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# Intracarotid infusion of hypertonic mannitol changes permeability of blood-brain barrier to methotrexate in rats[1]

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**KEY WORDS** mannitol; blood-brain barrier; methotrexate

## ABSTRACT

**AIM**: To study whether infusion of hypertonic mannitol through internal carotid artery could enhance methotrexate (MTX) concentration in rat cortex and to study the time-course of this process. **METHODS**: Hypertonic mannitol was infused into the rat left internal carotid artery, ten minutes later, MTX was injected from the left femoral vein (iv) or left common carotid artery (ia), and the concentration of MTX was assayed 1 h later. Rats were given MTX at different time interval after infusion of mannitol, and the concentration of MTX was assayed 1 h after drug administration. At the same time, the rat cortex's density was analyzed. **RESULTS**: After mannitol infusion, MTX's concentration in rat cortex increased 2.54 times (iv) and 3.41 times (ia) as compared to control, respectively. After 10 min, such effect reached its peak, and almost disappeared after 6 h. There was no significant change in rat cortex's density. **CONCLUSION**: Mannitol can make blood-brain barrier (BBB) reversibly permeable, and increase MTX concentration in brain without any obvious injury to the brain.

## INTRODUCTION

The blood-brain barrier(BBB) at the cerebrovascular endothelium prevents the passage of water-soluble non-electrolytes, electrolytes, and proteins from blood to brain. Tight junctions connect cerebrovascular endothelial cells and restrict diffusion between the cells[1]. However, in the treatment of brain tumors, BBB made the brain malignant tumor intact to water-soluble agents. Therefore, physicians usually choose drugs that are lipid soluble for CNS neoplasm. But many useful antineoplastic agents are not lipid soluble and are excluded by BBB.

If one could safely and transiently make the BBB permeable, water-soluble chemotherapeutic agents might reach the leading edge of the tumor. Hypertonic solutions, such as mannitol, can induce osmotic BBB opening. Such an effect does not produce functional neurological deficits, long-term brain edema, or brain pathology[2]. There are also reports about intracarotid administration of hypertonic solutions which increase the blood-brain barrier permeability to compounds of large molecular weight, such as Evans blue-albumin complex and smaller molecular agents, such as sumatriptan[3,4]. Therefore, inducing a change in BBB permeability opening, followed by injection of a water-soluble drug such as MTX, can enhance its brain uptake significantly compared with MTX administration without osmotic treatment.

In this paper, we studied how the MTX's concentration changed in rat cortex following osmotic opening of the BBB with intracarotid infusion of hyper osmotic mannitol. The results of MTX injection via different routes, femoral vein(iv) and internal carotid artery(ia), were also compared. Furthermore, in order to determine the optimum time of MTX administration after giving mannitol, the time-course of BBB opening induced by mannitol infusion was studied.

## MATERIALS AND METHODS

**Regents and instruments** MTX was purchased from Lianyungang Pharmaceutical Factory (№ 960817). Mannitol was obtained from Dongsheng Chemical Co, Wenzhou (№ 980329). Ethyl carbamate was obtained from Caoyang Chemical Co, Shanghai (Q/DBZL 10-91). All other chemicals were of AR grade. A fluorescence spectrophotometer from HITACHI mpf-4 was used.

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**Animals** Sprague-Dawley rats (♀, 230 ± 20 g, $n$ = 45, Shanghai Experimental Animal Center, Grade II) were used.

**The effect of mannitol on BBB's permeability** Adult female SD rats ($n$ = 20) were randomly allocated to four groups, each consisting of 5 animals. Rats were anesthetized with ethyl carbamate (1 g/kg). The left common carotid artery was exposed, and the left external carotid artery was ligated near the bifurcation. The rats of group 1 were given 0.9 % NaCl (8 mL/kg, 3 mL/min) from the left common carotid artery plus iv MTX (10 mg/kg) ten min later; group 2, ia 25 % mannitol plus iv MTX; group 3, ia 0.9 % NaCl plus ia MTX; group 4, ia 25 % mannitol plus ia MTX. One hour after MTX administration, all the rats were killed and samples of blood and cortex were obtained. All of the samples were frozen at −20 ℃ for MTX assay.

**Evaluation of the optimum time for MTX injection** Rats ($n$ = 25) were randomly divided into 5 groups. The operation was the same as above. The rats in group 1 were infused from the left common carotid artery at 3.0 mL/min with 0.9 % NaCl (8 mL/kg) instead of mannitol as control, ten minutes later, MTX were injected from the left common carotid artery (10 mg/kg). From group 2 to group 5, the time interval between giving mannitol and MTX was set at 10 min, 0.5 h, 2 h, and 6 h. One hour after MTX administration, the rats were killed and samples of serum and cortex were obtained. A little piece of cortex (about 1 $mm^3$) was clipped to determine its density according to a method in the literature[5]. All the serum and cortex samples were frozen at −20 ℃ for MTX assay.

**Methotrexate assay** MTX concentration was determined by fluorometric method[6]. The recovery of MTX from cortex was 86.3 % ± 4.4 % ($n$ = 15), the lowest limit of detection was 25 $ng \cdot g^{-1}$(protein). The recovery of MTX from serum was 96.1 % ± 2.3 % ($n$ = 15), the lowest limit of detection was 10 $mg \cdot L^{-1}$.

## RESULTS

**The effect of mannitol on BBB's permeability** In control animals (group 1), MTX level in serum was much higher than that in cortex (Tab 1). After mannitol infusion in the left internal carotid artery, MTX concentration (group 2) in left cortex increased 2.54 times as compared to control (group 1); if MTX was injected from the left internal carotid artery (group 4), its concentration increased to about 3.41 times as compared to control (group 3). The MTX concentration in left cortex after ia MTX was about 2.58 times higher than iv MTX (group 4 vs. group 2). Therefore, the effect of ia MTX is more obvious than that of iv MTX. At the same time, the right cortex's MTX concentration did not increase as much as the left one. But in group 4, its concentration also rose greatly compared with group 3 ($P$ < 0.05).

**Tab 1. MTX concentration in serum and cortex.** $n$ = 5. $\bar{x} \pm s$. $^bP < 0.05$ *vs* group 1. $^cP < 0.05$, $^fP < 0.01$ *vs* group 3. $^hP < 0.05$, $^iP < 0.01$ *vs* right cortex respectively.

| Group | Serum (mg/L) | MTX concentration Left cortex(ng/g) | Right cortex (ng/g) |
|---|---|---|---|
| 1 | 11 590 ± 61 50 | 230 ± 83$^h$ | 117 ± 63 |
| 2 | 22 737 ± 18 301 | 584 ± 233$^{bi}$ | 181 ± 98 |
| 3 | 14 586 ± 7 994 | 442 ± 203 | 176 ± 104 |
| 4 | 14 154 ± 4 079 | 1508 ± 616$^{fi}$ | 453 ± 178$^c$ |

*Group 1: NS plus iv MTX; Group 2: mannitol plus iv MTX;* Group 3: NS plus ia MTX; Group 4: mannitol plus ia MTX.

**Evaluation of the optimum time for MTX injection** Ten minutes after mannitol infusion, administration of MTX from the left common carotid artery increased its concentration to (1508 ± 617) ng/g ($n$ = 5) in left cortex, which was the highest concentration in the entire time course. Two hours later, MTX's concentration decreased to half of that concentration. After 6 h, the effect of mannitol disappeared completely. In the right cortex, although the change was not as obvious as the left, a similar trend could be identified (Fig 1).

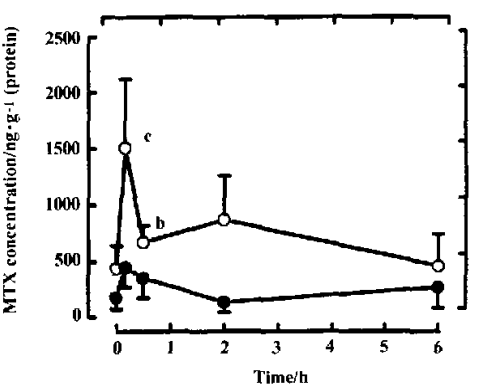


***Fig 1.* Change of MTX concentration in cortex after mannitol infusion. (○) MTX concentration in left cortex; (●) MTX concentration in right cortex.** $n$ = 5. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* control.

In this study, cortex's density did not change significantly. In the first ten minutes, the density of cortex dropped slightly. After 6 h, it returned to the normal level. All these changes were not significant (Fig 2).

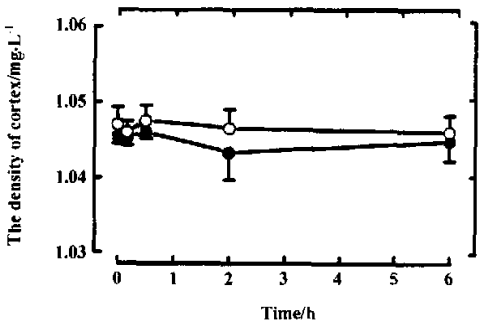


**Fig 2. Change of cortex density after mannitol infusion. $n = 5$. $\bar{x} \pm s$. (○) the left cortex; (●) the right cortex.**

## DISCUSSION

In the study of BBB, its permeability is often estimated by using computed tomographic (CT) densitometry values, Evan's blue dye staining on necropsy specimens, or the change of the intracellular pH of the brain[3,7]. Antineoplastic agents are seldom directly used as markers to indicate the permeability of BBB. However, using MTX as marker, it is convenient to assess BBB permeability quantitatively by measuring MTX concentration in the brain.

Although MTX is a classic antineoplastic agent, it is also a water-soluble drug which crosses the BBB poorly. It was reported that MTX's threshold concentration as an antitumor agent 456 ng/g[8]. But when MTX is used to treat brain tumor, it can not reach such concentration in brain without increasing its dose. However, the side effects of high-dose MTX therapy (HD-MTX therapy) are serious. There are numerous reports on acute hepatotoxicity after HD-MTX therapy[9-11]. From our results, it is clear that after mannitol infusion, both iv and ia administration can effectively increase MTX concentration in brain without an increase in dose. However, ia MTX's administration is more effective.

It is reported that intracarotid infusion of mannitol can cause cerebrovascular dilation and cell shrinkage, which stresses the cerebrovascular endothelium and possibly widens interendothelial tight junctions[12,13]. These factors might contribute to the opening of BBB and cause the augmentation of MTX in cortex. Intracarotid infusion itself has been reported to induce cerebral vasodilatation and cause BBB opening[14]. In group 1 of the first experiment, the concentration of MTX in the left and right cortex was significantly different ($P < 0.05$). Because no mannitol was given, such a difference could only be attributed to the increase in blood pressure and flow rate, which induced cerebral vasodilatation and caused more MTX to enter the cortex.

After passing through the brain and opening the barrier, the intracarotid solution gets diluted by plasma after undergoing one circulation. In our experiments, the rats tolerated the mannitol infusion very well. It is clear that the cortex's density did not change much after mannitol infusion. Higher MTX concentration in the left cortex suggests that hypertonic solution's effect on BBB is transient and regional. In fact, there is a report that the influence of mannitol on cerebral blood flow and cerebral metabolism is negligible[15].

In conclusion, MTX's concentration in cortex after administration of mannitol could be increased significantly without HD-MTX therapy. This is very helpful in brain tumor therapy, especially when such therapy needs some water-soluble antitumor agents with serious side effects at high doses.

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## 动脉内输注高渗性甘露醇改变大鼠血脑屏障对甲氨喋呤的通透性[1]

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**关键词** 甘露醇；血脑屏障；甲氨喋呤

**目的**：研究高渗性溶液开放血脑屏障能否提高甲氨喋呤在皮层内的含量及这一开放过程的时程变化. **方法**：1）大鼠颈内动脉注射甘露醇，10 分钟后分别从股静脉及颈内动脉给予甲氨喋呤，1 小时后测定脑皮层内浓度. 2）大鼠颈内动脉注射甘露醇，分别在不同时间间隔给予甲氨喋呤，给药 1 小时后测定脑皮层内药物浓度及脑皮层密度. **结果**：给予甘露醇后，静注甲氨喋呤和颈内动脉注射甲氨喋呤可以使相应侧脑皮层内浓度分别提高 2.54 倍和 3.41 倍. 此作用在给予甘露醇 10 分钟后达到最大，6 小时后完全消失；同时并不伴随明显的皮层密度改变. **结论**：甘露醇可逆性地开放血脑屏障，提高甲氨喋呤在脑皮层内的浓度，同时不会造成明显的脑损伤.

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