Travellers' tales

Providing grants to attend scientific meetings

Julie Crockett of the Department of Medicine and Therapeutics, University of Aberdeen, used a Biochemical Society Grant to go to Seattle for the 26th Annual Meeting of the American Society for Bone and Mineral Research.

"Being a large meeting there are always many interesting (topical and relevant) speakers, the top investigators in the field, " she said. "Although very large, the poster sessions are useful for speaking to people on a one-to-one basis. This year there seemed to be many posters of interest. We had a lot of interest in our own work as well."

As a member of the Bone Research Group in Aberdeen, Julie has been involved in elucidating the molecular mechanisms of action of bisphosphonate (BP) drugs. These drugs are inhibitors of bone resorption and are used in the treatment of metabolic bone diseases.

She and her colleagues have demonstrated that nitrogen-containing BPs reduce the activity of bone-resorbing osteoclasts by inhibiting the enzyme farnesyldiphosphate synthase, and thereby preventing protein prenylation. Non-nitrogen-containing BPs do not inhibit this enzyme and are metabolized intracellularly to toxic analogues of ATP.

More recently, she has been focussing on the route by which BPs are internalized into cells. The team at Aberdeen has synthesized a fluorescently-labelled BP analogue and demonstrated that BPs are internalized into vesicles by fluid-phase endocytosis. This stage of the internalization is Ca^{2+} dependent. Vesicular acidification is required for the BP to exit the vesicles and enter the cytosol. Since the exact route by which BPs enter cells has not been clarified, these studies are of great interest.

Julie presented a poster entitled 'Evidence for a specific recognition step involved in cellular uptake of bisphosphonates' with her colleagues Keith Thompson, Mike J. Rogers and Fraser P. Coxon from the Bone Research Group at the Institute of Medical Sciences, University of Aberdeen.

The exact mechanism by which BPs are internalized into cells remains unclear. Cellular uptake of BPs has been shown to be dependent on Ca^{2+} in RAW264 macrophages. Having previously shown that the non-nitrogen-containing BP clodronate antagonizes the effects of nitrogen-containing BPs in J774 macrophages *in vitro* and postulated that BP uptake takes place via a membrane-bound transport protein, the Bone Research Group investigated further by synthesizing a fluorescently-labelled analogue of alendronate (FL-ALN).

Using confocal microscopy, uptake of FL-ALN by J774 macrophages was detected in intracellular vesicles within 5 minutes of treatment. The pattern of uptake of FL-ALN was compared with specific markers of fluid-phase endocytosis [TRITC (tetramethylrhodamine β-isothiocyanate)–dextran], adsorptive endocytosis [wheat germ agglutinin–TAMRA (carboxytetramethylrhodamine)] and receptor-mediated endocytosis (transferrin–TAMRA). Only TRITC–dextran showed a marked degree of co-localization with FL-ALN, suggesting that a major component of BP uptake is mediated by fluid-phase endocytosis.

Using flow cytometry, J774 cells were found to markedly accumulate FL-ALN within 4 hours. In agreement with previous findings, molar excess of the non-nitrogen-containing BP clodronate dramatically inhibited the vesicular uptake of 100 μM FL-ALN. Co-treatment of J774 cells with FL-ALN and 250 μM clodronate inhibited uptake of FL-ALN by 90%. This inhibitory effect of clodronate was not due to inhibition of fluid-phase endocytosis, since clodronate did not affect the uptake of FITC–dextran.

Since BPs are non-hydrolysable analogues of pyrophosphate (PP_i), the Group hypothesized that PP_i may also compete with BPs for cellular uptake. Co-treatment of J774 cells with 250 μM PP_i decreased FL-ALN uptake by 76%. Due to the high affinity of BPs for divalent ions (such

Mount St Helen's fuming gently in Washington State.

as Ca^{2+}), we investigated whether the inhibitory effect of clodronate or PP_i was due to Ca^{2+} chelation. Whereas molar excess Ca^{2+} ions stimulated FL-ALN uptake, 500 μM EGTA (a Ca^{2+} chelator) inhibited FL-ALN uptake by 96%, which was reversed in the presence of 2 mM Ca^{2+}. Supplementation of 250 μM clodronate or PP_i with molar excess (1 mM) Ca^{2+} partially prevented the inhibitory effect of clodronate, but not PP_i, on FL-ALN uptake.

These observations show that cellular uptake of BPs can occur by fluid-phase endocytosis. However, there appears to be a specific recognition step prior to endocytosis that is dependent on Ca^{2+} and can be blocked by competition with PP_i or other BPs, such as clodronate .

After the conference, Julie was able to travel around Washington State. "The most spectacular sight was Mount St. Helen's 'semi-active'," she said.

Guidelines for Applications

The rules and regulations for applying for a Travel Grant are strict and can be viewed in full at www.biochemistry.org where you can also obtain an application form. The main points are listed here for your information:

1. The Travel Grants Committee meets six times a year with closing dates for applications of 1st January, 1st March, 1st May, 1st July, 1st September and 1st November. Applications received after these closing dates will not be considered. Furthermore, applications should be for meetings or visits which take place at least one month after a particular closing date.

2. One original plus four photocopies of a completed application with enclosures should be submitted by post to Alison McWhinnie, Assistant Director, Personnel and Administration, The Biochemical Society, Eagle House, 16 Procter Street, London W1CV 6NX. All parts of the form should be completed – if necessary writing "not applicable" for some sections. Applicants must be able to demonstrate that the most cost-effective form of transport and accommodation is to be utilized. Faxed and email applications will not be accepted.

3. New members may apply for their first Travel Grant after they have been a member of the British Biochemical Society for 1 year by the relevant closing date, although applicants will not be eligible if they have been awarded a Student Travel Grant within the last year. Furthermore, applicants will not be eligible if they have been awarded a Travel Grant from the Society during the previous 2 years.

4. Applications which do not comply with the full set of guidelines will not be considered.

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