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Pasireotide Is More Effective than Octreotide in Reducing Hepatorenal Cystogenesis in Rodents with Polycystic Kidney and Liver Diseases

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In polycystic liver (PLD) and kidney (PKD) diseases, increased cyclic adenosine monophosphate (cAMP) levels trigger hepatorenal cystogenesis. A reduction of the elevated cAMP by targeting somatostatin receptors (SSTRs) with octreotide (OCT; a somatostatin analog that preferentially binds to SSTR2) inhibits cyst growth. Here we compare the effects of OCT to pasireotide (PAS; a more potent somatostatin analog with broader receptor specificity) on: (1) cAMP levels, cell cycle, proliferation, and cyst expansion *in vitro* using cholangiocytes derived from control and PCK rats (a model of autosomal recessive PKD [ARPKD]), healthy human beings, and patients with autosomal dominant PKD (ADPKD); and (2) hepatorenal cystogenesis *in vivo* in PCK rats and *Pkd2*$^{WS25/-}$ mice (a model of ADPKD). Expression of SSTRs was assessed in control and cystic cholangiocytes of rodents and human beings. Concentrations of insulin-like growth factor 1 (IGF1) and vascular endothelial growth factor (VEGF) (both involved in indirect action of somatostatin analogs), and expression and localization of SSTRs after treatment were evaluated. We found that PAS was more potent (by 30%-45%) than OCT in reducing cAMP and cell proliferation, affecting cell cycle distribution, decreasing growth of cultured cysts *in vitro*, and inhibiting hepatorenal cystogenesis *in vivo* in PCK rats and *Pkd2*$^{WS25/-}$ mice. The levels of IGF1 (but not VEGF) were reduced only in response to PAS. Expression of SSTR1 and SSTR2 (but not SSTR3 and SSTR5) was decreased in cystic cholangiocytes compared to control. Although both OCT and PAS increased the immunoreactivity of SSTR2, only PAS up-regulated SSTR1; neither drug affected cellular localization of SSTRs. *Conclusion*: PAS is more effective than OCT in reducing hepatorenal cystogenesis in rodent models; therefore, it might be more beneficial for the treatment of PKD and PLD. (HEPATOLOGY 2013;58:409-421)

Polycystic liver (PLD) and kidney (PKD) diseases are genetic disorders linked to disturbances in many intracellular signaling pathways and cell functions.[1-3] One of the well-defined mechanisms involved in hepatorenal cystogenesis is increased accumulation of intracellular cyclic adenosine monophosphate (cAMP) that triggers cell hyperproliferation, cell cycle deregulation, and fluid secretion. Basal cAMP levels in cholangiocytes are maintained by the coordinated functioning of: (1) secretin receptors (activation of which by secretin increases cAMP); (2) somatostatin receptors ([SSTRs], activation of which by somatostatin inhibits cAMP); (3) adenylyl cyclases (crucial for cAMP production); and (4) phosphodiesterases (critical for cAMP degradation).[1,2]

Activation of SSTRs induces multiple transduction pathways and mediates several cellular functions; however, inhibition of cell proliferation is one of the major

Abbreviations: ADPKD, autosomal dominant PKD; ARPKD, autosomal recessive PKD; IGF1, insulin-like growth factor 1; PKD, polycystic kidney disease; PLD, polycystic liver disease; SSTR, somatostatin receptor; VEGF, vascular endothelial growth factor.

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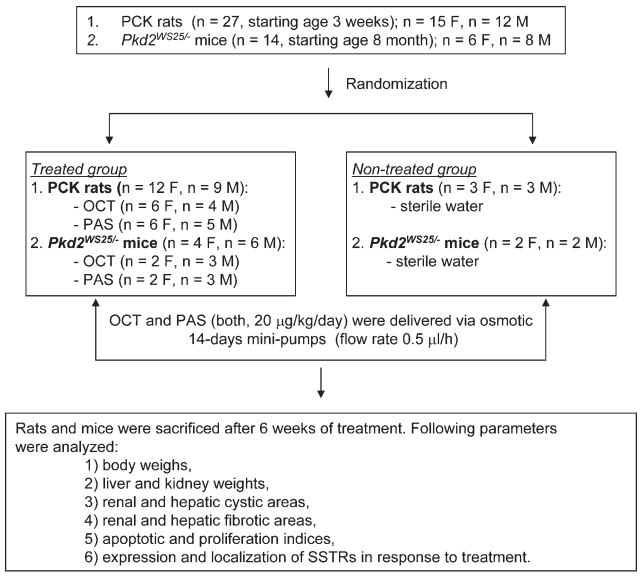

F – female, M - male

Fig. 1. Treatment protocol used to study the effects of OCT and PAS in PCK rats and *Pkd2*$^{WS25/-}$ mice.

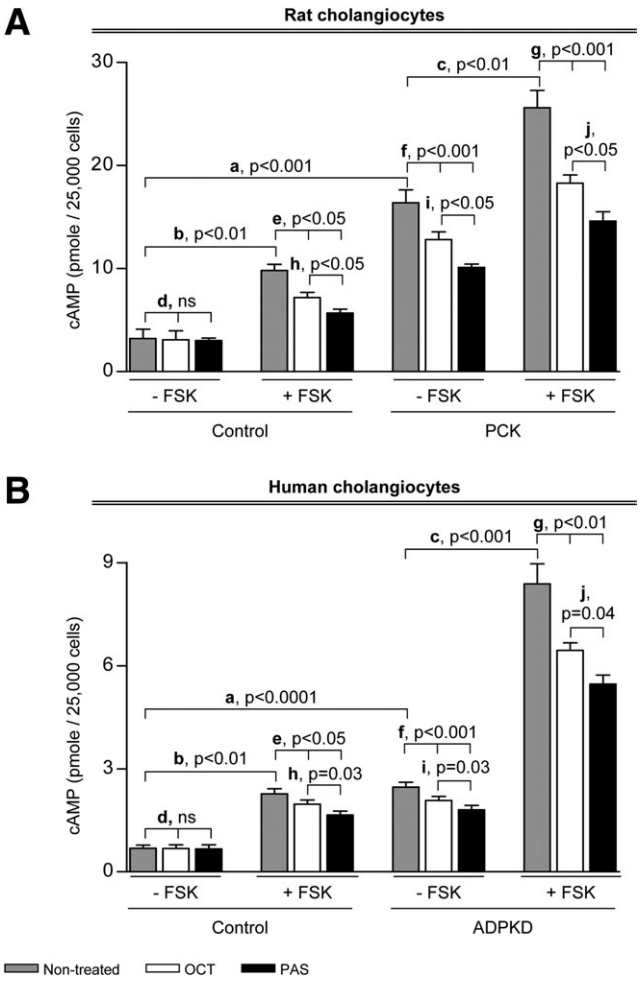

Fig. 2. PAS decreased cAMP accumulation more effectively than OCT. Levels of cAMP in PCK (A [a]) and ADPKD (B [a]) cholangiocytes were increased compared to respective controls. Forskolin (10^{-5}M) stimulated cAMP production in both rat (A [b and c]) and human (B [b and c]) cholangiocytes. OCT and PAS (both, 10^{-5} M) did not alter cAMP in control rat (A [d]) and human (B [d]) cholangiocytes under basal conditions (−FSK) but decreased it after addition of forskolin (+FSK, A [e] and B [e]). In PCK and ADPKD cholangiocytes, OCT and PAS inhibited cAMP in the absence (A [f] and B [f]) or presence (A [g] and B [g]) of forskolin. Note that PAS reduced cAMP accumulation to a higher extent than OCT (A [h, i, j] and B [h, i, j] (ns: not significant).

effects.[4,5] Cholangiocytes express all five SSTRs (i.e., SSTR1 through 5).[6,7] Importantly, targeting of SSTRs with synthetic somatostatin analogs in patients with PLD and PKD has limited but unequivocal benefit by reducing cyst growth.[3,7-10]

The natural SSTR ligand, somatostatin, is susceptible to proteolytic degradation and has a short half-life (~3 minutes), limiting its clinical utility.[5] Over the years, multiple stable somatostatin analogs have been developed. Octreotide (OCT; Sandostatin, SMS 201-95), a synthetic octapeptide with a half-life of 2 hours, was the first clinically introduced analog. We and others have tested the effects of OCT in preclinical and clinical trials in PLD and PKD.[7-11] However, despite beneficial results (i.e., symptom relief and cyst reduction), changes in liver and kidney volumes in patients are moderate; thus, additional pharmacologic approaches are needed.

OCT binds with high affinity to SSTR2 and SSTR3, with moderate affinity to SSTR5, and has no affinity to SSTR1 and SSTR4.[5,12,13] Because all five SSTRs coexist in cholangiocytes and renal epithelia,[6,7,14] somatostatin analogs with higher binding affinity to a broader range of SSTRs might be more effective. Recently, the cyclohexapeptide pasireotide (PAS, SOM-230) has been evaluated experimentally and clinically for the treatment of different pathological conditions.[12,13] PAS has a high affinity to SSTR1, SSTR2, SSTR3, and SSTR5 and a half-life of 12 hours.[12,13,15] Thus, we hypothesized that

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Conflict of interest: Partial financial support for this study was provided by Novartis USA (i.e., animal housing, cost of chemicals and supplies). Novartis was permitted to review the data before the article was written but the final decision on content and result interpretation was exclusively retained by the authors.

Additional Supporting Information may be found in the online version of this article.

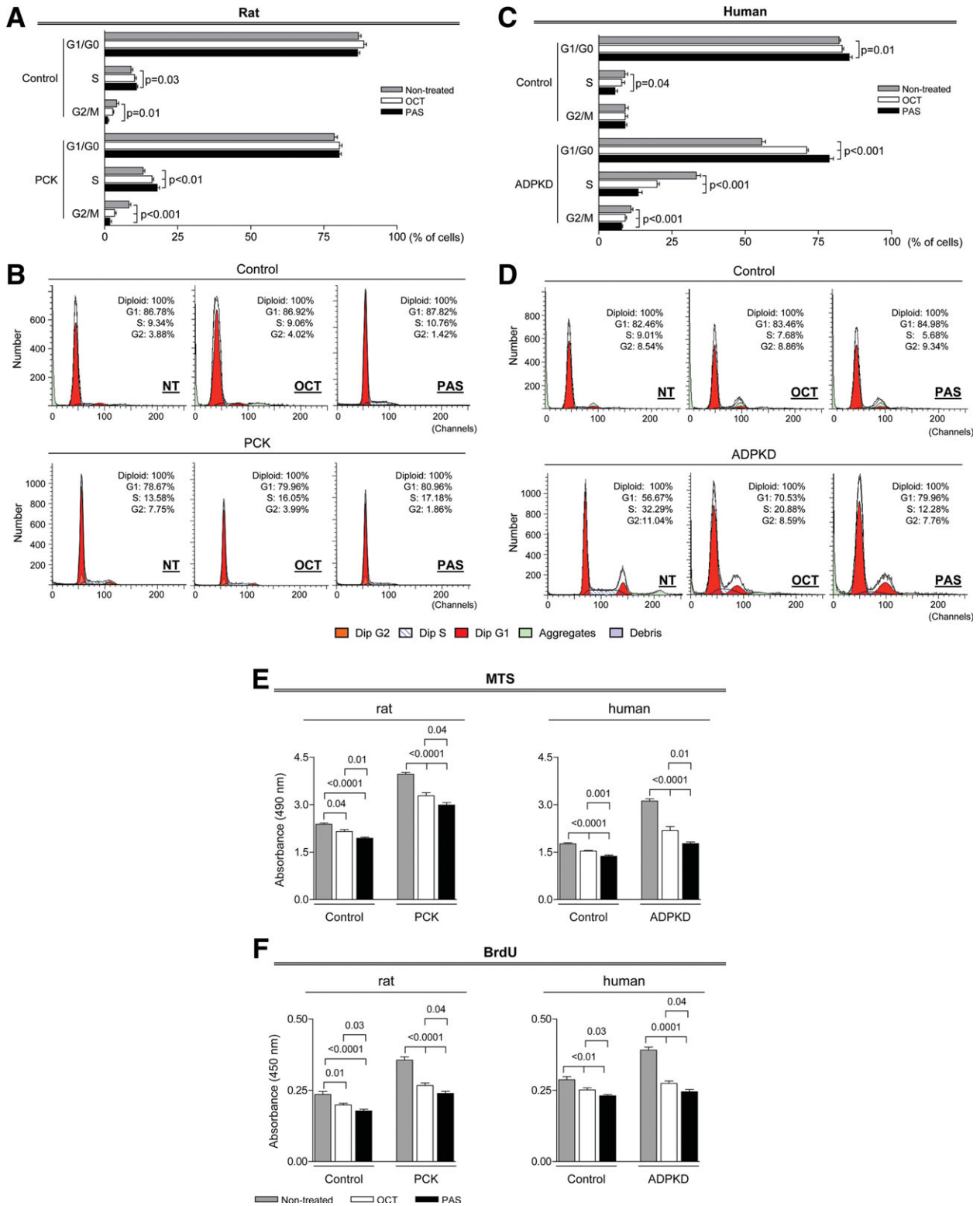


Fig. 3. OCT and PAS (both, 10^{-5}M) altered the cell cycle profile and reduced cholangiocyte proliferation. (A,C) The cell cycle distribution and (B,D) representative cell cycle profiles in rat and human cholangiocytes. In rat control cholangiocytes only PAS affected the cell cycle by increasing the cell number in S phase and decreasing it during G_2/M phase. In PCK cholangiocytes, both OCT and PAS increased percent of cells in S phase and reduced it in G_2/M phase. In human control cholangiocytes, OCT had no effects on the cell cycle, whereas PAS increased percent of cells in G_1 phase and decreased it in S phase. In ADPKD cholangiocytes, somatostatin analogs affected all phases of the cell cycle. (E,F) By MTS and BrdU assays, decreased proliferation of rat and human control and cystic cholangiocytes was observed in response to OCT and PAS, with PAS being more effective than OCT.

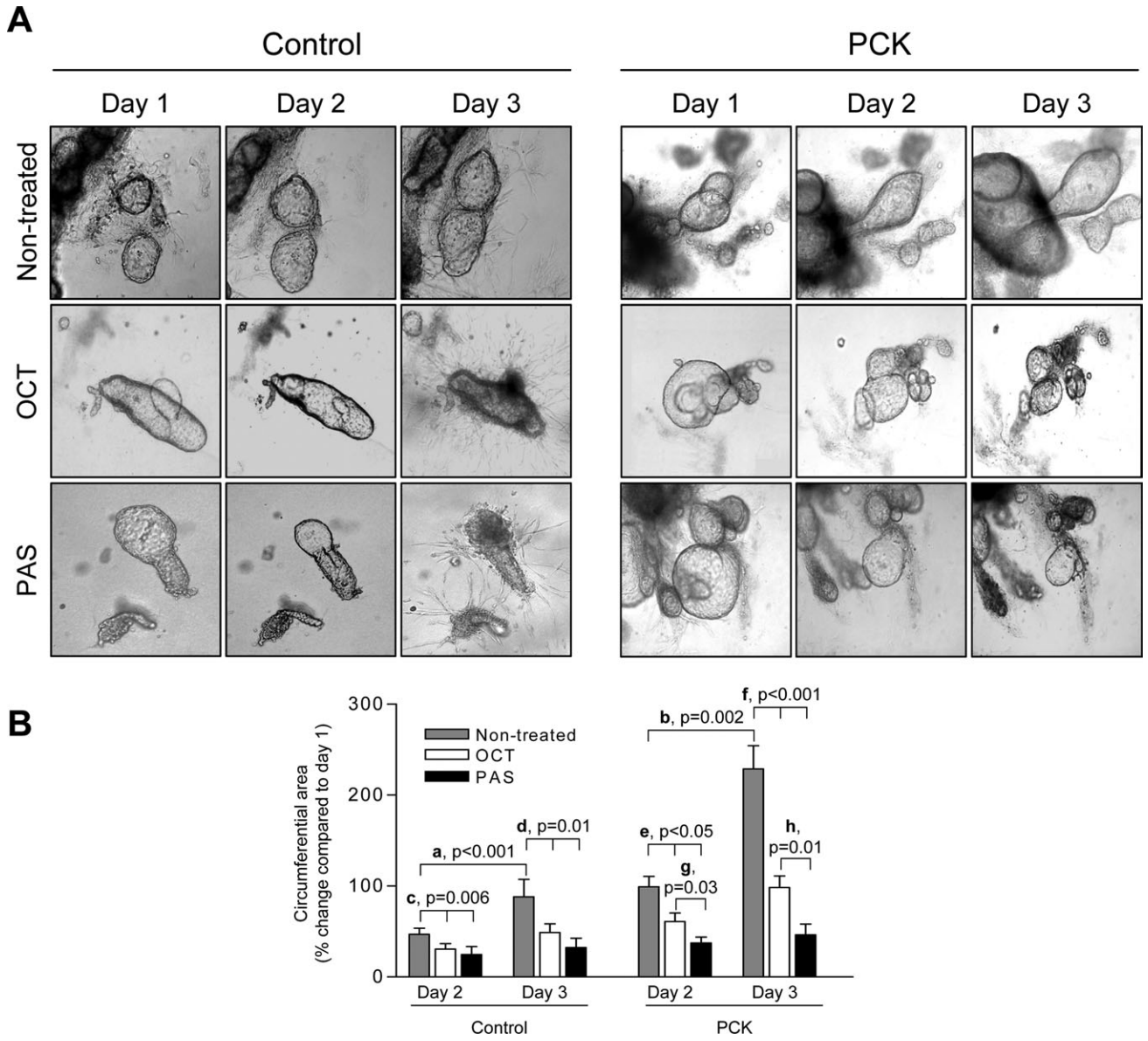


Fig. 4. PAS suppressed growth of hepatic cysts in 3D cultures more extensively than OCT. (A) Representative images of cystic structures formed by control and PCK bile ducts (×10). (B) In the absence of somatostatin analogs, both control (a) and PCK cysts (b) grow progressively, whereas OCT and PAS decreased their expansion (c-f). No differences in growth of OCT- and PAS-treated control cysts were observed, whereas expansion of PCK cysts was inhibited by PAS more greatly compared to OCT (g,h).

PAS should have more potent suppressive effects than OCT on cyst growth.

We used *in vitro* and *in vivo* experimental systems and models to compare the effects of OCT and PAS on cAMP levels, cell proliferation, cell cycle distribution, and hepatic cyst growth *in vitro*; and hepatorenal cystogenesis in two animal models of PLD and PKD, the PCK rat and *Pkd2*$^{WS25/-}$ mice. Expression of SSTRs in control and cystic cholangiocytes was assessed under basal conditions and after treatment. In addition, we examined concentrations of insulin-like growth factor 1 (IGF1) and vascular endothelial growth factor (VEGF) in response to OCT and PAS because these growth factors are linked to indirect inhibitory action of somatostatin analogs and are known to trigger hepatorenal cystogenesis by way of autocrine and paracrine mechanisms.[15-19]

We show that PAS has stronger suppressive effects on hepatorenal cystogenesis compared to OCT and thus might be more beneficial than OCT in patients with PLD and PKD.

Materials and Methods

Animals and Cell Cultures. Rats and mice were maintained on a standard diet and water *ad libitum*.

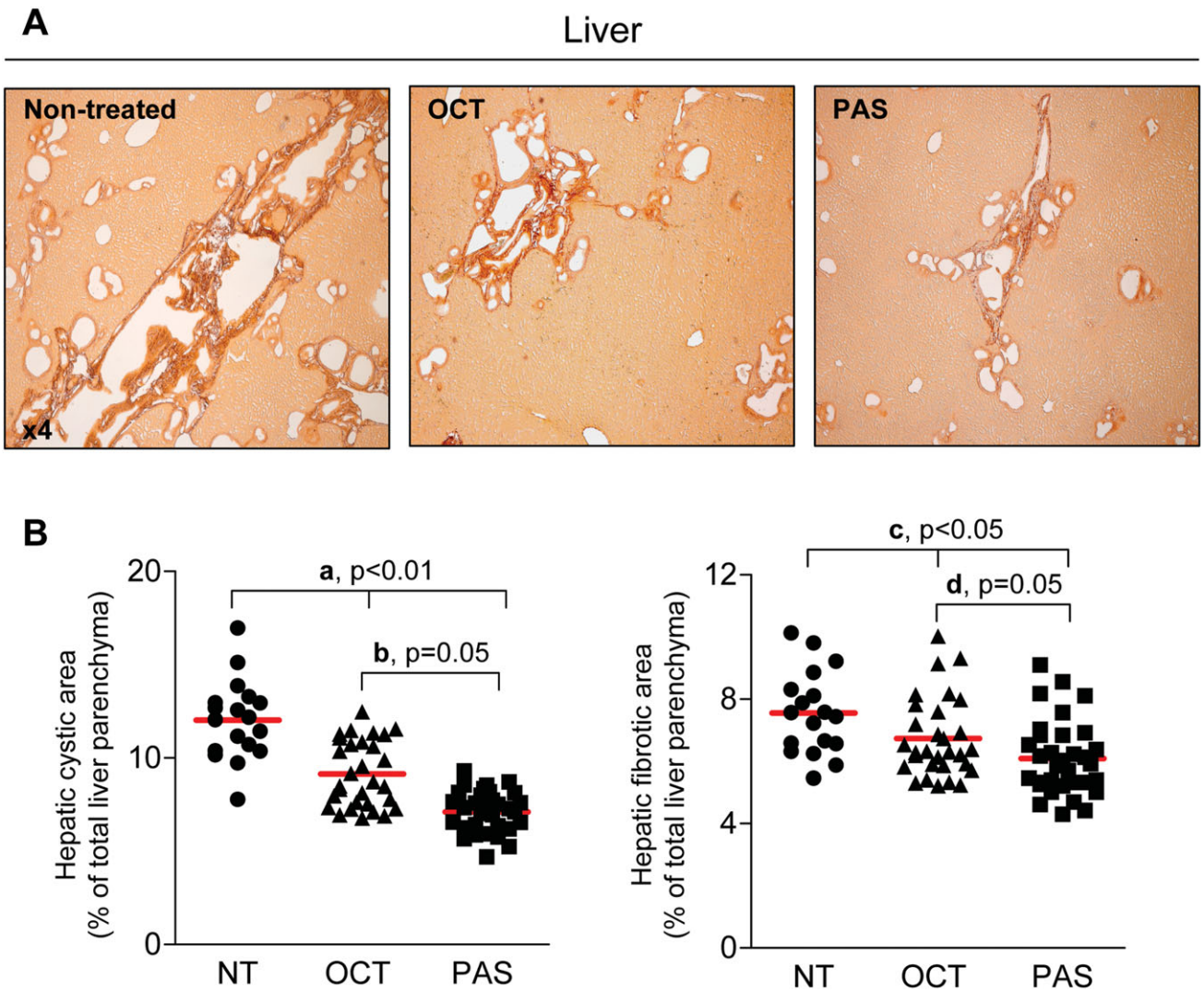


Fig. 5. PAS decreased hepatorenal cystogenesis in PCK rats to the higher degree than OCT. (A) Representative images of liver (picrosirius red staining) and (C) kidney (hematoxylin and eosin [H&E] staining) sections of nontreated and drug-treated PCK rats. Scatterplots show cystic and fibrotic areas of individual liver lobes (B; three liver lobes from each rat) and kidneys (D; two kidneys from each rat) analyzed. Hepatic and renal cystic (B [a] and D [e]) and fibrotic (B [c] and D [g]) areas were decreased after treatment. Note that PAS suppressed cystic (B [b] and D [f]) and fibrotic (B [d] and D [h]) areas in both organs more extensively than OCT.

After anesthesia (pentobarbital; 50 mg/kg) liver and kidney were fixed and paraffin-embedded. Cholangiocytes were isolated from control and PCK rats as described[20] and from liver transplant tissue of healthy human beings and patients with ADPKD (see Supporting Information for details). PAS and OCT were kindly provided by Novartis USA. The protocol was approved by the Mayo Institutional Animal Care and Use Committee (IACUC).

The treatment protocol is shown in Fig. 1. Rodents received PAS and OCT for 6 weeks. Drug doses were chosen based on our studies.[7] Somatostatin analogs were dissolved in sterile water and administered by way of osmotic mini-pumps (model 2002, Alzet Osmotic Pumps, Cupertino, CA). Pumps were implanted subcutaneously on the animal back under anesthesia with 1.5% isoflurane (Baxter, Deerfield, IL). They were replaced every 2 weeks; at this time, OCT and PAS concentrations were adjusted to the animal weight. Cystic and fibrotic areas were analyzed as described in the Supporting Information (also for additional experimental procedures).

Results

PAS Decreased Intracellular cAMP Levels to a Greater Extent than OCT. Under basal conditions (no forskolin), levels of cAMP in PCK and ADPKD cholangiocytes were higher ~5 and 4 times compared to respective controls (Fig. 2A,B). Forskolin

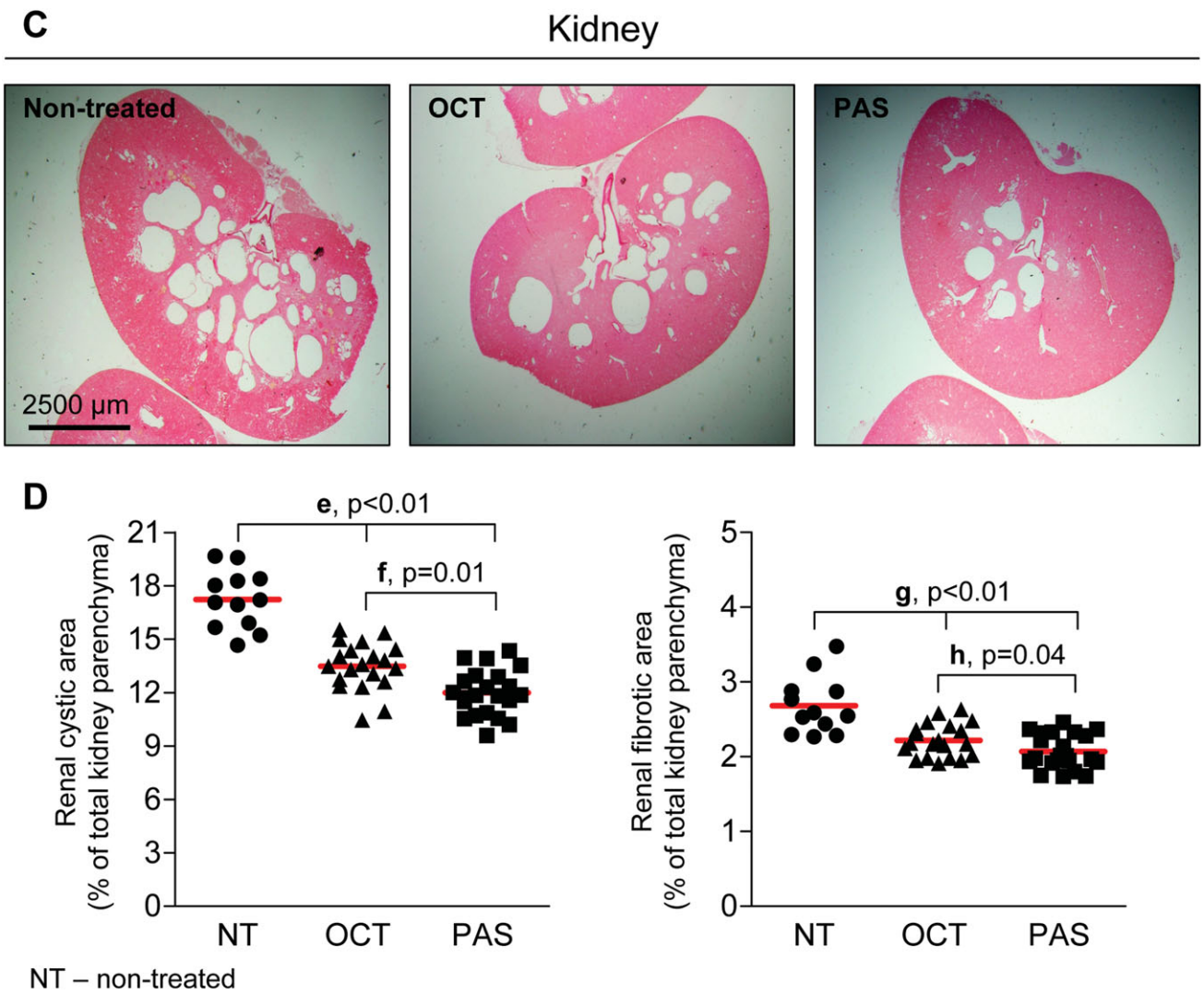


Fig. 5. (Continued)

increased cAMP production ~2 times in rat control and PCK cholangiocytes and ~3 times in human control and ADPKD cholangiocytes. Neither OCT or PAS affected cAMP accumulation in control rat and human cholangiocytes under basal conditions but suppressed it after forskolin stimulation. In contrast, in cystic PCK and ADPKD cholangiocytes, both somatostatin analogs, inhibited cAMP levels in the absence or presence of forskolin. Importantly, we observed more significant cAMP suppression by PAS than OCT (Fig. 2A,B).

OCT and PAS Affected the Cell Cycle. In rat control cholangiocytes, OCT had no effect on the cell cycle distribution, whereas PAS increased cell number in S phase from 9.07 ± 0.59% to 10.93 ± 0.46% and decreased it in G_2/M phase from 4.08 ± 1.82 to 1.06 ± 0.88% (Fig. 3A,B). In PCK cholangiocytes, OCT and PAS similarly affected the cell cycle profile by increasing the percentage of cells in S phase from 13.17 ± 1.48% to 16.27 ± 1.30% (OCT) and 17.99 ± 2.07% (PAS). In G_2/M phase, the number of cells was decreased from 8.29 ± 1.72% to 3.41 ± 1.33 in response to OCT and to 1.77 ± 0.62% in response to PAS (Fig. 3A,B).

OCT had no effects on the cell cycle progression in human control cholangiocytes, whereas PAS increased the number of cells in G_1 phase from 82.11 ± 0.54% to 85.50 ± 1.04% and decreased it in S phase from 8.88 ± 1.01% to 5.30 ± 0.89% (Fig. 3C,D). The number of ADPKD cholangiocytes during the cell cycle was: (1) elevated in G_1 phase from 55.66 ± 1.31 to 71.03 ± 0.55 (OCT) and to 78.69 ± 1.06 (PAS); (2) decreased in S phase from 33.31 ± 1.45 to 19.91 ± 0.79 (OCT) and to 13.47 ± 1.35 (PAS); and (3) decreased in G_2/M phase from 11.03 ± 0.65 to 9.07 ± 0.43 (OCT) and to 7.83 ± 0.34 (PAS) (Fig. 3C,D).

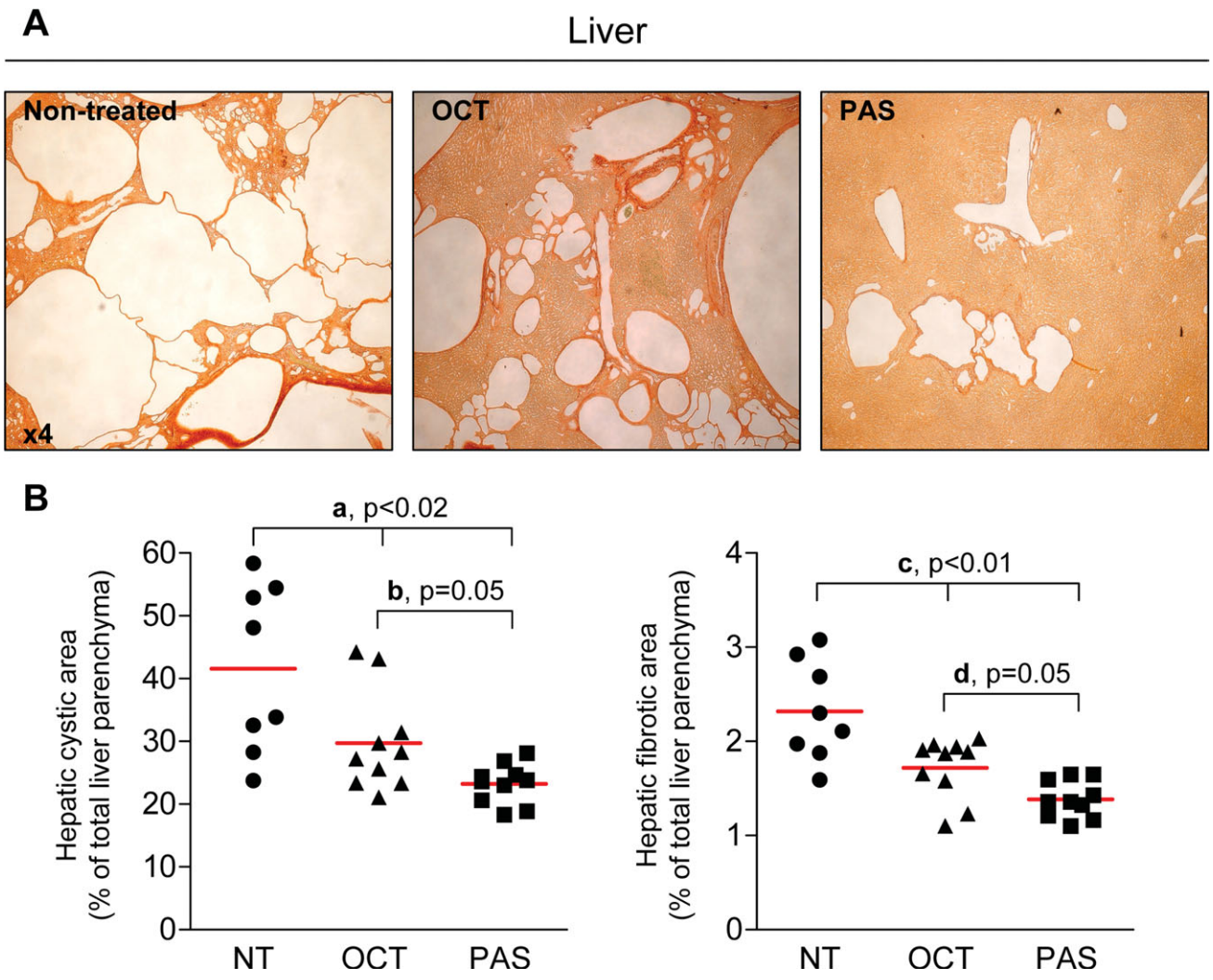


Fig. 6. PAS decreased hepatorenal cystogenesis in *Pkd2*$^{WS25/-}$ mice to a higher degree than OCT. (A) Representative images of liver (picrosirius red staining) and (C) kidney (H&E staining) sections of nontreated and drug-treated *Pkd2*$^{WS25/-}$ mice. Scatterplots show cystic and fibrotic areas of individual liver lobes (B; two liver lobes from each mouse) and kidneys (D; two kidneys from each mouse) analyzed. Hepatic and renal cystic (B [a] and D [e]) and fibrotic (B [c] and D [g]) areas were reduced by treatment. Note that PAS decreased cystic (B [b] and D [f]) and fibrotic (B [d] and D [h]) areas in both organs more extensively than OCT.

PAS Decreased Cell Proliferation More Effectively than OCT. Cell proliferation in response to somatostatin analogs was examined by 3-(4,5-dimethyl-thiazol-2yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) and bromodeoxyuridine (BrdU) assays. MTS assay demonstrated that in response to OCT, proliferation of rat control and PCK cholangiocytes was decreased by 9.6% and 16.8%, respectively, and in response to PAS by 18.6% and 24.3%, respectively (Fig. 3E). In human control and ADPKD cholangiocytes, OCT inhibited the rates of proliferation by 12.9% and 18.4%, respectively, and PAS by 21.9% and 33.7%, respectively (Fig. 3E). By BrdU assay, OCT and PAS suppressed, respectively, proliferation of: (1) rat control cholangiocytes by 15.5% and 24.4%; (2) PCK cholangiocytes by 25.1% and 32.9%; (3) human control cholangiocytes by 12.5% and 19.8%; and (4) ADPKD cholangiocytes by 29.8% and 38.5% (Fig. 3F). Importantly, PAS repressed cell proliferation to a higher extent than OCT in rat and human control and cystic cholangiocytes (Fig. 3E,F).

PAS Inhibited Expansion of Hepatic Cysts in 3D Matrices More Extensively than OCT. Under basal conditions, both control and PCK cysts expanded progressively over time (Fig. 4), although PCK structures grew to a greater extent. The circumferential area of control cysts enlarged by 92.07 ± 5.45% compared to a 228.50 ± 25.32% increase in PCK cultures. In response to OCT, the expansion of control and PCK cysts was decreased 1.6 and 2.3-fold, respectively; whereas PAS reduced enlargement

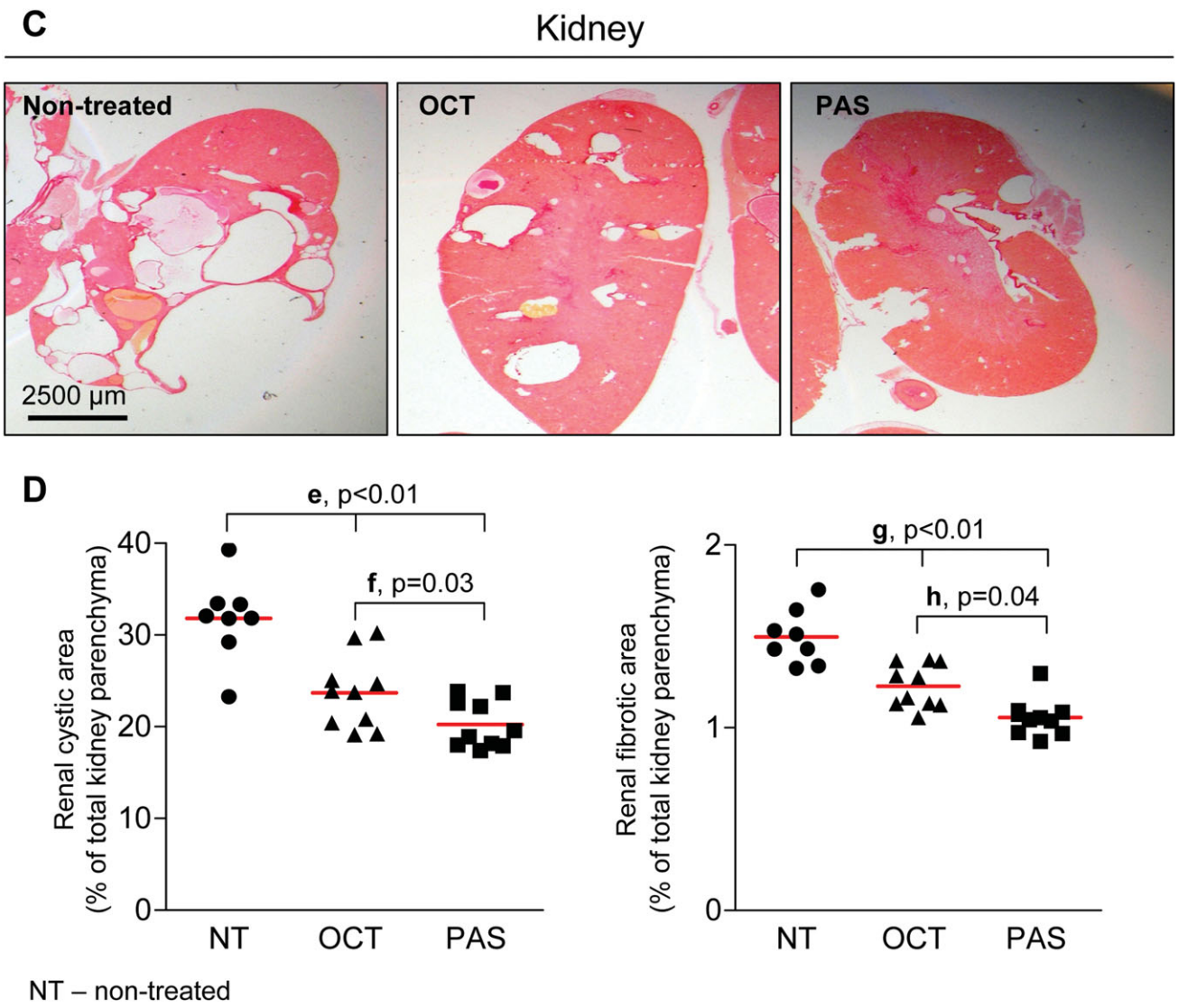


Fig. 6. (Continued)

of control and PCK cysts by 2.2- and 4.7-fold, respectively. No differences were observed between OCT- or PAS-treated control cysts; however, the suppressive effects of PAS on growth of PCK cystic structures were more noteworthy compared to OCT (Fig. 4).

PAS Reduced Hepatorenal Cystogenesis in Animal Models of PLD and PKD More Effectively than OCT. Both somatostatin analogs were well tolerated by PCK rats and *Pkd2*$^{WS25/-}$ mice.

In PCK rats, OCT and PAS reduced, respectively: (1) liver weights by 9% and 16%; (2) kidney weights by 7% and 14%; (3) hepatic cystic areas by 24% and 36%; (4) hepatic fibrotic areas by 10% and 19%; (5) renal cystic areas by 22% and 30%; and (6) renal fibrotic areas by 10% and 19% (Supporting Table 1; Fig. 5). In *Pkd2*$^{WS25/-}$ mice, OCT and PAS treatment decreased, respectively: (1) liver weights by 17% and 22%; (2) kidney weights by 16% and 20%; (3) hepatic cystic areas by 22% and 34%; (4) hepatic fibrotic areas by 13% and 25%; (5) renal cystic areas by 19% and 30%; and (6) renal fibrotic areas by 18% and 25% (Supporting Table 2; Fig. 6). Moreover, PAS decreased hepatic and renal cystic and fibrotic areas in PCK rats (Supporting Table 1; Fig. 5B,D) and *Pkd2*$^{WS25/-}$ mice (Supporting Table 2; Fig. 6B,D) to a greater extent than OCT.

IGF1 Levels Were Reduced After PAS Treatment. No changes in VEGF serum concentrations were observed in response to either OCT or PAS treatment in PCK rats. Serum levels of IGF1 were not affected by OCT, whereas PAS decreased it by 18% ($P < 0.05$) compared to control. Consistent with *in vivo* observation, suppressed secretion of IGF1 in

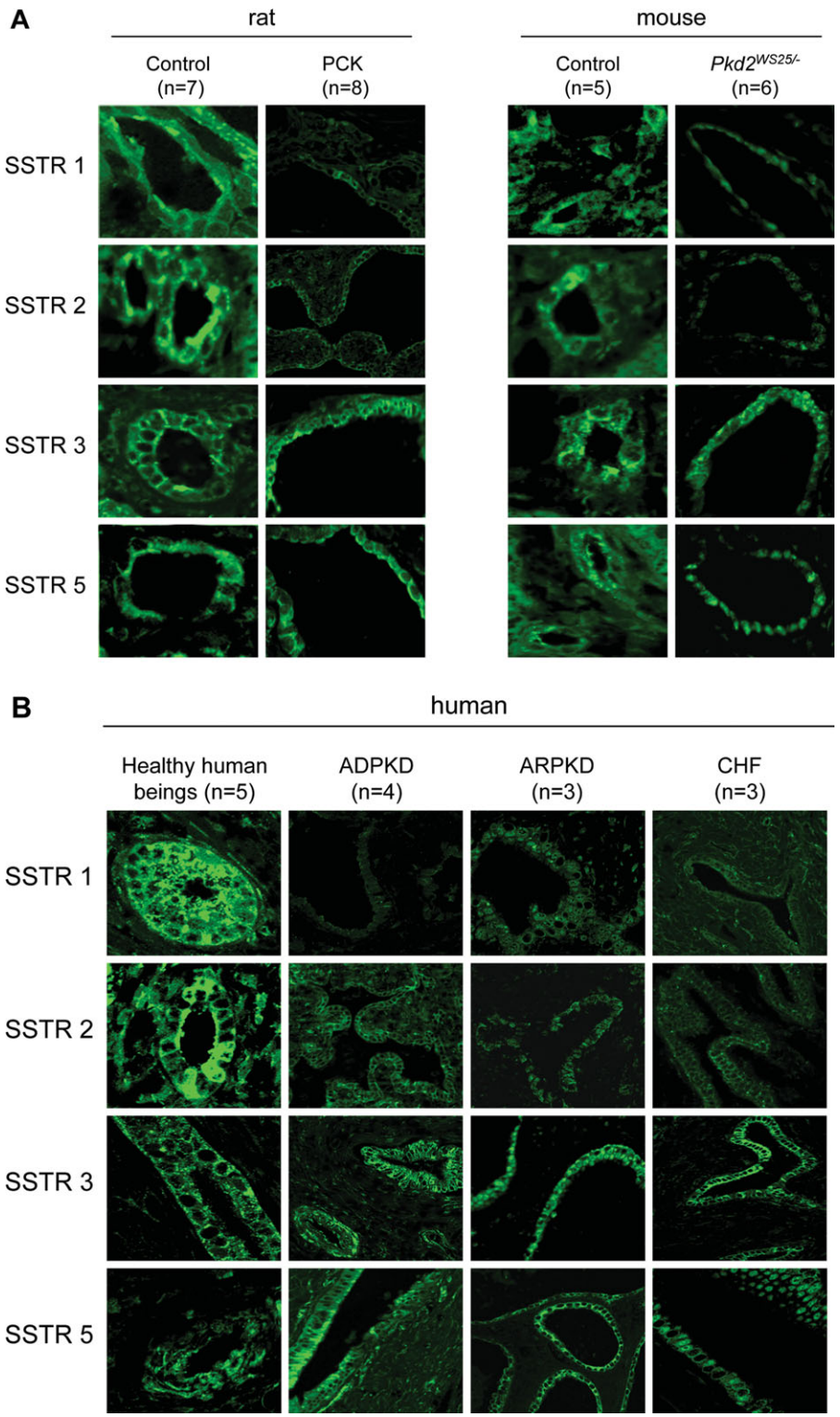
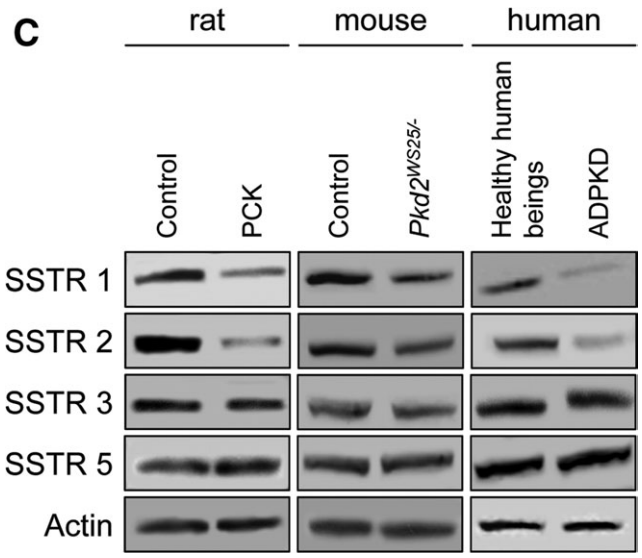


Fig. 7. Expression of SSTRs in rat, mouse, and human livers. Representative confocal images (A,B, ×63; SSTRs are in green) and western blots (C) show that SSTR1-3 and SSTR5 are expressed in cholangiocytes of control rats and mice, and healthy humans. Levels of SSTR1 and SSTR2 appear to be decreased in cystic cholangiocytes of PCK rats, *Pkd2*$^{WS25/-}$ mice, and in patients with PKD and PLD, whereas levels of SSTR3 and SSTR5 did not change compared to respective controls.

the presence of PAS (but not OCT) was found in cultured rat control (by 10.8%; $P < 0.03$) and PCK (by 21.5%; $P < 0.001$) cholangiocytes, and human control (by 11.2%; $P < 0.03$) and ADPKD (by 15.7%; $P < 0.01$) cholangiocytes (Supporting Fig. 1).



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SSTRs Are Differentially Expressed in Cystic Cholangiocytes. Expression of SSTR1, SSTR2, SSTR3, and SSTR5 (i.e., targets of OCT and/or PAS) were detected in cholangiocytes of control and PCK rats, control and *Pkd2*$^{WS25/-}$ mice, healthy human beings, and patients with PLD by confocal microscopy (Fig. 7A,B) and western blotting (Fig. 7C). Levels of SSTR1 and SSTR2 were decreased in cystic cholangiocytes, whereas expression of SSTR3 and SSTR5 did not change compared to control.

OCT and PAS Altered the Expression of SSTRs. Exposure to somatostatin analogs similarly affected expression of SSTRs in PCK rats and *Pkd2*$^{WS25/-}$ mice (Fig. 8A). OCT treatment increased levels of one of the SSTRs, SSTR2, whereas PAS treatment increased immunoreactivity of two SSTRs, SSTR1 and SSTR2, compared to nontreated cholangiocytes. This observation was also confirmed *in vitro* in cultured PCK cholangiocytes (Fig. 8B). In control PCK rats and *Pkd2*$^{WS25/-}$ mice, all four SSTRs mainly resided in the cytoplasm of cystic cholangiocytes and their distribution was not changed in response to either somatostatin analogs (Fig. 8A).

Discussion

The major findings described here relate to the relative potencies of OCT and PAS in hepatic and renal cystogenesis. Using *in vitro* and *in vivo* experimental models representing two forms of polycystic liver diseases, ARPKD and ADPKD, we show that PAS is more effective than OCT in: (1) reducing cAMP levels; (2) decreasing cell proliferation; (3) affecting cell cycle distribution; (4) suppressing growth of cultured hepatic cysts; and (5) inhibiting hepatorenal cystic disease in PCK rats and *Pkd2*$^{WS25/-}$ mice. We also found that: (1) expression of SSTR1 and SSTR2, but not SSTR3 and SSTR5, is decreased in cystic cholangiocytes of animal models and patients with PKD and PLD compared to their respective controls; (2) OCT and PAS treatment increases immunoreactivity of SSTR2 in cholangiocytes of PCK rats and *Pkd2*$^{WS25/-}$ mice, whereas SSTR1 is up-regulated only by PAS; (3) localization of SSTRs is not affected by treatment with either analog; and (4) the IGF1 concentration is decreased only in response to PAS, whereas VEGF is not affected by either somatostatin analog.

The effects of OCT and PAS on hepatorenal cystic disease are executed by way of activation of multiple SSTRs. PAS has high affinity to four SSTRs, with a median inhibitory concentration (IC_{50}) of 9.3 nM (SSTR1), 1.0 nM (SSTR2), 1.5 nM (SSTR3), and 0.16 nM (SSTR5).[12,13,21] OCT binds to three SSTRs displaying IC_{50} of 2.0 nM (SSTR2), 187 nM (SSTR3), and 22 nM (SSTR5).[21] PAS is more stable (i.e., 12-hour half-life) than OCT (i.e., 70-113 minutes).[12] The rationale for the superior efficacy of PAS is that it acts not only on SSTR2 but also on other SSTRs.[13,22,23] Because all five SSTRs are expressed in cholangiocytes and renal epithelial cells,[6,7,14] we performed this comparative study and, indeed, our results suggest that PAS should be more beneficial than OCT for the treatment of polycystic disease in humans.

Our data showing that OCT and PAS inhibit cAMP, decrease cholangiocyte proliferation, and affect the cell cycle machinery support previous observations by us and others.[7,24-26] Reduction of cAMP levels is triggered by activation of any of the SSTRs.[4,27] In contrast, the effects of somatostatin analogs on the cell proliferation and cell cycle machinery occur predominantly by way of SSTR2 and SSTR5 and, in some cases, SSTR1.[13,28,29] Activation of SSTR3 is also known to reduce proliferation and/or induce apoptosis.[4] Indeed, we found that OCT and PAS inhibited cAMP and cell proliferation in rat and human cystic cholangiocytes *in vitro* and decreased mitotic indices and increased apoptotic indices in rodent models of PLD and PKD. Moreover, the effects of PAS were consistently more potent than OCT. In line with our data, PAS has been shown by others to decrease cell proliferation and cAMP in several different cell lines to a greater extent than OCT.[17,21,24-26] We speculate that more potent PAS effects are likely related to the following. First, we observed the reduced expression of SSTR1 and SSTR2 in cystic cholangiocytes, whereas levels of

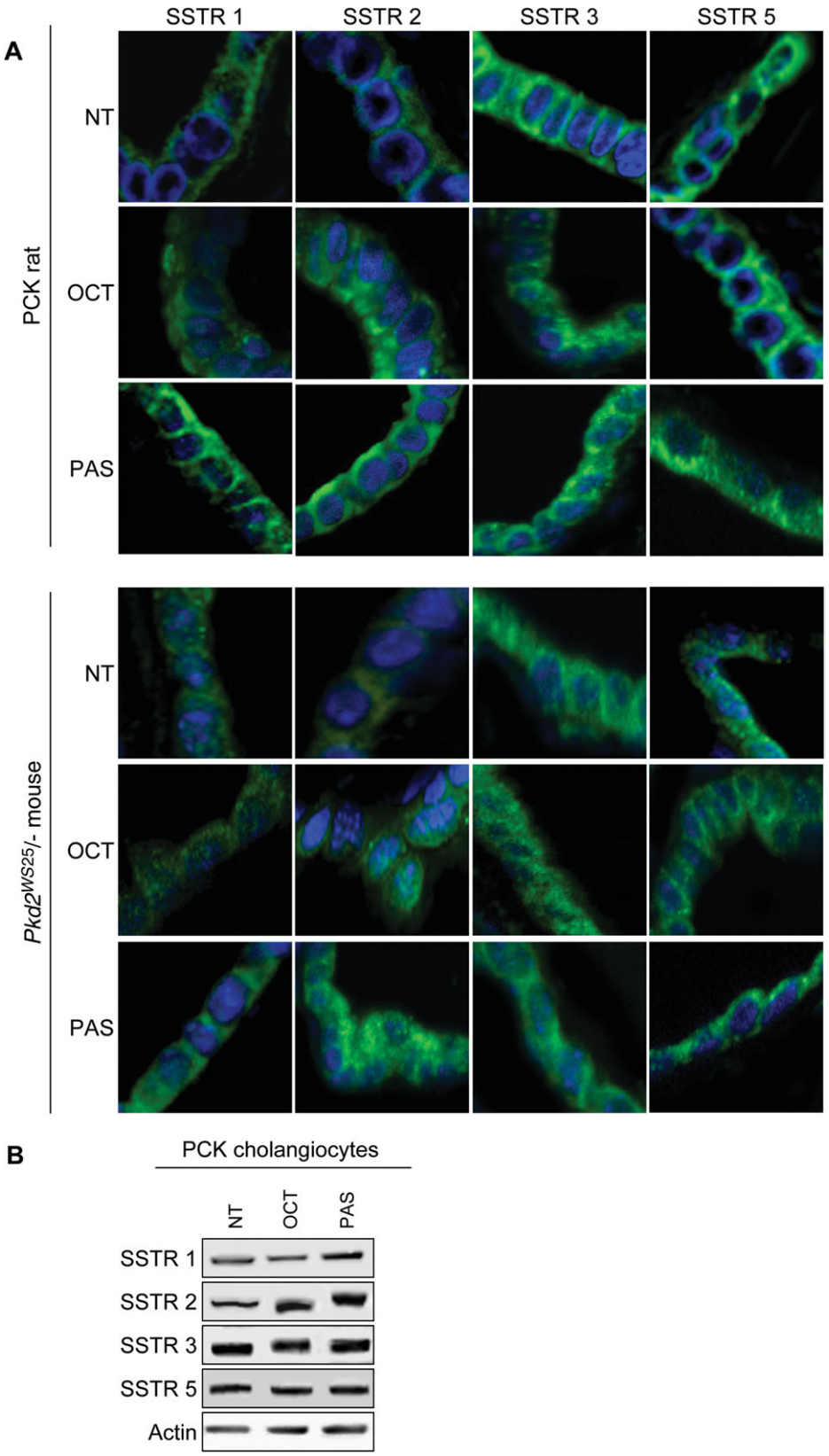

Fig. 8. Effects of OCT and PAS on expression and localization of SSTRs in PCK rats and *Pkd2*$^{WS25/-}$ mice. (A) Confocal images (×100) and western blots (B) show that OCT did not change the expression of SSTR1, SSTR3, and SSTR5, whereas PAS had no effects on SSTR3 and SSTR5. OCT treatment increased immunoreactivity of SSTR2, whereas PAS increased levels of both SSTR1 and SSTR2. All four SSTRs are localized in cytoplasm of cystic cholangiocytes under basal conditions and after treatment. SSTRs are in green, nuclei are stained with DAPI.

SSTR3 and SSTR5 were not affected. Second, SSTR2, SSTR3, and SSTR5 are targets of OCT and PAS, whereas SSTR1 is the target of PAS only. Third, the binding affinity of PAS to SSTR3 and SSTR5 is 5-fold and 39-fold, respectively, higher compared with OCT.[17]

OCT and PAS modulate their action both by way of direct (i.e., cell proliferation, apoptosis, and cell cycle regulation) and indirect effects. Indirect effects occur, in particular, through inhibition of secretion of IGF1 and VEGF.[13,15] Both growth factors are overexpressed in cystic cholangiocytes and have been implicated in hepatorenal cystogenesis influencing cyst growth by both autocrine and paracrine pathways.[3,16,18,19] In the present study, the VEGF concentrations were not affected by either drug. OCT also had no effect on IGF1 concentration, whereas PAS reduced it. This result is consistent with previous data and suggests that the observed greater action of PAS on hepatic cyst growth might also be linked to indirect action of PAS by inhibiting IGF1.[12,15,17]

We and others have previously reported that all five SSTRs are localized to rat and human cholangiocytes.[6,7] Our data showing the decreased levels of SSTR1 and SSTR2 (but not SSTR3 and SSTR5) in cystic cholangiocytes are novel. To this end, the most plausible explanation for the moderate therapeutic success seen in patients with PLD and PKD is that current somatostatin analogs target mainly SSTR2, the expression of which appears to be decreased in hepatic cysts.

Native somatostatin and its synthetic analogs have the ability to regulate the expression levels of SSTRs by as yet not well understood mechanisms.[21,30] It has been suggested that up-regulation of SSTRs results in a longer lasting functional responses to agonist exposure.[21] We showed that immunoreactivity of SSTR2 (in response to OCT and PAS) and SSTR1 (in response to PAS) is increased in cystic cholangiocytes. These changes in drug-induced receptor expression likely also contribute to the stronger suppressive effects of PAS because it binds to both SSTR1 and SSTR2. Finally, cytoplasmic localization of SSTRs which we detected in cystic cholangiocytes has also been observed in other tissues[31] and reflects the functional state of somatostatin receptors due to internalization after agonist exposure.[21]

In conclusion, the more potent effects of PAS compared to OCT on hepatorenal cystogenesis observed in this study are likely related to a combination of features of both the drug and the cystic cell phenotype including: (1) a broader range of SSTRs targeted by PAS; (2) a higher affinity of PAS to SSTR3 and SSTR5 (expression of which in cystic cholangiocytes is unchanged compared to control); and (3) the extended half-life of PAS. Our data suggest that PAS may be more effective for the treatment of PLD and PKD than OCT. A clinical trial (NCT01670110) to assess the effectiveness of PAS in hepatorenal cystogenesis in patients with ADPKD and ADPLD is now under way at our institution.

References

1. Strazzabosco M, Somlo S. Polycystic liver diseases: congenital disorders of cholangiocyte signaling. Gastroenterology 2011;140:1855-1859, 1859 e1851.
2. Masyuk T, Masyuk A, LaRusso N. Cholangiociliopathies: genetics, molecular mechanisms and potential therapies. Curr Opin Gastroenterol 2009;25:265-271.
3. Gevers TJ, Drenth JP. Somatostatin analogues for treatment of polycystic liver disease. Curr Opin Gastroenterol 2011;27:294-300.
4. Lahlou H, Guillermet J, Hortala M, Vernejoul F, Pyronnet S, Bousquet C, et al. Molecular signaling of somatostatin receptors. Ann N Y Acad Sci 2004;1014:121-131.
5. Fazio N, Cinieri S, Lorizzo K, Squadroni M, Orlando L, Spada F, et al. Biological targeted therapies in patients with advanced enteropancreatic neuroendocrine carcinomas. Cancer Treat Rev 2010;36 Suppl 3:S87-94.
6. Reynaert H, Rombouts K, Vandermonde A, Urbain D, Kumar U, Bioulac-Sage P, et al. Expression of somatostatin receptors in normal and cirrhotic human liver and in hepatocellular carcinoma. Gut 2004;53: 1180-1189.
7. Masyuk TV, Masyuk AI, Torres VE, Harris PC, Larusso NF. Octreotide inhibits hepatic cystogenesis in a rodent model of polycystic liver disease by reducing cholangiocyte adenosine 3′,5′-cyclic monophosphate. Gastroenterology 2007;132:1104-1116.
8. Caroli A, Antiga L, Cafaro M, Fasolini G, Remuzzi A, Remuzzi G, et al. Reducing polycystic liver volume in ADPKD: effects of somatostatin analogue octreotide. Clin J Am Soc Nephrol 2010;5:783-789.
9. Hogan MC, Masyuk TV, Page LJ, Kubly VJ, Bergstralh EJ, Li X, et al. Randomized clinical trial of long-acting somatostatin for autosomal dominant polycystic kidney and liver disease. J Am Soc Nephrol 2010; 21:1052-1061.
10. Ruggenenti P, Remuzzi A, Ondei P, Fasolini G, Antiga L, Ene-Iordache B, et al. Safety and efficacy of long-acting somatostatin treatment in autosomal-dominant polycystic kidney disease. Kidney Int 2005;68:206-216.
11. Chrispijn M, Nevens F, Gevers TJ, Vanslembrouck R, van Oijen MG, Coudyzer W, et al. The long-term outcome of patients with polycystic liver disease treated with lanreotide. Aliment Pharmacol Ther 2012;35: 266-274.
12. Schmid HA. Pasireotide (SOM230): development, mechanism of action and potential applications. Mol Cell Endocrinol 2008;286:69-74.
13. Appetecchia M, Baldelli R. Somatostatin analogues in the treatment of gastroenteropancreatic neuroendocrine tumours, current aspects and new perspectives. J Exp Clin Cancer Res 2010;29:19.
14. Bhandari S, Watson N, Long E, Sharpe S, Zhong W, Xu SZ, et al. Expression of somatostatin and somatostatin receptor subtypes 1-5 in human normal and diseased kidney. J Histochem Cytochem 2008;56: 733-743.
15. Weckbecker G, Briner U, Lewis I, Bruns C. SOM230: a new somatostatin peptidomimetic with potent inhibitory effects on the growth hormone/insulin-like growth factor-I axis in rats, primates, and dogs. Endocrinology 2002;143:4123-4130.
16. Alvaro D, Onori P, Alpini G, Franchitto A, Jefferson DM, Torrice A, et al. Morphological and functional features of hepatic cyst epithelium in autosomal dominant polycystic kidney disease. Am J Pathol 2008; 172:321-332.
17. Schmid HA, Brueggen J. Effects of somatostatin analogs on glucose homeostasis in rats. J Endocrinol 2011;212:49-60.

18. Fabris L, Cadamuro M, Libbrecht L, Raynaud P, Spirli C, Fiorotto R, et al. Epithelial expression of angiogenic growth factors modulate arterial vasculogenesis in human liver development. HEPATOLOGY 2008;47: 719-728.
19. Spirli C, Okolicsanyi S, Fiorotto R, Fabris L, Cadamuro M, Lecchi S, et al. Mammalian target of rapamycin regulates vascular endothelial growth factor-dependent liver cyst growth in polycystin-2-defective mice. HEPATOLOGY 2010;51:1778-1788.
20. Masyuk TV, Radtke BN, Stroope AJ, Banales JM, Masyuk AI, Gradilone SA, et al. Inhibition of Cdc25A suppresses hepato-renal cystogenesis in rodent models of polycystic kidney and liver disease. Gastroenterology 2012;142:622-633 e624.
21. Lesche S, Lehmann D, Nagel F, Schmid HA, Schulz S. Differential effects of octreotide and pasireotide on somatostatin receptor internalization and trafficking in vitro. J Clin Endocrinol Metab 2009;94:654-661.
22. Hofland LJ, van der Hoek J, Feelders R, van der Lely AJ, de Herder W, Lamberts SW. Pre-clinical and clinical experiences with novel somatostatin ligands: advantages, disadvantages and new prospects. J Endocrinol Invest 2005;28:36-42.
23. van Hoek M, Hofland LJ, de Rijke YB, van Nederveen FH, de Krijger RR, van Koetsveld PM, et al. Effects of somatostatin analogs on a growth hormone-releasing hormone secreting bronchial carcinoid, in vivo and in vitro studies. J Clin Endocrinol Metab 2009;94:428-433.
24. Martinez-Alonso M, Llecha N, Mayorga ME, Sorolla A, Dolcet X, Sanmartin V, et al. Expression of somatostatin receptors in human melanoma cell lines: effect of two different somatostatin analogues, octreotide and SOM230, on cell proliferation. J Int Med Res 2009;37:1813-1822.
25. Pasquali D, Rossi V, Conzo G, Pannone G, Bufo P, De Bellis A, et al. Effects of somatostatin analog SOM230 on cell proliferation, apoptosis, and catecholamine levels in cultured pheochromocytoma cells. J Mol Endocrinol 2008;40:263-271.
26. Adams RL, Adams IP, Lindow SW, Atkin SL. Inhibition of endothelial proliferation by the somatostatin analogue SOM230. Clin Endocrinol (Oxf) 2004;61:431-436.
27. Barnett P. Somatostatin and somatostatin receptor physiology. Endocrine 2003;20:255-264.
28. Pages P, Benali N, Saint-Laurent N, Esteve JP, Schally AV, Tkaczuk J, et al. sst2 somatostatin receptor mediates cell cycle arrest and induction of p27(Kip1). Evidence for the role of SHP-1. J Biol Chem 1999;274: 15186-15193.
29. Cordelier P, Esteve JP, Bousquet C, Delesque N, O'Carroll AM, Schally AV, et al. Characterization of the antiproliferative signal mediated by the somatostatin receptor subtype sst5. Proc Natl Acad Sci U S A 1997;94:9343-9348.
30. Hukovic N, Panetta R, Kumar U, Patel YC. Agonist-dependent regulation of cloned human somatostatin receptor types 1-5 (hSSTR1-5): subtype selective internalization or upregulation. Endocrinology 1996; 137:4046-4049.
31. Taniyama Y, Suzuki T, Mikami Y, Moriya T, Satomi S, Sasano H. Systemic distribution of somatostatin receptor subtypes in human: an immunohistochemical study. Endocr J 2005;52:605-611.