
12	—	—	—	0.21 $\pm$ 0.06	0.16 $\pm$ 0.04
15	—	—	—	—	0.06 $\pm$ 0.02

PSI, proximal small intestine; DSI, distal small intestine; '—', non-detectable.

**Tab 2. Concentration of DX in plasma after ig DXD50. Dose of conjugate was 5  $\mu\text{mol}/\text{kg}$ .  $n=6$ .  $\bar{x} \pm s$ .**

Time/h	Concentration of DX/ $\mu\text{g} \cdot \text{L}^{-1}$
1	—
2.5	—
3.5	—
4.5	10 $\pm$ 5
6	41 $\pm$ 14
9	59 $\pm$ 23
12	31 $\pm$ 12
15	10 $\pm$ 7

'—', non-detectable.

On the other hand, lymphocyte count in peripheral blood, spleen weight, and thymus weight of rat were greatly reduced by ig DX at the dose of 0.25  $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  ( $P < 0.05$  vs control). However, these were not influenced by the conjugate at the same dose (Tab 4). This indicated that immunosuppression induced by ig DXD50 was lower than that caused by DX.

**Tab 3. Effect of DXD50 on colonic ulceration, colon weight, and colonic MPO activity of ulcerative colitis rat.  $n=6$ .  $\bar{x} \pm s$ .  $^{\text{a}}P < 0.01$  vs control.  $^{\text{b}}P < 0.05$ ,  $^{\text{c}}P < 0.01$  vs model.  $^{\text{d}}P < 0.05$  vs DX.**

Group	Dose ( $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ )	Percent of ulcerative area (%)	CW/BW ( $\text{mg} \cdot \text{g}^{-1}$ )	Colonic MPO activity ( $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ )
Control	0	0	2.5 $\pm$ 0.2	40 $\pm$ 11
Model	0	27 $\pm$ 8 <sup>c</sup>	3.9 $\pm$ 0.7 <sup>c</sup>	228 $\pm$ 40 <sup>c</sup>
DX	0.25	18 $\pm$ 8 <sup>f</sup>	3.8 $\pm$ 0.6	99 $\pm$ 20 <sup>f</sup>
DXD50	0.05	18 $\pm$ 8 <sup>f</sup>	3.7 $\pm$ 0.6	225 $\pm$ 30
DXD50	0.25	12 $\pm$ 4 <sup>h</sup>	3.2 $\pm$ 0.4 <sup>di</sup>	93 $\pm$ 21 <sup>f</sup>
DXD50	1.25	9.7 $\pm$ 2.0 <sup>f</sup>	3.1 $\pm$ 0.3 <sup>c</sup>	44 $\pm$ 9 <sup>f</sup>

Percent of ulcerative area = ulceration area/total area of 8-cm colon. CW; colon weight; BW; body weight.

## DISCUSSION

It is well known that DX can be rapidly and almost completely absorbed in upper GI tract when free DX is simultaneously administered orally. This rapid and complete absorption of DX can easily cause systemic side effects of glucocorticoids such as adrenosuppression and immunosuppression, which are directly related to blood levels of exogenous glucocorticoids<sup>[8]</sup>. In order to reduce the absorption of DX in upper GI tract, a conjugate was synthesized by selecting succinic anhydride as a cross-linker to conjugate DX and dextran<sup>[4]</sup>.

The present experiment showed that the synthesized conjugate (DXD50) could selectively deliver DX to the colon. This implied that the absorption of the conjugate could be restricted from the small intestine, which resulted in the lowering of the blood DX concentration [AUC of DX was observed to be 11875  $\mu\text{g} \cdot \text{h}^{-1} \cdot \text{L}^{-1}$  (unpublished data), AUC of same dose of conjugate was 430  $\mu\text{g} \cdot \text{h}^{-1} \cdot \text{L}^{-1}$ ]. The systemic immunosuppression of glucocorticoids could thus be reduced.

Colonic MPO activity reflects the quantity of neutrophils in the tissue, and it is also an objective index for evaluating the inflammatory bowel disease<sup>[7,9]</sup>. When DXD50 was used to treat ulcerative colitis in rat, DXD50 had no effects on colonic MPO activity at the dose of 0.05  $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  whereas ulcerative area was significantly reduced at the same dose ( $P < 0.01$ , vs model). Similar discordant results between intestinal repair and MPO activity during treatment for colitis in rats have been reported by Fedorak *et al*<sup>[10]</sup>. This indicates that the facilitation by the synthesized conjugate at lower doses in repairing gross colonic ulceration was not a result of inhibition of neutrophil migration.

Tab 4. Effect of DXD50 on weights of thymus, spleen, and lymphocyte count in peripheral blood of ulcerative colitis rat.  $n=6$ .  $\bar{x} \pm s$ .  $^b P < 0.05$ ,  $^c P < 0.01$  vs control.

Group	Dose ( $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ )	TW/BW ( $\text{mg} \cdot \text{g}^{-1}$ )	SW/BW ( $\text{mg} \cdot \text{g}^{-1}$ )	$10^{-9} \times$ Lymphocyte ( $\text{L}^{-1}$ )
Control	0	$2.5 \pm 0.5$	$3.1 \pm 0.7$	$11 \pm 3$
Model	0	$2.2 \pm 0.4$	$3.1 \pm 0.8$	$14 \pm 4$
DX	0.25	$0.93 \pm 0.23^c$	$2.1 \pm 0.6^c$	$2.3 \pm 1.0^c$
DXD50	0.05	$2.4 \pm 0.3$	$3.2 \pm 0.6$	$13 \pm 4$
DXD50	0.25	$2.1 \pm 0.4$	$2.9 \pm 0.6$	$13 \pm 4$
DXD50	1.25	$1.43 \pm 0.26^b$	$2.3 \pm 0.4^b$	$4.0 \pm 1.0^c$

Percent of ulcerative area = ulceration area/total area of 8-cm colon. TW; thymus weight; BW; body weight; SW; spleen weight.

All these experimental results showed that the newly synthesized DXD50 could specifically deliver DX to the large intestine. The development of this conjugate may yield a promising agent for the treatment of human inflammatory bowel disease.

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prodrug accelerates healing of colitis in rats without adrenal suppression. Gastroenterology 1995; 108: 1688-99.

## 糖皮质激素连接物在大鼠胃肠道的转运及其对溃疡性结肠炎的治疗<sup>1</sup>

周四元, 梅其炳<sup>2</sup>, 刘莉, 郭欣, 邱博生, 赵德化, 曹之憲<sup>3</sup>

(第四军医大学药理学教研室, 西安 710032; <sup>3</sup>香港大学医学院药理系, 香港, 中国)

**关键词** 右旋糖酐; 结肠炎; 地塞米松; 三硝基苯磺酸

**目的:** 研究地塞米松(DX)-右旋糖酐(500 000)连接物(DXD50)在大鼠胃肠道的转运及其对大鼠溃疡性结肠炎的疗效。**方法:** 将DXD50给大鼠ig, 监测该DXD50在大鼠胃肠道不同部位释放DX的动力学过程及血药浓度变化。用三硝基苯磺酸诱导大鼠溃疡性结肠炎, 以结肠溃疡面积、结肠重量、结肠组织髓过氧化物酶、大鼠外周血淋巴细胞数、胸腺及脾脏重量为指标, 观察连接物的疗效。**结果:** 口服连接物后, DX主要分布在盲肠和结肠中。DXD50及DX  $0.25 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  可分别使大鼠溃疡面积缩小55.6%和33.3%, 同时结肠重量下降17.9%和2.6%。DXD50  $0.25 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  对大鼠外周血淋巴细胞数、胸腺及脾脏重量无影响, 而同剂等量的DX可引起大鼠外周血淋巴细胞数、胸腺及脾脏重量明显下降( $P < 0.05$  vs control)。**结论:** DXD50可将DX特异性地转运到结肠, 它是一种有潜力的治疗炎症性肠病药物。

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