Absorption, Metabolism, Distribution, and Excretion of a PEGylated Variant of Recombinant Factor FVIII Following Intravenous Administration to Rats

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Objective

FVIII concentrates are used in patients with hemophilia A to treat and prevent bleeding episodes. Multiple prophylactic administrations are necessary each week to maintain a FVIII level of at least 1% of normal. A longer acting FVIII concentrate would reduce the frequency of infusions. Baxter and Nektar are developing a recombinant FVIII (rFVIII) modified with polyethylene glycol (PEGylation) to achieve longer circulation (BAX 855).

The objective of this study was to investigate the absorption, metabolism, distribution and excretion of radiolabeled (tritiated) BAX 855 after a single intravenous dose to rats.

Methods

Radiolabeled (tritiated) PEG was conjugated to rFVIII in order to receive radiolabeled BAX 855. The chromogenic specific activity of the conjugate was 2088 IU/mg protein.

Sprague Dawley rats were assigned to three groups for this study. Group 1 (3M+3F) animals received 1 mg/kg dose for excrement analysis and terminal tissue and carcass evaluation. Group 2 (16M+16F) was also treated with 1 mg/kg to assess blood, tissue and carcass samples. Group 3 (4M+4F) received 2 mg/kg for whole body auto-radiography (WBA) of radiolabeled BAX 855. Blood, plasma, urine, feces and selected tissues were collected at time points up to 1008 h (6 weeks) and analyzed for total radioactivity (liquid scintillation counter model 2900, Packard) for at least 5 minutes or 100,000 counts.

One animal/sex/time point was killed 1, 8, 24, and 168 hours postdose for WBA. The carcasses were frozen and embedded in chilled carboxy-methyl-cellulose and frozen into blocks. A Leica CM 3600 cryomicrotome was used to generate sections of 40 µm and five levels of interest in the sagittal plane. A section set from each animal was prepared by mounting a representative section from each level of interest. Mounted sections were exposed on phosphorimaging screens along with tritium fortified blood for 7 days. Exposed screens were scanned using a Storm scanner.

The study was conducted in accordance with the Food and Drug Administration (FDA) Good Laboratory Practice Regulations.

Results

Radioactivity was quantifiable in all matrices analyzed in both males and females. The highest concentrations of radioactivity for tissues were observed in the 1-, 8-, 24-, and 168-h collections. The highest maximum concentrations of radioactivity were observed in the plasma, blood, mesenteric lymph nodes, spleen, liver, adrenal glands, and kidneys in both males and females.

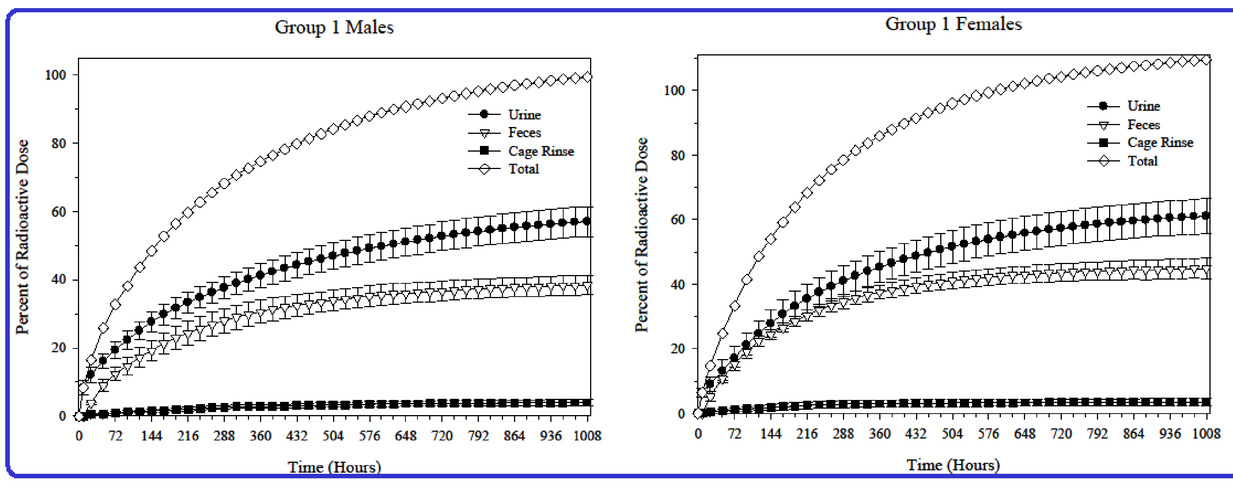

Fig. 1: Mean cumulative percent of radioactive dose in urine, feces, and cage rinse after a single intravenous administration of radiolabeled BAX 855 to male and female rats (Group 1, 1 mg/kg)

Elimination of radioactivity occurred primarily via urine. At 1008 h post-dose, urine, feces, and daily cage rinse corresponded to 51.9, 38.4 and 4.01% of the dose administered to males, and 55.7, 45.0 and 3.53% of the dose administered to females. The mean overall recoveries after 6 weeks were 97.4% in males and 107% in females.

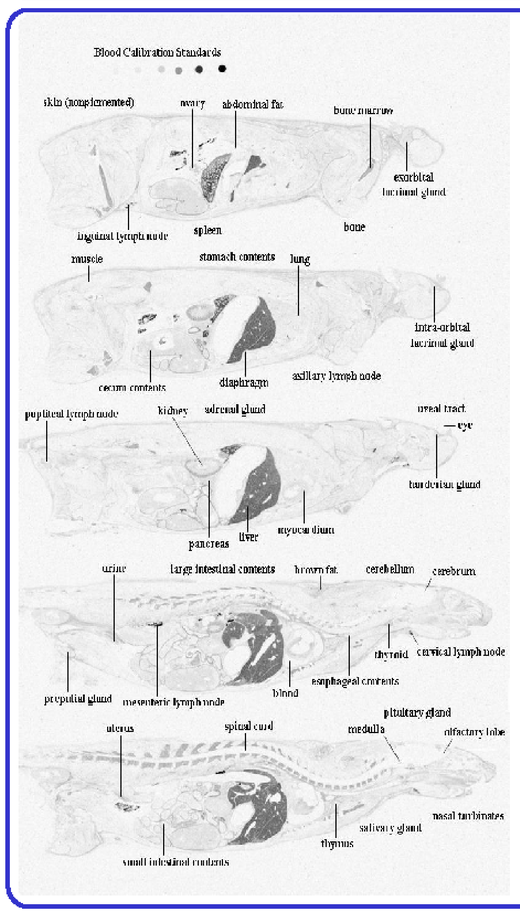

Fig. 3: Whole-body autoradiograph for a female rat 168 hours after a single intravenous administration of radiolabeled BAX 855 (Group 3, 2 mg/kg)

Radioactivity in the spleen analyzed by WBA presented a mottled appearance with most of the radioactivity in the red pulp. WBA derived values were consitent with data generated by liquid scintillation counting of tissue samples from animals designated to group 2. The highest maximum concentrations of radioactivity were observed in the plasma, blood, mesenteric lymph nodes, spleen, liver, adrenal glands, and kidneys in both males and females.

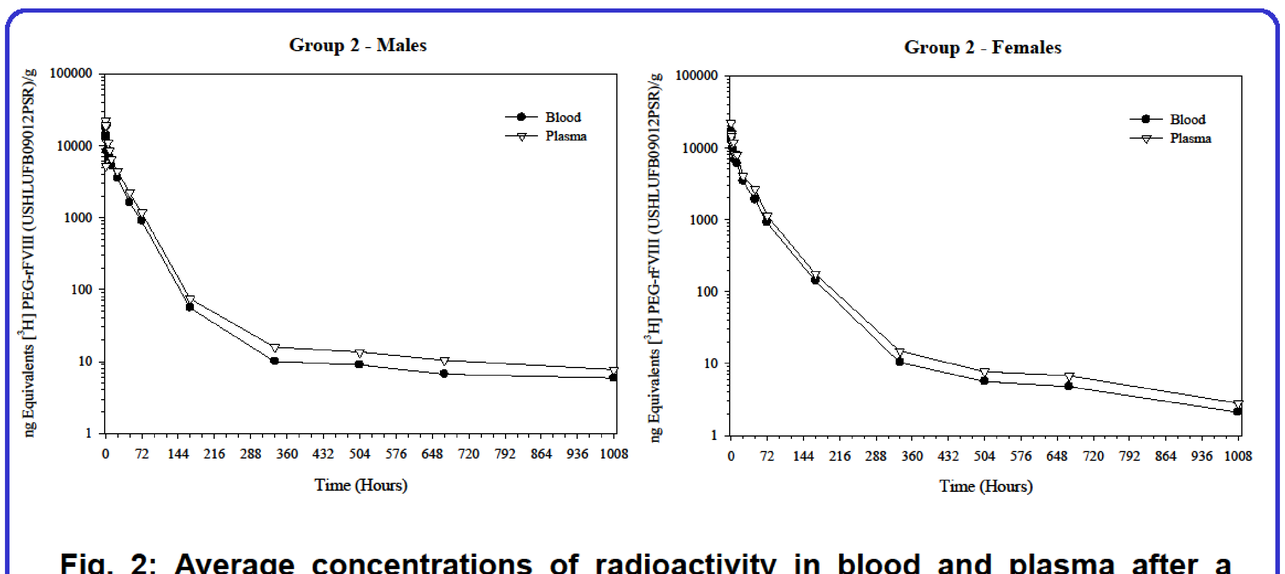

Fig. 2: Average concentrations of radioactivity in blood and plasma after a single intravenous administration of radiolabeled BAX 855 to male and female rats (Group 2, 1 mg/kg)

Radioactivity was eliminated from blood and plasma with half-lives of 827 and 655 h, respectively, in males, and 306 and 276 h, respectively, in females.

The average blood:plasma concentration ratios suggest that radiolabeled BAX 855-derived radioactivity does not have a strong association with the cellular component of blood.

Conclusion

- Radio-labeled PEG-rFVIII was distributed to the tissues analyzed without binding to the cellular blood components.
- Radioactivity was excreted quantitatively within 6 weeks via urine and feces.

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