In vitro Metabolism, CYP Inhibitory Potential and Transport Studies of SB 9200 – A Novel Broad-Spectrum Antiviral Agent

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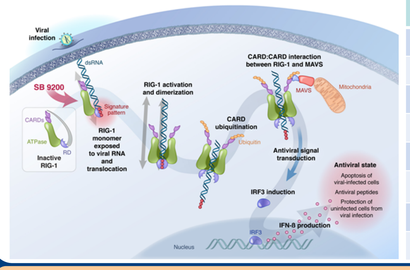
INTRODUCTION

Acute and chronic infections caused by RNA and DNA viruses constitute a major public health crisis affecting millions of people. A limited arsenal of antiviral agents exists, mostly directed against a specific viral target such as the polymerase or protease characteristic of each virus. There is also rapid emergence of resistance to direct-acting antiviral drugs and dose-limiting toxicity. Therefore, new anti-viral drugs are urgently needed. Ideally compounds with novel mechanisms of action that have a broader spectrum of antiviral activity and are agnostic to viral genotypes and resistant mutants will be needed. **SB 9200, an oral dinucleotide prodrug designed to target the liver, is a potent antiviral agent against HBV, HCV, RSV, and Norovirus.** Results of a Phase I clinical trial of SB 9200 in HCV-infected patients was recently reported (Thompson et al., EASL 2015, AASLD, 2015).

BACKGROUND

SB 9200 has novel mechanisms of action involving the activation/enhancement of cytosolic proteins involved in virus detection, resulting in activation of the IFN signaling cascade and induction of an antiviral state in cells. SB 9200 shows potent antiviral activity against wild-type and resistant HBV-variants in in vitro assays in chronically HBV-infected HepG2.2.15 cell lines. SB 9200 shows potent antiviral activity in chronically WHV-infected woodchucks. The pharmacokinetics, dose-ranging toxicity, and safety pharmacology studies of SB 9200 has also been conducted in rats and monkeys..

Activation of RIG-I by SB 9200



In Vitro Anti-HBV Activity of SB 9200 [EC50 for Inhibition of Viral Intermediates]

HBV strain	SB 9200, μM	3TC, μM	ADV, μM
Wild type	2.5	0.2	1.5
M204V	2.3	>100	1.8
M204I	3.0	>100	2.0
L180M	2.1	5.3	2.1
L180M/M204V	3.1	>100	2.2
N236T	2.8	0.2	7.5

3TC = Lamivudine; ADV = adefovir dipivoxyl

OBJECTIVE

The objective of this study was to evaluate the in vitro metabolism of SB 9200 through determination of serum conversion half-life from SB 9200 to SB 9000, cytochrome P450 inhibitory activity (CYP), and transport characteristics of SB 9000 in vitro.

METHODS

SB 9200 was incubated with pooled mixed gender human, beagle dog, Sprague-Dawley rat and cynomolgus macaque liver microsomes and S9 fractions at 1 and 10 uM.
The CYP inhibitory potential of the compounds was evaluated using a high throughput assay involving CYP isoforms. Transporter studies were carried out using Caco-2 cells or MDCK-II cells transfected with the appropriate plasmids encoding the transporter.

RESULTS

In the presence of liver microsomes, the prodrug SB 9200 was converted with a $t_{1/2}$ ca. 1hr to the active SB 9000, which was found to be metabolically stable with no observed sulfation or glucuronidation. Furthermore, the compounds did not affect the enzymatic activities of Cytochrome P450 (CYP) isoforms. SB 9000 was found to be a substrate for OATP1B1, OATP1B3, OAT1, and OAT3 expressed in MDCK-II cells.

RESULTS

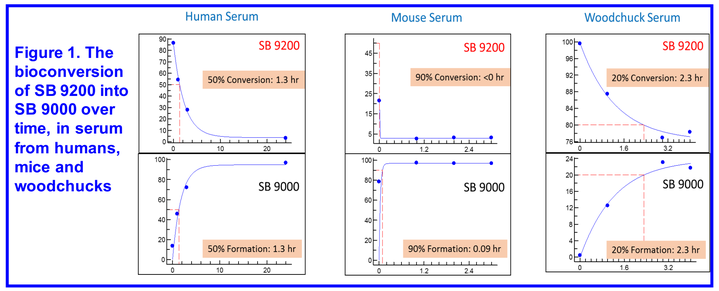


Figure 1. The bioconversion of SB 9200 into SB 9000 over time, in serum from humans, mice and woodchucks

Table 1. In Vitro metabolic stability of SB 9200 using human, dog, rat, and monkey liver microsomes.

		% of Test Article Remaining vs T = 0 min [a]							
		Human		Dog		Rat		Monkey	
Time (min)		1 μM Mean	10 μM Mean	1 μM Mean	10 μM Mean	1 μM Mean	10 μM Mean	1 μM Mean	10 μM Mean
SB 9200	15	74.2	79.7	68.7	76.3	76.5	91.2	32.3	40.3
	30	55.6	63.5	45.9	51.4	61.7	79.2	11.4	16.9
	60	32.7	37.5	24.1	26.8	38.8	54.9	1.8	3.0
SB 9200 with HI[b]-Microsomes	60	94.4	93.9	93.0	94.7	90.8	89.0	93.6	93.9
SB 9200 No NADPH	15	80.3	79.3	68.0	72.9	83.1	79.6	54.5	58.1
	30	63.0	59.3	50.3	51.9	72.2	78.7	29.6	31.0
	60	35.9	33.1	24.0	26.9	53.3	57.1	8.6	8.9

[a] % of test article remaining at T=0 min is 100%.
[b] HI= heat inactivated. Aliquots from incubations with heat-inactivated microsomes were removed at 0 and 60 min only.

Table 2. In Vitro metabolic stability of SB 9000 using human, dog, rat, and monkey liver microsomes.

		% of Test Article Remaining vs T = 0 min [a, d]							
		Human		Dog		Rat		Monkey	
Time (min)		1 μM Mean±SD	10 μM Mean±SD	1 μM Mean±SD	10 μM Mean±SD	1 μM Mean±SD	10 μM Mean±SD	1 μM Mean±SD	10 μM Mean±SD
SB 9000	15	93.5±6.0	100.5±3.4	97.9±4.4	99.0±5.5	106.9±3.0	107.2±13.0	97.9±8.8	99.9±4.8
	30	93.6±1.5	99.7±4.8	99.0±5.0	99.2±5.5	104.9±3.2	108.1±3.2	102.6±8.6	96.7±9.2
	60	94.9±8.4	100.2±6.2	101.0±7.6	105.2±7.2	110.6±11.7	106.7±5.9	104.7±9.3	95.0±15.3
SB 9000 with HI[b]-Microsomes	60	101.7±5.7	100.9±7.5	99.4±5.4	100.7±4.9	98.2±6.0	98.5±8.1	102.0±3.6	106.9±15.4
SB 9000 No NADPH	15	96.2±7.1	99.0±4.0	92.7±2.5	94.6±2.4	103.9±6.1	87.0±10.5	94.8±7.0	95.6±6.6
	30	94.3±4.9	98.2±7.6	95.2±5.9	99.1±9.1	100.2±6.4	90.9±7.8	95.1±13.6	93.6±7.5
	60	88.3±3.4	102.1±5.2	87.8±4.3	92.0±11.8	101.1±5.2	103.9±17.6	93.4±9.1	99.4±8.7
SB 9000 with HI[b]-Microsomes	60	98.8±NA[c]	103.8±NA[c]	99.1±NA[c]	104.0±NA[c]	96.3±NA[c]	119.3±NA[c]	106.6±NA[c]	90.0±NA[c]

[a] % of test article remaining at T=0 min is 100%.
[b] HI= heat inactivated. Aliquots from incubations with heat-inactivated microsomes were removed at 0 and 60 min only.
[c] NA= Not Applicable. No SD when n=2.
[d] Experiment performed twice due to data variability.

Table 3. Effect of SB 9200 and SB 9000 on in vitro CYP activity in human liver microsomes.

		CYP Activity[a] (% of Control)						
Test Article/Inhibitor	Conc (μM)	CYP1A2 (25 μM Phenacetin)	CYP2B6 (25 μM Bupropion)	CYP2C9 (10 μM Diclofenac)	CYP2C19 (20 μM Mephenytoin)	CYP2D6 (10 μM Bufuralol)	CYP3A4 (50 μM Testosterone)	CYP3A4 (4 μM Midazolam)
		Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
SB 9200	1	91.0±6.1	104.0±2.8	95.2±2.1	86.7±2.6	98.2±1.9	93.7±2.6	102.2±0.6
	10	95.5±3.1	105.3±2.2	96.1±1.0	88.8±5.1	99.8±1.8	95.8±1.1	101.0±0.7
SB 9000	1	101.6±1.2	107.8±2.1	98.0±1.4	96.3±4.3	103.2±2.1	99.3±0.9	102.6±1.1
	10	95.9±5.5	102.4±7.0	97.7±1.9	94.4±2.6	98.4±1.0	98.4±3.4	103.8±1.5
Furafylline (CYP1A2)	10	36.1±2.5	100.2±6.2	100.5±3.3	90.7±8.2	98.6±4.7	98.9±3.5	99.8±3.1
ThioTEPA (CYP2B6)	10	101.3±1.9	41.8±0.8	99.9±2.0	94.5±4.0	99.7±2.7	89.0±0.9	95.7±0.7
Sulfaphenazole (CYP2C9)	3	97.2±7.5	96.4±6.4	29.9±1.4	98.5±5.7	98.3±5.5	98.4±3.0	97.0±4.0
Nootkatone (CYP2C19)	10	95.9±1.3	51.6±1.7	91.7±2.9	67.3±6.7	99.7±2.8	88.1±1.8	94.6±4.9
Quinidine (CYP3A4)	2	96.4±1.8	100.4±4.7	98.6±2.7	92.8±3.6	12.8±0.2	91.6±2.0	91.6±2.0
Ketoconazole (CYP3A4)	5	88.2±2.0	84.7±0.0	96.6±2.1	85.8±1.4	99.0±2.6	7.7±0.4	19.3±0.4

[a] Values represent mean of triplicate determinations ± SD.

RESULTS

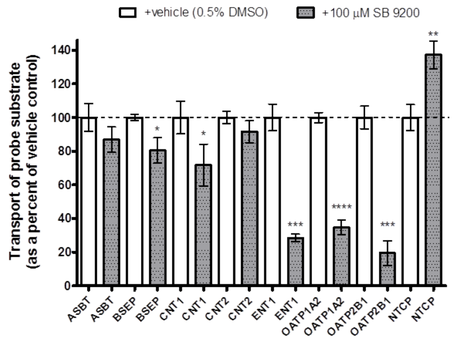


Figure 2. Inhibition of human transporters by SB 9200. The mean and standard deviation of triplicate samples is shown. Statistical analysis: Student's t-test significance: *(P<0.05), **(P<0.01), ***(P<0.001), **** (P<0.0001).

Table 4: The % inhibition of probe transport substrate in the presence of 100 μM SB 9000

Transporter	Function	SB 9000	Transport
OATP1B1	Exclusively on hepatocytes – influx	24.5%	Yes
OATP1B3	Exclusively on hepatocytes – influx	21.7%	Yes
OAT1	Tubular secretion in kidney	16.6%	Yes
OAT3	Tubular secretion in kidney	38.5%	Yes

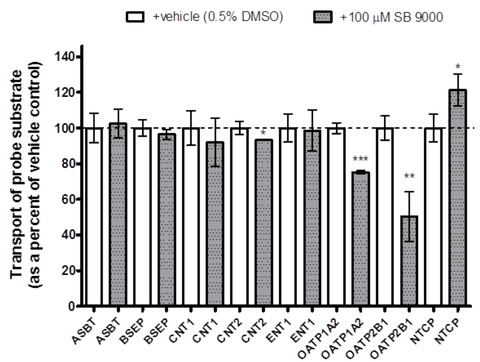


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Table 5: The % inhibition of probe substrates by 100 μM SB 9200 or SB 9000 in various transporter assays

Transporter	Function	SB 9200	SB 9000
CNT1	Intestinal uptake and tubular secretion	28.3%	ND
CNT2	Intestinal uptake and tubular secretion	ND	6.5%
ENT1	Hepatic, renal, intestinal and BBB uptake	71.5%	ND
OATP1A2	Luminal influx small intestine and hepatocytes	65.2%	25%
OATP2B1	Luminal influx small intestine and hepatocytes	80.5%	49%
BSEP	Efflux hepatocyte to bile	19.6%	ND
NTCP	Exclusively on hepatocytes – influx	(37.32%)*	(21.2%)*

ND = no detectable inhibition
* = an increase

CONCLUSIONS

Our studies show that the SB 9200 prodrug is efficiently converted to the active SB 9000, which has no inhibitory effect on CYP isoforms. SB 9000 appears to be a substrate for organic anion transporters that might facilitate absorption via active transport.

- ❑ SB 9200 was metabolized, in the presence of liver microsomes, likely due to esterases, not CYP enzymes. No metabolism of SB 9000 was detected.
- ❑ OATP1B1/OATP1B3 are basally located hepatocyte transporters involved in transporting molecules from portal blood, which suggests SB 9000 is likely taken up by the liver via this route.
- ❑ Transport-mediated uptake of compounds into the liver is a saturable process, which is consistent with results observed in our rat studies, where high concentrations were observed in rat livers and did not increase in a dose proportional manner. (April 18th poster # 047).

ACKNOWLEDGEMENTS

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