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Liposomal DTPA as a good strategy for enhancing Pu decorporation regardless of treatment regimen.

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1 Introduction

Internal contamination with plutonium (Pu), either in the context of accidental occupational exposure, after a natural disaster affecting nuclear facilities, or as a result of possible terrorist use, continues nowadays to be a potential hazard. The only practical way to reduce Pu body burden and the associated radiation risks is decorporation by chelation therapy, which consists in administering a saline solution of the chelating agent diethylenetriaminepentaacetic acid (DTPA used as Ca or Zn trisodium salt). Unfortunately, the very short biological half-life (90-99% excreted within 24 hours) and the too low ability to enter cells of DTPA seriously limit its efficacy for Pu decorporation.

The encapsulation of DTPA within liposomes, i.e., artificial lipid spherules, provides a greater cell penetration and a longer retention of DTPA in tissues, thereby enhancing its ability to mobilize various deposited metals including Pu, compared to free DTPA. Most of the decorporation studies have assessed the efficacy of liposomal DTPA on animals injected with polymers/colloids of Pu.

The present study aimed at assessing the efficacy of 110nm-sized liposomes encapsulating DTPA in rats injected with a soluble form of Pu which mimics the partitioning of dissolved Pu following its absorption into the blood from the primary site of contamination (lung or wound site depending on the route contamination).

2 Materials and Methods

Unilamellar conventional liposomes (DSPC/Cholesterol/DSPE 69:30:1) were used to encapsulate a solution of DTPA as Na_3Ca-DTPA (25mM).

Rats were contaminated by intravenous administration of the soluble citrate form of ^{238}Pu (7 to 10.3kBq) and received or not an injection of DTPA either as marketed free form or entrapped within liposomes (5 µmol/kg). Treatment schedules started at one hour (prompt treatment) or at seven days (delayed treatment) after contamination and were given as a single injection or repeated injections. A prophylactic single treatment was also tested given at three days before Pu exposure.

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Excreta were collected and organs of interest (liver, bone and spleen mainly) were excised at euthanasia for measurement of residual Pu alpha activity. The comparative effects of liposomal and free DTPA at similar doses were examined in terms of fecal and urinary Pu overexcretion and limitation of Pu burden in tissues from rats receiving the various treatment regimens.

3 Results

The prompt treatment (1 hour) with liposomal DTPA at 5µmol.kg^{-1} prevented significantly by about 75%, 52%, and 77% Pu deposition in liver, bone and spleen as compared to controls, respectively. Thus, this galenic form of DTPA reduced Pu retention by 2.5-, 1.6- and 3-fold in liver, bone and spleen, respectively, compared with free DTPA at similar dose. Similar to data for tissues, liposomal DTPA had a significantly greater effect on urinary elimination of Pu compared with free DTPA during at least one week after prompt treatment. Whereas Pu level excretion in feces increased in the order no treatment < free DTPA < liposomal DTPA at early times, this order was reversed at later times. The total fecal excretion does not seem to be enhanced by DTPA treatments but only accelerated compared to no treatment, and in a faster way with liposomal DTPA than with free DTPA.

For delayed administration (7 days), liposome-entrapped DTPA decreased hepatic, skeletal and splenic Pu levels by, respectively 68, 39 and 74% of the levels in untreated control rats (only 19, 12 and 24% with a similar dose of free DTPA). Concerning urinary and fecal Pu excretions after delayed DTPA treatment, similar observations as those referred to the early treatment have been made.

In addition, four injections of liposomal DTPA begun at 7 days and spaced at 3-day intervals further improved the removal of Pu especially from bone (-55% as compared to untreated rats) compared to a single injection (-39% as compared to untreated rats).

Lastly, a treatment by liposomal DTPA given three days before Pu exposure prevented Pu accumulation at seven days by about 47%, 29% and 63% in the liver, bone and the spleen, respectively, compared to untreated control rats, which shows an efficacy of a prophylactic treatment with liposomal-encapsulated DTPA.

4 Discussion/Conclusion

DTPA formulated in our liposomes can significantly improve Pu decorporation from systemic tissues regardless of the treatment regimen applied.

As the expected fate of liposomes is their capture by phagocytic cells in organs of the mononuclear phagocytic system, the higher effectiveness of liposomal DTPA in removing Pu from the liver and the spleen, as compared to free DTPA, was undoubtedly related in part to a far greater accumulation of DTPA molecules in phagocytic cells of these tissues. However, the early increased Pu excretion in feces following liposomal DTPA treatment suggests that intracellular chelation of Pu can also take place in other cells than phagocytic cells, such as hepatocytes. The success of a prophylactic treatment with liposomal DTPA given three days prior Pu exposure shows a persistence of DTPA molecules in tissues over several days, thereby providing another advantage to liposome-encapsulated DTPA over free DTPA in chelating intracellular Pu.

Thanks to this high and persistent accumulation of DTPA in soft tissues when delivered as liposomal form, these tissues certainly act as reservoirs for DTPA. DTPA molecules that are slowly released from them into the blood stream may largely contribute to a sustained extracellular action of DTPA on Pu still deposited on bone surfaces, contrary to free DTPA which has a very short blood half-life. A contribution of DTPA molecules slowly lost from

liposomes during their blood circulation to the bone Pu decorporation is not excluded but it is assumed to be marginal because of the high stability of our liposomes.

As a consequence, the advantage of liposomal DTPA over marketed free DTPA would be not only directly but also indirectly due to the better cell penetration of DTPA when loaded within liposomes.

To conclude, encapsulation of DTPA in suitable liposomes is therefore a good therapeutic strategy for improving delivery, and thereby enhancing decorporation efficacy for transuranic actinides such as plutonium. As compared to free DTPA, this galenic formulation could be advised for both early and delayed chelation therapy. Besides, the sustained action of DTPA when encapsulated within liposomes suggests a great efficacy of a treatment regimen consisting of spaced repeated administrations.

Results presented here were obtained in the frame of the Orano-Inserm-CEA collaboration.