Assessment of Radioactive Residues Arising from Radiolabel Instability in a Multiple Dose Tissue Distribution Study in Rats

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Our study objectives were: To quantitatively determine the effect of radiolabel instability on terminal phase radioactive tissue residues in a multiple dose tissue distribution study. To quantitatively compare tissue residue artifacts (non-drug-related radioactivity) from the acetamide radiolabel site to the oxazolidinone radiolabel site. To conduct a definitive multiple dose tissue distribution study using the better of the two radiolabeled compounds. We compared the excretion and tissue distribution in rats of [14C]linezolid, radiolabeled in two different locations, after 7 consecutive once-daily [14C] oral doses. The radiolabels were in the acetamide (two carbon) and oxazolidinone (isolated carbon) functional groups. Terminal phase tissue residue and excretion data were compared to data from rats dosed orally with [14C]sodium acetate. Drug-related radioactivity was excreted rapidly over 24 h. After a single dose, the acetamide and oxazolidinone radiolabel sites both gave 3% of dose as exhaled 14CO2. After 7 daily [14C] oral doses, terminal phase radioactive tissue residues were higher from the acetamide radiolabel, relative to the oxazolidinone radiolabel, and were primarily not drug-related. In the definitive tissue distribution study, low concentrations of drug-related radioactivity in skin and thyroid were observed. We conclude that although small amounts of radiolabel instability do not significantly affect single dose tissue radioactivity Cmax and AUC, artifacts arising from radiolabel instability can prolong the apparent terminal phase half life and complicate study data interpretation. When possible, it is always preferable to use a completely stable radiolabel site.

Key words tissue; distribution; excretion; artifact; International Congress for Harmonization (ICH); rat

Linezolid ((S)-N-[[3-[3-fluoro-4-(4-morpholinyl)phenyl]-2-oxo-5-oxazolidinyl]methyl]-acetamide, ZYVOXTM, PNU-100766, Fig. 1) is the first of a new class of antibiotics, the oxazolidinones. Linezolid is approved in the United States and other countries worldwide, for the treatment of gram positive bacterial infections.10) The oxazolidinones are synthetic compounds that selectively inhibit the initiation phase of bacterial protein synthesis.2—5) The disposition of [14C]linezolid has been studied in humans6) and in mice, rats, and dogs.7)

Until 1995, multiple dose tissue distribution studies in rats were routinely conducted as part of the preclinical ADME section of most Japanese NDAs. In 1995, International Congress for Harmonization (ICH) guidelines were adopted that defined four specific circumstances under which these studies should still be considered.8) The following condition seemed to apply to linezolid: “When single dose distribution studies suggest that the apparent half life of the test compound (and/or metabolites) in organs or tissues significantly exceeds the apparent half life of elimination phase in plasma, and is more than twice the dosing interval in the toxicity studies.”9)

Fig. 1. Metabolic Fate of the Radiocarbon from the Two Chemically-Distinct Radiolabel Sites of Linezolid

The scheme shows the loss of radioactivity as a one carbon fragment from the oxazolidinone ring radiolabel (3% of dose), or as acetate from the acetamide radiolabel (3% of dose). The fate of the two radioactive byproducts is distinct. While both sites result in terminal phase radioactive tissue residues that are not drug-related, the acetate radiolabel has potential for higher incorporation into endogenous metabolic pathways, as it is not excreted by direct exhalation.

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The plasma half life of linezolid in rats was 1 h and the plasma radioactivity terminal half-life was 25 h, the latter half life was approximately equal to twice the daily dosing interval in rat toxicity studies. Although there was no evidence of toxicity problems that may have been related to low concentrations of terminal phase radioactivity, we undertook the present study to better understand the radioactivity half life in plasma.

To examine the persistence of low concentrations of radioactivity in rat plasma in rats dosed with acetamide-radiolabeled linezolid, we compared the multiple radioactive dose excretion and tissue distribution of two distinct radiolabel sites in \[^{14}C\]linezolid (Fig. 1), to orally administered \[^{14}C\]-sodium acetate. The better of the two possible radiolabel locations was then selected for a definitive good laboratories practice (GLP)-regulated study on terminal phase excretion and tissue distribution of radioactivity.

**MATERIALS AND METHODS**

Linezolid (PNU-100766) was synthesized by Pharmacia. Acetamide radiolabeled linezolid contained 6.2 MBq/mg (167.82 \(\mu\)Ci/mg or 56.61 mCi/mmol) of radioactivity with radiochemical purity >99.18% by HPLC. Oxazolidinone ring radiolabeled \[^{14}C\]linezolid contained 6.2 MBq/mg (163.24 \(\mu\)Ci/mg or 55.07 mCi/mmol), with a radiochemical purity 100% by HPLC. Acetic acid, sodium salt \([1-{^{14}C}]\) (Moravek Biochemicals Inc., Brea, CA, U.S.A.) lot number 400A-016-055, 2035 MBq/mmol (55 mCi/mmol), radiochemical purity 99.9% by HPLC was used in this study.

This study was conducted according to established regulations or guidelines pertaining to the care and use of animals, including the Federal Animal Welfare Act (Pub.L. 89-544), the Guide for the Care and Use of Laboratory Animals (ILAR, NRC, 1996), accreditation standards established by AAALAC, and the AVMA Panel on Euthanasia (JAVMA, March 1, 2001).

**Preliminary Experiments. Single Dose Radiolabel Stability Studies** Preliminary single dose radiolabel excretion studies were conducted to determine major excretion routes and radiolabel stability. An acetamide radiolabeled aqueous solution formulation of linezolid 344 kBq/g (9.3 \(\mu\)Ci/g and 2.5 mg/g) was dosed at 25 mg/kg and 3.7 MBq/kg (100 \(\mu\)Ci/kg) to four male Sprague Dawley rats (224–235 g). Urine and feces were collected for 120 h and exhaled \(^{14}\)CO\(_2\) was collected from 2 rats for 72 h in an alkaline \(^{14}\)CO\(_2\) trapping tower. Radioactivity recovery results have been summarized elsewhere.\(^7\)

The oxazolidinone ring radiolabel analogue was synthesized for use in multiple dose distribution studies. A preliminary single dose radiolabel excretion study was conducted. An oxazolidinone radiolabeled suspension formulation of linezolid (ingredients described below, 740 kBq/g (20 \(\mu\)Ci/g and 5.1 mg/g)) was dosed at 25 mg/kg and 3.7 MBq/kg (100 \(\mu\)Ci/kg) to three male Sprague Dawley rats (221±5 g). Urine, feces and exhaled \(^{14}\)CO\(_2\) were collected for 72 h and analyzed for radioactivity.

**Multiple Dose Formulations** The oral suspension vehicle consisted of 1% w/v microcrystalline cellulose and carboxymethylcellulose sodium NF low viscosity (Avicel RC-591), 5% w/v polysorbate 80 NF food grade, (50 mCi) sodium acetate USP granular, pH was adjusted to 4.47 with hydrochloric acid or sodium hydroxide.

Radiolabel locations are shown in Fig. 1. The acetamide labeled \[^{14}C\]linezolid formulation concentration was 5.4 mg linezolid per g formulation containing 186 kBq/g (5.03 \(\mu\)Ci/g). The specific activity was 34.4 kBq/mg (0.93 \(\mu\)Ci/mg). The oxazolidinone (carbamate) \[^{14}C\]linezolid formulation concentration was 4.8 mg linezolid per g dose formulation containing 188 kBq/g (5.09 \(\mu\)Ci/g). The specific activity was 38.9 kBq/mg (1.05 \(\mu\)Ci/mg).

The \[^{14}C\]sodium acetate formulation was 4.187 mg sodium acetate (including buffer acetate) per g dose solution containing 24.1 kBq/g (0.6524 \(\mu\)Ci/g). The specific activity was 5.8 kBq/mg (0.156 \(\mu\)Ci/mg) drug. Since acetate is an endogenous compound and is rapidly metabolized, absolute concentrations of acetate are not relevant; radioactivity is nonetheless expressed as acetate equivalents to allow comparison to linezolid data.

**Multiple Dose Excretion and Tissue Distribution Studies** Part A: Five male Sprague Dawley rats received 7 daily oral bolus doses of acetamide labeled \[^{14}C\]linezolid at approximately 25 mg/kg/rat/d (925 kBq/kg/d or 25 \(\mu\)Ci/kg/d).

Part B: Two rats received 7 daily oral bolus doses of oxazolidinone ring labeled \[^{14}C\]linezolid at approximately 25 mg/kg/rat/d (925 kBq/kg/d or 25 \(\mu\)Ci/kg/d). Part C: Five male rats received 7 daily oral bolus doses of approximately 18.5 kBq/d (0.5 \(\mu\)Ci/d) of \[^{14}C\]sodium acetate for seven consecutive days. Only the radioactive dose is relevant to the sodium acetate dosimetry, since acetate is an endogenous compound.

Parts A and C: One rat was sacrificed at 168 h (day 8 of the study or 24 h after the last dose), two rats were sacrificed at 312 h (day 14 of the study or 7 d after the last dose) and two rats were sacrificed at 480 h (day 21 of the study or 14 d after the last dose). In Part B: both rats were sacrificed at 480 h (day 21 of the study or 14 d after the last dose).

**Sample Collection** Whole blood samples were collected from CO\(_2\) anaesthetized animals by cardiac puncture. Aliquots were removed for radioanalysis and the remainder of blood was centrifuged and plasma was harvested. Urine was collected over dry ice/wet ice at 24 h intervals from day 1 of dosing to day of sacrifice. The samples were stored frozen until analysis. Feces were collected over dry ice/wet ice at predose and at 24 h intervals from day 1 of dosing to day of sacrifice. The samples were stored frozen until analysis. The following tissue samples were excised at time of sacrifice: skin, white fat, adrenal glands, kidneys, epididymis, testes, thyroid/parathyroid, bone marrow from the right femur, whole blood, plasma, brain and liver. Carcasses were collected at time of sacrifice and stored frozen.

**Sample Preparation and Analysis** Duplicate aliquots of whole blood or feces or tissue homogenate were weighed into tared combustion cones, air dried, combusted and analyzed by liquid scintillation counting (LSC). Duplicate aliquots of plasma or urine were placed in a tared scintillation vial containing approximately 10 ml Ultima-Gold® and the weights recorded. Radioactivity was measured by direct LSC. The skin, fat, adrenal gland, kidney, epididymis, testes, thyroid/parathyroid and bone marrow samples were weighed into tared combustion cones, air dried, combusted and analyzed by LSC.
Radioanalytical Methods Radioactivity was measured by LSC in a Packard Tri-Carb Model 2300TR liquid scintillation counter (Packard Instruments, Meriden, CT, U.S.A.). A Packard Model 307 sample oxidizer equipped with a Packard Oximate 80 robot was used to combust the tissue homogenate samples completely to carbon dioxide and water. The resulting $^{14}$CO$_2$ was trapped in 9 ml Carbo-Sorb$^\circ$ E and diluted with 10 ml Permafluor$^\circ$ E+ scintillation cocktail. The $[^{14}C]$ activity was quantified by LSC.

Definitive Study. Test Substance The $[^{14}C]$linezolid (oxazolidinone radiolabel site) formulation had a final concentration of 5.4 mg linezolid per g dose solution containing 203.9 kBq/g (5.51 $\mu$Ci/g) of dose and a specific activity of 37.8 kBq/mg (1.0203 $\mu$Ci/mg) of drug. The radiochemical purity of the $[^{14}C]$linezolid dose formulation was determined by radiometric-HPLC to be 100%.

Animals Forty-four Sprague–Dawley rats (22 males and 22 females, 205–225 g) were purchased from Charles River Laboratories, Kingston, NY, U.S.A. After dosing, 8 rats (4 male and 4 female, group 6) were housed individually in metabolism cages. Groups 1—5 and 7 extra rats were housed communally by sex and group number, in shoebox cages with a raised stainless steel flooring over corncob bedding. The bedding was changed daily during the multiple dosing time period. Animals were not fasted prior to dose administration.

Study Design The study was designed to provide an assessment of the excretion and tissue distribution of radioactivity related to oxazolidinone ring labeled $[^{14}C]$linezolid in male and female Sprague–Dawley rats after a single oral dose or seven consecutive daily oral doses. Six rats (3 male and 3 female, Group 1 only) received a single oral bolus of $[^{14}C]$linezolid at approximately 25 mg/kg. Thirty-eight rats (Group 2—7, 19 male and 19 female) received multiple (once a day for 7 d) oral bolus doses of $[^{14}C]$linezolid at approximately 25 mg/kg/rat/d. Group 1 rats were sacrificed at 24 h after dosing; at which time, selected tissues were excised. Group 2 rats were sacrificed at 168 h (day 8 of the study or 24 h after the last dose). Group 3 rats were sacrificed at 240 h (day 11 of the study or 4 d after the last dose). Group 4 rats were sacrificed at 312 h (day 14 of the study or 7 d after the last dose). Group 5 rats were sacrificed at 480 h (day 21 of the study or 14 d after the last dose). In Group 6, urine and feces were collected at 24 h intervals throughout the entire study. The Group 6 rats were sacrificed at 480 h. Group 7 rats were dosed as spares, for use if needed.

Male rats received 24.4—26.8 mg/kg and female rats received 24.4—28.4 mg/kg of $[^{14}C]$linezolid. A multiple (once a day) bolus dose of the drug was given by gastric intubation. Blood samples were collected from CO$_2$ anaesthetized animals by cardiac puncture. Urine was collected at room temperature at 24 h intervals from day 1 of dosing to day of sacrifice. Feces were collected at room temperature at 24 h intervals from day 1 of dosing to day of sacrifice. The following tissue samples were excised at time of sacrifice: adrenal glands, bladder (rinsed), blood, bone (tibia), bone marrow, brain, epididymis, eyes, white fat, brown fat, large intestine (rinsed), small intestine (rinsed), stomach (rinsed), combined stomach and intestinal tract contents (GI contents), Harderian gland, liver, lungs, lymph node (mesenteric), kidney, muscle (gastrocnemius), myocardium, pancreas, pituitary, plasma, prostate, skin (shaved dorsal), spleen, testes, thymus and thyroid/parathyroid. The following additional tissue samples were excised from female animals at time of sacrifice: blood, ovary, uterus and plasma. Carcasses were collected at time of sacrifice and stored frozen.

Duplicate aliquots of whole blood were weighed into tared combustion cones, air dried at room temperature, combusted and analyzed by LSC. Duplicate aliquots of plasma were placed in a tared scintillation vial containing approximately 10 ml Ultima-Gold$^\circ$ and weighed. The urine samples were weighed and duplicate aliquots of urine (plus cage rinse) were weighed into tared scintillation vials containing approximately 10 ml Ultima-Gold$^\circ$. Radioactivity was measured by direct LSC.

Feces, liver, brain and carcasses were each homogenized with approximately 3—5 volumes of water and weighed. Tissue or feces homogenates were weighed in duplicate into tared combustion cones, air dried at room temperature, combusted and analyzed by LSC.

Statistical analysis of distribution and excretion data was calculated using DEBRA$^\text{TM}$ for Windows, version 5.0 (1997 LabLogic Systems Ltd.). The DEBRA 5 program was used to convert the concentration of radioactivity in the analyzed sample to $\mu$g-eq/g. The dpm value for each aliquot (after background subtraction of blank matrix dpm and correction for efficiency in the combusted samples) was divided by the aliquot weight to give dpm/g. The concentrations were determined from the mean dpm of the sample and the specific activity of the drug ($\mu$Ci/mg). Dosimetry, terminal phase ARE (amount remaining to be excreted) and approximate terminal elimination and half-life data was calculated using Microsoft Excel 97 (Microsoft Corp., Seattle, WA, U.S.A.).

RESULTS

Preliminary Studies. Radiolabel Instability and Its Effect on Radioactivity Excretion in Single Dose Studies Preliminary single dose radiolabel excretion studies were conducted to determine major excretion routes and radiolabel stability. The acetamide radiolabel analogue of linezolid gave a total 0—120 h recovery of 98.9±1.43%, comprised of radioactivity in urine (73.1±1.8%), feces (23.6±1.14%), carcass (0.6±0.1%) and $^{14}$CO$_2$ (2.7%, n=2). Much later, when it was suspected that terminal phase radioactive residues in plasma from the acetamide radiolabel were not drug-related, the oxazolidinone ring radiolabel analogue was synthesized. This radiolabel site was chosen based on ease of preparation, and the presumption of better in vivo radiolabel stability at the oxazolidinone ring carbon. A preliminary single dose radiolabel excretion and stability study was conducted. The oxazolidinone radiolabel analogue of linezolid gave a total 0—72 h recovery of 94.6±4.15%, comprised of radioactivity in urine (67.7±3.2%), feces (23.1±3.4%), carcass (0.6±0.2%), cagewash (0.2±0.1) and $^{14}$CO$_2$ (3.0±0.3%). The observed 3% loss to $^{14}$CO$_2$ was unexpected, but is chemically rational for a carbamate-containing ring system. Rather than embark upon the synthesis and testing of a fluorophenyl ring radiolabel, we chose to investigate the hypothesis that $^{14}$CO$_2$ from the oxazolidinone label site would be directly exhaled, rather than incorporated into tissues. This feature would render the oxazolidone ring radiolabel suitable for the definitive multi-
Multiple Dose Excretion and Tissue Distribution Studies Comparing Two Label Sites to Sodium Acetate  

The preliminary studies compared the excretion and tissue distribution in rats of these two radiolabel sites in [14C]linezolid, after 7 consecutive once daily [14C] oral doses. Terminal phase tissue residue and excretion data were compared to data from rats dosed with [14C]sodium acetate.

Table 1 compares the cumulative terminal excretion of radioactivity after administration of acetamide labeled [14C]-linezolid, oxazolidinone (carbamate) labeled [14C]linezolid, and [14C]sodium acetate. Recovery of linezolid-related radioactivity in excreta is effectively complete at 24 h after the last (7th) daily dose. In accord with radiolabel stability studies showing 3% loss to 14CO2, total radioactivity recoveries for radiolabeled linezolid were between 93 and 95% of dose. For orally dosed [14C]sodium acetate, the recovered radioactivity 14d after the last radioactive dose (9—13% in excreta, tissue and carcass), indicates that only approximately one-tenth of [14C]acetate consumed by intermediary metabolism is retained in the carcass or excreted. The rest, represented by the unrecovered dose (approximately 89%), is presumed to have been lost as exhaled 14CO2.

Figure 2 compares terminal phase amount-remaining to be excreted (ARE) plots (percent of dose remaining in body versus time) for the two radiolabeled linezolid analogues with radioactive sodium acetate. Parallel plots show that the terminal excretion half-lives are relatively uniform for all three compounds. This can be taken as indirect evidence that radiolabel loss to intermediary metabolism is a major contributor to terminal phase elimination half-life of radiolabeled linezolid. Importantly, this long lived radioactive residue, in its entirety, represents much less than 1% of the radioactive linezolid dose.

Figure 3 compares radioactivity concentrations (µg-eq/g) in 12 different tissues at 14d after the last dose. Both linezolid radiolabel sites are compared to radioactive concentration from orally dosed radioactive sodium acetate. At an equivalent dose (925 KBq/kg, (25 µCi/kg)) and equivalent loss to 14CO2 (3%), the acetamide radiolabel left higher tissue residues than the oxazolidinone radiolabel. Skin and thyroid stand out as potential sites of drug-related radioactivity retention and the oxazolidinone label site most clearly indicates this. Cycling of released radioactivity through diverse endogenous pathways is suggested by the difference in absolute tissue concentrations between the two radiolabel sites. The lower incorporation of oxazolidinone-derived radioactivity, relative to the acetamide radiolabel indicated that the oxazolidinone radiolabel site was the best choice for a definitive study.

Definitive GLP-regulated Study  
The study was designed to provide an assessment of the terminal phase excretion and tissue distribution of radioactivity related to oxazolidinone ring labeled [14C]linezolid in male and female Sprague–Dawley rats after a single oral dose or seven consecutive daily oral doses.

The overall cumulative excretion of radioactivity at 14d after the last dose is summarized and compared to preliminary study data in Table 1. Recovery of radioactivity in urine and feces was near complete by 168 h (24h after the last dose) at 88.5±1.36% of dose (n=8). By 14d after the last dose, total recovery had increased by only 1.47%. Since approximately 3% of the radiolabeled linezolid dose was lost as 14CO2 from the oxazolidinone radiolabel site, the total 7 d recovery was approximately 93—94% of the administered dose. Radioactive CO2 was not determined in this study due to the 21 d duration sample collection.

Urine radioactivity accounted for 60.4±3.22% of dose, feces accounted for 29.6±3.10% of dose and the carcasses contained 0.49±0.37% of the dose. The terminal phase ARE half life that accounts for the elimination of the last approximately 0.1% of dose, based on a two point determination from 240—480 h, was 36 and 43 h in male and female rats, respectively.
Tissue concentration data are presented in Table 2. At the first dose nadir (24 h after the first of 7 daily doses), the highest concentrations of radioactivity were observed in the large intestine, GI tract contents, skin, thyroid and bladder (1.647 mg-eq/g or less). The near complete 24 h excretion of drug-related radioactivity was also shown in a single dose distribution study, where tissue concentrations at 24 h were maximally 4% (in thyroid), of the single intravenous dose radioactivity concentration measured at 20 min post dose.7)

At 168 h (24 h after last dose), the highest concentrations of radioactivity were observed in the thyroid, GI tract contents, large intestine, skin, small intestine, Harderian gland (not present in humans) and adrenal gland (2.959 µg-eq/g or less).

Tissue concentrations declined between 168 and 312 h to below 100 ng-eq/g in all tissues except thyroid (609 ng-eq/g), skin (215 ng-eq/g), large intestine (117 ng-eq/g) and prostate (109 ng-eq/g).

Tissue concentrations at 480 h (14 d) after the last dose were below 60 ng-eq/g, except in thyroid (1.019 µg-eq/g), and skin (0.298 µg-eq/g). Terminal phase radioactivity is not entirely drug-related, and the total carcass burden represented 0.49±0.37% of the administered dose.

DISCUSSION

Despite relatively rapid and complete excretion of radioactivity in rats over the 0—24 h post dose interval, linezolid radiolabeled in the acetamide substituent had a plasma radioactivity terminal half-life of 25 h, and the parent drug terminal half-life was only 1 h.7) In our opinion, this was due to a combination of the duration of detectability of two different analytical methods, and a small amount of radiolabel loss to intermediary metabolism, thus the radioactivity causing the 25 h plasma half life was not drug-related.

There are three observations in this study that, taken together, indicate that the 25 h plasma radioactivity half life was an artifact related to radiolabel instability. Firstly, after an IV bolus, the drug-related radioactivity (up to 97% of dose) was rapidly excreted, leaving residual concentrations in all tissues that were uniformly very low at 24 h post dose, maximally 4% of the 20 min measured Cmax.7) Secondly terminal phase ARE half lives were similar for both radiolabel sites and for orally administered radioactive sodium acetate, possibly indicating the nascent turnover of endogenous radio-carbon. Thirdly, terminal phase tissue residues were higher for the acetamide radiolabel, which is more likely to enter intermediary metabolism as a lipid precursor than 14CO2-derived bicarbonate from the oxazolidinone radiolabel.

Based on these observations, we concluded that acetamide radiolabeled linezolid afforded terminal phase tissue residues that were related to the 3% of dose that was lost as acetate-derived 14CO2. Therefore, while suitable for single dose excretion and distribution studies, this radiolabel site was not an optimal choice for a multiple dose tissue distribution study, since terminal phase residues were largely not drug-related. Ninety percent of orally administered radiolabeled sodium acetate was lost as [14C]CO2 shortly after dosing. Extrapolating from this, we speculate that a 3% [14C]CO2 evolu-
tion from the linezolid acetamide label therefore may indicate that approximately 0.3% of radioactivity cycled as long half life endogenous compounds.

Oxazolidinone ring labeled linezolid was chosen for use in the definitive GLP multiple dose distribution study, based on a lower tissue radioactivity concentrations in the terminal phase. In the definitive study, skin and thyroid were the only two tissues that retained terminal phase radioactivity that may, in part, be drug-related. Given that the duration of linezolid therapy is generally less than two weeks, and that there are no adverse effects related to the thyroid in clinical or toxicity studies, no clinical significance can be inferred from the persistence of trace level radioactive residues in thyroid tissue of the rat.

In conclusion, although small amounts of radiolabel instability (<5%) do not significantly affect single dose tissue \( C_{\text{max}} \) and AUC, artifacts arising from radiolabel instability can prolong the apparent terminal phase half life and complicate study data interpretation. When possible, it is always preferable to use a completely stable radiolabel site.

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**REFERENCES**


