New Technology for Continuous Intravenous Infusion via the Central and Portal Veins in the Rat

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NISHIHIRA, T., KOMATSU, H., ENDO, Y., SHINEHA, R., SAGAWA, J., NAKANO, T., HOSHINO, A., YOSHIDA, K. and MORI, S. New Technology for Continuous Intravenous Infusion via the Central and Portal Veins in the Rat. Tohoku J. Exp. Med., 1994, 173 (2), 275-282 —— Total parenteral nutrition via the central vein is a technique used extensively in basic and clinical research. Recent research has also focused on the administration of various drugs and nutrients via the portal vein. To date, however, no technique which would permit prolonged continuous infusion simultaneously through both the central vein and the portal vein in the rat has been reported. The development of such a technique would open up new possibilities for utilizing the advantages of each of these two routes and contribute to progress in metabolic and nutritional research. To establish such a technique, the authors implemented several unique improvements such as the application of clamps to minimize bleeding during catheter insertion and an increase in the number of sutures to prevent catheter dislodgment. With these improvements, it was possible to continuously administer specified doses of infusion solution in both veins for 6 days in 156 of 158 rats (99%) in the main experiment. We herein describe the techniques used and some of the results obtained with this experimental system. —— portal vein infusion; dual infusion system; fatty liver; amino acid imbalance; valine-depleted solution

Techniques for continuous infusion via the central vein (CV), employed for total parenteral nutrition (TPN), have already been established in unanesthetized and unrestrained rats (Steiger et al. 1972). Although frequently employed in experimental studies on metabolism and nutrition, previously reported techniques represent unphysiological forced feeding, because nutrients tend not to pass through the portal vein (PV). Recently, however, techniques for continuous infusion via the PV have been established which more closely approximate metabolic routes normally occurring in oral feeding (i.e., nutrients are metabolized
initially in the liver because infusion is administered via the PV, the physiological route for transport of nutrients) and which provide higher metabolic efficiency than TPN (Shirotani 1984; Nishihira et al. 1988). None of the techniques reported thus far have enabled continuous infusion via both the CV and PV. The availability of such a technique, permitting the infusion of different substances via the CV and PV (e.g., the infusion of specific drugs or nutrients targeted for the liver via the PV concurrent with TPN of high calorie amino acid solution via the CV) is expected to broaden the possibilities for research on metabolism and nutrition.

In this study, continuous infusion via the CV and PV was performed in rats for 6 days. This communication primarily reports the methodology of the experimental technique and describes the results obtained using this experimental system. The system was assessed by administering TPN by a continuous infusion of valine-depleted amino acid imbalance solution, found to have an antitumor effect by the authors (Nishihira et al. 1988; Nishihira et al. 1993a, b) into the CV, concurrent with infusion of low doses of valine into the PV to prevent fatty liver, the most unfavorable side effect of this anticancer therapy. In this report, we focus on the correlation between the levels of valine in systemic blood and the administered doses of this amino acid imbalance solution.

**Materials and Methods**

**Animals**

Male Crj: Donryu rats (body weight, 250–300 g) were purchased at 8 weeks of age and allowed about 1 week for acclimatization. From the night before the operation, only water was provided.

**Catheter preparation**

The CV catheter was prepared by cutting the tip of a 50-cm-long silicon rubber catheter (Silascon, Dow Corning Asia Co., Tokyo; inner diameter, 0.5 mm; outer diameter, 1.00 mm) at an angle of approximately 45° and subjecting it to gas sterilization.

The PV catheter was prepared by cutting the tip of a 10-cm-long silicon rubber catheter (Silastic, Dow Corning Asia Co., Tokyo; inner diameter, 0.5 mm; outer diameter, 0.64 mm) at approximately a 45° angle, and connecting the opposite end to a thicker silicon rubber catheter (Silascon, Dow Corning Asia Co., Tokyo; inner diameter, 0.5 mm; outer diameter 1.00 mm), 100 cm in length. Both catheters were connected to a 1-cm section of the barrel catheter of a 24 G indwelling needle (Saflow-C, Thermo Co., Tokyo). The connection was held in place with adhesive (Bond-Alon Alpha, Toha Gousei Kagaku Co., Tokyo). The end of this catheter was connected to a blunted 22 G butterfly needle for intravenous injection (Thermo Co.). The completed catheter was finally subjected to gas sterilization.
Catheter placement

Under pentobarbital anesthesia (Nembutal, 40 mg/kg, i.p., Dainabot, Chicago, IL, USA), the neck of the rat was shaved and disinfected with povidone iodine (Isodine Solution, Meiji Seika Co., Tokyo). An incision, about 2 cm in length, was made in the right cervical region, and about 1 cm of the jugular vein was exposed from the proximal portion of the clavicle towards the head by means of forceps. The distal end of the jugular vein was ligated, and a small hole was made in the center of the exposed vessel by means of micro-scissors. The CV catheter was inserted for about 2 cm towards the superior vena cava and ligated from the point of entry in four places on the cardiac side. The neck was then sutured.

Next, the abdomen was disinfected similarly to the neck and opened by a median incision extending for about 3 cm. Sterilized gauze was applied and the cecum was pulled out from the abdomen. The cecal vein (the part of the cecal vein extending about 1 cm toward the cecum from the junction of the ileal vein and cecal vein) was exposed from its overlying capsule and fat by forceps, and its proximal end was ligated. Bleeding from the PV and ileal vein was prevented by application of neurosurgical vascular clamps. Then a specialized needle (prepared by heating a 27 G injection needle with a gas burner, flattening the tip with a small hammer, and bending the needle to a 90° angle at about 3 mm from the tip) was slightly raised while pushing gently to create a small opening in the vein. The PV catheter was inserted into this opening for about 1 cm until it reached the clamps. The clamps were then removed, and the catheter was further inserted by means of the right hand for about 3 cm towards the PV, while bleeding was controlled by applying pressure with the thumb and index finger of the left hand. The tip of the catheter was thereby placed inside the PV. Immediately after insertion, the catheter was ligated at 4 places on the side of the opening towards the liver. The inside of the abdominal cavity was washed with physiological saline solution; a small dose (100 mg/kg) of synthetic penicillin, piperacillin sodium (Pentcillin, Toyoma Chemical Industries, Tokyo), was injected i.p., and the abdomen was sutured. The CV and PV were ligated with 5-0 silk thread and the skin was ligated with 4-0 silk thread. Catheterization of the CV and PV in the rat is schematically represented in Fig. 1.

Infusion technique and grouping for infusion experiments

The rats were transferred to metabolic cages, and the infusion solutions were continuously administered via the CV and PV with the rats in an unrestrained state for 6 days. Two microinjection pumps (Proportional Assay Delta Pump, Watson-Marlow, Cornwall, England) were used to administer the infusion solutions. The mean rates of infusion per rat were 250 mg/kg/day via the CV and 50 ml/kg/day via the PV. The mean number of calories supplied was 270 kcal/kg/
day. A schematic representation of a rat receiving infusion solution via the CV and PV is presented in Fig. 2.

For the infusion experiments, the rats were broadly allocated into 3 groups: (1) a control group which received 10% complete amino acid preparation via the CV and lactated Ringer's solution via the PV; (2) a group which received a valine-depleted amino acid imbalance preparation via the CV and lactated Ringer's solution also via the CV; (3) a group which received a valine-depleted amino acid imbalance preparation via the CV and lactated Ringer's solution containing various amounts of valine (equivalent to 5%, 10%, 25%, 50%, 75% and 100% of the valine contained in the amino acid preparation given to the control group). After completion of the treatment, the rats were exsanguinated from the abdominal aorta and necropsied. The liver was removed. The plasma aminogram was determined and the liver was examined histopathologically by conventional methods.

During treatment with the infusion solutions, free access was allowed to water, but all food was withheld.
RESULTS

In pilot experiments, the infusion solution could not be successfully administered for 6 days in 9 (50%) out of 18 rats. The reasons for this were massive bleeding during insertion of the PV catheter, dislodgment of the catheter, and entanglement of the PV catheter around the protective coil due to the spontaneous activity of the rats, sometimes resulting in the catheter being severed. Several improvements were therefore devised for the main experiment: (1) Neurosurgical clamps were employed to minimize bleeding during insertion of the PV catheter. (2) The number of sutures applied at the insertion points of the CV and PV catheters was increased to avoid leakage of infusion solution and catheter dislodgment. (3) The PV catheter was disentangled from the protective coil at a mean interval of 4 hr. (4) A catheter equivalent in thickness to the CV catheter was used for the distal end of the PV catheter from the subcutaneous tunnel to prevent the catheter from being severed or other accidents due to entanglement.

These improvements allowed the specified doses of infusion solution to be administered in 156 (99%) of 158 rats in the main experiment.

Next, the results obtained in this experimental system will be described. The development of fatty liver was prevented in rats continuously administered
with valine-depleted amino acid imbalance solution via the CV and with a concurrent continuous infusion of only valine via the PV, even at the lowest dose level of valine (equivalent to 25% of the valine contained in the amino acid preparation given to the control group) (Table 1). On the other hand, the plasma aminogram derived from peripheral blood samples demonstrated low valine concentrations (Fig. 3). Mean plasma valine concentrations in the experimental groups are shown and are expressed as a percent of the mean value concentration in the control group (regarded as 100%). The mean value and standard deviation

Table 1. Pathological examination of the liver

<table>
<thead>
<tr>
<th>Group</th>
<th>Yellow-whitish change (Positive rate)</th>
<th>Enlargement (Positive rate)</th>
<th>Fatty vacuoles (Positive rate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0/10 (0%)</td>
<td>0/10 (0%)</td>
<td>5/10 (50%)^{++}</td>
</tr>
<tr>
<td>0%</td>
<td>8/10 (80%)^{+++}</td>
<td>6/10 (60%)^{++}</td>
<td>9/10 (90%)^{+++}</td>
</tr>
<tr>
<td>5%</td>
<td>6/10 (60%)^{+++}</td>
<td>4/10 (40%)^{++}</td>
<td>10/10 (100%)^{+++}</td>
</tr>
<tr>
<td>10%</td>
<td>0/10 (0%)</td>
<td>0/10 (0%)</td>
<td>6/10 (60%)^{+++}</td>
</tr>
<tr>
<td>25%</td>
<td>0/10 (0%)</td>
<td>0/10 (0%)</td>
<td>5/10 (50%)^{++}</td>
</tr>
<tr>
<td>50%</td>
<td>0/10 (0%)</td>
<td>0/10 (0%)</td>
<td>6/10 (60%)^{++}</td>
</tr>
<tr>
<td>75%</td>
<td>0/10 (0%)</td>
<td>0/10 (0%)</td>
<td>6/10 (60%)^{++}</td>
</tr>
<tr>
<td>100%</td>
<td>0/10 (0%)</td>
<td>0/10 (0%)</td>
<td>5/10 (50%)^{++}</td>
</tr>
</tbody>
</table>

Grade: ±, very slight; +, slight; ++, moderate; ++++, marked.

Fig. 3. Mean valine concentrations in the experimental groups expressed as a percent of the mean value concentration in the control group (regarded as 100%). Plasma valine concentrations were significantly lower in the groups treated with ≤50% of the control valine concentration via the PV (i.e., groups infused via the PV with ≤50% of the valine concentration administered via the CV in the control group). **p<0.01 vs. control.
DISCUSSION

The administration of infusion solution via the PV was first performed by Fine et al. (1945) who found that arterial blood infused into the PV was effective in patients with hemorrhagic shock. Subsequently, from a nutritional standpoint, portal nutrition was reported to offer advantages over TPN in terms of improving amino acid utilization, promoting albumin synthesis, stabilizing blood sugar and serum osmotic pressure, and maintaining a favorable nitrogen balance (Joyeux 1974; Piccone et al. 1980; Fairman et al. 1983; Bozzetti et al. 1993). However, the infusion of high calorie solutions into the PV has also been reported to be detrimental with regard to nitrogen balance and fatty degeneration of the liver (King et al. 1983).

Infusion of solutions via the PV alone is thus associated with some unresolved problems. With such problems in mind, the authors designed and developed a technique for continuous, simultaneous infusion via the CV and PV which makes it possible to administer different types of infusion solutions via each route to take advantage of their respective positive features. This technique can hopefully be employed in metabolic and nutritional research.

Several improvements over the prototype design were made in this experiment. Consequently, favorable results were obtained, with the success rate being 99% in 6-day infusion experiments. This improved system employed a thin catheter, with an outer diameter of 0.64 mm, for insertion into the PV to minimize interference by blood flow from the ileal vein. On the other hand, the catheter had to be disentangled from the protective coil at regular intervals, which required considerable time and effort.

The authors were able to prevent the development of fatty liver by continuously infusing valine-depleted amino acid imbalance solution through the CV concurrent with the infusion of low concentrations of valine solution via the PV. At the same time, the valine concentration of the peripheral blood was low, demonstrating that a valine-depleted state could be maintained systemically. The above results indicated that it is possible to prevent valine deficiency, specifically in the liver, by supplementation of valine via the PV. Hepatocytes were found to have had an adequate intake of valine, thereby apparently preventing the development of fatty liver. Presently, the authors are performing experiments designed to elucidate lipid metabolic aspects of mechanisms involved in the prevention of fatty liver. In addition, other metabolic and nutritional studies are now in
progress using the CV/PV continuous infusion technique described in this paper to assess antitumor activity in rats with cancer and the prevention of fatty liver under identical experimental conditions.

References


