In Vivo Metabolism-Based Discovery of a Potent Cholesterol Absorption Inhibitor, SCH58235, in the Rat and Rhesus Monkey through the Identification of the Active Metabolites of SCH48461

MARGARET VAN HEEK, CONSTANCE F. FRANCE, DOUGLAS S. COMPTON, ROBBIE L. MCLEOD, NATHAN P. YUMIBE, KEVIN B. ALTON, EDMUND J. SYBERTZ and HARRY R. DAVIS, JR.

Department of CNS and Cardiovascular Research (M.V.H., C.F.F., D.S.C., E.J.S., H.R.D.), Department of Allergy (R.L.McL.), Department of Drug Metabolism and Pharmacokinetics (N.P.Y., K.B.A.), Schering-Plough Research Institute, Kenilworth, New Jersey

Accepted for publication June 30, 1997

ABSTRACT

SCH48461 is a selective and highly potent inhibitor of cholesterol absorption. In rats, SCH48461 is rapidly and completely metabolized in the first pass through the body. To compare the activity of the metabolites of SCH48461 with SCH48461 itself, an intestinally cannulated, bile duct-cannulated rat model for cholesterol absorption was developed. SCH48461 inhibited the absorption of cholesterol by 70%, whereas bile containing the metabolites of SCH48461 (henceforth, “metabolite bile”) inhibited the absorption by greater than 95%. Very little of the recovered radioactive dose of SCH48461 was located in the intestinal lumen (7%) or wall (4%), whereas 85% appeared in bile. However, in rats treated with metabolite bile, 62% of the dose remained in the lumen, 13% was associated with the wall and only 24% reappeared in bile, which suggests that the activity of the metabolite bile may be related to its higher retention in the intestinal wall. Rats treated with metabolite bile had 64% and 84% less drug-related radioactivity in their plasma and livers, respectively, compared with animals treated with SCH48461, which indicates that the metabolites are systemically less available than SCH48461. The metabolites in bile were separated by high-performance liquid chromatography; the most active fraction in the bile duct-cannulated rat model was identified by mass spectrometry as the glucuronide of the C4-phenol of SCH48461. The other fractions had moderate or no activity. Through the identification of the most active biliary metabolites of SCH48461 in the rat, we have discovered SCH58235, a novel cholesterol absorption inhibitor which is 400 times more potent than SCH48461 in the cholesterol-fed rhesus monkey.

Many years of investigation have shown that dietary cholesterol intake and plasma cholesterol levels are positively associated with the risk of atherosclerosis (National Research Council, 1989). Lowering plasma cholesterol by dietary and/or pharmacological manipulation has been shown to decrease the incidence of death by coronary artery disease as well as total morbidity (Scandinavian Simvastatin Survival Study Group, 1994). The level of plasma cholesterol in the body is affected by biosynthesis of cholesterol, removal of cholesterol from the circulation, the absorption of dietary cholesterol and the reabsorption of cholesterol from the bile. Reducing the absorption of dietary and biliary cholesterol would prevent the entry of exogenous and endogenously synthesized cholesterol into the body and potentially lower plasma cholesterol levels. SCH48461 has been shown to be a potent hypocholesterolemic agent in cholesterol-fed hamsters, rats, rabbits, dogs and cynomolgus and rhesus monkeys (Burnett et al., 1994; Salisbury et al., 1995; Sybertz et al., 1995) and lowers LDL cholesterol in humans (Bergman et al., 1995). It is known that SCH48461 prevents the absorption of cholesterol in the intestine, but the precise molecular mechanism of action is not known. It was therefore essential to develop in vivo models to elucidate SAR, because in vitro systems were not available to conduct standard SARs.

Preliminary experiments in our laboratory have determined that predosing rats with SCH48461, even as little as 1 hr before giving 14C-cholesterol, led to a significantly greater inhibition of cholesterol absorption than in animals which were simultaneously given SCH48461 and 14C-cholesterol. We hypothesized that this could be simply because the

ABBREVIATIONS: SAR, structure-activity relationship; ACAT, acyl-CoA:cholesterol acyltransferase; RP-HPLC, reverse phase-high pressure liquid chromatography; TLC, thin layer chromatography; LC, liquid chromatography; MS, mass spectrometry; HMG-CoA, hydroxymethylglutaryl coenzyme A.
SCH48461 was reaching the site of action before cholesterol, or that SCH48461 had to undergo metabolism before becoming more active. We have found that SCH48461 undergoes both phase I and phase II metabolism resulting in several polar glucuronide conjugates. We identified the metabolites of SCH48461 and determined which of the metabolites was the most active in inhibiting cholesterol absorption. This directed our SAR to discover SCH58235 (Rosenblum et al., 1995), a potent, metabolically stable cholesterol absorption inhibitor.

Materials and Methods

Collection of control and 3H-SCH48461 metabolite bile. Male Sprague-Dawley rats weighing 300 to 400 g were used to generate donor bile. After an overnight fast, rats were anesthetized (inactin, 0.1 mg/kg i.p.) for the duration of each study, were bile duct-cannulated (Waynforth, 1980) and were fitted with a cannula into the small intestine just below the pyloric valve. For the bile duct cannulation, PE 50 tubing was inserted into the common bile duct and ligated in place. For the cannulation of the small intestine, a catheter (Surflo i.v. catheter (18G × 2”), Terumo Medical Corporation, Elkton, MD) was inserted through the fundus of the stomach, advanced 1 cm beyond the pylorus and ligated in place. For the cannulation of the small intestine, a catheter (Surflo i.v. catheter (18G × 2”), Terumo Medical Corporation, Elkton, MD) was inserted through the fundus of the stomach, advanced 1 cm beyond the pylorus and ligated in place. Three milliliters of control emulsion (Tso et al., 1980) consisting of 2.23 mg/ml L-phosphatidylcholine and 11.8 mg/ml triolein in 19 mM sodium taurocholate (Sigma Chemical Co., St. Louis, MO) buffer (pH 6.4) was delivered to each rat as a bolus via the intestinal catheter into the small intestine of control bile donors, and bile was collected at approximately 0.5 to 1.0 ml/hr. For the generation of bile containing SCH48461 metabolites (referred to as "metabolite bile"), unlabeled SCH48461 and 3H-SCH48461 were included in the emulsion described above. To generate metabolite bile, 3 ml of this emulsion was placed into the intestines of metabolite bile donors as described above. Control and metabolite bile were collected for up to 5 hr, and bile for each group was pooled. From the specific activity of the 3H-SCH48461 in the emulsion, and the radioactivity recovered in the metabolite bile, the concentration of metabolite(s) in the SCH48461 metabolite bile was calculated. In general, most SCH48461 metabolite bile pools were approximately 0.6 nM (equivalent to 0.25 mg/ml SCH48461).

Determination of cholesterol absorption inhibitory activity and tissue distribution of 3H-metabolite bile versus 3H-SCH48461. Twenty-four rats weighing approximately 300 g each were sorted into four groups (n = 6/group). The intestines and bile ducts of 18 rats were cannulated as described above. The intestines, but not bile ducts, of the remaining 6 rats were cannulated. One group (Control; cannulated) of bile duct-cannulated rats received 2.5 ml of control bile via the intestinal cannula. An equivalent amount of 3H-SCH48461 was added to control bile to equal the specific activity of the metabolite bile described above; 2.5 ml of this bile (2 mg/kg 3H-SCH48461) was placed into the small intestines of one group of bile duct-cannulated rats (SCH48461; cannulated) and the group of bile duct-intact rats (SCH48461; intact). The latter group was included to ensure that use of bile as a delivery vehicle for SCH48461 did not interfere with the compound’s ability to inhibit the absorption of cholesterol. The third group of bile duct-cannulated rats received 2.5 ml (equivalent to 2 mg/kg SCH48461) of the metabolite bile (Metabolite; cannulated). After delivery, bile was collected from each rat at 0.5-h intervals.

One hour after the bile doses were delivered, 3 ml of the triolein, phosphatidylcholine, sodium taurocholate emulsion (as described above) containing 3 mg cholesterol and 1 μCi 14C-cholesterol (NEC; Boston, MA) was delivered to each rat as a bolus via the intestinal cannula. Bile collection at 0.5-h intervals continued. Ninety minutes after the cholesterol emulsion was delivered, the rats were sacrificed. Blood was collected and plasma was separated by centrifugation at 2000 rpm for 15 min at 4°C. Entire luminal contents were collected by rinsing the intestines with 50 ml of saline. Entire small intestines (mucosa and muscle layer) from pylorus to cecum (100–120 cm) and livers were collected, minced and extracted with 2:1 (v/v) chloroform/methanol. Triplicate aliquots of intestinal luminal contents, intestinal wall extracts, plasma, liver extracts and bile fractions were analyzed for 3H and 14C radioactivity. Data are expressed as mean ± SD. Total radioactivity in tissues observed between groups would reflect real differences rather than a discrepancy in recovery. Recovery of both radioactivity labels was >85% in all groups.

Separation of metabolites of SCH48461, analysis of cholesterol absorption inhibitory activity and identification of the most active metabolite. Forty-five milliliters of a SCH48461 biliary metabolite pool was processed by solid-phase extraction (Sep-Pak Vac 35 cc tC18; Waters, Milford, MA). Preparative mode HPLC separation of bile metabolites recovered after solid-phase extraction was performed with two Waters model 590 pumps, a Waters 991 Photodiode Array Detector and an Inertsil (20 × 250 mm) PREP-ODS reversed-phase column (Jones Chromatography USA, Inc., Lakewood, CO). The HPLC flow rate was 10 ml/min, and the elution solvents were 0.2 M ammonium acetate (pH 6) and methanol. A nonlinear elution gradient (Waters Expert-Ease gradient no. 9) was programmed initially at 65% methanol/35% ammonium acetate and was changed to 100% methanol during the next 45 min. Five percent of the total flow was diverted for monitoring radioactivity and UV. Individual radioactive peaks were collected manually, and the methanol was removed in vacuo (Buchi Rotavapor; Flawil, Switzerland). Metabolites contained within individual HPLC fractions were de-salted and concentrated with Sep-Pak C18 (1 cc) cartridges. One fraction (fraction 6) of the metabolite bile extract was further purified by preparative TLC (UNIPLATE Taper; Analtech; Newark, DE) with an ethylacetate/isopropanol/water/NH4OH (100:70:32:8 v/v/v/v) solvent system. This TLC-purified sample was analyzed by thermospray LC/MS. The LC used was a Waters 600 multisolvvent delivery system with a U6K injector and a Waters 600 MS system controller. The column used was an Inertsil C8 (4.6 × 150 mm), a reversed-phase column (GL Sciences, Inc., Rockford, IL). The MS used was a Finnigan TSQ 70B. In a series of experiments, the methanolic extract of metabolite bile and the HPLC fractions described above were tested for their ability to inhibit the absorption of cholesterol in the bile duct-cannulated, intestinally cannulated rat model. The doses were equalized after determining radioactivity in the bile extract and the fractions. Aliquots of methanol extracts and fractions were dried under N2 and were resolubilized in control bile before administration to recipient rats.

Determination of ID50 of metabolite bile and SCH58235 in rats. Dose-response characteristics to graded doses of biliary metabolites of SCH48461 (0.2-13 mg equivalents/kg) and SCH58235 (0-0.03 mg/kg) in bile duct-intact, intestinally cannulated rats (n = 5-11/group) were evaluated to determine the dose at which metabolite bile inhibits the absorption of cholesterol by 50% (ID50).

Dose response of SCH58235 versus SCH48461 in cholesterol-fed rhesus monkeys. In a series of experiments, dose responses of SCH58235 and SCH48461 were conducted in cholesterol-fed rhesus monkeys as described previously in detail (Salisbury et al., 1995). Rhesus monkeys (n = 5-6 group) were fed 150 g/day of a diet containing 0.25% (w/w) cholesterol, 15% (w/w) hydrogenated coconut oil and 7.5% (w/w) olive oil with or without SCH58235 or SCH48461 at various doses. Plasma cholesterol levels were determined weekly for 3 weeks by the cholesterol oxidase method (Wako Pure Chemicals Industries, Osaka, Japan).

All animal studies were conducted in an AAALAC accredited facility following protocols approved by the Schering-Plough Research Institute’s Animal Care and Use Committee. The procedures were performed in accordance with the principles and guidelines.
established by the National Institutes of Health for the care and use of laboratory animals.

**Chemical structures.** Schering compound structures referred to in this text are shown in figure 1.

## Results

**Determination of cholesterol absorption inhibitory activity and tissue distribution of \(^3\)H-biliary metabolites of SCH48461 versus \(^3\)H-SCH48461.** Previous experiments determined that no intact SCH48461 (see structure, fig. 1) could be found in the bile of bile duct-cannulated rats that had been given an intraduodenal dose of SCH48461 and that the bile contained several different metabolites of SCH48461 (N. Yumibe, personal communication). Bile duct-cannulated rats receiving \(^3\)H-SCH48461 (2 mg/kg) in bile had 70% less \(^{14}\)C-cholesterol radioactivity in plasma than controls, whereas the metabolite bile inhibited \(^{14}\)C-cholesterol absorption by greater than 95% in bile duct-cannulated rats at the same dose (fig. 2A). Intact rats that received \(^3\)H-SCH48461 in bile also had 95% less \(^{14}\)C-cholesterol in plasma, which demonstrated that: 1) bile was an appropriate delivery vehicle for SCH48461 and 2) when SCH48461 was metabolized and delivered back to the intestine via the bile duct, the new metabolites were as active in inhibiting cholesterol absorption as placing metabolite bile directly into the intestine. These differences were also reflected in the \(^{14}\)C-cholesterol found in the livers (fig. 2B).

The amount of drug-derived radioactivity found in plasma and liver in the three groups which received \(^3\)H-SCH48461 or \(^3\)H-metabolite bile is shown in figure 3. Bile duct-cannulated rats that were given \(^3\)H-metabolite had 64% and 84% less radioactivity in their plasma (fig. 3A) and livers (fig. 3B), respectively, than the amount of \(^3\)H found in those tissues of bile duct-cannulated rats given \(^3\)H-SCH48461 during the 2.5 hr of the study. Plasma and liver \(^3\)H in intact rats was somewhat higher than in bile duct-cannulated rats given \(^3\)H-SCH48461; this is likely because, in the intact rats, the compound was delivered back to the intestine via the bile and reabsorbed.

The tissue distribution of \(^3\)H in bile duct-cannulated rats suggests a mechanism by which the metabolite bile might be more active than SCH48461 in inhibiting cholesterol absorption. In bile duct-cannulated rats given metabolite bile (fig. 4A), substantially more drug-related radioactivity remained in the lumen of the intestine (62% of the radioactivity recovered) and the intestinal wall (13%) than in the intestinal lumen (7%) and wall (4%) of bile duct-cannulated rats treated with \(^3\)H-SCH48461 in bile (fig. 4B). Only 24% of the recovered radioactivity was found in the bile of bile duct-cannulated rats given \(^3\)H-metabolite bile compared with 85% in the \(^3\)H-SCH48461-treated rats. These data suggest that one or more of the metabolites associate more avidly with the intestinal wall, thereby leaving more of the pharmacologically active species in the intestinal lumen and wall than SCH48461. Subsequently, less metabolite(s) appear in

![Fig. 1. Chemical structures of SCH48461, SCH53695 and SCH58235.](image)

![Fig. 2. Inhibition of \(^{14}\)C-cholesterol appearance in plasma (A) and liver (B) of rats given a bolus of either control bile, SCH48461 in bile or \(^3\)H-biliary metabolites of SCH48461. All rats (n = 6/group) were intestinally cannulated. All rats except those in the “SCH48461; intact” group were bile duct-cannulated. The doses (which contained unlabeled and \(^3\)H-compound as described under “Materials and Methods”) were delivered into the intestines via the intestinal catheters. The doses were the equivalent of 2 mg/kg of SCH48461. After 1 hr, an emulsion containing unlabeled and \(^{14}\)C-cholesterol was delivered into the intestines. After 1.5 hr, the rats were sacrificed and \(^{14}\)C-cholesterol in plasma (A) and liver (B) were determined. Values are mean ± S.E.M.](image)
plasma, liver and bile. SCH48461, on the other hand, appears to leave the intestinal lumen rapidly, cross the intestinal wall, travel through the blood and liver and be excreted in the bile as newly formed metabolites. In the intact animal, these metabolites would then be able to re-enter the intestinal wall and further prevent cholesterol absorption.

Separation of metabolites, analysis of cholesterol absorption inhibitory activity and identification of the most active metabolite. To determine whether one or all of the metabolites found in the rat bile were responsible for the inhibitory activity, a pooled volume of metabolite bile was processed by a combination of solid-phase extraction, preparative RP-HPLC and TLC as described under “Materials and Methods.” After solid-phase extraction, more than 90% of the drug-related radioactivity in the bile was recovered. The biliary extract was subjected to preparative RP-HPLC (recovery of radioactivity was 94.6%) which resulted in the separation of several radioactive fractions (fig. 5A). As observed before, these comprised glucuronide-conjugated metabolites. Equal doses (0.1 mg/kg) of the total extract, fractions 2 to 6, as well as pooled material from fractions 8 and 9 were then tested in the bile duct-cannulated rat model for cholesterol absorption inhibitory activity (fig. 5B). There was insufficient material to test the remaining fractions. Fraction 6 had the greatest inhibitory activity; fractions 2 and 3 had significant activity, whereas fractions 4, 5 and 8+9 had no inhibitory activity. Fraction 6 was therefore further purified by TLC. A single UV absorbing radioactive band was recovered from the plate and submitted for mass spectral analysis. Another sample from this purified isolate was incubated (37°C) for 16 hr with bovine β-glucuronidase. HPLC analysis of the hydrolysate substantiated the appearance of only the C-4 phenol of SCH48461 (SCH53695, see fig. 1). These findings, which were consistent with a glucuronide conjugate of SCH53695, were further corroborated by LC/MS mass spectral data (fig. 6). Equal doses (0.025 mg/kg) of the purified fraction 6 (glucuronidated form) and SCH53695 were then tested in the bile duct-cannulated rat model for cholesterol absorption inhibitory activity (fig. 7). Both fraction 6 and SCH53695 inhibited cholesterol absorption far more than the bile extract itself. More importantly, these data show that
fraction 6, which is the glucuronidated form of SCH53695, is the most potent metabolite of SCH48461.

From these data, and other in vitro and in vivo data not described herein, a large chemical synthesis program evolved to discover a more potent backup compound for SCH48461 (Clader et al., 1996; Dugar et al., 1996; Kirkup et al., 1996; McKittrick et al.). A benzylic hydroxyl group was added to SCH53695 and several of the sites that were readily metabolized in SCH48461 were blocked with fluorines resulting in SCH58235 (fig. 1).

**In vivo dose-response comparisons: determination of ID₅₀ values in rats and rhesus monkeys.** A comparison of dose responses of SCH58235 versus the metabolites of SCH48461 in bile duct-intact rats is shown in figure 8A. The ID₅₀ for SCH58235 in this acute model of cholesterol absorption was calculated to be 0.0015 mg/kg compared with 0.05 mg/kg for the metabolites of SCH48461, a 33-fold increase in potency. A comparison of dose responses for SCH58235 versus SCH48461 in the cholesterol-fed rhesus monkey are shown in figure 8B. The ED₅₀ for SCH58235 was determined to be 0.0005 mg/kg compared with 0.2 mg/kg for SCH48461. It can be concluded that SCH58235 is 400 times more potent than SCH48461 in inhibiting cholesterol absorption in the cholesterol-fed rhesus monkey.

**Discussion**

To assess the cholesterol absorption inhibitory activity of the metabolites of SCH48461, as well as the tissue distribution and systemic availability of the metabolites, we used a bile duct- and intestinally cannulated rat model. The most active metabolite was identified by mass spectrometry to be the glucuronidated form of SCH53695, which is the C-4 phenol of SCH48461 (fig. 1). From these experiments and others not described herein, a potent second generation cholesterol absorption inhibitor, SCH58235, was discovered.
was found to be 400 times more potent in inhibiting cholesterol absorption than SCH48461 in the cholesterol-fed rhesus monkey.

Although extensively studied at Schering-Plough Research Institute, the mechanism of the cholesterol absorption inhibition of this class of compounds is not known. Two possible candidates, ACAT and the pancreatic lipases (Salisbury et al., 1995; Sybertz et al., 1995) are not inhibited by these compounds. In addition, recent publications describing knock-out mice have indicated that intestinal cholesterol absorption in mice lacking ACAT (Meiner et al., 1996) or cholesterol esterase (Howles et al., 1996) is not altered significantly. The cholesterol absorption inhibitors do not sequester bile acids as cholestyramine does. Nor do these cholesterol absorption inhibitors precipitate cholesterol or inhibit HMG-CoA reductase activity (Salisbury et al., 1995). Most likely, these compounds interfere with the uptake of cholesterol into the intestinal wall by a novel, yet undiscovered mechanism. Although absorption of cholesterol has been investigated extensively, the molecular mechanism by which this occurs is still poorly understood (Wilson and Rudel, 1994). The cholesterol absorption inhibitors discovered at Schering-Plough Research Institute will potentially help elucidate the mechanism by which cholesterol is absorbed.

SCH58235 is now in development. Its predecessor, SCH48461, was shown to reduce LDL cholesterol in humans by 15% (Bergman et al., 1995). Whether SCH58235 will have a greater effect in humans on LDL cholesterol reduction is yet to be determined. Presently, HMG-CoA reductase inhibitors (the statins) are widely prescribed for both primary and secondary intervention as monotherapy to lower cholesterol, and they are generally well tolerated (Grundy, 1988). However, reports indicate that many patients with hypercholesterolemia are not receiving any drug therapy, or may not be achieving sufficient cholesterol reduction with the statins alone (Cohen et al., 1991; Giles et al.; 1993; Marcelino and Feingold, 1996). Cholestyramine, a bile acid sequestrant, is prescribed in combination with the statins to further reduce LDL cholesterol. However, gram quantities of cholestyramine must be consumed for efficacy, and it is well known that patient compliance is often poor because of unpleasant side effects. SCH58235 has been shown to be synergistic with the statins (Davis et al., 1995). This combination therapy may prove to be very effective in lowering cholesterol dramatically in severely hypercholesterolemic humans. Monotherapy of SCH58235 may also be an effective primary intervention for the patients with moderate hypercholesterolemia who are unable to lower their plasma cholesterol by dietary modification.

In summary, we have discovered a very potent cholesterol absorption inhibitor by use of a novel in vivo mechanism-based animal model to determine SARs. Efficacy and potency of SCH58235 in humans awaits the outcome of clinical trials.

Acknowledgments

We acknowledge Lizbeth Hoos, Daniel McGregor, John Cook and Grace Gruela for their technical assistance. We also thank Drs. Duane Burnett, Stuart Rosenblum and John Clader for synthetic compounds and helpful discussion.
Typical cholesterol levels increase from 150 mg/dl to 280 mg/dl after 3 weeks on this therapy. Plasma cholesterol levels of rhesus monkeys given the indicated doses of SCH58235 and SCH48461 admixed in the cholesterol-fed rhesus monkeys. Cholesterol-fed rhesus monkeys were given the indicated doses of SCH58235 and SCH48461 admixed in the emulsion containing 14C-cholesterol as described under "Materials and Methods." (A) Dose response of SCH58235 versus cholesterol absorption in rats (A) and inhibition of hypercholesterolemia in rhesus monkeys (B) by cholesterol absorption inhibitors. (B) Dose response of SCH58235 versus SCH48461 in cholesterol-fed rhesus monkeys. Cholesterol-fed rhesus monkeys were given the indicated doses of SCH58235 and SCH48461 admixed in the diet for 3 weeks. Data shown are plasma cholesterol levels after 3 weeks of drug/dietary treatment. Plasma cholesterol levels of rhesus monkeys not treated with a cholesterol absorption inhibitor would typically increase from 150 mg/dl to 280 mg/dl after 3 weeks on this high-fat/high-cholesterol diet. Values are mean ± S.E.M.

Fig. 8. In vivo dose-response comparisons: Inhibition of cholesterol absorption in rats (A) and inhibition of hypercholesterolemia in rhesus monkeys (B) by cholesterol absorption inhibitors. (A) Dose response of SCH58235 versus SCH48461 in cholesterol-fed rhesus monkeys. Cholesterol-fed rhesus monkeys were given the indicated doses of SCH58235 and SCH48461 admixed in the diet for 3 weeks. Data shown are plasma cholesterol levels after 3 weeks of drug/dietary treatment. Plasma cholesterol levels of rhesus monkeys not treated with a cholesterol absorption inhibitor would typically increase from 150 mg/dl to 280 mg/dl after 3 weeks on this high-fat/high-cholesterol diet. Values are mean ± S.E.M.

References


Send reprint requests to: Margaret Van Heek, K15–2-2600, Schering-Plough Research Institute, 2015 Galloping Hill Rd., Kenilworth, NJ 07033.