Plasma Level Monitoring of Nasal Salmon Calcitonin in the Rat by a Heterogeneous Two-site Enzyme Immunoassay

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The experimental and clinical effectiveness of nasal salmon calcitonin (SCT) for treatment of osteoporosis in humans has been well established, but none is known yet about the pharmacokinetic property in relation to therapeutic efficacy, especially when used in a therapeutic dose range. This preclinical study was designed to evaluate such a property, first of all in rats, using a novel heterogeneous two-site enzyme immunoassay that has allowed us to evaluate the pharmacokinetic property of parenteral SCT in rats due to the high sensitivity (the detection limit = 2 pg of SCT/ml of plasma). It was found that as early as 10 min after the nasal dosing of 1.25, 5, or 20 U/rat, the SCT immunoactivity became detectable in plasma and thereafter it waned rapidly with time. Hypocalcemia developed in a dose-dependent manner, but with a delay of ~20 min from the peak of the

immunoactivity and lasted hours. The pharmacokinetic parameters measured for the doses (1.25, 5, and 20 U/rat) were as follows; the AUCs (pg·hr/ml) = 20.8, 89.0, and 189, and the MRTs (min) = 52, 54, and 45, respectively. The results appear to suggest: (1) the unexpected quick transfer of nasal SCT into and from the circulation, (2) a delayed onset of hypocalcemia and possibly its anti-osteopenic action, both of which may last longer, (3) that keeping the plasma SCT above the *in vitro* anti-osteoclastic level (~1 pM) only for a few hours per 2 days would be enough for inducing the distinct anti-osteopenic effect in rats, and (4) the feasibility of designing the clinical study as to the pharmacokinetics and pharmacodynamics of nasal SCT on humans. *J. Clin. Lab. Anal.* 11:129–131, 1997. © 1997 Wiley-Liss, Inc.

Key words: bioavailability; AUC; MRT; hypocalcemic effect

INTRODUCTION

Salmon calcitonin (SCT), one of the standard peptide therapeutics for diffuse bone diseases, i.e., osteoporosis and Paget's disease, has been clinically applied by the parenteral injections (intramuscular or subcutaneous) (1–3). Lately, a nasal application formula tends to replace the parenterals especially for the chronic use in senile and postmenopausal osteoporosis, due to a lower incidence of annoying side effects (vomiting, nausea, and flushing) with the ease for self-administration (4–6). Not only clinically but also experimentally, however, no direct study has been reported so far about the pharmacokinetic property of nasal SCT given in the prescribed dose range, mainly due to the lack of a reliable assay method of picomolar level of SCT (7, 8). Very recently, we have succeeded in developing a heterogeneous two-site enzyme immunoassay for SCT with the detection limit of picomolar level (9). In the study reported here, we attempt directly to estimate the bioavailability of nasal SCT given in such a therapeutic dose range to rats that were proved to prevent the ovariectomy-induced osteopenia (10, 11) and to provide the

pre-clinical data based on which one can make a protocol for clinical studies in humans.

MATERIALS AND METHODS

Wistar male rats (10 weeks old) were purchased from Japan SLC (Shizuoka). SCT (SMC 20-51, 4,220 U/mg, Sandoz Pharm., Basal, Switzerland) was dissolved in saline containing 0.05% benzalkonium chloride (pH: 3.5–4.0) and directly applied onto the left nasal mucous membrane of rat by a micropipet (50 µl/rat) under Nembutal anesthesia (35 mg/kg *i.p.*, Abbott Lab., North Chicago, IL). Blood (0.5 ml) was collected from the femoral vein and mixed with heparin (5 U/tube, Shimizu Pharm., Shimizu, Japan) in a sampling tube. Sampling was limited to twice per rat and made at –60, 10,

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Received 7 May 1996; Accepted 27 August 1996

30, 60, 180, or 360 min after administration in such a manner that every time pair would be covered. After centrifugation, plasma samples were stored at -70°C . SCT concentration was measured by a heterogeneous two-site of enzyme immunoassay method as described (9) and total calcium concentration as described by Gitelman (12). The parameters of bioavailability, AUC (area under the concentration-time curve), and MRT (mean residence time), were calculated using a linear trapezoidal equation (13, 14). All data were the means $\pm$ S.E. The significance of difference was assessed by Kruskal-Wallis test and $P < 0.05$ or less taken as significant.

RESULTS

Within 10 min after the nasal dosing, the peptide was found to appear in plasma (Fig. 1A). Hypocalcemia followed the appearance with a delay of ~ 20 min (Fig. 1B). The peptide peak was short-lived, whereas the hypocalcemia lasted hours. Seemingly, there was no overt correlation between the rise-and-fall pattern of SCT in plasma and the time course of hypocalcemia, and the duration rather than the magnitude of hypocalcemia appeared to be related to the doses of SCT. Two parameters for the bioavailability, AUC and MRT, were calculated from the data in Figure 1A and summarized in Table 1. In general, the AUC, the extent of bioavailability increased in a dose-dependent manner, whereas the MRT, the rate of bioavailability, was constantly around 50 min with every dosage.

DISCUSSION

This is the first report about the bioavailability of nasal SCT given in a therapeutic dose range. The quick transition into the circulation and a short-lived plasma peak were the characteristics defined for this application route, indicating a short stay of the peptide in the circulation. The AUC calculated for nasal SCT was about one-fourth of that of subcutaneous SCT described in our previous report (9), whereas the MRTs for both routes were comparable. The detection limit of SCT by the enzyme immunoassay employed herein was 2 pg/ml, at least fivefold lower than those reported with RIA so far (7, 8) and possibly of more importance. The limit is closer to the minimum effective concentration (≤ 1 pM = 3.4 pg/ml) of SCT that inhibits pit formation by osteoclasts in vitro (15, 16). In addition, recently we have determined the ED_{50} (7.5 U/rat/2 days) for the in vivo anti-osteopenic effect of nasal SCT from the experiment in which SCT (5–20 U/rat) were given to ovariectomized rats on alternate days for 3 weeks (10). As seen in Figure 1A, the plasma peak after intranasal application of SCT (5 U/rat) was short-lived and within 3 hr fell to the undetectable level. Thus it is considered that the measurable anti-osteopenic effect would develop by maintaining the plasma SCT level above in vitro effective concentration only for a few hours per 2 days. However, the hypocalcemic effect, the most sensitive in vivo indicator of SCT biological effects, lasted longer than the plasma immuno-

Fig. 1. Concentrations of SCT (A) and Ca (B) in plasma after intranasal administration of SCT (1.25 U/rat: circle, 5 U/rat: diamond and 20 U/rat: square). Each value indicates the mean $\pm$ S.E. of five determinations. * and

** denote significant differences to the value before administration ($P < 0.05$ and $P < 0.01$).

TABLE 1. Parameters for the Bioavailability of Intranasal SCT

Dose U/rat	AUC pg.hr/ml	MRT min
1.25	20.8	54.1
5	89.0	50.3
20	186	46.0

activity, although the effect ensued with a delay as noted for other application routes (1, 9). From the time response curve of hypocalcemia in Figure 1B, the minimum requirement for the anti-osteopenic effect of SCT may be the dose that can induce hypocalcemia lasting longer than 6 hr, although the hypocalcemic effect does not simply reflect the action on the bone.

In conclusion, by employing a novel sensitive enzyme immunoassay, the bioavailability of nasal SCT given in an anti-osteopenic dose range to rats was first directly measured; it was first evidenced that keeping the plasma SCT level above the anti-osteoclastic one for a few hours per 2 days would be enough to induce a distinct anti-osteopenic effect in rats, and now one can make a protocol for the clinical study as to the pharmacokinetic and pharmacodynamic property of SCT when being prescribed as the anti-osteoporotics for human patients. In fact, a study from the protocol is now underway using an EIA system with a sub-picomolar detection limit with which the pharmacokinetics of a human therapeutic dose can be easily followed for hours (unpub. obs.).

ACKNOWLEDGMENTS

This work was supported in part by a Grant-in-Aid for Scientific Research (06557136) from the Ministry of Education, Science, Sports and Culture of Japan.

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