Kinetic analysis and comparison of uptake, distribution, and excretion of $^{48}$V-labeled compounds in rats


Faculty of Pharmaceutical Sciences, and Tri-University Meson Facility, The University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z1

Setyawati, I. A., K. H. Thompson, V. G. Yuen, Y. Sun, M. BatteLL, D. M. Lyster, C. Vo, T. J. Ruth, S. Zeisler, J. H. McNeill, and C. Orvig. Kinetic analysis and comparison of uptake, distribution, and excretion of $^{48}$V-labeled compounds in rats. J. Appl. Physiol. 84(2): 569–575, 1998.—Vanadium has been found to be orally active in lowering plasma glucose levels; thus it provides a potential treatment for diabetes mellitus. Bis(maltolato)oxovanadium(IV) (BMOV) is a well-characterized organovanadium compound that has been shown in preliminary studies to have a potentially useful absorption profile. Tissue distributions of BMOV compared with those of vanadyl sulfate (VS) were studied in Wistar rats by using $^{48}$V as a tracer. In this study, the compounds were administered in carrier-added forms by either oral gavage or intraperitoneal injection. Data analyzed by a compartmental model, by using simulation, analysis, and modeling software, showed a pattern of increased tissue uptake with use of $^{48}$V-BMOV compared with $^{48}$VS. The highest $^{48}$V concentrations at 24 h after gavage were in bone, followed by kidney and liver. Most ingested $^{48}$V was eliminated unabsorbed by fecal excretion. On average, $^{48}$V concentrations in bone, kidney, and liver 24 h after oral administration of $^{48}$V-BMOV were two to three times higher than those of $^{48}$VS, which is consistent with the increased glucose-lowering potency of BMOV in acute glucose lowering compared with VS.

vanadium; compartmental modeling; simulation, analysis, and modeling software; diabetes; insulin mimic

Despite its ubiquitous presence in mammalian tissues (26), vanadium is an ultratracer element, the essentiality of which has not yet been conclusively demonstrated. Homeostatic mechanisms ensure low levels of absorption of dietary vanadium (30) and rapid clearance of vanadium from the bloodstream (31). Numerous studies have demonstrated the insulin-mimetic actions of vanadium both in vitro and in vivo (for reviews, see Refs. 27, 29, and 35). The most common physiologically relevant ions of vanadium are vanadate [VO$_3^-$; V(V)] and vanadyl [VO$_2^{2+}$; V(IV)] (8). In vitro, VO$_3^-$ and/or VO$_2^{2+}$ has been shown to increase glucose uptake and to stimulate glycogen synthesis in rat diaphragm, liver, and fat cells (39), to enhance glucose transport and oxidation in rat adipocytes and skeletal muscle (9, 15, 34), and to inhibit lipolysis (16) and activate lipogenesis (36) in rat adipocytes.

A key advantage of vanadium over insulin is its effectiveness when administered orally (20). Unfortunately, poor absorption from the gastrointestinal (GI) tract into the blood, coupled with doses close to toxic levels for glucose- and lipid-lowering effects, has hampered efforts to develop vanadium compounds as therapeutic adjuncts for treatment of diabetes mellitus (19, 29, 33). Although both VO$_2^{2+}$ (10) and VO$_3^-$ (17) have recently shown positive results in limited clinical trials in humans, the search is underway for better absorbed and less toxic vanadium compounds (27).

One such compound that has been synthesized in our laboratories and has undergone extensive testing over the last several years is bis(maltolato)oxovanadium(IV) (BMOV) (Fig. 1) (25). BMOV can be readily synthesized (7) by combining vanadyl sulfate (VS) and maltol (3-hydroxy-2-methyl-4-pyrone), an approved food additive in both Canada and the US. Potentially useful properties of BMOV include significant water solubility, neutral charge, and lipophilicity, a combination designed to enhance GI absorption, probably through a passive diffusion process. BMOV is effective in lowering blood glucose at a lower dose than VS and does not show evidence of toxicity over a 6-mo period of administration in streptozotocin-diabetic rats (13, 42). Both oral gavage and intraperitoneal (ip) administrations indicate that BMOV is two to three times more potent than its parent compound, VS, in bringing about acute glucose lowering in this experimental model of insulin-dependent diabetes (41).

Because BMOV displays favorable chemical and physiological properties, we were interested in its biodistribution after oral or ip administration. To this end, we prepared BMOV and VS, each containing $^{48}$V as a tracer. $^{48}$V has a half-life of 16 days and decays to nonradioactive $^{48}$Ti by means of positron and gamma emission ($\gamma = 511, 983, 1,312$ KeV). $^{48}$V was isolated as a tracer. $^{48}$V has a half-life of 16 days and decays to nonradioactive $^{48}$Ti by means of positron and gamma emission ($\gamma = 511, 983, 1,312$ KeV). $^{48}$V was isolated as a tracer. $^{48}$V has a half-life of 16 days and decays to nonradioactive $^{48}$Ti by means of positron and gamma emission ($\gamma = 511, 983, 1,312$ KeV). $^{48}$V was isolated as a tracer. $^{48}$V has a half-life of 16 days and decays to nonradioactive $^{48}$Ti by means of positron and gamma emission ($\gamma = 511, 983, 1,312$ KeV). $^{48}$V was isolated as a tracer.
MATERIALS AND METHODS

Three sets of experiments were run: an initial oral gavage (experiment A), a second oral gavage experiment in which excreta were collected (experiment B), and an ip set (experiment C).

Animals and diets. Male Wistar rats (University of British Columbia Animal Care Unit, Vancouver, BC), weighing between 190 and 220 g, were housed in polycarbonate cages. During experiments A and C, rats were housed three per cage.

For experiment B, animals were transferred to individual metabolic cages immediately after administration of the compound. These cages were equipped with fecal and urinary collection containers. The animals were kept in a room maintained at 22–25°C in a 12:12-h light-dark cycle and were cared for in accordance with the principles and guidelines of the Canadian Council on Animal Care. A commercial diet (Purina rat chow, Ralston Purina, St. Louis, MO) and tap water were offered ad libitum for several days before and throughout the experimental period.

Materials. Maltol was purchased from Sigma Chemical (St. Louis, MO) or Pfizer (Veltol, New York, NY), and VO2 ·3H2O was from Aldrich Chemical (Milwaukee, WI). With the exception of drinking water, all water was deionized (Barnstead D8902 and D8904 cartridges) and distilled (Corning MP-1 Megapure Still). 48V, in the form of non-carrier-added 48VS, was prepared as described in Zeisler and Ruth (43).

Preparation of carrier-added 48V-labeled complexes. In the initial oral and ip experiments (experiments A and C, respectively), carrier-added 48VS was prepared by mixing an appropriate volume of 48VS (in 0.01 M H2SO4) with 0.2 mmol (50 mg) of VO2 ·3H2O. Immediately before administration of the solution, the pH was adjusted to between 3.5 and 4 by dropwise addition of 1 M NaOH. The total volume was then increased to 12 ml with the addition of water.

Carry-added 48V-BMOV in the initial oral and ip experiments (A and C) was synthesized as follows: to the mixture of 0.2 mmol (50 mg) of VO2 ·3H2O and 3–4 eq of maltol was added an appropriate volume of 48VS (in 0.01 M H2SO4). With rapid stirring, 0.5 ml of 1 M NaOH was added dropwise to the suspension at 80°C. This temperature was maintained for another 10 min so that the reaction mixture was almost dry. 48V-BMOV was isolated as a gray-green solid. Immediately before administration, it was dissolved in 16 ml of water.

In the preparation of 48V-BMOV in the subsequent oral gavage administration (experiment B), 0.08 mmol (18 mg) of VO2 ·3H2O and 4 eq of maltol were mixed in water. After dissolution of the solid, 0.8 ml of 0.2 M NaOH was added dropwise over a 10-min period. To this mixture was added an appropriate amount of 48VS (in 0.005 M H2SO4). The pH of the solution was finally adjusted to between 3.5 and 4 with 0.2 M NaOH before gavage. 48VS in experiment B was prepared by dissolving 0.07 mmol (15 mg) of VO2 ·3H2O and 48VS (in 0.005 M H2SO4) in water. The pH of this solution was adjusted to 4 by adding 0.2 M NaOH.

Biodistribution studies. In experiment A, 48V-BMOV (3.8 mg V) or 48VS (2.6 mg V), 0.012 mmol/animal and containing 200 µCi/animal of each compound, was administered by oral gavage. In experiment C, 48V-BMOV (1.9 mg V) or 48VS (2.2 mg V), 0.006 and 0.010 mmol/animal, respectively, and containing 100 µCi 48V for BM0V/rat or 150 µCi 48V for VS/rat, was administered by ip injection. In these two experiments (A and C), rats (n = 3/time point) were euthanized, and tissue and blood samples were collected at time points of 15 and 30 min and 1, 4, and 24 h after administration. In experiment B, 48V-BMOV (3.2 mg V, 0.010 mmol) or 48VS (2.0 mg V, 0.009 mmol), containing 170 or 50 µCi of 48V/rat, respectively, was administered, also by oral gavage. Collection times in experiment B were 2, 6, 10, 14, 19, and 24 h after administration.

At each time point in all studies, three rats were anesthetized with halothane, blood was obtained from the carotid artery, and the anesthetized animals were then killed by cervical dislocation. Tissues were collected and placed in weighed receptacles. Liver, kidney, muscle, spleen, lung, testis, bone, heart, and brain samples were rinsed in normal saline, weighed, and blotted dry before freezing. In the second oral gavage study (experiment B), urine, fecal, stomach, and GI tissue samples were also collected. All tissue samples were stored frozen at −20°C until counted for 48V activity. The activity was recorded in a well gamma counter (Canberra Packard, Cobra II, Auto-gamma/3-in. crystal). The percentage of administered dose (%AD) was calculated as a fraction of the “standard counts” for that time period, determined by counting the equivalent volume of radiolabeled solution with the same set of samples (Fig. 2, as %AD/organ). In this carrier-added study, calculating %AD corrects for variation of the “standard counts” for that time period, determined by counting the equivalent volume of radiolabeled solution with the same set of samples (Fig. 2, as %AD/organ). In this carrier-added study, calculating %AD corrects for variation of the “standard counts” for that time period, determined by counting the equivalent volume of radiolabeled solution with the same set of samples (Fig. 2, as %AD/organ).

Kinetic modeling. Simulation of 48V uptake, distribution, and excretion was carried out by using SAAM II software (version 1.0.2) (4, 5). Data, in %AD/g tissue, from the initial oral gavage study (experiment A) were used to modify a model of vanadium metabolism published previously (28). The fractional transfer coefficients (kij), where i and j are compartments, derived from experiment A were then used as a starting point for modeling the data from the second oral gavage study (experiment B). Initial values were varied by iteration until a consistent set of parameters was obtained for each compound, with the greater variability of the second data set for each compound tested having been accounted for.

Experiment C, with the highest overall coefficient of variation (CV; 0.9), was modeled last as an extension of the combined oral gavage paradigm (37). In accordance with standard modeling nomenclature (14), kij represents the fraction of material moving from compartment j to compartment i per unit time (h). Residence time is equivalent to the inverse of kij for those compartments having only one egress; for blood, residence time is defined as 1/k11, where compartment 1 represents blood (Fig. 3) (22). Absorption of vanadium 24 h postadministration was calculated as (intake – fecal excretion) as a percentage of administered oral dose (22).

RESULTS

Overall, for oral administration the highest concentrations of 48V (expressed as %AD/g wet wt of tissue) were seen in kidney, followed by bone, blood, liver,
The concentrations in the above tissues did not exceed 0.6 %AD/g at any one time point (see below). In most other tissues, the concentration did not exceed 0.1 %AD/g of oral dose. The concentration of $^{48}$V in bone exceeded that of kidney at 24 h after oral gavage. CVs for experiment B were considerably higher than those for experiment A (average CVs of 0.3 and 0.5 for experiments A and B, respectively). IP injection of $^{48}$V-labeled compounds (experiment C) resulted in a higher apparent uptake in kidney and bone (not exceeding 1.6 %AD/g tissue), with little change in uptake for most other tissues between 4 and 24 h after injection, compared with either of the oral gavage experiments.

The relative concentrations of $^{48}$V in bone and kidney, spleen, and heart (see Fig. 2 for data as %AD/organ). The concentrations in the above tissues did not exceed 0.6 %AD/g at any one time point (see below). In most other tissues, $^{48}$V concentration did not exceed 0.1 %AD/g of oral dose. The concentration of $^{48}$V in bone exceeded that of kidney at 24 h after oral gavage. CVs for experiment B were considerably higher than those for experiment A (average CVs of 0.3 and 0.5 for experiments A and B, respectively). IP injection of $^{48}$V-labeled compounds (experiment C) resulted in a higher apparent uptake in kidney and bone (not exceeding 1.6 %AD/g tissue), with little change in uptake for most other tissues between 4 and 24 h after injection, compared with either of the oral gavage experiments.

For tables of overall data from experiments A–C, order NAPS Document 05438 from NAPS, c/o Microfiche Publications, PO Box 3513, Grand Central Station, New York, NY 10017.
after ip administration were also considerably higher than those after an oral dose (see below).

Soft tissue uptake from oral gavage of $^{48}$VS and $^{48}$V-BMOV, as %AD/organ (Fig. 2), on the basis of average total tissue or organ weights, was greatest overall in liver > kidney > spleen > heart > testes > lung. The highest $^{48}$V content at 24 h after oral gavage was seen in bone, liver, and kidney (Fig. 2); however, muscle also took up appreciable amounts of $^{48}$V (Fig. 2) because of the high percentage of body weight (45%) represented by muscle.

A 13-compartment model (including urinary and fecal "sinks") adequately described the data for both $^{48}$V-BMOV and $^{48}$VS carrier-added doses by oral gavage (Fig. 3). High variation in the data, both within data sets and between experiments A and B, precluded a more complex model (14). Hence, the tissues represented (see Fig. 3) were chosen on the basis of previous studies showing uptake into these tissues (12). Remaining tissues sampled (heart, brain, spleen, lung, pancreas, and muscle) were included in a "lumped" compartment, labeled as compartment 7. A kinetic heterogeneity in kidney was apparent, with the data fitting best to a two-compartment kidney. The three-compartment GI represents a delay between oral input and fecal output and does not signify physiological differences between these compartments (38).

Compartmental analysis revealed a pattern of increased uptake from an oral dose of $^{48}$V-BMOV compared with that of $^{48}$VS. The greatest difference in uptake was seen in liver, which had a four times greater uptake of $^{48}$V-BMOV relative to $^{48}$VS. The schematic of the model (Fig. 3) shows oral input proceeding through a three-compartment GI, with absorbed $^{48}$V taken up into the blood and from there being distributed to short- and long-stay tissues. Unabsorbed $^{48}$V was excreted via the feces, whereas excretion of absorbed $^{48}$V was via the urine through the second kidney compartment. Recirculation of $^{48}$V is possible through both biliary secretion from the liver and resecretion back to blood from the second kidney compartment. Simulations of tracer disappearance by using the combined data sets, in %AD/g tissue, are presented in Fig. 4 ($^{48}$VS and $^{48}$V-BMOV). Most tissues showed a gradual uptake, peaking at 2–6 h after gavage, with a decline thereafter; however, bone, liver, and kidney uptakes remained high at 24 h, indicating continued accumulation or slow clearance.

Apparent absorption of $^{48}$V-BMOV was greater than that of $^{48}$VS. The primary route of elimination was via the feces. On the basis of compartmental analysis of combined data sets (experiments A and B), 75% of $^{48}$VS and 62% of $^{48}$V-BMOV were excreted unabsorbed in the feces within 24 h after oral gavage. Although the time frame of this experiment was too short to accurately determine absorption, the model-predicted apparent vanadium absorption was 25% for $^{48}$VS and 38% for $^{48}$V-BMOV. These values are most likely a considerable overestimate of vanadium absorption because they are based on estimates from 24-h data sets only. The model-predicted absorption values were adequate to indicate a trend toward greater absorption of $^{48}$V-BMOV compared with $^{48}$VS, ~1.5 times greater.

The bone-to-kidney-to-liver ratios of $^{48}$V concentrations (in %AD/g) 24 h after oral gavage predicted by the model were 0.3265:0.1163:0.082 for $^{48}$V-BMOV and
0.1131:0.0856:0.0212 for 48VS (Table 1). Thus the proportions of 48V taken up by bone, kidney, and liver after 48V-BMOV treatment were ~3, 1.4, and 4 times, respectively, greater than those after 48VS treatment. Averaging the increased uptake into liver, kidney, and bone resulted in a ratio of 48V-BMOV to 48VS uptake of 2.7. With total tissue weight accounted for, the principal uptakes of 48V with use of an oral dose of BMOV vs. that of VS were 0.82 vs. 0.21% in liver, 0.23 vs. 0.17% in kidney, 1.14 vs. 0.67% in muscle, 3.55 vs. 2.73% in blood, and 8.62 vs. 2.99% in bone, on the basis of model-predicted compartmental masses at 24 h after gavage.

Residence times of 48V (and the fractional transfer coefficients on which they were based) for the tissues modeled are given in Table 2. The shortest residence times were in blood; the longest in bone. Residence times in blood were calculated to be 7 min for 48V-BMOV and 5 min for 48VS; in bone, residence times of 31 days for 48V-BMOV and 11 days for 48VS were calculated.

**DISCUSSION**

These results clearly demonstrate the increased tissue uptake from an oral dose of 48V-BMOV compared with that of 48VS. This is in accordance with comparisons in tissue uptake between iron sulfate and the iron maltol complex, tris(maltolato)iron(III) (2, 3, 23). These studies have shown that, by forming a stable, neutral complex with Fe3+, maltol enhanced Fe3+ absorption across the rat small intestine in vivo and that not only was the Fe3+ held in soluble form but it was also efficiently carried into the mucosa. At the same time, regulatory processes to prevent Fe3+ overload were not bypassed. In our study, complexation of labeled vanadium(IV) with maltol resulted in enhanced uptake and slower excretion of 48V-BMOV compared with 48VS (Fig. 4, Tables 1 and 2). Absorption was also greater with 48V-BMOV than with 48VS. This greater absorption and tissue uptake are also consistent with the increased pharmacological potency in lowering in vivo blood glucose levels seen previously (41).

The long residence time of 48V in bone (11.1 days) with use of 48VS and 31.3 days with use of 48V-BMOV) predicted by our model is in agreement with earlier studies (1, 28). Subcutaneous injection of rats with 48V-VO3– (1.8 mg V/kg) analyzed with a two-compartment model for each tissue (1) predicted a half-life for bone of 376 h (15.6 days), which would be equivalent to a residence time of 22.6 days (t1/2=ln2RT, where RT is residence time). Prediction of a previous vanadium model (28) (assuming that the unspecified long-stay tissue in the model of Patterson et al. (28) represents bone) was >104 h, or ~42 days, also reasonably close to the residence times predicted by our model. Variability within data sets, and between the first and second oral gavage studies (experiments A and B), resulted in high error terms for the fractional transfer coefficients representing movement of vanadium between compartments and, as a consequence, also for calculated residence times.

In another study, at 24 h postinjection, %AD (of 48V-vanadyl chloride added to ammonium metavanadate) was 1.25% in blood, 17.9% in bone, and 2.65% in muscle (1) compared with 3.55% in blood, 8.62% in bone, and 1.14% in muscle with use of 48V-BMOV in our study. In Conklin et al. (11), oral gavage of 48V-V2O5 (40 µg/rat, ~0.3 mg/kg) resulted in 0.65 %AD in bone 3 days after dosing. The low solubility of V2O5 compared with VO3–, VO2+, or BMOV is probably the cause of this low localization; however, the longer time frame is also a factor. Hamel and Duckworth (18) predicted longer residence times than we calculated for a variety of soft tissues (bone vanadium was not measured) in rats on the basis of patterns of uptake and redistribution of low levels of dietary vanadium, as detected by neutron-activation analysis over a longer time period. The significant difference in dose and the chronic, rather than acute, nature of the study may explain this discrepancy. The researchers (18) also observed that 2.7% of a gavage dose was in the blood (total) at 24 h, which is in good agreement with our calculations.

The kinetic heterogeneity in kidney predicted by our model suggests that the longer-stay kidney compartment may represent stored vanadium, whereas the short-stay kidney compartment may represent a vana-

**Table 1. Relative concentrations of 48V 24 h after oral gavage**

<table>
<thead>
<tr>
<th></th>
<th>Liver (%)</th>
<th>Kidney (%)</th>
<th>Bone (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>48VS</td>
<td>0.0212</td>
<td>0.0856</td>
<td>0.1131</td>
</tr>
<tr>
<td>48V-BMOV</td>
<td>0.082</td>
<td>0.1163</td>
<td>0.3265</td>
</tr>
<tr>
<td>48V-BMOV/48VS</td>
<td>3.9</td>
<td>1.4</td>
<td>2.9</td>
</tr>
</tbody>
</table>

Percent administered dose (%AD: as %AD/g tissue) predicted by model (Fig. 3) at 24-h time point. Average %AD of 48V-bis (maltolato)oxovanadium(IV) (BMOV)/Vanadyl sulfate-48 (48VS) for liver, kidney, and bone was 2.7.

**Table 2. Fractional transfer coefficients and residence times for 48V in compartmental model**

<table>
<thead>
<tr>
<th></th>
<th>48VS</th>
<th>48V-BMOV</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Transfer Coefficients</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>k1,2 (kidney1–kidney2)</td>
<td>0.75 ± 0.28</td>
<td>0.26 ± 0.05</td>
</tr>
<tr>
<td>k1,4 (liver–plasma)</td>
<td>0.26 ± 0.09</td>
<td>0.15 ± 0.04</td>
</tr>
<tr>
<td>k1,5 (testes–plasma)</td>
<td>0.63 ± 0.13</td>
<td>0.29 ± 0.10</td>
</tr>
<tr>
<td>k1,6 (bone–plasma)</td>
<td>0.0038 ± 0.0022</td>
<td>0.0028 ± 0.0011</td>
</tr>
<tr>
<td>k1,13 (kidney2–plasma)</td>
<td>4.9 ± 2.3</td>
<td>0.63 ± 0.50</td>
</tr>
<tr>
<td>k3,13 (kidney2–urine)</td>
<td>7.4 ± 0.7</td>
<td>5.5 ± 0.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>48V</th>
<th>48V-BMOV</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Residence Times</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RT1 (blood*)</td>
<td>5.0 min</td>
<td>7.0 min</td>
</tr>
<tr>
<td>RT2 (kidney1)</td>
<td>4.9 min</td>
<td>9.7 min</td>
</tr>
<tr>
<td>RT4 (liver)</td>
<td>3.8 h</td>
<td>6.8 h</td>
</tr>
<tr>
<td>RT5 (testes)</td>
<td>1.7 h</td>
<td>3.4 h</td>
</tr>
<tr>
<td>RT6 (bone)</td>
<td>11 days</td>
<td>31 days</td>
</tr>
<tr>
<td>RT13 (kidney2†)</td>
<td>1.3 h</td>
<td>3.8 h</td>
</tr>
</tbody>
</table>

Fraction of vanadium pool moving from compartment i to compartment j per unit time (h) = ± SE for variation between simulation runs is shown. Residence time (RT) is calculated as inverse of k1 from that compartment. Kidney1 and kidney2, kinetically distinct compartments that could be either physiologically or biochemically distinguishable, with no anatomic separation implied; numbers, compartment numbers in model (Fig. 3). *RT1 = 1/2k1,1. +RT2 = 1/2k1,13.
KINETIC VANADIUM MODEL

Received 17 July 1997; accepted in final form 24 September 1997.

The authors gratefully acknowledge the Medical Research Council of Canada for an operating grant (C. Orvig and J. H. McNeill) as well as the Natural Sciences and Engineering Research Council of Canada (I. A. Setyawati and Y. Sun) and Angiotech Pharmaceuticals (K. H. Thompson) for personnel support. We also thank Prof. K. W. Riggs for helpful discussions.

Address for reprint requests: C. Orvig, Medicinal Inorganic Chemistry Group, Chemistry Dept., The Univ. of British Columbia, Vancouver, BC, Canada V6T 1Z1 (E-mail: ORVIG@chem.ubc.ca).

Received 17 July 1997; accepted in final form 24 September 1997.

REFERENCES


