Mechanisms of Ageing and Development 130 (2009) 652–655

Contents lists available at ScienceDirect

Mechanisms of Ageing and Development

journal homepage: www.elsevier.com/locate/mechagedev

Short communication

A method for oral administration of hydrophilic substances to *Caenorhabditis elegans*: Effects of oral supplementation with antioxidants on the nematode lifespan

Ayumi Shibamura, Takanori Ikeda, Yoshikazu Nishikawa *

Department of Interdisciplinary Studies for Advanced Aged Society, Osaka City University Graduate School of Human Life Science, 3-3-138 Sugimoto, Sumiyoshi-ku, Osaka 558-8585, Japan

ARTICLE INFO

Article history:
Received 9 May 2009
Received in revised form 19 June 2009
Accepted 26 June 2009
Available online 4 July 2009

Keywords:
Longevity
Caenorhabditis elegans
Antioxidant
Liposome
Oral administration

ABSTRACT

Numerous studies using *Caenorhabditis elegans* have used a protocol in which chemicals are orally delivered by incorporating them into the nematode growth media or mixing them with the food bacteria. However, actual exposure levels are difficult to estimate as they are influenced by both the rates of ingestion into the intestine as well as absorption from the intestinal lumen. We used liposomes loaded with the hydrophilic fluorescent reagent uranin to test oral administration of water-soluble substances to *C. elegans*. Ingestion of liposomes loaded with fluorescent dye resulted in successful oral delivery of chemicals into the intestines of *C. elegans*. Using liposomes, oral administration of hydrophilic antioxidants (ascorbic acid, N-acetyl-cysteine, reduced glutathione, and thioproline) prolonged the lifespan of the nematodes, whereas the conventional method of delivery showed neither fluorescence nor longevity effects. Our method efficiently and quantitatively delivers solutes to nematodes.

Caenorhabditis elegans is a free-living bacteriophagous nematode that has played an important role in biological research. Despite increased use of *C. elegans* in a variety of studies, there is no efficient method to administer chemicals orally. When chemicals need to be administered to nematodes, they are either dissolved in the nematode growth medium (NGM) or the solution is poured onto the *Escherichia coli* strain OP50 (OP50), which is the international standard food of nematodes on NGM plates. *C. elegans* takes up liquid containing suspended particles (bacteria), and then spits out much of the liquid, while retaining the particles (Avery and Thomas, 1997). This feeding behavior is likely to be inefficient for ingestion of solutions.

We hypothesized that nematodes would be able to take up liposomes, similar to their ingestion of bacteria. In this study, we used liposomes loaded with the hydrophilic fluorescent reagent uranin to test oral administration of water-soluble substances to *C. elegans*, and compared the efficiency of liposome-mediated delivery with conventional methods.

Dietary supplements of antioxidants were previously reported to have positive effects on longevity (Brown et al., 2006; Kampkötter et al., 2008; Melov et al., 2000; Wilson et al., 2006; Wu et al., 2002), while other studies reported controversial results (Bass et al., 2007; Goldstein et al., 1993; Keaney et al., 2004; Larsen and Clarke, 2002). Water-soluble antioxidants were administered using both the new and conventional methods to compare the effect on lifespan of nematodes and on host defense against salmonella infection. Using our new method, we showed marked effects of antioxidants on the lifespan of *C. elegans*.

C. elegans Bristol strain N2 were propagated on NGM with standard techniques (Stiernagle, 1999). Liposomes (25 μl) containing 50 μg of uranin were spread on the surface of a peptone-free NGM (mNGM) plate (12.5 ml of mNGM, 6 cm diameter) with OP50 (10 mg/plate), and nematodes were allocated onto the plate. Three hours later, nematodes showed fluorescence in the lumen of the mouth to the pharynx, and beyond the pharyngeal bulb to the anus. The fluorescence was observed both in the lumen and intestinal cells (Fig. 1A). Nematodes appeared to ingest liposomes even in the presence of OP50, showing that nematodes did not selectively avoid liposomes. The amount of fluorescent dye absorbed in 3 h was high compared with worms administered dye by conventional methods (Fig. 1B and C). The conventional method required 100-fold more uranin (5 mg/plate) to stain worms than the liposome method.

Liposomes are tiny vesicles made of phospholipids and are likely to be a useful drug delivery system. For example, the lipid bilayer can fuse with tumor cell membranes, thus delivering anticancer drugs into tumor cells. Furthermore, liposomes made in a particular size range can be easily phagocytosed or endocytosed

* Corresponding author. Tel.: +81 6 6605 2910; fax: +81 6 6605 2910.
E-mail address: nisikawa@life.osaka-cu.ac.jp (Y. Nishikawa).

0047-6374/$ – see front matter © 2009 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.mad.2009.06.008

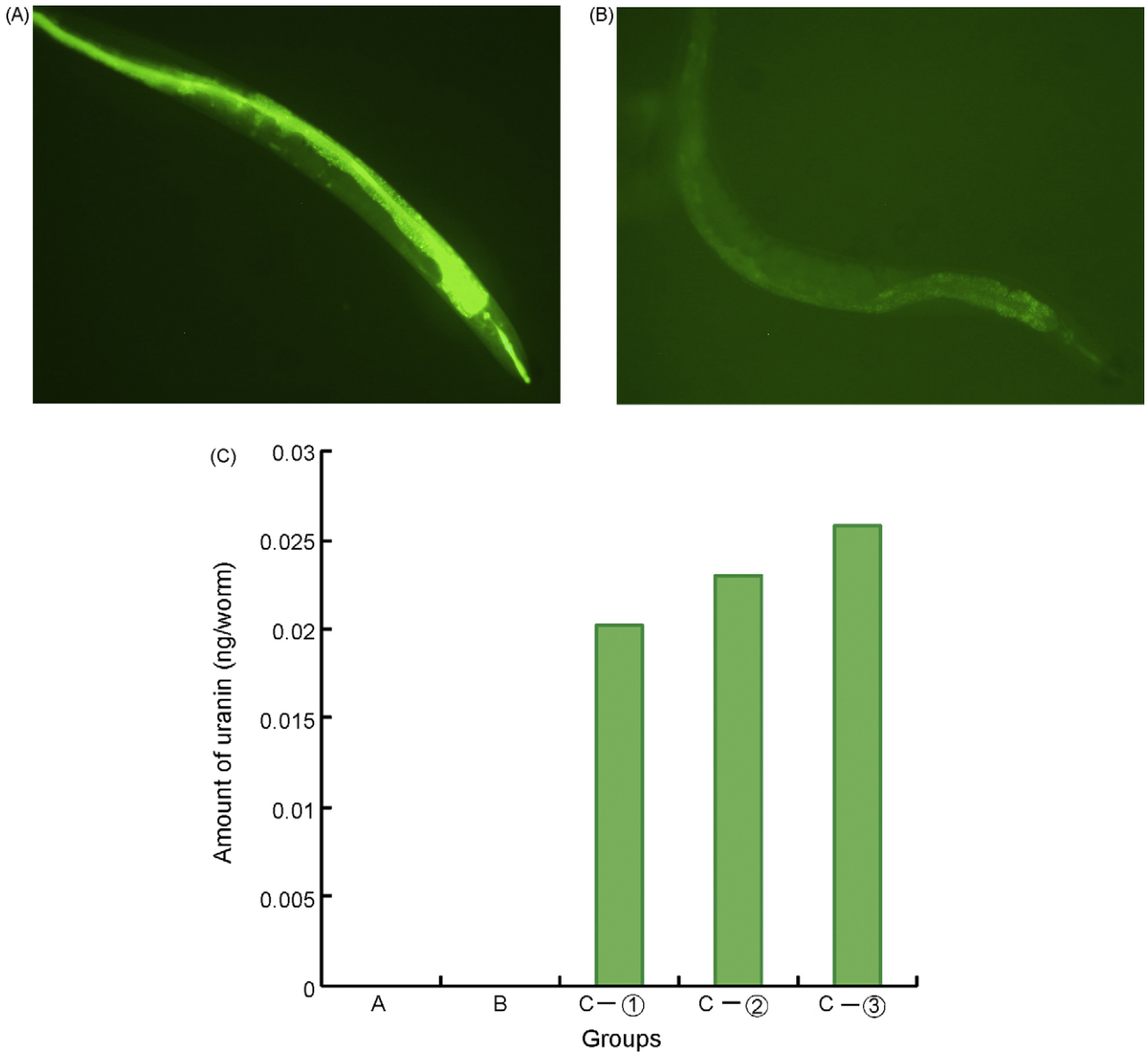

Fig. 1. Nematode fluorescence after oral administration of uranin. To monitor nematode ingestion of liposomes containing chemical solutions, the water-soluble fluorescent compound uranin was used. Uranin was dissolved in distilled water (2 mg/ml). L-α-Phosphatidylcholine was added to the solution at 48 mg/ml, and liposomes were produced by mixing the solution at 65 °C through a Nucleopore track-etched membrane (pore size 0.1, 1.0, or 5.0 μm, Whatman, Inc., Newton, MA) using a Mini-Extruder (Avanti Polar Lipids, Inc., Alabaster, AL). For comparison, 25 μl of uranin solution was dissolved into the mNGM directly or the solution was poured onto the mNGM plate with OP50. (A) A nematode fed OP50 and 25 μl of liposomes containing uranin shows clear fluorescence along the digestive tract. (B) A nematode fed OP50 on a uranin-containing mNGM plate is not fluorescent. (C) Amount of uranin recovered from worms. Groups A and B were fed OP50 on mNGM in which uranin was directly dissolved into the medium or poured onto the plate, respectively. Groups C-1 to C-3 were fed OP50 and liposomes with diameters of 0.1, 1.0, and 5.0 μm, respectively. Nematodes were fed on OP50 *ad libitum* for 3 h, and then collected, counted, and washed three times with M9 buffer. Nematodes were then placed in a 0.5-ml microtube containing 20 μl of M9 buffer and mechanically disrupted with a microtube pestle. The volume was adjusted to 100 μl with M9 buffer, and fluorescence of the supernatant was analyzed using a spectrofluorophotometer. Standard curves were generated by plotting fluorescence intensity against the concentration of uranin. The amount of uranin ingested and absorbed by a worm during the 3 h was calculated by dividing the total uranin recovered from worms by the number of worms.

and their contents can be released into target cells. However, we did not expect liposomes to play such a function in our system. For administration to nematodes, liposomes were used simply as artificial cells.

Previous results have suggested that *C. elegans*, a bacteriophagous nematode, could not adapt to ingestion of solutions (Avery and Thomas, 1997). Additionally, we think that hydrophilic substances may diffuse into the mNGM in the absence of liposomes, preventing worms from sucking up a solution containing the chemicals of interest. In support of these ideas, 100-fold more uranin was required to retain the desired concentration on the surface of mNGM. Thus, we hypothesized that liposomes could work as efficient carriers by mimicking bacterial particles and preventing absorption of the chemicals into the mNGM.

Liposomes are an excellent vehicle for quantitative oral administration of hydrophilic chemicals to *C. elegans*. If all the dye in the ingested liposomes were recovered from the assay, the amount of uranin ingested by a nematode in 3 h would be 20–25 pg or 10–12 pl of solution, even in the presence of OP50. Although axenic culture of *C. elegans* in nutritious liquid media has been established (Vanfleteren, 1980), worms appeared to grow slowly compared with those fed OP50 on mNGM. We hypothesized that this was partly due to inefficient intake of the solution and are now planning to incorporate the media into liposomes to make artificial bacteria that will substitute for OP50 on mNGM.

The lifespan of nematodes that ingested liposomes containing ascorbic acid, N-acetyl-cysteine, glutathione, or trimethadione was longer than that of worms that ingested control liposomes