Pharmaceutical Research, Vol. 7, No. 2, 1990

Report

Effect of Molecular Weight on the Lymphatic Absorption of Water-Soluble Compounds Following Subcutaneous Administration

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Received May 1, 1989; accepted August 11, 1989

The lymphatic absorption of four water-soluble compounds with different molecular weights (MW) was determined by measuring their cumulative recovery in lymph draining from the site of s.c. administration in sheep. The cumulative recoveries (% of dose, mean ± SD; $N = 3$) were 4.0 ± 1.5 (5-fluoro-2′-deoxyuridine, MW 246.2), 21.0 ± 7.1 (inulin, MW 5200), 38.6 ± 6.7 (cytochrome *c*, MW 12,300), and 59.5 ± 7.7 [human recombinant interferon (rIFN) alpha-2a, MW 19,000], respectively. Our data show that in the investigated MW range, there is a linear relationship between the molecular weight and the proportion of the dose absorbed lymphatically. An increase in molecular weight results in an increased lymphatic absorption. Molecules with MW > 16,000 are absorbed mainly by the lymphatics which drain the application site. The knowledge gained in this investigation may help to improve the mode of administration and therapeutic efficacy of endogenous proteins whose targets are lymphoid cells (e.g., interferons, interleukins). Practical implications for the clinical use of such proteins are discussed.

KEY WORDS: Water-soluble compounds; subcutaneous administration; lymphatic absorption; molecular weight; sheep.

INTRODUCTION

Following s.c. administration a drug can be transported to the general cardiovascular pool either by the blood capillaries or by the lymphatics. For small molecules, up to 1 kDa, the blood capillary wall diffusivity is very high and represents a small barrier to drug transport (1). However, permeability of macromolecules through the blood capillary is low, and therefore, direct movement into the blood is restricted. Hence, soluble macromolecules were found to enter the bloodstream indirectly by way of lymphatic vessels. Lewis (2) injected horse serum s.c. into dogs and detected it in thoracic duct lymph after 40 min and in blood after 3.5 hr. Field and Drinker (3) also demonstrated that horse or dog serum was absorbed mainly by the lymphatics in the dog when injected subcutaneously. Weinstein and coworkers (4) proved that after s.c. injection, monoclonal antibodies enter local lymphatic capillaries and pass to the draining lymph nodes, where they bind to target cells. Antibody not bound there passed to more distant nodes. If still not removed from the lymph flow, antibody entered the bloodstream through the thoracic duct. Our laboratory showed that following i.d. or s.c. administration rIFN alpha-2a is absorbed mainly by the lymphatics that drain the application site (5).

These observations demonstrate that the lymphatic vessels are important for the absorption of macromolecules injected into the s.c. space. However, the required molecular size was unknown for preferential transport by the lymphatics to the general cardiovascular pool following s.c. administration. The present work evaluates the effect of molecular weight on lymphatic absorption of water-soluble compounds following s.c. application.

MATERIALS AND METHODS

Materials

Cytochrome *c* (from Sheep Heart, Type XII, MW 12,327) and inulin (from Dahlia Tubers, MW 5200) were purchased from Sigma, St. Louis, MO. [^{3}H]Inulin (769 μCi/mg, MW 5200) was obtained from Amersham International, Amersham, UK. Fluorodeoxyuridine, 5-[6-^{3}H] (58.8 mCi/mg, MW 246.2) and cytochrome *c* [methyl-^{14}C] methylated (29 μCi/mg, MW 12,300) were purchased from NEN Research Products, Boston, MA. 5-Fluoro-2′-deoxyuridine (FUDR) and human serum albumin-free lyophilized rIFN alpha-2a (MW 19,000) containing 18×10^6 U per vial (Lot No. G FER 13 031) were from F. Hoffmann-La Roche, Basle, Switzerland. The amount of rIFN alpha-2a is expressed as international units (U) of antiviral activity determined in the vesicular stomatitis virus MDBK cell assay. The specific activity

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0724-8741/90/0200-0167$06.00/0

of the pure unformulated substance was 2–4 × 10^8 U/mg protein (protein purity of >98%). Details of the products and purification procedures are described elsewhere (6). All other reagents used were of analytical grade.

Animals and Surgical Procedures

White Alpine and Black Jura sheep of both sexes, aged 2–4 years, were obtained from Versuchsbetrieb Sennweid, Olsberg.

The cannulation of the efferent duct of the popliteal lymph node was done as described elsewhere (7). After the operation sheep were maintained in metabolism cages and were fed on commercial pellets, and lymph was collected as described elsewhere (8).

Lymphatic Absorption Studies

The absorption studies were performed using the lymph cannulated sheep model as described elsewhere (5). This system allows the collection of peripheral lymph draining directly from the injection site. Experiments were started 2–3 days after surgery. After collecting a blank lymph sample the test compounds including appropriate amounts of labeled substances were injected s.c. into the lower part of the lymph cannulated leg using a special injection device. The following four test solutions were injected: (I) 3.6 × 10^6 U lyophilized rIFN alpha-2a dissolved in 2 ml water; (II) 1 mg cytochrome *c* mixed with 0.5 μCi [*methyl*-^{14}C]cytochrome *c* in 1 ml 10 m*M* PBS, pH 7.4; (III) 0.5 mg inulin mixed with 15 μCi [^{3}H]inulin in 1 ml 10 m*M* PBS, pH 7.4; (IV) 0.34 mg FUDR mixed with 15 μCi [6-^{3}H]FUDR in 1 ml 10 m*M* PBS, pH 7.4. Lymph was collected continuously in heparinized tubes (5 U heparin/ml lymph). Collection flasks were changed every hour for the first 8 hr after injection and then at the end of the experiment at 24 hr. After centrifugation (200*g*, 10 min, 4°C) all lymph plasma samples were kept at 4°C until assayed.

Analysis of rIFN Alpha-2a

The concentration of rIFN alpha-2a in lymph plasma was measured by an enzyme-linked immunosorbent assay, as described by Gallati (9).

Determination of Radioactivity

To 50-μl lymph plasma samples 2 ml of liquid scintillation cocktail (Emulsifier Scintilator 299, Packard Instruments International, Zürich, Switzerland) was added, and the radioactivity present was determined by liquid scintillation spectrometry (Liquid Scintillation Analyzer 2000 CA Tri-Carb, Packard). Efficiency of counting was determined by comparison with an external standard.

RESULTS

The effect of molecular weight on the cumulative recovery of four water-soluble compounds in lymph draining from the site of s.c. administration was determined. The recovery in lymph was calculated as the product of the concentration in lymph and the volume of lymph collected for each time interval and was expressed as a percentage of the administered dose. The cumulative recoveries determined were 4.0 ± 1.5 (FUDR; MW 246.2), 21.0 ± 7.1 (inulin, MW 5200), 38.6 ± 6.7 (cytochrome *c*, MW 12,300), and 59.5 ± 7.7% (rIFN alpha-2a, MW 19,000), respectively (mean ± SD, N = 3) (Fig. 1). No activity was found in the cell pellets recovered by centrifugation. As shown in Fig. 2 there was a linear relationship between the molecular weight, in the range investigated, and the proportion of the dose lymphatically absorbed. The line drawn in Fig. 2 is the best fit by linear regression analysis calculated with the four mean values. The points have a correlation coefficient r of 0.998 ($p < 0.01$).

DISCUSSION

Our data indicate that there is a linear relationship between the molecular weight of a drug and the proportion of the dose absorbed by the lymphatics which drain the site of s.c. application in the investigated MW range. Molecules with MW > 16,000 are absorbed mainly by the lymphatics, since more than 50% of the administered dose was recovered in lymph. Compounds with MW < 1000 were hardly absorbed at all by the lymphatic vessels. An increasing tendency toward lymphatic absorption was determined for molecules with MW between 1000 and 16,000. These findings can be compared with data published previously by Muranishi and co-workers (10). They evaluated the absorption route of FITC-labeled dextrans (FD) of various molecular weights following rectal administration in rats. They found that small amounts of the administered FD dose can pass the epithelium of the rectum with the help of absorption enhancer. After this passage FDs with MW < 10,000 were transferred into both blood and lymph, whereas FDs with MW > 20,000 preferentially entered the lymphatic capillaries.

The different access of macromolecules from the s.c. space to blood and lymphatic capillaries may be related to structural differences between blood and lymphatic endothelial cell lining. The endothelia lining the blood capillaries have a continuous and uninterrupted subendothelial basement membrane. In contrast, terminal lymphatics do not possess this membrane. The lack of a basal lamina around

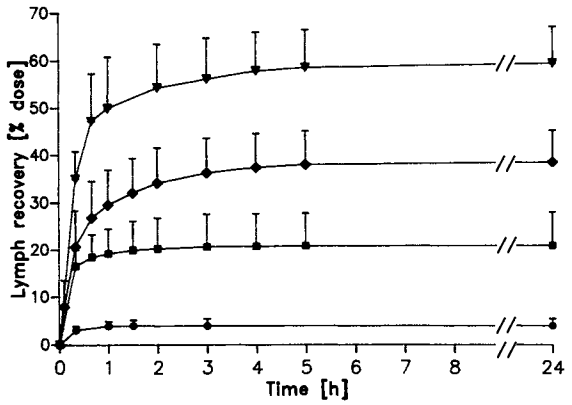

Fig. 1. Cumulative recovery of rIFN alpha-2a (▼), cytochrome *c* (◆), inulin (■), and FUDR (●) in the efferent lymph from the right popliteal lymph node following s.c. administration into the lower part of the right hind leg. Each point and bar show the mean and SD of three experiments performed in three separate sheep.

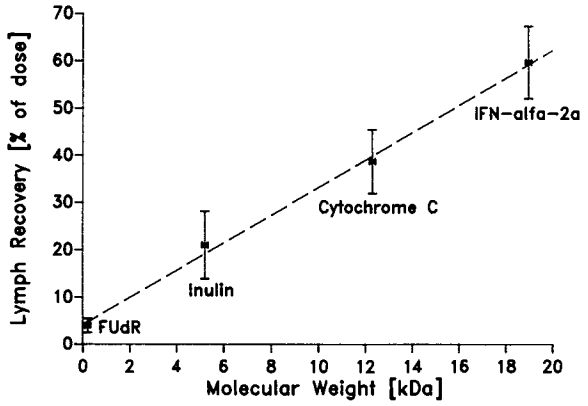

Fig. 2. Correlation between the molecular weight and the cumulative recovery of rIFN alpha-2a (MW 19,000), cytochrome *c* (MW 12,300), Inulin (MW 5200), and FUDR (MW 246.2) in the efferent lymph from the right popliteal lymph node following s.c. administration into the lower part of the right hind leg. Each point and bar show the mean and SD of three experiments performed in three separate sheep. The line drawn is the best fit by linear regression analysis calculated with the four mean values. The points have a correlation coefficient r of 0.998 ($p < 0.01$).

lymphatic capillaries may be of major functional importance in facilitating access of interstitial macromolecules to the lymphatic system (11). In addition, adjacent endothelial cells of terminal lymphatics may have gaps from 20 to more than 100 nm. These gaps make it possible that macromolecules and even large particles of up to perhaps 1-μm diameter may enter the lymphatics (1,12).

The results presented could be of importance for the practical use of endogenous proteins produced by recombinant DNA technology. Poste (13) proposed that effective therapy with these proteins requires a delivery system that leads to a distribution of the drug in the body approximating the natural distribution. In this regard, one has to distinguish between endocrine and autocrine/paracrine molecules. Endocrine agents are released into the bloodstream at the site of production and are then distributed within the blood to reach their target tissues at distant sites. Therefore systemic administration such as i.v. application is a suitable way to administer such drugs. In contrast, most paracrine/autocrine mediators act only over limited regions and are often rapidly inactivated to prevent their action at distant sites. In addition, many of the paracrine/autocrine mediators act in a cascade with complicated interactions between the individual mediators, leading to positive and negative feedback regulations. As a result site-directed delivery with a release profile which mimics the complex chronopharmacological behavior of such cascades should be used for optimal therapy. Interferon and other lymphokines, such as IL-2, can be considered as paracrine agents. Their physiological action is limited mostly to the lymphoid system. Therefore, Bocci has proposed that lymphatic delivery of lymphokines might improve their therapeutic efficacy (14,15). Based on our results and Bocci's proposition, the subcutaneous administration of endogenous mediators with MW above 16,000 and whose targets are lymphoid cells may represent a suitable delivery strategy. This should be borne in mind when planning further clinical studies with proteins whose targets are lymphoid cells such as interferons and interleukins.

ACKNOWLEDGMENTS

We thank Miss L. Dudler and Miss E. Infanger for skilled technical assistance and Miss G. Völk for typing the manuscript. We also wish to acknowledge Dr. H. Gallati for the measurement of the concentration of rIFN alpha-2a in lymph.

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