ABSTRACT:

Bis[1-(Ethoxycarbonyl)propyl]5-acetylamino-2,4,6-triiodoisophthalate (NC 68183) was designed as a new computed tomography imaging agent. The purpose of this study was to determine the pharmacokinetics and metabolism of NC 68183 in conscious rats and in the isolated perfused rat liver. Animals were i.v. dosed at 69 and 690 mg of iodine/kg. Blood samples were collected at 5, 15, 30, and 60 min, and 7 days after dosing. Tissue samples (liver, kidney, and spleen) were taken at 60 min and 7 days after dosing. NC 68183 was cleared from blood in first order kinetics following an i.v. administration of 69 mg I/kg. The volume of distribution (Vss) at steady state and elimination half-life (t1/2) were estimated as 24 ml and 11 min. The clearance of NC 68183 from blood was changed to zero-order kinetics following administration of 690 mg/kg, and its elimination rate was 16 µg l/min/mg. The liver and spleen were the only tissues to have the nanoparticle residue at day 7 following administration. NC 68183 (75 mg of agent, 35 mg of I) was injected into the isolated perfused rat liver system. Bile flow increased from 1.0 to 1.3 µl/min/g liver following administration. The biliary excretion rate maximum was estimated as 11 µg/min/g liver. The metabolite was identified using liquid chromatography/mass spectrometry as a monocarboxylic acid product, which exclusively excreted into the bile in a soluble iodinated metabolite. Pharmacokinetics data suggested that NC 68183 primarily resides in the blood pool following an i.v. administration with a plasma half-life appropriate for blood pool imaging.

X-ray computed tomography (CT) is an essential tool for detection and evaluation of tumors and other focal lesions. Presently available contrast materials for CT are water-soluble, freely diffusible agents administered by a bolus injection. Two characteristics that would be useful in a CT contrast agent are prolonged vascular enhancement and tissue-specific, hepatosplenic opacification. Neither is possible with current extracellular fluid agents, which rapidly extravasate (1–2 min) from the vascular to interstitial space following i.v. administration (Wegener, 1983; Swanson et al., 1990). Bis[1-(Ethoxycarbonyl)propyl]5-acetylamino-2,4,6-triiodoisophthalate (NC 68183) was designed and synthesized as a new CT imaging agent to provide prolonged vascular radiopacity with enhancement of normal liver parenchyma sufficient for differentiation of liver lesions. The chemical structure was shown at Fig. 1. Preclinical CT imaging studies in both normal and VX2 tumor-bearing rabbits demonstrated that NC 68183 nanoparticle provided prolonged (15–30 min) opacification of the blood followed by adequate contrast enhancement between the tumor tissue and normal liver parenchyma as well as splenic opacification. Furthermore, in these imaging studies, NC 68183 appeared to be cleared from the body quickly as blood and organ opacification had returned to precontrast levels by 48 to 72 h (E.R. Bacon and G.L. McIntire, unpublished data).

NC 6813 compound has a molecular mass of 829 and formulated as nanoparticles with an average size less than 300 nm. Previous studies have shown that the urinary excretion is negligible (G. Shackleton, J. Allen, J. Johnson and W. Blazak, unpublished data). Although hepatobiliary clearance is suspected, it is not clear how the iodinated nanoparticle is metabolized and eliminated in the body. The liver, especially Kupffer cells representing reticuloendothelial system (RES), plays an important role in the clearance of foreign particles from blood plasma by phagocytosis (te Koppele et al., 1991; Stiskal et al., 1996). The isolated perfused rat liver (IPRL) has been shown as an...
excellent model for determining hepatic metabolism and clearance (Liu and Thurman, 1992; Liu et al., 1996; Nolting et al., 1997). Therefore, the objectives of these studies are to determine the pharmacokinetics and biodistribution of the nanoparticulate in the rat, and hepatic disposition in the IPRL.

Experimental Procedures

Materials. NC 68183 was synthesized and formulated in the Department of Pharmaceutical Analytical and Chemical Sciences, Nycomed Amersham (Fig. 1). NC 68183 nanoparticles were prepared according to the method described by Bacon et al. (1997). Poloxamer 338 (BASF, Parsippany, NJ) was used as the surfactant in preparation of nanoparticles. Poloxamer 338 consists of copolymer of ethylene oxide and polypropylene oxide. The size (<300 nm) of the nanoparticle was set as the criteria for formulation. The mean size of the nanoparticle was determined to be 140 ± 6 (S.D.) by light scattering using a Horiba 910 (Horiba, Irvine, CA). The solubility of the nanoparticle in water is very limited (<10 μg/ml). Pentobarbital was purchased from The Butler Company (Columbus, OH), and ketamine and xylazine were obtained from Phoenix Pharmaceuticals (St. Joseph, MO). Other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO).

Animals. Male Sprague-Dawley rats (Taconic Farms, Germantown, NY), weighing from 350 to 450 g, were used throughout. The rats had free access to Rodent Diet (PMI Certified No. 5002; PMI Nutrition International, Inc., Brentwood, MO) and water, and were maintained on a 12-h automatically timed light/dark cycle.

Pharmacokinetics. Rats were anesthetized with a combination of 100 mg/kg ketamine and 20 mg/kg xylazine i.p. Rats were catheterized using double catheters into the jugular vein using a modified method (Niederhuber et al., 1982). A surgical incision was made on the ventral side of the neck to expose the jugular vein. The jugular vein was cannulated with a micro-renethane catheter (Braintree Science, Braintree, MA) that was fed under the skin and exteriorized at the back of the neck. The rats were appropriately restrained and monitored in metabolic cages. The rats were allowed at least 2 days to recover from surgery. NC 68183 was prepared as 15% (w/v%) suspension of which 46% of the agent is X-ray active as iodine. Animals were dosed at 1 (69 mg I/kg) and 10 ml/kg (690 mg I/kg) via the jugular vein catheter by a bolus injection. The blood samples were collected from the catheter at 5, 15, 30, and 60 min, and 7 days following an i.v. injection of the iodinated agent. At the end of the experiment, animals were euthanized; the spleen, liver, and kidneys were removed. The tissue samples were mixed with PBS (1:1 w/v) and homogenized before analysis. The blood and tissue homogenates were analyzed for iodine content of the agent using X-ray fluorescence (XRF). The WinNonlin program was used to estimate pharmacokinetic parameters.

XRF. The amount of iodine (milligrams per milliliter) was determined using XRF. Because iodine is very stable in the nanoparticle, the iodine concentration presumably reflects the concentration of NC 68183 nanoparticle. Rat tissue and blood samples weighing between 0.50 and 0.80 g and an equal amount of 0.01 M PBS were mixed using a tissue homogenizer (Turrax). One milliliter of each mixed sample was added to an autosampler cup and analyzed for iodine using XRF (Lab-X 3000; Oxford Instruments) using a calibration curve of NaI solutions as standards. One Housfeld unit was defined as 17.9 ± 0.8 mg I/ml and the mean residence time was calculated as 17.9 ± 0.8 mg I/min/ml and the mean residence time was 16.3 ± 1.2 min. The t1/2 and clearance were estimated as 11.3 ± 0.8 min and 1.5 ± 0.07 ml/min, respectively. The Vss was approximately 24 ml, which is very close to the estimated total blood volume of the rat (~7% of body weight) (Davies and Norris, 1993). Tissue distribution of the nanoparticle was determined using XRF. No iodinated compound was detected in urine, kidney, and spleen at day 7 (Fig. 3). The liver is the only organ/tissue to have detected iodinated compound (~2% of injected dose).

The blood clearance of the iodinated nanoparticle appeared to be saturated following a bolus injection of 690 mg I/kg into the rats (Fig. 2, middle). No apparent tissue distribution phase was observed. The slope of nanoparticulate blood concentration following administration of 690 mg I/kg was different from 69 mg I/kg (Fig. 2, bottom). Nanoparticles were cleared from blood in zero order kinetics at a rate of 16 μg I/ml/min. Approximate 80% of injected dose remained in the blood 60 min following administration of this dose. Assuming 30 μl of blood per gram body weight, the iodinated compound was completely cleared from blood plasma at day 7. At day 7, liver and spleen were the only tissues which had detectable iodinated compound (Fig. 3, bottom).

Hepatic Metabolism and Biliary Excretion. To characterize how the iodinated nanoparticle is disposed from the liver, an IPRL model was used to determine its hepatic uptake, metabolism, and elimination. Basal bile flow increased about 30% (P < 0.05) from 1 to 1.3 μl/min/g liver following a bolus injection of 75 mg (0.5 ml) of NC 68183 into the reservoir. The clearance of NC 68183 compound from the perfusate following a bolus injection showed biphasic kinetics (Fig. 4). Following administration, the metabolite was detected as

HPLC and Liquid Chromatography/Mass Spectrometry (LC/MS) Analysis. The perfusate (100 μl) and bile samples (20 μl) were separately mixed with 600 μl of ethyl acetate for extraction. The organic extract was dried down and resuspended with 100 μl 50% acetonitrile + 50% distilled water. The extracted samples were eluted from a C8 column (30 × 406 mm; 5 μm) using a Waters HPLC system with an isotropic solution of 35% acetonitrile in Nanopure water, and 0.1% trifluoroacetic. NC 68183 compound and its metabolite were quantitated based on the area under curve of the standards at 290 nm. The extracted bile and perfusate samples were further analyzed using a LCQ system (Finnigan Mat, San Jose, CA) with a full scan range from 400 to 1,700 m/z (mass charge ratio).

Data Analysis. The WinNonlin program (Pharsight, Cary, NC) was used to generate the pharmacokinetic parameters, the area under the plasma curve, the mean residence time, the t1/2, and the volume of distribution at steady state (Vss). The nanoparticulate concentration in the blood samples was fitted with a single-compartment model, and the biliary excretion rate maximum in the IPRL was generated from a fitted noncompartment model. All data were expressed as means ± S.E. of three replications of animals or livers per group.
early as 10 min from bile, and biliary excretion rate maximum (11 mg/min/g liver) was achieved at 40 min following the administration.

Approximately 13% of the injected dose was eliminated from the liver during 120-min perfusion (Fig. 4).

NC 68183 compound was identified as a single peak with retention time of 10 min using HPLC and further characterized as a molecular mass of 829 using LC/MS (Fig. 5). The major metabolite of NC 68183 was identified as a monocarboxylic acid (MCA) product with a molecular mass of 801 (Fig. 6). The metabolite MCA product of the nanoparticulate was excreted in bile in an iodinated and soluble form. This metabolic product was discovered exclusively in the bile. No parent compound was recovered in the bile. These data clearly indicate that iodinated nanoparticles are extracted by the liver, metabolized by the hepatic esterase, and excreted into the bile in a soluble metabolic form.

**Discussion**

This study characterized disposition of the iodinated-containing nanoparticle from the body. NC 68183 nanoparticle primarily resided in the blood pool following an i.v. administration and mainly cleared by RES in spleen and liver. Hepatobiliary excretion was the major determinant in elimination of the nanoparticulate from the body. The iodinated nanoparticles were extracted from the perfusate/plasma by
the liver and metabolized into a MCA product, which was excreted into bile as an iodinated metabolite.

The characteristic anatomic and functionary features of the liver allow the organ to directly extract the insoluble iodinated nanoparticulate from blood. The liver is an organ to have high blood flow (10–16 ml/min in rat) and large surface of the hepatocyte membrane. Sinusoidal domains in the liver lack a basement membrane and possess pores of 100 to 1000 nm in size (Gabriele, 1993), thus allowing the nanoparticulate to freely access hepatocytes and Kupffer cells from Disse space. Kupffer cells belong to the RES and are primarily located at the sinusoidal domains of the liver. Kupffer cells account for about 2% of volume fraction of the total cell population in the liver (Blouin et al., 1977). The interesting finding is that the metabolite of NC 6813 was exclusively found in bile, not in perfusate, suggesting that hepatocytes are involved in its elimination. Based on the size of NC 6813 nanoparticle, it is unlikely for hepatocytes to directly take up nanoparticles from plasma/perfusate. How do the nanoparticles enter into hepatocytes? Two possible explanations for

**FIG. 4.** Hepatic clearance of the nanoparticulate and biliary excretion of its metabolite in the IPRL following a bolus injection of 75 mg of NC 68183. The data are expressed as the mean ± S.E. of three rat livers.

**FIG. 5.** LC/MS analysis of perfusate sample from the IPRL following a bolus injection of 75 mg NC 68183. NC 68183 was identified as molecular mass of 829.3 in perfusate (m/z, inset).
this finding are discussed below. Kupffer cells contain a great variety of enzymes such as phosphatases and esterases, and have a distinct intracellular environment. These factors may facilitate dissolving NC 68183 nanoparticles. Presumably, the initial limited soluble compound of NC 68183 could be rapidly taken up by hepatocytes from plasma, whereas massive nanoparticles in plasma are phagocytosed by Kupffer cells where nanoparticles could convert to soluble form. The soluble compound of NC 68183 could be released back to the sinusoidal domain and Disse space from Kupffer cells and rapidly taken up by hepatocytes. NC 68183 compound undergoes a hydrolysis of ester bond in hepatocytes via hepatic esterases and is excreted into bile. Alternatively, the ester bond of NC 68183 could be cleaved in Kupffer cells. The hydrolytic product is delivered back to sinusoidal domains and Disse space from Kupffer cells, and rapidly taken up by hepatocytes and excreted into bile. As a result, the concentration of metabolites in the outflow of perfusate could be too low to be detected. Only the parent compound can be detected in the perfusate.

As shown in Fig. 4, the iodinated nanoparticle was rapidly taken up by the liver and hydrolyzed into an iodinated MCA product (MW 801), which was excreted into bile. MCA was a soluble form in bile and can be detected from bile at 10 min following administration. MCA retained the three iodine in its structure. This excreted iodinated metabolite may account for the choleretic effect (30% increase in bile flow) due to its osmotic action. The iodinated metabolite was vectorially eliminated from the liver because it was found exclusively in bile, not in the perfusate. Approximately 13% of the injected dose was cleared from the liver during 120-min perfusion. These results indicate that the hepatobiliary excretion serves as an important route in elimination of the nanoparticulate. Because the biliary canalicular membrane serves as a barrier to separate the intracellular compartment of hepatocytes from bile, it is unlikely for the molecule like MCA (MW 801) to diffuse from intracellular compartment of hepatocytes to bile. Based on its vectorial elimination and anionic moiety, MCA could be excreted into bile via a carrier-mediated transport such as cMOAT/MRP2.

CMOAT/MRP2 has been identified from the canalicular plasma membrane in the liver (Meier, 1995; Müller and Jansen, 1997) and accounts for transport of a great variety of endogenous compounds and xenobiotics and their metabolites (Oude Elferink and Jansen, 1994; Kartenbeck et al., 1996; Paulusma et al., 1996). These compounds include the GSH S-conjugates of chlorodinitrobenzene and BSP, leukotriene C4 (LTC4), estriol-17β-D-glucuronide (E217G), and unmetabolized antibiotic ceftriaxone (Oude Elferink et al., 1995; Vore et al., 1996, 1997). These studies clearly indicate the broad substrate specificity of cMOAT/MRP2 for anionic compounds.

Based on our data, it is apparent that disposition of iodinated nanoparticulate is different from iron containing nanoparticles. Superparamagnetic iron oxide particles (SIOP) are a class of MRI nanoparticles containing iron (Okon et al., 1994). SIOP are initially phagocytosed by RES (mainly in spleen), where SIOP are degraded into free iron and other noniron components. Free iron was released from RES into blood and eventually taken up by hepatocytes via intraportal or intrahepatic transport. Unlike SIOP, NC 68183 is metabolized in the liver and eliminated as an iodinated form via the biliary route. Because NC 68183 is an iodinated compound, free iodine could be a potential toxic substance in the body if iodine released from the aromatic ring of either the parent compound or the metabolites. Biliary excretion of iodinated metabolite could avoid/minimize this potential iodine-mediated toxicity.

In vivo pharmacokinetic data clearly indicated that the $V_{ss}$ (24 ml) was in good agreement with the estimated total blood volume of the rat (~7% of body weight). The blood clearance of NC 68183 nano-
particle followed a single compartment model, indicating a negligible tissue distribution. As a result, the nanoparticulate was assumed to be primarily confined in the blood pool and cleared by RES in the liver following an i.v. administration. This pharmacokinetic feature of NC 68183 allows the iodinated nanoparticulate to generate an opaque imaging in blood pool under X-ray for differentiation of focal lesions from normal tissues. The contrast imaging window can be prolonged by adjusting doses to generate the appropriate iodine concentration in blood. The systemic clearance of the iodinated nanoparticulate can be saturated by increasing the administered dose. The hepatic esterase and biliary excretion may represent the rate-limiting step in disposition of the nanoparticulate. Unlike other CT agents, NC 68183 primarily resides in the blood pool following an i.v. administration with an appropriate plasma half-life to generate acceptable blood pool imaging. Nanoparticles can be eliminated as an iodinated metabolite from the liver into bile.

References
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