

CENTER FOR DRUG EVALUATION AND RESEARCH

Application Number 21-324

PHARMACOLOGY REVIEW(S)

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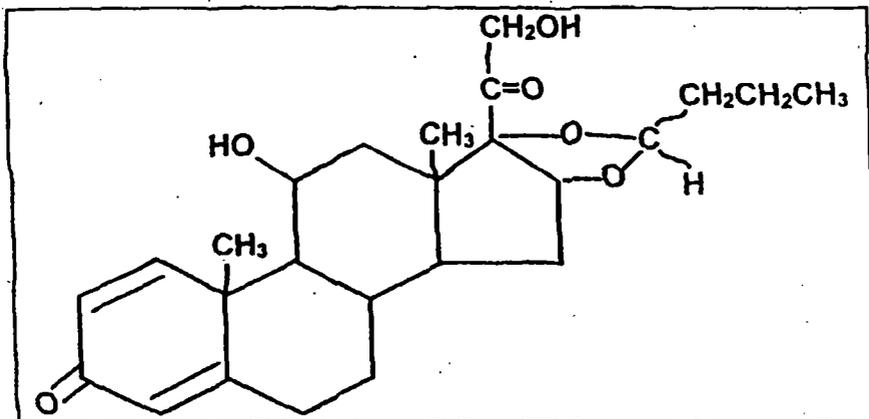
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Date of Review: June 25, 2001

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA.
(Original Summary)

Drug: ENTOCORT™ Capsules (budesonide modified-release capsules; 3 mg).
The chemical name of budesonide is 16 α , 17 α -butylidenedioxypregna-1, 4-diene-11 β , 21-diol-3, 20-dione.



$C_{27}H_{34}O_6$

M.W. = 430.5

Category: Glucocorticosteroid.

Proposed Marketing Indication: For the treatment of mild to moderate active Crohn's Disease involving the ileum and /or ascending colon.

Dose: The dose of budesonide CIR capsules is 9 mg once a day.

PRECLINICAL STUDIES AND THE TESTING LABORATORIES:

Type of Study	Study #	Lot #	Lab	Page # of Review
Pharmacology				5
<u>Absorption, Distribution, Metabolism and Excretion (ADME)</u> Rats (oral, intratracheal) Mice (oral, I.V) Monkeys (oral, plain and CIR capsules)				14
<u>Acute Toxicity Study:</u>				
<u>Rat:</u> Oral S.C	850-RD 0052 850-RD 0052	G01,LW18,LW21 G01,LW18,LW21	AB Astra, Sweden AB Astra, Sweden	25
<u>Mouse</u> Oral S.C.	850-11 D 30 850-11 D 30	M121/C 1249 28	AB Astra, Sweden AB Astra, Sweden	25
<u>Subacute/Subchronic/Chronic Toxicity Study:</u>				
<u>Rat:</u> One-Month Oral Toxicity Study 3-Month Drinking Water Toxicity Study 3-Month S.C. Toxicity study 26-Week S.C. Toxicity Study	75111 610-146 850 T 1817 850-01 T1233	MP 1 64/79 (791214) Mikr 23 F17-19,LW156-158, MP4	AB Astra, Sweden AB Astra, Sweden AB Astra, Sweden	26 26 26 27
<u>Dog:</u> 1-Month Oral Toxicity Study	75103	MP 1	AB Astra, Sweden	27
<u>Rabbit:</u> 1-Month S.C. Toxicity Study	850-01 T 1190	F1, F2	AB Astra, Sweden	27
<u>Monkey</u> 1-Month Toxicity Study with CIR Capsules 26-Week Toxicity Study with CIR Capsules	T2507 T2760	DRC1 1525-1 DSE 1 P525-1 DRE 2		28 29
<u>Reproductive Toxicity</u> <u>Fertility and Reproductive Performance (Segment I)</u> Rat (S.C)	850-01 T 833	LW74-76		32
<u>Teratogenicity (Segment II)</u> Rat (S.C.) Rabbit (S.C)	850-01 T 720 850-01 T 721	F9, F10, F15 F9, F10, F15		32 33
<u>Perinatal/Postnatal (Segment III)</u> Rat (S.C)	850-01 T 834	LW74-74		33

<u>Carcinogenicity Studies:</u>				
<u>Mouse</u>				
91-Week (drinking water)	610-163	64/79 (791214)	--	33
<u>Rat</u>				
104-Week (drinking water)	610-162	64/79 (791214)	--	36
104-Week (drinking water; male rat)	610-180	44	--	39
<u>Mutagenicity</u>				
Ames Test	850-801T1198	64/79, 28	--	48
Rat Hepatocyte DNA Repair Assay	850-01 T 1688	28	--	48
Mouse Lymphoma (TK ⁺) Assay	850-01 T 1627	28	--	48
Human Lymphocyte Chromosome Aberration Assay	850-01 T 1626	28	--	48
Mouse Bone Marrow Micronucleus Test	850-01 T 1208	--	--	48
Sex-Linked Recessive Lethality Test	850-01 T 1654	28	--	48
<u>Mutagenicity of 21-dehydrobudesonide</u>				
Ames Test	SR99289-01	202/99	Astra Zeneca	51
In Vitro Human Lymphocyte Chromosomal Aberration Assay	SR99319-01	202/99	Astra Zeneca	53
In vivo Mouse Micronucleus Test	SR99292-01	202/99	Astra Zeneca	56
Rat liver UDS Assay	SR00131-01	202/99	Astra Zeneca	57

The following studies were submitted as part of NDA 20-233 for Rhinocort Nasal Inhaler (HFD-570): Pharmacology, ADME studies in mice, rabbits and dogs, oral and s.c. acute toxicity studies in mice and rats, subacute/subchronic/chronic toxicity studies: one month oral, 3-month s.c., and 6-month s.c. toxicity studies in rats; 1-month s.c. toxicity study in rabbits; 1-month oral toxicity study in dogs; 3-month oral (drinking water) dose-ranging studies in mice and rats; oral drinking water carcinogenicity studies in rats (104-week) and mice (91-week), repeat drinking water carcinogenicity study in male rats (104-week); Segment I s.c. fertility and general reproductive performance study in rats, Segment II s.c. teratology study in rats, Segment II s.c. teratology study in rabbits, Segment III s.c. prenatal and postnatal study in rats, genotoxicity studies: Ames test, L5178Y/TK mouse lymphoma gene mutation assay, in vitro chromosomal aberration assay in human lymphocytes, in vivo mouse micronucleus test and sex-linked recessive lethality test in *Drosophila melanogaster*. The studies were reviewed by Dr. Conrad Chen (HFD-570), and the pharmacology review of NDA 20-233 is incorporated.

The following studies were submitted as part of _____ for Budesonide CIR capsules: pharmacology, ADME studies in rats, pharmacokinetic studies in male cynomolgus monkeys and dogs, in vitro pharmacokinetics, a single dose inhalation toxicity study of budesonide and terbutaline mixture in mice, rats and dogs, a 6-week s.c. general toxicity study of budesonide and terbutaline mixture in rats, a 6-week oral inhalation general toxicity study of budesonide and terbutaline mixture in dogs, a 4-week oral toxicity study in monkeys, a 26-week oral toxicity study in monkeys; mutagenicity of 21-dehydrobudesonide using Ames test, *in vitro* chromosomal aberration assay using human peripheral blood lymphocytes, *in vivo* mouse micronucleus test by the i.v. route and *in vivo* rat liver unscheduled DNA synthesis assay. The studies were reviewed earlier and the pharmacology reviews of _____

incorporated. The single dose inhalation toxicity study of budesonide and terbutaline mixture in mice, rats and dogs, the 6-week s.c. general toxicity study of budesonide and terbutaline mixture in rats, and the 6-week oral inhalation general toxicity study of budesonide and terbutaline mixture in dogs are not relevant to the present NDA and were not incorporated in the review.

The following new studies were submitted as part of the present NDA, and are reviewed: published pharmacology studies on budesonide, covalent binding of budesonide in liver and brain from male rats, irreversible binding of budesonide and other steroids to liver and brain from rats, *in vitro* formation and degradation of 21-aldehydes of budesonide and cortisol in liver preparations from human, rat and mouse, *in vitro* metabolism of budesonide epimers in the male and female rat liver and brain, and exploratory mutagenicity studies of 21-aldehyde of budesonide, related glucocorticoids and other keto aldehydes.

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PHARMACOLOGY:

1. Glucocorticosteroid (GCS) properties**A. Affinity for glucocorticoid receptor**

The affinity for the glucocorticoid receptor in rat skeletal muscle of budesonide was compared with other reference glucocorticoids. Budesonide, a 16 α , 17 α -acetal type of glucocorticoid, had twice the affinity of other acetal compounds (i.e. triamcinolone acetonide and prednisolone acetonide.) Budesonide had approximately 8 and 200 times the affinity of dexamethasone and hydrocortisone, respectively. Two main metabolites of budesonide, 6 β -OH-budesonide and 16 α -OH-prednisolone, on the other hand, showed a very low affinity for the glucocorticoid receptor.

The receptor affinity of budesonide was 3 times that of beclomethasone-17 α , 21-dipropionate (BDP), comparable to that of beclomethasone-17 α -propionate (BMP), and 3 times that of beclomethasone.

The relative affinity of budesonide and prednisolone for glucocorticoid receptor was also determined using cytosol from rat hepatocytes. The relative affinity of budesonide for this receptor was found to be 17 times more than that of prednisolone (0.06 0.01, unit not mentioned).

B. Local anti-inflammatory activity and systemic effect of locally applied GCS.

GCS were incorporated into implanted cotton pellets and the proliferation of these cotton pellets into granuloma was measured in rodents. The effects of GCS to involute the thymus were measured in these tests as their systemic effects. As shown in the following table, budesonide demonstrated an improved ratio between local anti-inflammatory potency at application site and potency to involute the thymus as compared to other GCS.

GCS	Relative potencies		
	Inhibition of granuloma formation (=local)	Thymus involution (=systemic)	Local/systemic
IAI:			
Budesonide	1	1	1
Triamcinolone acetonide	0.1	1.2	0.1
RSI:			
Budesonide	1	1	1
BDP	0.1	1	0.1

Anti-inflammatory Activity: Budesonide caused a dose-dependent inhibition of T-cell proliferation and significantly decreased the expression of interleukin -2 (IL-2) receptors.

Therefore, budesonide may exert some anti-inflammatory effect by down regulating IL-2 receptor and allergen-specific immune response. In vitro studies have demonstrated that budesonide down-regulated allergen-induced T-cell activation and the release of cytokine factors for eosinophil by inhibiting the release of IL-3 and IL-5. The following table shows the EC-50 (GCS effective conc.) to inhibit the proliferative response of T-lymphocytes to mitogen:

Substance	No. of Tests	EC-50 (nM)	Relative Potency Compared to Budesonide
Budesonide	4	2.45	1
D5519	4	0.139	20
S1316	4	0.046	55
Fluticasone Propionate	4	0.044	57
Tipredane	4	3.85	0.7
Tixocortol Pivalate	3	>>10	Not detectable

Budesonide caused inhibition of lipopolysaccharide (LPS)-induced tumor necrosis factor (TNF)- α release from rat alveolar macrophages. The ED-50 (50% reduction in TNF- α release) of different glucocorticoids are shown below:

Substance	ED-50 (nM)
Prednisolone	5.0
Hydrocortisone	5.0
Dexamethasone	0.90
D5477	0.22
Budesonide	0.16
D5519	0.12
S1316	0.10

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C. Other systemic GCS activities.

Budesonide, like other GCS, has the ability to inhibit gains in body growth or body length and in adrenal or spleen weights, to reduce the white blood cell count, and to lower the plasma cortisol.

D. Effects on lung anaphylaxis.

Studies in guinea-pigs, sheep and rats demonstrated that one or a few doses of budesonide (by intraperitoneal injection, intratracheal instillation or inhalation) inhibited IGE-mediated bronchial anaphylaxis (measured as increase of pulmonary resistance.)

E. Effects on mediator release.

Pretreatment with budesonide has been demonstrated to reduce the formation/release of several important anaphylactic and inflammatory mediators. It is proposed that these effects contribute to the anti-anaphylactic and anti-inflammatory efficacy of budesonide.

Release process stimulus used	Budesonide treatment	Block of substance-induced release	(% reduction)
Guinea-pig lung in vitro (IGE-mediated)	5 mg/kg i.p. 20 hrs before challenge	Histamine LTD ₄ LTB ₄ PAF-acether	(65) (50) (55) (100)
Guinea-pig lung in vitro (IGE-mediated)	50 mg/kg i.p. 20 hrs before challenge	ERS-A (=LTC ₄ -E ₄)	(60)
Human lung tissue in vitro passively sensitized (IGE-mediated)	10 ⁻⁷ mol/l over night	Histamine	(45)
Human basophile (anti IGE)	10 ⁻¹⁰ -10 ⁻⁸ mol/l over night	Histamine	(22-68)
Human alveolar macrophages (opsonized zymosan)	10 ⁻⁴ mol/l over night	LTB ₄ Total chemotactic activity	(100) (100)
Human eosinophils (opsonized Sephadex)	10 ⁻⁶ mol/l overnight	Eosinophil cationic protein	(88)
Thymocytes (mitogen)	10 ⁻⁶ mol/l over night	Lymphoblast transformation	(50)

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F. Vascular permeability

Budesonide as well as other GCS inhibits the vascular permeability at the endothelial level caused by histamine, bradykinin, LTB₄, and other stimulus (e.g. ischemia-induced vascular leakage in hamster cheek pouch.) This inhibition of vascular permeability probably contributes to the anti-inflammatory efficacy of budesonide in animal models.

G. Study with skin.

Budesonide ointment (0.025%) counteracted the edema provoked by a contact hypersensitivity reaction (with 1% picryl chloride.) The same ointment reduced the UVB-induced rise of epidermal DNA-synthesis by 40%. This anti-proliferative action leads to retarded wound healing in normal skin.

Effect of Oral Budesonide and Prednisolone on Allergen Challenge-Induced Plasma Exudation in the Rat Ileum *In Vivo*:

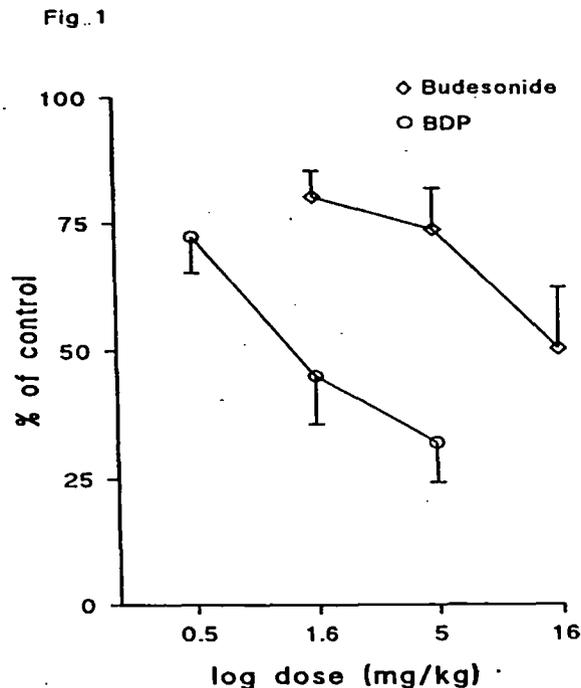
Method: Male Sprague-Dawley rats were sensitized to produce IgE by intraperitoneal injection of ovalbumin mixed with Al(OH)₃. Three to five weeks after sensitization, rats were treated with either budesonide (0.1 mg/kg) or prednisolone (1, 3.3, and 10 mg/kg) or saline by oral gavage. Radiolabeled (¹²⁵I) human serum albumin was used as a plasma tracer. Blood samples were collected from the carotid artery at 10, 60, 120, and 180 minutes after the administration of the radioactive tracer. A 10-cm segment of ileum was catheterized for ileal lavage. The lavage fluid and the plasma samples were analyzed for radioactivity in a gamma counter. Thymi were also dissected after experiment for examination as a marker of the systemic effect of the corticosteroids.

Result: Budesonide significantly reduced the allergen-induced plasma exudation at 0.1 mg/kg dose level whereas prednisolone was effective at 10 mg/kg dose. Budesonide produced less systemic effect compared to prednisolone, as budesonide did not reduce the thymus weight at 0.1 mg/kg dose. Oral (gavage) budesonide was found to be 30 times more potent than prednisolone in reducing ileal inflammation.

Effect of Budesonide (BUD) and Beclomethasone Dipropionate (BDP) on Stress Induced Plasma Cortisol in Guinea-Pigs:

Method: Animals were pretreated intrathecally with 0.5, 1.6, and 5 mg/kg of BDP and 1.6, 5, and 16 mg/kg of BUD. Thirty minutes before blood collection, animals were stressed by exposure to ether for 2 minutes. Thirty minutes after stress induction, blood was collected by heart puncture. Plasma cortisol level was estimated by radioimmuno assay kit (RIA-kit).

Result: A dose-related inhibition of plasma cortisol level was observed after administration of BDP and BUD. However, BUD was found to be less effective in reducing the cortisol level compared to BDP. BUD was 0.1 times as potent as BDP. The following figure (from vol. 1, Page 90 of sponsor's submission dated February 28, 1996) demonstrates the relative potencies of BDP and BUD:



Effects of Oral and Intrarectal D5519 in Oxazolone-Induced Colitis: Comparison with Budesonide:

Method: Rats were sensitized by dermal exposure to oxazolone to induce colitis. The animals were treated from the day after the challenge for four consecutive days with D5519 (orally: 30, 300 nmol/kg; Rectally: 10, 30, 100, and 300 nmol/kg) and budesonide (orally: 30, 100, 300 nmol/kg; Rectally: 30, 100, 300 nmol/kg) either orally or intrarectally. After sacrifice, colon was isolated for measurement and myeloperoxidase (MPO) activity. Systemic effects were also determined by weighing thymus.

Result: Budesonide and D5519 suppressed oxazolone-induced colon inflammation as evidenced by histopathologic measurements (decreased edema). The potency of the test compounds varied depending on the route of administration. Results of this study showed that when administered

orally, D5519 was found to be 2.5 times more potent than budesonide for treatment as well as systemic effects. However, intrarectally, D5519 was found to be equipotent as budesonide in reducing colon edema (wet weight) but the D5519 showed a higher potency in inhibiting MPO activity than budesonide. The systemic effect of D5519 was found to be 3 times more than that of budesonide when administered rectally.

Effects of Plain and Controlled-Ileal-Release Budesonide Formulations in Experimental Ileitis (Boyd AJ. et al, Scand J Gastroenterol 1995; 30: 974-81).

The effects of plain and controlled-ileal-release (CIR) budesonide on trinitrobenzene sulfonic acid (TNBS)-induced intestinal inflammation in hamsters were examined after oral administration of the drug. The doses used were 200 and 800 µg/kg/day (plain budesonide), or 200 µg/kg/day (CIR budesonide). The pre-treatment groups received the drug or placebo 2 days before the induction of inflammation and the post-treatment groups received the drug from Day 7 after the induction of inflammation. The animals were killed after 2 weeks and inflammation was assessed by histologic examination of the intestine and by measuring mastocytosis and myeloperoxidase activity.

Two weeks after TNBS treatment, ileal segments showed clear histologic evidence of inflammation and increased levels of tissue myeloperoxidase activity (control segments, 0.300±0.001 units/g; TNBS-treated segments, 9 units/g). Treatment with 200 µg/kg/day of plain budesonide had no effect on the intestinal inflammation as assessed by histological examination; the 800 µg/kg/day dose caused a decrease in the histologic appearance of inflammation in both pre- and post- treatment groups. This dose also caused a significant decrease in the mast cell numbers (43% and 27% decreases in pre- and post-treatment groups respectively) as compared with controls. Treatment with the CIR formulation also caused an improvement of the histologic appearance of inflammation, decreased the number of mast cells (31.7% and 36% in pre- and post- treatment groups respectively). Treatment with both formulations of budesonide (800 µg/kg/day plain and 200 µg/kg/day CIR) caused decreases in the myeloperoxidase activities of the inflamed intestines. Treatment with 800 µg/kg/day of plain budesonide caused 87.7% and 62.2% decreases in the myeloperoxidase levels in the pre- and post treated animals respectively; the 200 µg/kg/day dose of the CIR formulation caused 77.8% and 67.8% decreases of the enzyme activity respectively. Thus, oral CIR budesonide was effective in reducing trinitrobenzene sulfonic acid induced inflammation in experimental animals.

Oxazolone-induced Colitis in Rats: Effects of Budesonide, Cyclosporin A and 5-Aminosalicylic Acid (Ekstrom GM, Scand J. Gastroenterol 1998; 33: 174-179).

The effects of budesonide and other anti-inflammatory agents were examined in oxazolone-induced colitis in rats. Dark Agouti rats were skin-sensitized with oxazolone and further challenged with intra-rectal oxazolone. The animals were treated with the drugs twice: the day before and the day after the challenge. The following drugs were used: budesonide (430 µg/kg intra-rectally), 5-ASA (21.5 mg/kg intrarectally), cyclosporin-A (15 mg/kg orally) and prednisolone (20 µmol/kg i.p.).

In the sensitized rats challenged with intra-rectal oxazolone, there were marked inflammation of the distal colon (inflammation score, 2.3±0.4 vs. 0.6±0.3 in unchallenged rats) that was associated with

increased colon weights (51%) and myeloperoxidase (700%) activity. Treatment with budesonide, cyclosporin or prednisolone caused significant reductions of the colonic weights and myeloperoxidase activities, while 5-ASA had no effect. Budesonide treatment caused about 21% and 47% reductions of the colonic weight and myeloperoxidase activities respectively.

Plasma Exudation, Hyperemia, and Epithelial Permeability in Rats with Oxazolone-Induced Colitis: Modulatory Effects of Budesonide (Ekstrom GM and Anderson SE, Scand J Gastroenterol 2000; 35: 190-197).

The effects of budesonide on the inflammatory changes in oxazolone-induced colitis in the rat colon were examined after topical administration of the drug. Colitis was induced by intra-rectal application of oxazolone after previous sensitization. The doses of budesonide used were 25.8 and 129 µg/kg.

In oxazolone challenged animals, there were mucosal inflammation of the colon with increased plasma exudation, hyperemia and epithelial permeability. Treatment with 25.8 µg/kg budesonide did not cause any attenuation of the absorptive permeability in the colon while the 129 µg/kg dose caused attenuation of the permeability changes. Budesonide (129 µg/kg) caused significant reductions of the plasma exudation and abolition of the hyperemia in the oxazolone-challenged animals. Thus, in oxazolone-induced colitis in rats, budesonide reduced the inflammation by decreasing permeability, plasma exudation, and increased blood flow.

Topical Anticolitic Efficacy and Selectivity of the Glucocorticoid Budesonide in a New Model of Acetic Acid-Induced Acute Colitis in The Rat (Fabia R et al, Aliment Pharmacol Ther 1994; 8: 433-441).

The effects of budesonide on acetic acid-induced colitis in sprague-Dawley rats were examined after local instillation (10^{-6} or 10^{-8} M) or s.c. administration (0.5, 0.75 or 1.0 mg/kg) of the drug. The treatment started on the day after acetic acid instillation and continued on Days 2 and 3. The effect of the drug on the plasma exudation into the colonic lumen was measured using 125 I-labeled albumin as a tracer. The animals were sacrificed on the fourth day after colitis induction and the colonic segments were examined microscopically and the tissue myeloperoxidase activity was determined.

Acetic acid induced colitis caused significant increases in total morphological scores, tissue myeloperoxidase activity (u/g) and plasma exudation (µl/min/g) into the colonic lumen of the rats as compared with the controls, and treatment with local budesonide caused significant reduction of all the three parameters (total morphological scores- control 1.5 ± 0.2 , acetic acid 14.8 ± 0.8 , budesonide 3.5 ± 0.4 ; myeloperoxidase activity- control 83.8 ± 5.5 , acetic acid 258.6 ± 23.4 , budesonide 109.1 ± 8.3 ; plasma exudation- control 0.28 ± 0.05 , acetic acid 1.68 ± 0.18 , budesonide 0.56 ± 0.08). Subcutaneous budesonide also caused significant improvements of the morphologic scores and the plasma exudation values in acetic acid-induced colitis in rats.

Effects of Systemic and Topical Steroids on Expression and Function of NF- κ B and Growth in Intestinal Epithelial Cells (Goke MN et al, Gastroenterology 1999; 116: G3838).

The effects of budesonide and prednisolone on expression and DNA binding activity of (nuclear factor-kappaB) NF- κ B and proliferation and apoptosis were analyzed in intestinal cells (IEC-6). The concentrations of the steroids used were 10^{-10} to 10^{-4} M. DNA binding activity of NF- κ B was determined by electrophoretic mobility shift assays and the expressions of NF- κ B and I κ B $_{\alpha}$ were assessed by Western blotting.

Stimulation of IEC-6 cells with budesonide and prednisolone caused a dose- and time- related inhibition of the DNA binding activity of the NF- κ B. The steroids caused a slight increase in the cell proliferation at low doses while at high doses there was significant inhibition of cell proliferation. High concentrations of budesonide induced apoptosis in IEC-6 cells, but prednisolone had no effect. Thus, budesonide may exert its anti-inflammatory effects by modulation of the transcription factor NF- κ B.

Effects of Local Budesonide Treatment on The Cell Mediated Immune Response in Acute and Relapsing Colitis in Rats (Palmen MJ et al, Dig Dis Sci 1998; 43: 2518-2525).

The effects of budesonide and dexamethasone on acute experimental colitis and on T cells in thymus and spleen were examined in rats. The effect of budesonide was also examined on relapsing colitis in rats. Colitis was induced by intra-colonic administration of 2,4,6-trinitrobenzene sulfonic acid (TNBS; 30 mg/animal in ethanol). Relapse was induced 5 weeks after the initial induction of colitis by an intra-peritoneal booster dose of TNBS (30 mg/animal in 0.5 ml saline). The glucocorticosteroids (150 μ g) were administered intra-rectally on days 1, 4 and 6 after induction of acute colitis or relapse.

All TNBS-treated animals developed both clinical and histopathological symptoms of colitis, such as diarrhea and transmural inflammation with or without ulceration. Treatment with budesonide in acute and relapsing colitis resulted in a reduction of microscopic damage (damage score was reduced from 4.2 to 0.3 and 0.46 on days 13 and 16 respectively) and decreased the numbers of macrophages and neutrophils in the colon. Dexamethasone was less effective than budesonide. Dexamethasone, but not budesonide reduced the number of T cells in the thymus. Budesonide treatment in relapsing colitis resulted in a mean damage score in the colon (from 0.81 to 0.21). The study suggests that budesonide is more effective in the treatment of acute experimental colitis in rats and budesonide did not cause general suppression of T cells.

Immunopharmacology of Budesonide Induced Inhibition of Pro-Inflammatory Cytokine Secretion by IBD Mononuclear Phagocytes (Schreiber S. et al., Gastroenterology 1996; 110: 1011).

The immunopharmacology of budesonide induced deactivation of peripheral monocytes/intestinal macrophages were examined in biopsy specimens of human intestinal luminal propria in vitro. The inhibition kinetics of the pro-inflammatory cytokines (IL-1 β , IL-6, IL-8) and IL-1ra release in culture were assessed by Northern Blot.

Budesonide inhibited the release of proinflammatory cytokines by intestinal lumina propria mononuclear cells (LMPNC) and peripheral monocytes in a dose-dependent manner; no differences were observed between IBD patients and normal subjects. Budesonide was approximately 10 times more potent

in inhibiting the pro-inflammatory cytokines than dexamethasone. Treatment of the intestinal LMPNC or monocytes with 5×10^{-5} M budesonide caused complete inhibition of the pro-inflammatory cytokine release for up to 60 hours. The authors concluded that because of its prolonged inhibition of the pro-inflammatory cytokines, once daily dosing of budesonide may be sufficient for the treatment of IBD.

Other pharmacological effects.

Budesonide in doses up to 125 $\mu\text{g}/\text{kg}$ i.v. in cats did not affect the arterial blood pressure, the heart rate or the ECG. Acute i.v. injection of 87.6 $\mu\text{g}/\text{kg}$ of budesonide to cats did not affect the pulmonary resistance or compliance.

Budesonide did not reduce sodium secretion and urinary volume in sodium- and water-loaded and adrenalectomised rats. Budesonide has a low mineralocorticoid activity.

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ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION (ADME) STUDIES:**A. Absorption.**

In the oral studies, the approximate bioavailability was 35% in mouse, 32% in rat, and 18% in dog ethanol/saline solution of budesonide. In an inhalations study in rat exposure to 5 and 500 $\mu\text{g}/\text{kg}$ ^3H -budesonide produced between 37-81% of deposit of radioactivity in the upper respiratory and gastrointestinal tract at 30 min. post-dose. Only 0.7-2.0% was recovered from the lung. In experiments using isolated perfused rat lungs, 45% of budesonide administered via the airways was absorbed within 30 min. The remaining fraction was bound to some lung tissue compartment and released slowly into the circulatory system.

After the percutaneous administration of ^3H -budesonide, 85%, 44%, and < 1% of the applied dose was recovered from the treated area at 5 min., 6 hours, and 96 hours post-dose, respectively. Considerable amounts of radioactivity (3-13%) were present in the skin of the treated area 96 hours after the start of the application.

After rectal administration of ^3H -budesonide to anesthetized rats, the drug was rapidly absorbed as judged from the concentration of radioactivity in plasma.

B. Distribution.

The intravenous studies in animals showed that budesonide was extensively distributed into tissues. Uptake in endocrine organs such as the adrenal cortex and reproductive glands was noted. By using ^3H -budesonide, radioactivity was found in the fetuses and placenta of pregnant animals. Low radioactivity was noted in the brain and spinal cord. The most brain radioactivity was attributable to unchanged budesonide. Partition ratios of radioactivity between the blood cells and the plasma were from 0.3 to 1.2.

Plasma protein binding in the rat and dog was found to be about 90%.

C. Metabolism.

Budesonide is biotransformed in liver to become 16 α -hydroxyprednisolone, 6 β -

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hydroxybudesonide, 23-hydroxybudesonide, and Metabolite III (A-ring reduced compound.) No metabolism was observed in the brain, skin and lung tissue. No significant difference was observed in *in vivo* and *in vitro* metabolism of budesonide. In the mouse liver, epimer 22 R budesonide was biotransformed 5-6 times as fast and in human liver, about twice as fast as epimer 22S budesonide. No such pronounced differences in the metabolic rate between the epimers were found in the rat liver. After incubation of the 22R epimer with human liver homogenate for 120 min., less than 2% of 3H-budesonide remained intact. Pretreatment of rats with budesonide for 13 weeks did not affect the rate of budesonide biotransformation by the liver homogenate.

The biological activity of budesonide and its metabolites was compared in the rat ear edema test. The relative potencies were: budesonide, 1; 6 β -hydroxybudesonide, 0.007; and 16 α -hydroxyprednisolone, 0.0005. The relative glucocorticoid receptor affinities were: budesonide, 1; 6 β -hydroxybudesonide, 0.008; and 16 α -hydroxyprednisolone, 0.004. 23-Hydroxybudesonide was not available for pharmacological testing. However, the receptor affinity of 24-hydroxybudesonide was 0.02. The receptor affinity of A⁶-budesonide, a minor metabolite, was 0.2. In summary, it appeared that the biotransformation of budesonide would produce about a 100-fold drop in glucocorticoid activity.

D. Excretion.

Excretion via feces was the major route of elimination of ³H-budesonide in the rat and dog after various routes of administration. This finding indicated an excessive biliary excretion of budesonide. In the rabbit, about equal amounts of radioactivity were eliminated in urine and feces. Analysis of urine and bile samples revealed only traces of unchanged budesonide and demonstrated an extensive biotransformation of budesonide. In man, budesonide is excreted in urine and feces in the form of its inactive metabolites. It goes through an extensive first pass hepatic degradation. After oral administration in man, peak plasma level is achieved in 1-2 hours. The plasma half-life of budesonide ranges from 2 to 3 hours after intravenous administration.

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Pharmacokinetic Study in Male Cynomolgus Monkeys (Study # 850-TO-0132)

Methods: Eight male Cynomolgus monkeys were given a single i.v. dose of budesonide solution (0.025 mg/kg), oral dose of budesonide plain (1 or 10 mg/kg) or oral dose of budesonide CIR (1 or 10 mg/kg). Between each administration there was a 7-day washout period. Blood samples were collected from femoral vein at 5 (i.v.), 45 min, 1.5, 3, 5.5, 8 and 10 hours after drug administration for measuring plasma levels of the drug. The budesonide concentration in plasma was determined by liquid chromatography and mass spectrometry method.

Results: Oral absorption of budesonide from micronized plain capsules was limited (oral bioavailability = 1.7-2.3%), T_{max} reached within 1.5-3.0 hr and the $t_{1/2}$ was 2.44-3.37 hr. The oral

absorption of budesonide from micronized controlled ileal release (CIR) capsules was also limited (oral bioavailability = 0.9-1.4%), T_{max} reached within 3 hr and $t_{1/2}$ was 2.91-3.49 hr. Although oral bioavailability was low, irrespective of the formulation AUC values increased linearly with dose. The extremely low oral bioavailability (about 1-2%) could be due to extensive first pass elimination and/or incomplete absorption.

Pharmacokinetic Parameters in Male Rats*					
	I.V.	Oral (1 mg/kg)		Oral (10 mg/kg)	
	(0.025 mg/kg)	Plain	CIR	Plain	CIR
Cl (ml/min/kg)	42.9 (33.9)	---	---	---	---
MRT (hr)	0.97 (1.47)	3.92 (4.20)	5.77 (7.05)	4.52 (5.47)	6.53 (7.17)
V_{ss} (L/kg)	2.51 (2.99)	---	---	---	---
$t_{1/2}$ (hr)	0.89 (0.99)	2.48 (2.44)	2.91 (3.37)	2.31 (2.39)	3.46 (3.49)
T_{max} (hr)	---	1.5 (1.5)	3.0 (3.0)	3.0 (3.0)	3.0 (3.0)
AUC _{0-∞} (mcg/L x hr)	10.42 (12.40)	7.74 (8.37)	4.14 (4.64)	90.4 (90.2)	54.1 (55.2)
MAT (hr)	---	2.95 (2.81)	4.80 (5.58)	3.55 (4.0)	5.56 (5.70)
F (%)	---	2.0 (1.7)	1.1 (0.9)	2.3 (1.8)	1.4 (1.1)

Cl = plasma clearance

MRT = mean residence time

MAT = mean absorption time

V_{ss} = steady state volume of distribution

F = systemic bioavailability

plain = micronized budesonide in capsules

CIR = budesonide controlled ileal release capsules

* = pooling equal volumes of plasma, collected at the same time from 4 animals (# 147, 149, 151 & 153)

() = results obtained from the pooled plasma of animals (# 155, 157, 159 & 161)

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1. [³H]-Budesonide: Absorption, Metabolism and Excretion in the Rat Following Oral and Intrathecal Administration:

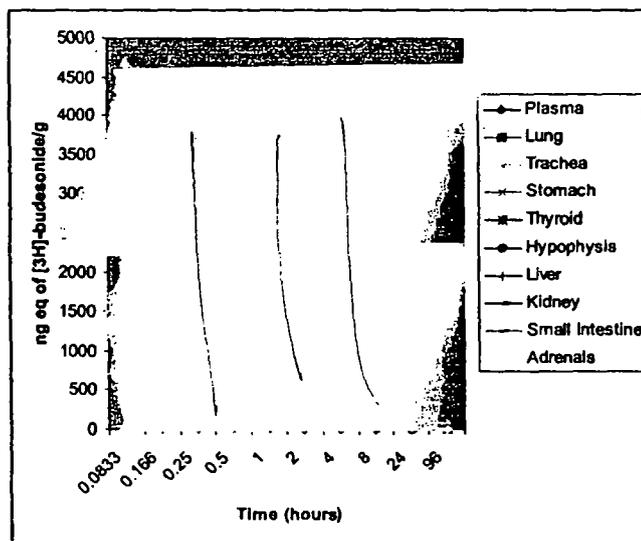
Methods: ADME of [³H]-budesonide was examined in male rats following a single oral (50 µg/kg) or intrathecal (100 µg/kg) administration of [³H]-budesonide. Plasma concentrations of the parent compound were determined by high performance liquid chromatography (HPLC) method. The distribution of [³H]-budesonide was investigated after intrathecal administration.

Absorption: The pharmacokinetic parameters of budesonide are shown in the following table:

Mean (of 3 animals) Plasma Radioactivity Pharmacokinetic Parameters in Male Rats		
Route	Oral	Intrathecal
Dose Administered	13.32 µg	33.85 µg
T _{1/2} (hr)	10.99 (3.857)	2.518 (0.91)
C _{max} (ng equiv/ml)	4.079 (1.526)	7.367 (41.4)
T _{max} (hr)	2.5 (0.500)	1.028 (0.083)
AUC _(0-∞) (ng equiv.hr/ml)	18.79 (1.282)	16.81 (13.5)

Values in parentheses represent the plasma pharmacokinetic parameters of the parent compound.

Distribution: Tissue distribution of [³H]-budesonide was examined following a single intrathecal dose (100 µg/kg) of [³H]-budesonide in male rats. The following figure shows the concentration of radioactivity in different organs of male rats following a single intrathecal administration of 100 µg/kg of [³H]-budesonide:



As shown in the above figure, maximum radioactivity was found in the trachea and stomach. Whole body autoradiographic study was also conducted to examine the distribution of radioactivity in rats after a single intrathecal dose of 0.56 µmol/kg of [³H]-budesonide. Very

high radioactivity was found in thyroid, choroid plexus, bile, lung, liver, nasal and gastric mucosa, salivary and lacrimal glands, pancreas, pituitary, pineal body, adrenal cortex and part of stomach contents at 10 min after drug administration. Almost all the radioactivity was eliminated at 96 hr.

Metabolite Profile: The following table shows the concentration of major metabolites in the plasma following a single intrathecal administration of 50 and 100 µg/kg [³H]-budesonide to male rats. However, the reason for selecting the intrathecal route for these distribution studies was not explained.

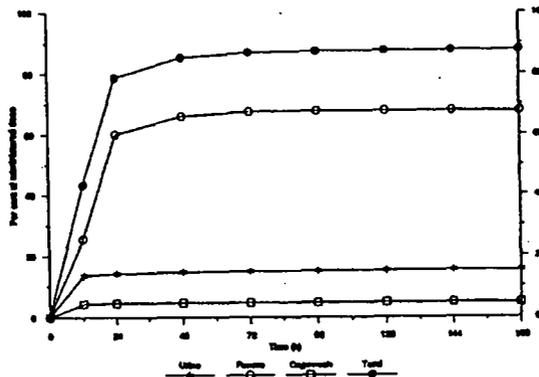
Metabolite	Percent Radioactivity					
	50			100		
Dose (µg/kg)						
Time	15 min	1 hr	4 hr	5 min	1 hr	4 hr
16 α-hydroxyprednisolone	1.89	4.63	0.51	2.78	3.26	1.92
6 β-hydroxybudesonide	0.65	1.08	0.10	4.01	1.31	1.13
Budesonide	0.26	0.46	0.06	37.09	2.95	1.70

The following table shows the concentrations of major metabolites in the urine following a single intrathecal administration of 50 and 100 µg/kg [³H]-budesonide to male rats:

Metabolite	Percent Radioactivity	
	50	100
Dose (µg/kg)		
Time (hour)	12	12
16 α-hydroxyprednisolone	18.74	18.20
6 β-hydroxybudesonide	0.72	0.66

Excretion Profile: The following graphs (from volume 4, page 201 of sponsor's submission) show the mean excretion of radioactivity following single intrathecal administration of 50 and 100 µg/kg of [³H]-budesonide:

Mean cumulative excretion of radioactivity following a single oral administration of (³H)-budesonide at a nominal dose level of 50 µg/kg body weight (mean values from 3 animals)



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In summary, rate of absorption of [³H]-budesonide after oral administration was found to be moderate as C_{max} of the parent compound was achieved at 0.5 h post-dose. Peak plasma radioactivity (4.079 ng eq/ml) was attained at 2.5 hr post-treatment and the radioactivity slowly declined over time. After intrathecal administration, C_{max} (7.36 ng eq/ml) was attained at 1.028 hr post-dose and thereafter, radioactivity declined rapidly (T_{1/2} = 2.518 hr) over time. The highest amount of radioactivity was localized in the trachea (site of administration) and stomach. Moderate concentrations of radioactivity were found in liver, lungs, kidney and small intestine. Metabolism studies revealed two metabolites (16 α -hydroxyprednisolone and 6 β -hydroxy-budesonide) of budesonide. There was no evidence of formation or elimination of glucuronide or sulphate conjugates. Fecal elimination was the major route of excretion (67.8%).

Covalent Binding of Budesonide in Liver and Brain from Male Rats (Report no. 350-RD-0145)

To investigate whether budesonide is metabolized to reactive products in the liver and brain, the co-valent binding of budesonide to rat tissue protein was examined. Co-valent binding to proteins was used as a measure of formation of reactive products. Male rat liver and brain tissue homogenates (10 mg protein/ml) were incubated with ³H (1, 2)-budesonide (1 μ M) in Tris buffer at pH 7.4. The incubations were run at 0°C and 37°C and aliquots were withdrawn at 0, 10, 30 and 60 minutes. After precipitation and washing, the radioactivity in the samples was determined by liquid scintillation counting.

Incubation of rat liver and brain homogenates with radiolabeled budesonide for 10, 30 and 60 minutes did not cause any time-dependent increase in the binding of the radioactivity to the tissue proteins. There were very low bindings of the radiolabel to rat liver and brain proteins during the 60 minutes incubation period. The binding to the liver proteins was higher at 37°C as compared with that at 0°C, while the reverse was the case for brain proteins. Thus, there is a possibility that budesonide is converted to reactive products that can bind to tissue proteins. The relative binding of budesonide to rat liver and brain proteins at 0°C and 37°C is shown in the sponsor's Table below.

TABLE 1. Covalent binding of budesonide to protein of liver and brain from male rat.

	pmol/mg protein per 60'	
	0°C	37°C
liver	0.009 \pm 0.006	0.186 \pm 0.148
brain	0.138 \pm 0.082	0.064 \pm 0.057

Values represent means \pm SE from 3 experiments.

Irreversible Binding of Budesonide and Other Steroids to Liver and Brain from Male and Female Rats In Vitro (Report no. 850-RD-0160)

To determine the extent of reactive products formed from the metabolism of budesonide, the irreversible binding of budesonide to tissue macromolecules of the rat liver and brain S9 fractions were examined. The R and S epimers of both ring-labeled (1, 2-³H budesonide) and acetal chain-labeled (22, 23, 24, 25-³H-budesonide) budesonide were used in the study. In addition, the binding of several other steroids, such as ³H-corticosterone, ³H-triamcinolone acetonide, ¹⁴C-testosterone and ³H-prednisolone to the rat liver and brain S9 fractions were also examined. S9 fractions of the male and female rat liver and brain were prepared after centrifugation of the tissue homogenates at 9000 g for 20 minutes and incubated with the radio-labeled compounds at 37°C in the presence or absence of 2 mM NADPH. The bound and unbound radioactivities were separated by filtration through cellulose filters

Incubation of the 22R epimer of budesonide (tritiated at the acetal chain) with different concentrations of rat liver S9 fractions (37°C, 60 min) showed the maximum total binding at 7 mg/ml of protein, and approximately 2% of the substrate was irreversibly bound to the macromolecules. Addition of NADPH to the incubates, increased binding by about 4-fold. The 22R epimer was bound more than the 22S epimer; however, no difference was observed between ring-labeled and acetal chain-labeled budesonide. The other steroids examined also showed irreversible binding, and no apparent differences were observed between them. The extent of binding of different budesonide epimers and other steroids to rat liver and brain S9 fractions are shown in the Table below.

Relative binding of different steroids to S9 fractions from male and female rat liver and brain

Liver		Male	Female
	22R-acetal chain-labeled	1.0	1.1
22S-acetal chain-labeled	0.6	0.2	
22R-ring-labeled	0.9	0.8	
22-S-ring-labeled	0.7	0.2	
Triamcinolone acetonide	1.6	N.D	
Corticosterone	0.3	0.3	
Testosterone	0.8	N.D	
Prednisolone	0.6	N.D	
Brain	22R-acetal chain-labeled	0.6	0.6
	22S-acetal chain-labeled	0.3	0.3
	22R-ring-labeled	0.7	0.6
	22-S-ring-labeled	0.4	0.5
	Corticosterone	0.2	0.3

N.D., Not done

In Vitro Formation and Degradation of 21-Aldehydes of Budesonide and Cortisol in Liver Preparations from Human, Rat and Mouse (Study no. GHM-2000-00145)

The sponsor submitted an interim report on the *in vitro* formation and degradation of 21-aldehydes of budesonide and cortisol in liver preparations from human, rat and mouse. [³H]-labeled budesonide, cortisol, semiacetal of the 21-aldehyde of budesonide and its congener were incubated with human, male rat and male mouse liver cytosol and mitochondria and the samples were analyzed by liquid chromatography (LC)-radiometry or LC-MS.

There was no 21-aldehydes formation during incubation of [³H]-budesonide or [³H]-cortisol with human, rat and mouse cytosol preparations in the presence of NAD or NADP; incubation with human and mouse mitochondria in the presence of NADPH resulted in the formation of the aldehydes of budesonide. There was no detectable 21-aldehyde of cortisol during the incubation. Incubation with rat mitochondria was also associated with the formation 21 aldehyde of budesonide. There were 3% (at 5 μmol/L budesonide) to 5% (at 2 μmol/L budesonide) conversions of budesonide to the 21-aldehyde by human mitochondria in 5 minutes. In mouse and rat mitochondria, 1% and 0.2% of the compound was converted to the 21-aldehyde (at 5 μmol/L). When NAD or NADP were used as co-factors, there were no 21-aldehyde formation. The 21-aldehyde of budesonide was also formed in human microsomes, in concentrations higher than that in mitochondria. At 5 μmol/L budesonide concentration, 19% of the compound was converted to 21-aldehyde in 5 minutes. The formation of 21-aldehyde of budesonide was also observed in mouse and rat mitochondria.

Incubation of the 21-aldehydes of budesonide and cortisol with human or mouse cytosol with NADPH as co-factor, resulted in a rapid reduction of the aldehyde to the parent compound. Incubation of the 21-aldehyde of budesonide with human mitochondria also caused metabolism of the aldehyde; however, the product was a complex mixture (a small part was budesonide and the carboxylic acid). The half-lives of the 21-aldehyde of budesonide ranged from 5-7 minutes in human mitochondria (with NADPH). The sponsor stated that the formation of the carboxylic acid from the aldehyde was a minor pathway, but no data was provided. The apparent K_M and V_{max} parameters of metabolism of 21-aldehydes of budesonide and cortisol in human, mouse and rat liver cytosol is shown in the Table below.

K_M and V_{max} parameters of metabolism of 21-aldehydes of budesonide and cortisol in human, mouse and rat liver cytosol

Species	Metabolism of 21-aldehyde of budesonide		Formation of budesonide	
	K_M (μmol/L)	V_{max} (mmol/mg protein x min)	K_M (μmol/L)	V_{max} (mmol/mg protein x min)
Human	11	1	15	1
Mouse	7	0.6	17	0.8
Rat	9	0.8	7	0.7
Species	Metabolism of 21-aldehyde of Cortisol		Formation of Cortisol	
	K_M (μmol/L)	V_{max} (mmol/mg protein x min)	K_M (μmol/L)	V_{max} (mmol/mg protein x min)
Human	10	2	27	2
Mouse	15	3	79	7
Rat	18	6	456	114

The sponsor investigated the formation and degradation of 21-aldehydes of budesonide and cortisol in human, mouse and rat liver after incubation of the [³H]-labeled steroids with liver mitochondria and cytosol with NADPH. There was formation of 21-aldehydes of budesonide during incubation of budesonide with the mitochondria from human, rat and mouse. The concentration of the 21-aldehyde was the highest in the human liver mitochondria, followed by mouse and rat. Budesonide was also converted to the aldehyde by human microsomes; the rate of conversion by microsomes was higher than that by mitochondria. The 21-aldehyde was converted back to budesonide during incubation with human liver cytosol or mitochondria.

In Vitro Metabolism of the ^3H -Budesonide Epimers in the Male and Female Rat Liver and Brain
(Report no. 850-RD-0138)

To evaluate the sex differences in the rat in the biotransformation of budesonide epimers, the degradation of epimers 22R and 22S of ^3H -budesonide by the male and female rat liver and brain 9000 g supernatant fraction were examined in vitro. ^3H (1,2)-budesonide (specific activity 89.4 mCi/mg) was diluted with unlabeled compound to a specific activity of 200 $\mu\text{Ci}/\mu\text{mol}$. The two epimers were obtained by high performance liquid chromatography (HPLC) separation. The 9000 g supernatant supernatant fractions were obtained by centrifugation of the homogenates at 4°C. The supernatant fractions of the liver and brain were incubated with the budesonide epimers at 37°C and aliquots were taken at different time points. Relative concentrations of the parent compound and the metabolites in the incubates were determined by HPLC analyses.

More than 91% of the radioactivity was recoverable in the extraction process. The six metabolites of 22R epimers were designated as MR1, MR2, MR3, MR4, MR5 and MR6 and the five metabolites of 22S epimers were designated as MS1, MS2, MS3, MS4 and MS5. Three of the metabolites were identified as 16 α -hydroxyprednisolone (MR1), epimers of 6 β -hydroxybudesonide (MR4, MS2). Five metabolites (MS2 – MS5, MR5 and MR6) lost their UV absorptivity at 254 nm. The degradation of the epimers of ^3H -budesonide was 6-8 times faster in male than female rat liver fraction. The degradation of the budesonide epimers followed a first order kinetics as shown in the sponsor-provided Figure below.

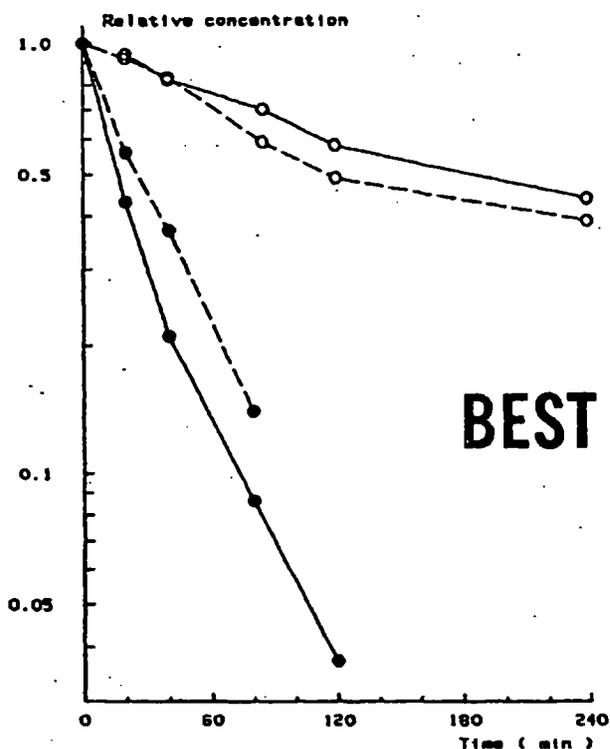


Fig. 2. The degradation of epimer 22R (—) and epimer 22S (---) of ^3H -budesonide in male (e) and female (o) rat liver 9000 g supernatant fraction. Initial concentration of each compound 5×10^{-8} mol/L.

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The half-lives (minutes) of degradation of 22R and 22S epimers of budesonide by the male and female rat liver supernatant fractions are shown in the Table below.

Half-lives (minutes) of epimer 22R and epimer 22S of ^3H -budesonide after incubation with male and female rat liver 9000 g supernatant fraction.

	Males	Females
22R ^3H -budesonide	26	206
22S ^3H -budesonide	29	169

When each epimer was incubated with a mixture of brain and liver supernatant fractions, the degradation rates and the metabolic patterns could not be distinguished when compared with incubation with only liver. To determine the sex differences in the metabolism of budesonide epimers, the metabolism of 22R and 22S ^3H -budesonide epimers by the male and female rat liver 9000 g supernatant fractions were examined. The metabolism of both epimers was 6-8 times faster in by the male rat liver fractions as compared with the females. The degradation of the budesonide epimers by rat liver followed a first order kinetics.

1. Formation of Fatty Acid Conjugates of Budesonide by Human Lung and Liver Microsomes, and Hydrolysis of the Conjugates by Lipase *In Vitro*:

Method: Human lung and liver microsomes were incubated with [^3H]-budesonide for 60 min, either in the presence or absence of co-enzyme A (CoA, 1 mM) and adenosine triphosphate (ATP, 5 mM). The samples were withdrawn at 5, 10, 15, 30, and 60 min intervals for liquid chromatography-mass spectroscopy (LC-MS) analysis of fatty acid conjugates. Porcine pancreatic lipase was used for hydrolysis of fatty acid conjugates of budesonide.

Results: Human lung and liver microsomes formed fatty acid conjugates with budesonide. This conjugation was dependent on the presence of CoA and ATP. Major metabolites identified following incubation of budesonide with lung and liver microsomes include budesonide oleate, palmitate, linoleate, palmitoleate and arachidonate. However, no quantitative measurements of these conjugates were done.

2. Budesonide is Metabolized by Cytochrome P450 3A (CYP3A) Enzymes in Human Liver:

In this study, two major metabolites (16 α -hydroxyprednisolone and 6 β -hydroxybudesonide) of budesonide were identified by incubating budesonide (10 μM) with human liver microsomes in the presence of compounds (ketoconazole IC-50 = 0.1 μM , troleandomycin = 1 μM , erythromycin, cyclosporin, etc) known to interact with different isoforms of cytochrome P450. CYP3A was found to be the specific isoform involved in the metabolism of budesonide as there was a strong correlation between metabolite formation and testosterone 6 β -hydroxylation (r : 0.98 and 0.95), a marker for CYP3A. Budesonide metabolism was not inhibited either by CYP2C inhibitor (sulfaphenazole, mephenytoin and tolbutamide) or by antibodies against CYP1A subfamily. However, budesonide metabolism was inhibited by antibodies against CYP3A.

3. Protein Binding of Budesonide in Plasma from Human, Rat, Mouse, Dog, and Rabbit:

The purpose of this study was to determine the unbound fraction of budesonide in human, rat, dog, rabbit and mouse plasma samples. The unbound fraction of [³H]-budesonide was determined in the above samples by incubating [³H]-budesonide with plasma samples (5 ml) at 1, 10 and 100 nmol/L concentrations followed by ultrafiltration. The results were found to be independent of budesonide concentration in plasma. The range of average unbound fraction was found to be 12.8-14.5%, 7.7-8.4%, 10.4-11.2%, 12.5-15.3% and 14.0-14.2% in human, rat, dog, rabbit and mouse, respectively.

4. Distribution *In Vitro* of Budesonide in Whole Blood from Different Species:

The blood cell/plasma concentration ratios (C_{bp}/C_p) of budesonide were examined in whole blood from human, dog, rat, mouse, and rabbit by incubating [³H]-budesonide (0.1, 1.0 and 10 nmol/L) with blood samples (10 ml) at 37°C for 30 minutes. The results were found to be independent of budesonide concentration. C_{bp}/C_p concentration was found to be 0.75, 0.54, 0.41 in rabbit, human and dog, respectively. The ratio between whole blood and plasma (C_b/C_p) was determined as 0.81, 0.71, 0.78, 0.90 and 0.85 in human, dog, rat, rabbit and mouse, respectively.

Budesonide is Metabolized by Cytochrome P450 3A (CYP3A) Enzymes in Human Liver (Jonsson *et al*, *Drug Metab Disp* 1995; 23(1): 137-42).

To identify the isoform of cytochrome P450 involved in the metabolism of budesonide, budesonide was incubated with microsomes from ten different human liver samples where the different CYP activities had been rank ordered. A strong correlation between the formation of the two budesonide metabolites, 16 α -hydroxy prednisolone and 6 β -hydroxybudesonide and testosterone 6 β -hydroxylation (a marker of CYP3A) was observed ($R=0.98$ and 0.95). When budesonide was incubated with the human liver microsomes in the presence of compounds that interact with different isoforms of CYP, ketokonazole was found to be the strongest inhibitor of budesonide metabolism ($IC_{50} \sim 0.1 \mu M$), followed by troleandomycin ($IC_{50} \sim 1 \mu M$), erythromycin and cyclosporin, all substances known to be metabolized by CYP3A. Substances known to be metabolized by CYP2C (sulfaphenazole, mephenytoin and tolbutamide) and CYP2D6 (Quinidine) did not specifically inhibit the metabolism of budesonide. The formation of the budesonide metabolites was also inhibited by CYP3A antibody but not by CYP1A antibody or control immunoglobulin G, thus confirming that budesonide is metabolized by CYP3A subfamily of enzymes.

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BEST POSSIBLE COPY**TOXICOLOGY:****Acute Toxicity Studies:****Toxicity after single administration of budesonide to mice and rats**

Species	Strain	Number of animals and sex	Route of admini-	Test compound	LD ₅₀ after 3 weeks + s.e.m. mg/kg
Mouse	WBI	30M	s.c.	Budesonide	35 ± 18
Mouse	WBI	30M	s.c.	Beclo-methasone dipropionate	>100
Mouse	WBI	24M + 26F	p.o.	Budesonide	>800
Mouse	WBI	8M + 8F	p.o.	Beclo-methasone dipropionate	>800
Mouse	WBI	30M	s.c.	Budesonide, racemate	>100
Mouse	WBI	30M	s.c.	Budesonide, epimer 22S	-250
Mouse	WBI	30M	s.c.	Budesonide, epimer 22R	32 - 66
Rat	Sprague-Dawley	54M	s.c.	Budesonide	15.1 ± 4.4
Rat	Sprague-Dawley	60M	s.c.	Flucinolone acetate	8.1 ± 2.8
Rat	Sprague-Dawley	36M	s.c.	Budesonide	20.3 ± 7.1
Rat	Sprague-Dawley	30F	s.c.	Flucinolone acetate	-9
Rat	Sprague-Dawley	18M	p.o.	Budesonide	-400
Rat	Sprague-Dawley	18M	p.o.	Flucinolone acetate	>200

* Control groups which received the vehicle for budesonide were included in all studies.

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Repeat Dose Toxicity Studies:**Rat****(a) One-month oral study.**

SD rats, six/sex/group, dose: 0, 0.05, 0.5, and 50 mg/kg/day, (also triamcinolone acetonide group, 5 mg/kg/day), by gastric tube for one month. Increased mortality was seen in the high dose group. Atrophy of the adrenal gland and lymphoid system, gastric ulceration, and intestinal bleeding were found in the treated groups. A dose related decrease of lymphocytes was also observed.

(b) Three-month drinking water study.

CD-rats, 10/sex/group, dose: 0, 10, 50, 200, and 700 $\mu\text{g}/\text{kg}$ daily for 13 weeks. The purpose of this study is to establish dose levels for a carcinogenicity study. No death occurred during the study. A significant decrease in terminal body weight, growth rate, total food consumption with

changes in organ weights was observed at 200 and 700 $\mu\text{g}/\text{kg}/\text{day}$ groups. A reduction in body-weight gain slightly less than 10% was noted at a dose level of 50 $\mu\text{g}/\text{kg}/\text{day}$. Hunched appearance, thinness, salivation, rough haircoat, alopecia, and stomach lesions were observed mainly in the high dose groups.

(c) Three-month subcutaneous study with young rats.

SD rats, 15/sex/group, dose: 0, 0.2, 2.0, and 20 $\mu\text{g}/\text{kg}/\text{day}$ by S.C. route (also triamcinolone acetonide group, 20 $\mu\text{g}/\text{kg}/\text{day}$). Young rats, at an age of 6-7 days, were used in the study. No clinical signs of adverse effects were seen in low and mid dose groups. Two males and two females in the high dose budesonide group and one male and two females in the triamcinolone acetonide group died, respectively, after 10-16 days of drug administration. Alopecia, decreased body weight gain and decreased food consumption were observed in the high dose groups. In the high dose groups, typical glucocorticoid effects including decreased cellularity of bone marrow, increase in extramedullary hemopoiesis in the spleen, and lipid depletion of the adrenal cortex were seen. Atrophy of lymphoid organ and thymus, GI hemorrhage, etc. were also found. These findings were more prominent in the triamcinolone acetonide group. It appeared that the depressive effects of budesonide on the lymphoid system in young rats were more accentuated than in the adult rats.

(d) Twenty-six-week subcutaneous study.

Wistar rats, 15/sex/group, dose: 0, 5, 20, and 80 $\mu\text{g}/\text{kg}/\text{day}$ by subcutaneous injection.

A dose related decrease in body-weight gain was seen in males from all three dose groups; a similar decrease was seen in females in mid- and high dose groups. Typical corticosteroid effects (reduction in lymphocytes, atrophy of lymphoid organs, and lower adrenal weights, etc.) were seen mostly in the mid- and high dose groups.

Treatment with 5 $\mu\text{g}/\text{kg}/\text{day}$ budesonide S.C. was relatively non-toxic in this study.

(e) Twenty-six-week subcutaneous study.

Wistar rats, 10/sex/group, dose: 0, 0.01, 0.1, and 5 $\mu\text{g}/\text{kg}/\text{day}$ by subcutaneous injection.

In this study, the absolute and body-weight-relative adrenal weight of 5 $\mu\text{g}/\text{kg}$ male group was lower than that of the control group. There were no other treatment related changes observed. It was concluded that the 5 $\mu\text{g}/\text{kg}/\text{day}$ dose level was relatively non-toxic. At 0.1 $\mu\text{g}/\text{kg}/\text{day}$ dose level, budesonide failed to show any effect in this 26-week subcutaneous study.

Dog**(a) One-month oral study.**

Beagle dogs, 1/sex/group, 7-8 months old at the start, dose: 0, 0.01, 0.1, and 1.0 mg/kg, also 1 mg/kg BDP (beclomethasone dipropionate.) The body weight of the dogs averaged from 9.5 to 12 kg at the start of the study. The test and reference compound (BDP) were suspended in a solution of 0.75 g Metocel and 0.04 g Tween 80 in 100 ml water to make up concentrations of 0.2 mg/ml, 2 mg/ml, and 20 mg/ml. Atrophy of the adrenal gland and lymphoid system occurred with both corticosteroids at 1 mg/kg. Increased glycogen storage in the liver was also observed. These effects were also observed in the mid-dose budesonide group but were relatively minor in the low-dose budesonide group. No obvious changes besides these typical corticosteroid effects were reported in the study.

Rabbit**(a) One-month subcutaneous study.**

New Zealand White Rabbits, 3/sex/group, dose: 0, 25, and 100 $\mu\text{g}/\text{animal}$ by subcutaneous injection daily for one month. The test compound was dissolved in a solution containing Tween 80 and Metocel. The body weight of rabbits at the beginning of the study averaged 3.5 to 5 kg. The bruising or swelling around the site of injection were similar in both control and test animals. In most high dose group animals and some low dose group animals, the following changes were noted: (1) a proportion of cells in adrenal zona fasciculata showed a reduction in size related to a loss of cytoplasm, (2) regression to the thymus, and (3) minimal hyperplasia of endometrium body in females.

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4-Week Oral Toxicity Study in Monkeys
(Study # 850-T0-0129)

In the 4-week preliminary oral (CIR capsule controlled ileal release capsule) toxicity study in monkeys doses of 100, 330 and 1000 mcg/kg/day were used. In this study various toxicological parameters (clinical signs, body weight, food consumption, hematology and blood chemistry tests, organ weight and histopathology) were measured. The highest tested dose was no effect dose. In this study, plasma levels of the drug were also monitored at 45 min, 1.5, 3.0, 5.5, 8 and 24 hours after drug administration on days 1 and 28 of the study. The AUC values increased with increasing dosages and there were no accumulation of the drug after repeat administration and there were no sex differences.

Parameters	Low Dose		Mid Dose		High Dose	
	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28
AUC (mcg/L x hr)	0.344	0.297	1.244	1.480	3.917	3.453
T _{max} (hr)	3	3	3	3	3	3

Limit of detection = 0.043 mcg/L

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26-Week Oral Toxicity Study in Monkeys
(Study # 850-TO-0133)

Testing Laboratories: _____

Study Started: September 23, 1992

Study Completed: December 17, 1993 (report date)

GLP Requirements: A Statement of Compliance with GLP regulations was included.

Animals: 18-39 Months old male and female Cynomolgus monkeys (2.1-3.0 kg).

Drug Batch No.: 1525-1-DSE 11 and P525-1-DRE 2

Methods: In this study dose selection was based on 4-week preliminary oral toxicity study in which doses of 0.1, 0.33 and 1 mg/kg/day were used. In this preliminary study the highest tested dose was the no effect dose. In the present study, sponsor has selected 0, 0.5, 2 and 5 mg/kg/day dose levels. The high dose level was limited due to the number of capsules needed to be administered daily. Groups of monkeys (4/sex) were given orally (CIR capsules) budesonide at daily doses of 0, 0.5, 2.0 and 5.0 mg/kg/day for 26 weeks. All animals were observed daily for clinical signs and mortality. Body weights were recorded weekly and food intakes daily. Ophthalmoscopic examinations were performed on all animals at pre-test, during weeks 13 and 26 of the study. Blood samples were collected from femoral vein at pre-test, and just before drug administration during weeks 6, 13 and 26 of the study for hematology and serum chemistry tests. Overnight urine samples were also collected at the above mentioned time period for urinalysis. Additionally, blood samples for measuring plasma drug levels were collected from all

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animals at 3 hour after drug administration on day 1, weeks 4, 13 and 26 of the study. At the end of study period all surviving monkeys were sacrificed and subjected to complete necropsy and histopathological examinations.

Results:

1. Observed Effects: None
2. Mortality: One male monkey from control group died due to an accident in dosing.
3. Body Weight/Food Consumption/Water Consumption: At the end of treatment period body weight gains were reduced by 52% and 44% in mid and high dose treated monkeys respectively when compared to the control values. In females, body weight gains were reduced by 59%, 45% and 99% (no gain in body wt.) in low, mid and high dose treated groups respectively. Treatment had no significant effect on food intakes.
4. Hematology/Coagulation/Bone Marrow: No treatment related effects were seen.
5. Blood Chemistry/Urinalysis: Serum cortisol levels were decreased by 24% and 38% in mid and high dose treated monkeys (both sexes) respectively when compared to the control values. In high dose group serum glucose levels were increased by 18% compared to control values. No treatment related effects were seen on urinalysis.
6. Vital Signs/Physical Examination/Ophthalmic Examination: No treatment related effects were seen.
7. Organ Weights: Lung weights were increased by 13-14% in treated animals (both sexes), and liver weights were increased by 13% in high dose group when compared to the control values. Additionally, in high dose group (both sexes), thymus and adrenal weights were reduced by 19% and 31% respectively when compared to their respective control values.
8. Gross Pathology: No treatment related effects were seen.
9. Histopathology: Reduced cellularity of the thymus was seen in 2 out of 4 high dose treated male monkeys. Additionally, reduced cortical width of the adrenals was seen in 1/4 low dose treated females, 2/4 each mid dose treated male and female monkeys and 2/4 males and 3/4 females treated with high dose.

10. Plasma Levels (report # 850-RD-0341): Plasma levels of budesonide increased with increasing dosage and there were no accumulation of the drug after repeat administration.

Plasma Levels at 3 hr Post Dose (mcg/L)				
Duration	Sex (M/F)	0.5 mg/kg/day	2 mg/kg/day	5 mg/kg/day
Day 1	M	0.426	1.295	3.788
	F	0.387	1.713	2.846
Week 4	M	0.310	1.153	3.082
	F	0.435	1.619	2.200
Week 13	M	0.422	1.421	4.950
	F	0.284	1.830	2.359
Week 26	M	0.495	1.804	4.055
	F	0.418	1.950	3.310

In 26-week oral (CIR capsules) toxicity study in monkeys, doses of 0, 0.5, 2.0 and 5.0 mg/kg/day were used. In males, body weight gains were reduced by 52% and 44% at mid and high dose respectively, while in females body weight gains were reduced by 59%, 45% and 99% (no gain in body weights) at low, mid and high dose levels respectively. Decreased body weight can not be used for assessing safety because it is related to the effects of glucocorticoid. Additionally, typical glucocorticoid toxicities (decreased serum cortisol, increased serum glucose levels, decreased thymus and adrenal weights) were also seen in some of the high dose treated monkeys. However, all these effects are typical corticosteroid effects, and no additional adverse effects were seen in this study. The highest tested dose (5.0 mg/kg/day) can be considered a well tolerated dose.

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Reproduction Studies.**A. Segment I study in rats.**

Rats of the Sprague-Dawley strain, male: treated for 9 weeks pre-mating and through mating, female: treated for 2 weeks pre-mating and continued up to 21 days post partum, dose: 0, 5, 20, and 80 $\mu\text{g}/\text{kg}/\text{day}$ subcutaneously. Males and females from the same dose level group were mated in the study.

No obvious effects were observed in the control and the low dose groups in terms of maternal and fetal findings. In the mid-dose group, the food consumption and body weight gain was decreased during the gestation. Besides, both prenatal viability and the viability of the young at birth and during lactation was significantly reduced.

In the high dose group, these findings were more pronounced.

B. Segment II study in rats.

Rats of the Sprague-Dawley strain, 20 pregnant/group, dose: 0, 20, 100, and 500 $\mu\text{g}/\text{kg}/\text{day}$ (also triamcinolone acetonide group at 500 $\mu\text{g}/\text{kg}/\text{day}$) from days 6 to 15 of pregnancy by subcutaneous administration. Although the animals from the low dose group did not show any major adverse effects (except decrease in food consumption and body weight gain,) the animals in the mid-dose group showed some increases in fetal loss and fetal abnormalities.

In the high dose group, the general condition of animals was poor (piloerection, drowsiness, decreased food consumption and body weight gain.) Fetal abnormalities were significantly increased in this group. The main findings were umbilical eversion and reduced ossification of skull bones in combination with some sternal or vertebral defects. Of the animals in the triamcinolone acetonide group, 14 out of 18 died before gestation day 21 and none of the surviving rats had any living fetuses.

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D. Segment II study in rabbits.

New Zealand white rabbit, 14-15 pregnant/group, dose: 0, 5, 25, and 125 $\mu\text{g}/\text{kg}/\text{day}$ (also flucinolone acetone 125 $\mu\text{g}/\text{kg}/\text{day}$) from days 6 to 18 of gestation by subcutaneous administration. In the low- and mid-dose groups food consumption and body weight gains was decreased. Visceral abnormalities were observed in one low-dose group fetus and two mid-dose group fetuses. The frequency of skeletal anomalies was increased in the mid-dose group. The most anomalies were skull and vertebral defects. The type of defects was characterized as signs of delayed development. In the high-dose group and flucinolone acetone group, all does aborted at the end of gestation. The litter size and fetal loss was similar in the control, low-, and mid-dose groups.

E. Segment III study in rats.

Sprague-Dawley rats, 20 pregnant/group, dose: 0, 5, 20, and 80 $\mu\text{g}/\text{kg}/\text{day}$ from day 15 of pregnancy to day 21 post partum by subcutaneous route. Slight prolongation of gestation period was seen in the high-dose group. No drug effect on parturition, nursing and lactation was observed. Body weight gains were decreased in the mid- and high-dose group dams. Litter size at birth was decreased in the high-dose group. The viable young at days 7 and 21 post partum were significantly decreased. The mean pup weights were smaller in the high-dose group. No adverse effects were observed in the low-dose group in this study.

CARCINOGENICITY STUDIES:**91-week drinking water study in mice.**

CD-1 mice, 50/sex/group, dose: 0, 10, 50, and 200 $\mu\text{g}/\text{kg}/\text{day}$ for 91 weeks, (the control group had 100/sex/group), conducted by

from Feb. 26, 1981 to Dec. 2, 1982. Cumulative survival data revealed that there was a dose-dependent decrease in survival in the males. Cumulative female survival data revealed no significant trend or group differences. The sponsor reported a marked drop in survival for all male and female groups at approximately weeks 47 and 48 which coincides with the outbreak of viral infection in the study colony. The sponsor stated that in order to determine if increased mortality during the infection masked a treatment-related effect on survival, cumulative survival data were also analyzed excluding all female mice which died during weeks 47 and 48. The sponsor reported that no significant trend or difference between the groups. The sponsor's analysis of survival data and other data will be reviewed by a FDA statistician later. The deaths of mice during weeks 46 and 47 were identified as by infection to Sendai virus through determination of serum antibody titers. The incidence of alopecia was slightly increased in the high-dose males and females after the first 26 weeks of study. Mean food consumption and mean body weights of treated groups were comparable to the control group. Mean water consumption for

treated groups were significantly different than the corresponding control group at several intervals during the study, but no trend or relationship to treatment can be established. Mean weekly compound consumption was variable during the study. However, averages over the course of the study were within 3% of target for all groups, according to the sponsor's calculations.

Groups:	Males				Females	
	2	3	4	2	3	4
Average Compound Consumption (mg/kg/day):	9.94	48.91	190.81	9.84	49.63	199.99
Percent Desired Compound Consumption:	99.4	97.8	99.4	98.4	99.3	100.0

No significant differences were noted in organ weights between treated and control groups for either sex. No changes in gross pathology which were attributable to the drug treatment were observed. The lung changes (principally dark red lungs), noted for many of the female mice found dead between weeks 27 and 52, were explained as the results of the viral infection and not drug treatment. No histopathologic evidence of carcinogenicity of budesonide in mice was reported. The incidence of various neoplasia was generally similar in the control and treated groups according to the sponsor's analysis.

The FDA statistician's analysis of the same data showed that there was a statistically significant (at 0.05 level) linear trend in lung alveolar/bronchiolar carcinoma - multiple ($p=0.0103$, incidence: 0,2,1,3) in male mice. There were marginally significant positive linear trends in multiple organs malignant lymphoma, histiocytic ($p=0.0523$, incidence: 2,0,1,3) and stomach squamous cell carcinoma ($p=0.0667$, incidence: 0,0,0,2) in female mice.

FDA statistician also evaluated the validity of study by survival rates and MTD. It was found that the terminal survival rates were 55%, 56%, 40%, and 30% for males and 46%, 50%, 56%, and 42% for females.

It seems that there were not sufficient male and female mice living long enough to get an adequate exposure to the drug and to be at risk of forming late-developing tumors. There was a statistically significant linear trend in the intercurrent mortality rate in male mice, but not in female mice. Based on the decreased body weight gain, the high dose was close to MTD for male and female mice.

Tumor Incidence Rates
Male Mice, Lung-Alveolar/Bronchiolar Carcinoma - Multiple

Weeks	Control		Low		Medium		High	
	T	N	T	N	T	N	T	N
0-50	0	21	0	4	0	9	0	12
51-80	0	16	0	15	0	16	0	17

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81-91	0	8	0	3	1	5	0	4
terminal	0	55	2	28	0	20	3	15
Total	0	100	2	50	1	50	3	50

**Tumor Incidence Rates
Female Mice, Multiple Organ Malignant Lymphoma, Histiocytic**

Weeks	Control		Low		Medium		High	
	T	N	T	N	T	N	T	N
0-50	0	38	0	15	0	15	0	15
51-80	0	3	0	3	0	5	0	8
81-91	1	13	0	5	1	2	2	4
terminal	1	46	0	25	0	28	1	21
Total	2	100	0	50	1	50	3	50

**Tumor Incidence Rates
Male Mice, Stomach Squamous Cell Carcinoma**

Weeks	Control		Low		Medium		High	
	T	N	T	N	T	N	T	N
0-50	0	38	0	15	0	15	0	15
51-80	0	3	0	5	0	5	1	8
81-91	0	15	0	5	0	2	0	6
terminal	0	46	0	25	0	28	1	21
Total	0	100	0	50	0	50	2	50

Notes: T: Number of necropsies with the above tumor.
N: Number of necropsies.

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104-week drinking water study (#610-162) in Sprague-Dawley rats. CD rats, 36 days-old, 100/sex/control, 50/sex/treated, dose: 0, 10, 25, and 50 $\mu\text{g}/\text{kg}/\text{day}$ in drinking water for 104 weeks.

This study was conducted by ... from March 1981 to March 1983. The report was originally submitted under IND ... and IND ... but was not received by HFD 140 or HFD-160. The same report was submitted under IND ... and was reviewed on April 20, 1988. The above mentioned pharmacology review should be referred.

The test material was dissolved in ethanol and dosing solutions were prepared in tap water as dilutions of the stock-solution. The water consumption and hence the compound consumption was measured weekly. However, the plasma drug levels were not determined in the study.

Based on the reduction in body weight gain during the 104 weeks, the high dose level, 50 $\mu\text{g}/\text{kg}/\text{day}$, probably has attained the MTD based upon the carcinogenicity study guideline (i.e. up to 10% reduction in body weight gain.)

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Survival was comparable in all groups. Gross pathology results indicated a slight increase in incidence of subcutaneous masses and alopecia in treated groups. No statistically significant differences or treatment-related trends were noted for organ weight data for either sex. Microscopic examinations revealed that there were statistically significant increases in astrocytomas (high-dose males), primary hepatocellular neoplasms (mid- and high-dose males), and fibroadenomas and combined primary neoplasms of the mammary gland (high-dose females) compared to concurrent control group incidences. A statistically significant decrease in splenic pigment (probably hemosiderin) was detected in both sexes in the mid- and high-dose groups, and in the low-dose males. The incidences of brain gliomas (astrocytomas) were reported as 1, 0, 1, 6 (1%, 0%, 2%, 12%) in males and 0, 0, 0, 0 in females in the original report. However, the sponsor revised the report in a subsequent amendment (after conducting a serial sectioning) as 2, 0, 3, 8 (2%, 0%, 6%, 16%) in males and 1, 0, 0, 0 (1%, 0%, 0%, 0%) in females. In the original reports, the incidences of astrocytoma and oligodendroglioma were combined by the sponsor. The incidences of liver neoplasms and mammary neoplasms were also shown in the following table.

Summary of Important Histomorphologic Observations
Combined Scheduled and Unscheduled Deaths

Sex Group: Number:	Male				Female			
	-1- 100	-2- 50	-3- 50	-4- 50	-1- 100	-2- 50	-3- 50	-4- 50
Spleen (SP) Number that can be examined:	98	49	49	50	100	48	50	50
Pigment								
-)	12	7	9	9	3	1	3	1
1)	20	21	19	20	12	6	7	15
2)	34	13	14	14	14	11	14	23
3)	22	4	7	2	38	18	18	7
4)	7	2	0	3	27	9	5	3
5)	3	2	0	0	4	3	2	1
7)	98	49	49	50	100	48	49	50
Brain (BR) Number examined:	100	49	50	50	100	30	27	50
-M- Astrocytoma	1	0	1	5	0	0	0	0
-M- Oligodendroglioma	0	0	0	1	0	0	0	0
Liver (LI) Number examined:	100	49	50	50	100	35	33	50
-focus(1)/area(s) of cell alteration	40	18	17	24	34	8	10	25
Primary Hepatocellular Neoplasms								
-B-Neoplastic nodule	2	0	2	3	6	1	0	4
-B-Neoplastic nodule, multiple	0	1	1	2	1	1	2	0
-M-Hepatocellular carcinoma	2	2	6	2	1	0	2	2
-M-Hepatocellular carcinoma, multiple	1	0	0	1	0	0	0	0
Animals with one or more								
Primary Hepatocellular Neoplasms	4	3	9	8	8	2	4	6
Mammary Gland (MG) Number examined:					100	50	50	50
Primary Mammary neoplasms								
-B-Fibroadenoma(s)					27	22	15	23
-M-Carcinoma					14	4	7	6
-B-Cystadenoma					0	0	0	3
-B-Adenoma					0	3	1	0
Animals with one or more								

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Primary Mammary Neoplasms

33 26 19 29

* For statistical evaluation, the number examined for mammary tumors was taken to be the total number of animals in the group that were examined grossly for tissue masses.

- > = Finding Not Present
- P> = Finding Present
- 1> = Minimal
- 2> = Slight
- 3> = Moderate
- 4> = Moderately Severe
- 5> = Severe
- TL> = Total

In the amendment, the incidences of astrocytoma and other histopathological findings were reported in the following table.

TABLE ADDENDUM 3C
 *** PAIR/TOX SYSTEM OUTPUT ***
 104-WEEK DRINKING STUDY IN RATS

TABLE INCLUDES: SEX=ALL, GROUP=ALL, SCREEN=ALL, WEEK=ALL DEATH=ALL, FIND=ALL, SUBSET=Y	--- NUMBER OF ANIMALS ---								
	SEX:	MALE				FEMALE			
	GROUP:	-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
ORGAN AND FINDING DESCRIPTION	NUMBER:	100	50	50	50	100	50	50	50
** TOP OF LIST **									
BRAIN (BR)	NUMBER EXAMINED:	100	49	50	50	100	50	49	50
	NOT REMARKABLE:	87	41	43	34	74	30	28	35
--N-ASTROCYTOMA		2	0	3	7	1	0	0	0
--N-OLIGODENDROGLIOMA		0	0	0	1	0	0	0	0
--N-GRANULAR CELL TUMOR		0	0	0	1	1	0	0	0
--N-MENINGIOMA		1	0	0	0	0	0	0	0
--GLIOSIS		0	0	1	1	0	1	1	1
--PERIVASCULAR MONONUCLEAR INFILTRATION		1	0	1	1	0	0	0	1
--X-MALIGNANT LYMPHOMA (SEE "MULTIPLE ORGANS" FOR CLASSIFICATION)		1	0	0	0	0	0	0	0
--VENTRAL CONGESTION		5	8	3	2	17	18	21	13
--CONGESTION/HEMORRHAGE		0	0	0	2	0	0	0	0
--MENINGEAL MONONUCLEAR INFILTRATION		2	0	0	1	1	0	0	0
--FOCAL MINERALIZATION		3	0	1	4	2	0	0	0
--ENCEPHALOMALACIA		0	0	0	1	1	0	1	2
--MENINGOENCEPHALITIS		0	0	0	0	1	2	0	3
--BACTERIA		0	0	0	0	2	1	0	0
--VENTRICULAR DILATION		0	1	0	0	5	0	0	0
--I-PITUITARY CARCINOMA		0	0	0	0	2	0	1	1
--PIGMENT		0	0	0	1	2	0	0	1
--FOCAL NEUTROPHIL INFILTRATION		0	0	0	0	1	1	0	0
--THROMBUS(1)		0	0	1	0	0	1	0	1
--FOCAL NECROSIS		0	0	0	1	0	1	0	0
** END OF LIST **									

According to FDA statistician's analysis, there was significant (at 0.05 level) linear trend in brain astrocytoma (original standard section: p=0.0024; serial step section: p=0.0007) and primary hepatocellular neoplasms - animals with one or more (p=0.004) in male rats. there were significant (at 0.05 level) positive linear trends in primary mammary fibroadenoma (p=0.0309), primary mammary cystadenoma (p=0.0076) and primary mammary neoplasms - animals with one or more - (p=0.0126) in female rats. In mid- and high-dose

female groups, a decrease in hematocrit, hemoglobin, and erythrocyte count was found during a certain period in the study. In the high-dose male group, a slight increase in alkaline phosphatase levels was seen from week 13 to week 104. No differences between the groups were noted in the ophthalmological findings.

(i) 104-week drinking water study in male rats with budesonide and reference compounds. (#610-180)

Sprague-Sawley male rats (Ctrl: CDBR), 100/group, dose: 0, 0, 50 (budesonide), 400 (prednisolone,) and 15 (triamcinolone acetonide, reduced to 10 at week 10 and to 5 at week 22) µg/kg/day for 104 weeks in drinking water (prepared with ethanol solution and tap water.) Satellite groups (20 males/group) were used for clinical pathology studies. The study was conducted from Aug. 6, 1985 to Aug. 14, 1987 by

The study completion date was listed as April 8, 1988. The summary report of this study was submitted under IND on Feb. 24, 1988 and was reviewed by FDA on April 22, 1988. IND was under the sponsorship of Astra Clinical Research Associates, Inc., Hopkinton, Massachusetts. Survival was decreased for all treated groups as compared to both control groups. Survival in the budesonide group was significantly reduced.

Unscheduled Deaths*
Weeks 1-104

Group:	1	2	3	4	5	6	7	8	9
Dose Level (µg/kg/day):	0.0	0.0	50	400	5	0.0	50	400	5
	Control	Control	(B)	(P)	(TA)	Control	(B)	(P)	(TA)
	Number of Animals								
Found Dead	19	23	38	32	26	1	6	6	9
Nonblind sacrifices	12	14	18	15	17	4	3	7	3
Accidental death		1	1	3	1			1	1
Total number of deaths	31	38	57	50	44	5	9	14	13

B = Budesonide
P = Prednisolone
TA = Triamcinolone Acetonide

Group mean body weights and body weight changes of all groups receiving steroids were significantly decreased compared to two control groups. Total leucocyte and lymphocyte values were also lower in the steroid treated groups. Alopecia and malocclusion of teeth were increased in the steroid treated groups. The biological significance of the latter finding was not known. Absolute spleen weights were decreased in all groups receiving steroids with statistical significance in the groups receiving prednisolone and triamcinolone acetonide. Absolute brain weights were similar in all groups, but relative brain

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weights were increased in steroid treated groups (with significance in groups receiving prednisolone and triamcinolone acetonide.) This significant increase in relative brain weights reflects the significant decrease in terminal body weights. Food consumption for groups receiving steroids tended to be lower than controls. Water consumption was generally comparable among treated and control groups. No report of drug plasma levels during the study was found in the submission. Originally, only the brain was examined histopathologically. The samples were prepared by . and then forwarded to the sponsor for microscopic examination (by Dr. .) The hematoxylin and eosin stained slides of brain tissue were sectioned only at three routine levels (Further serial sectioning of brain tissue was recommended by FDA and later conducted by Dr. see report under (k).) Dr. reported that the glioma incidences and the other findings in the brain were similar between control and treated groups.

TABLE 1. SUMMARY TABLE OF MICROSCOPIC FINDINGS IN THE BRAINS - MAIN STUDY

FINDINGS	GROUP				
	1 Control	2 Control	3 Dexamethasone	4 Prednisolone	5 Triamcinolone Acetonide
No. Examined	99	100	100	100	100
Glioma	1	1	2	2	1
Granular Cell Tumor	0	0	1	1	0
Malignant Lymphoma	1	0	0	0	0
Pituitary Adenoma Remnants	1	4	0	1	0
Focal Gliosis	1	1	1	0	2
Chor. Plax. Hyperplasia	0	0	0	0	1
Focal Hemorrhage	1	2	1	1	2
Bacterial Microabscesses	0	0	1	0	1

TABLE 2. SUMMARY TABLE OF MICROSCOPIC FINDINGS IN THE BRAINS - SATELLITE STUDY

FINDINGS	GROUP			
	6 Control	7 Control	8 Dexamethasone	9 Prednisolone
No. Examined	20	20	20	19
Glioma	0	1	1	0
Malignant Lymphoma	1	0	0	0
Myeloid Leukemia	0	0	0	1
Pituitary Carcinoma	1	0	0	0
Pituitary Adenoma Remnants	0	0	1	0
Hypophysial Stalk Cyst	1	0	0	0

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Focal steatosis	0	0	1	0
Focal hemorrhage	0	0	0	2

The histopathological evaluation of male rat livers was later conducted by . in April 1989. As shown in the following table, the combined incidences of hepatocellular adenomas and hepatocellular carcinomas were statistically increased in all treated groups.

The grades and incidences of eosinophilic foci and basophilic foci were slightly higher and/or slightly increased in the treated groups. The portal inflammation and bile duct proliferation was reduced in the treated groups. This was probably caused by a glucocorticoid effect related to anti-inflammatory or immunosuppressive action.

Table 1.
Male Rat Livers
Dexamethasone and Reference Compounds (Study MJA 618-188)

Group	1	2	3	4	5
Number examined	100	100	100	100	100
Hepatocellular adenoma	4	3	10*	15**	8
3+ or higher	2	1	3	4	3
mean grade	2.2	2.7	2.2	2.1	2.2
Hepatocellular carcinoma	3	1	4	6	5
3+ or higher	3	0	2	2	2
mean grade	3.0	2.0	2.3	2.3	2.4
Total tumors	7	4	14**	21**	13*
Focal eosinophilic alteration	20	18	14	17	17
3+ or higher	1	3	3	3	5
mean grade	1.2	1.6	1.8	1.8	1.8
Focal basophilic alteration	23	20	26	31	26
3+ or higher	2	3	6	7	10
mean grade	1.4	1.6	2.0	1.9	2.3
Bile duct proliferation	57	57	14	5	18
Portal inflammation	60	54	19	1	20

one-sided fishers exact tests, group 3-5 v. combined 1 and 2.

- $P < 0.05$
- $P < 0.01$

Key to Individual Animal Diagram

Grades:

- 1 = slight
- 2 = slight to moderate
- 3 = moderate
- 4 = moderate to severe
- 5 = severe
- NI = within normal limits

) 104-Week drinking water study in Fischer-344 rats.

CDF male rats (Fischer 344,) 28 days old, 75/group, dose: 50 (budesonide,) 0, 400 (prednisolone,) and 15 (triamcinolone acetonide) $\mu\text{g}/\text{kg}/\text{day}$. However, the dosing adjustments were made according to the following table.

Time and Level of Steroid Dose Changes*

Drug	Time of dose change	New dosage level ($\mu\text{g}/\text{kg}/\text{day}$)
Budesonide	11 weeks	25
Prednisolone	7 weeks	600
	78 weeks	400
	99 weeks	0
Triamcinolone acetonide	10 days	7.5
	11 weeks	4

* Dose levels were changed after consultation with officials because of mean weight losses exceeding targeted 10%, concerns about mortality, or in the case of Prednisolone stoppage, actual increased mortality over controls.

The study was conducted by

from Sept. 26, 1985

to Oct. 7, 1987.

The study report was originally submitted under IND on Feb. 24, 1988 and was reviewed by FDA on April 22, 1988. There was no significant difference in the mortality rate when the budesonide group and triamcinolone acetonide group were compared to the control group. The prednisolone group had a higher mortality rate than the control group in the study.

All three of the steroid treated groups had significantly higher organ weight to body weight ratio for the brain and adrenal glands. In the budesonide group, the ratio for liver was only marginally higher than the control; the ratio for spleen was comparable to the control. The changes of organ weight to body weight ratio in steroid treated groups were partially caused by the decrease in body weight of the treated groups. The histopathological examination of the brain and spinal cord showed only three glioma in the CNS. It was concluded that steroids did not lead to increased incidences of gliomas in Fischer rats.

Primary Brain Lesions

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Group 1 Sulfonamide	Group 2 Control	Group 3 Prothidolone	Group 4 Tiamololone acetamide
0/75	0/75	0/75	1/74

* Gliomas only, does not include 1 hemorrhage and 1 brainstem calcification in group 1, 1 hemorrhage and 1 pituitary adenoma in group 2, 2 hemorrhages, 1 pituitary adenoma and 1 granular cell tumor in group 3, 1 hemorrhage, 1 ossified mass and 1 pituitary adenoma in group 4; includes 1 small glioma in group 4.

Chart 7. Primary Spinal Cord Tumors

Group 1 Sulfonamide	Group 2 Control	Group 3 Prothidolone	Group 4 Tiamololone acetamide
1/74*	0/75	0/75	1/74

* Gliomas only, does not include 1 hemorrhage in group 1, 2 hemorrhages in group 2, 2 hemorrhages and 1 meningeal metastasis in group 3, 1 hemorrhage in group 4; includes 1 cervical glioma in group 1 and 1 dorsal glioma in group 4.
x One spinal cord was not saved at the time of necropsy in group 1.

The gross pathological examination showed not treatment related changes. However, the report stated that these tissues were not examined microscopically per protocol of the study.

Gross Pathology

Type	Group 1	Group 2	Group 3	Group 4
Hemorrhage-brain	4	1	7	5
Hemorrhage-stomach/intestine	11	4	6	6
Enlarged Spleen	10	16	7	16
Stomach tumors/nodules	2	0	0	2
Intestinal tumors/nodules	2	4	1	3
Testicular tumors/growth	2	2	0	1
Kidney tumors/nodules	1	2	0	1
Liver tumors/nodules	1	0	3	4
Enlarged Pituitary	1	0	2	5
Subcutaneous tumors/nodules/cyst	15	12	16	5
Enlarged liver	0	1	0	0
Bladder tumors/nodules	0	0	1	2
Lung tumors/nodules	0	0	2	0
Ascites tumors	0	0	1	1
Diaphragm tumors/nodules	0	0	1	0
Mesentery tumor/nodule	0	0	0	1

Enlarged salivary gland

In this study, the water consumption and drug plasma levels were not determined.

In November of 1988, the GLP audit of this study was conducted by a pharmacologist from the Division of Scientific Investigation and a FDA field investigator (see a memo dated Feb. 27, 1989 by Dr. Frances O. Kelsey, Director of HPD-340.) It was advised that this study not be used in the safety assessment of budesonide because of several serious regulatory deficits. The three most significant were: 1) failure to provide Quality Assurance monitoring; 2) failure to accurately assess the amount of drug administered; and 3) the omission of data from or the failure to properly record data in the log books.

) Addendum to carcinogenicity study #610-180 (see (I).)

Brain tissue in paraffin blocks from study #610-180 was processed by multiple step sectioning for further histopathological examination. This examination was conducted by

in September, 1992. Five micron sections were taken through the entire block and every tenth section was stained with Hematoxylin and Eosin for examination. The original report of study #610-180 was based upon the three coronal sections of the brain (a. the frontal cortex and basal ganglia, b. the parietal cortex and diencephalon, and c. the cerebellum and pons.) Since study #610-162, which showed budesonide-treatment-related gliomas was based upon the multiple step sectioning, a similar sectioning was recommended for study #610-180 in order to obtain a meaningful comparison of study #610-162 and study #610-180. In the original report of study #610-180, no corticosteroids-induced gliomas were found for budesonide, prednisolone, or triamcinolone acetonide.

The following table compares the microscopic findings of brains from the control male group and the budesonide male group through standard three (3) sectioning and multiple step sectioning. Although some numerical changes in histopathological findings were observed after the multiple step sectioning as compared to the standard three (3) sectioning, it was concluded that no budesonide-related brain neoplasms were discovered in study #610-180.

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September 17, 1972

STUDY 8516-108

TABLE 4. SUMMARY OF MICROSCOPIC FINDINGS IN THE BRAINS COMPARING CONTROL GROUP 18 WITH SUBSEQUENT 3M GROUP

FINDINGS	IN CONTROL (3 section)		IN CONTROL (8/16 section)		Grand Total 3 Section and 8/16 Section		IN SUBSEQUENT (3 Section)		IN SUBSEQUENT (8/16 Section and 3 Section)		Grand Total 3 Section and 8/16 Section	
	Examining Pathologists	Total	Examining Pathologists	Total	Examining Pathologists	Total	Examining Pathologists	Total	Examining Pathologists	Total	Examining Pathologists	Total
Number of brains examined per group	99		99				100		100			
Glioma	84876, 84878	2	84876, 84878	2	2		84903	1	84903	1		1
Granular Cell Tumor		0	84879, 84883, 84884, 84882	4	4		84909, 84907	2	88819, 88829, 84910	3		4
Malignant Lymphoma	See footnote 3											
Pituitary Adenoma Benign		0	84877	1	1		84907	1	84903, 84905	2		3
Pituitary Adenoma	84889	1	84884	1	2			0		0		0
Focal Gliosis		0		0	0			0		0		0
Chr. Plac. Hyperplasia		0		0	0			0	84908	1		1
Focal or Subarachnoid Hemorrhage		0		0	0		84907	1	84882	1		2
Bacterial Microabscesses		0		0	0		84902	1	84902	1		1

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STUDY 9510-100

TABLE 4 (cont.). SUMMARY OF MICROSCOPIC FINDINGS IN THE BRAINS COMPARING CONTROL GROUP TO WITH MEGESTROL IN GROUP

FINDINGS	IN CONTROL (3 SECTION)		IN CONTROL (STOP SECTION)		Grand Total 3 Section and STOP SECTION IN CONTROL		IN MEGESTROL (3 SECTION)		IN MEGESTROL ((STOP SECTION) STOP EXAMINING PATHOLOGISTS SEE/DOE)		Grand Total 3 Section and SECTION IN MEGESTROL	
	Examining Pathologists SEE/DOE	Total	Examining Pathologists SEE/DOE	Total		Total	Examining Pathologists SEE/DOE	Total	Examining Pathologists SEE/DOE	Total		Total
Subarachnoid infiltrate, questionable leukemic or malignant lymphoma	04062,	1	04062	1	1	0	0	0	0	0	0	0
Bizarre intravascular cells--not primary brain tumor--relative perivascular and subarachnoid hemorrhage vs. tumor emboli		0		0	0	04001,	1	04001	1	1	1	1
Multiple diffuse infarcts		0		0	0		0	04072	1	1	1	1
Focal necrosis		0		0	0		0	04004	1	1	1	1
Neuritic tumor vs. pituitary adenoma		0		0	0	04005	1	04005	1	1	1	1
Focal lymphocytic proliferation inflammatory, not neoplastic		0		0	0		0	04003	1	1	1	1

Explanation of footnotes

- Control animal 04064 was diagnosed in our original interpretation in the single slide-3 section study as gliosis (likely on the edge of an infarct). With multiple stop section examination, it was clearly a small glioma.
- By comparison of the stop sections of 04002-14 with the original single 04003 slide-3 section study, the diagnosis of pituitary adenoma remnants could be made. With the single slide-3 section study only and no general autopsy or gross description, no diagnosis could have been made in a single section.

NDA 21-324

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September 17, 1992

STUDY 0490-100

TABLE 4 (cont'd), SUMMARY OF MICROSCOPIC FINDINGS IN ED BRAINS COMPARING CONTROL GROUP ON WITH SUBCUTANEOUS IN GROUP

Explanation of footnotes continued

1. In the original single slide-3 section study of animal 049062, findings of generalized enlargement of lymph nodes and spleen. slide-3 section examination of the brain on slide 049062, but with the lesion present in multiple slides of the step section study, it was clear that 049062 had a subarachnoid leukemia or malignant lymphoma in the subarachnoid space. Diagnosed malignant lymphoma with access to general autopsy could not be sure of a neoplastic lesion on the single slide-3 section examination.
4. The tumor diagnosed in the original 049057 single slide-3 section study, was not present in the step section study and re-examination of all slides of both sectionings led to the conclusion that 049057 single slide-3 section was a normal structure.
5. The pituitary adenoma remnants in 049037 single slide-3 section study on re-examination is still considered pituitary adenoma remnants. Nevertheless, on none of the step sections of 049037 or 049037-1A are there any pituitary adenoma remnants.
6. On re-examination of 049031 single slide-3 section, the same bizarre cells present in the step section are present, although fewer in number. On contacting for the general autopsy results, it was reported that 049031 had a large thyroid gland tumor on the left side of the head. It was likely has metastasized to the brain and is responsible for the brain findings in both the single slide-3 section and the step section study. The tumor was so large, the animal had to be euthanized at study week 100.

Mutagenicity studies.**A. Ames test**

Using the dose range of 1 μg to 10,000 μg budesonide per plate, the results from Ames Salmonella/microsome test were negative.

B. Mouse lymphoma test.

Budesonide was not mutagenic at the thymidine kinase locus in L5178Y mouse lymphoma cells under the conditions tested.

C. DNA-repair test using rat hepatocyte primary cell culture. Budesonide did not induce DNA repair (at the highest nontoxic concentration of 5×10^{-9} mg/ml) in the rat hepatocyte primary culture DNA-repair assay.**D. Chromosome aberration test.**

Budesonide was evaluated regarding its ability to induce structural chromosome aberrations in human lymphocyte culture with and without metabolic activation (S9-fraction of rat liver extract.) The results were negative under the experiment conditions used.

E. Mouse micronucleus test.

Budesonide was evaluated for its ability to induce micronuclei in polychromatic erythrocytes from mouse bone marrow. Under the condition of this assay, no dose related response was observed.

F. Sex-linked recessive lethal test in *Drosophila melanogaster*.

This test measures recessive lethal mutations in the X-chromosome, which represents about 20% of the entire genome of the fly. No mutagenic potential of budesonide was found in this test.

Exploratory Mutagenicity Studies of 21-Aldehyde of Budesonide, Related Glucocorticoids and Other Keto Aldehydes

The 21-aldehyde of budesonide (21-dehydrobudesonide) is formed from budesonide in the presence of water. It is also formed after incubation of budesonide with human and animal liver microsomes *in vitro*. As certain aldehydes have been found to possess genotoxic and carcinogenic properties, the sponsor examined the mutagenic potential of 21-dehydrobudesonide in Ames tests. The mutagenic potential of 21-dehydrocortisol was examined by the mouse lymphoma TK assay. The sponsor did not submit the full reports of the studies, and only summarized reports were submitted.

Ames Tests:

Methods: The Ames tests were performed using either the plate incorporation or the pre-incubation method with tester strains TA100, TA102 and TA104. Pre-incubation was for 60 minutes at 37°C. In some tests the enzymes glyoxalase I and II and 21-dehydrosteroid dehydrogenase were included. The aldehydes were pre-incubated with enzyme and co-factors for 60 minutes before addition to the bacteria. To find whether aldehydes are formed in aqueous solution to induce mutagenic effects, budesonide and prednisolone were dissolved in a mixture of DMSO and water and incubated for 4 days before adding to the bacteria.

Results: The amounts of 21-aldehyde of budesonide formed at the end of the incubation period were 1.4%, 1.0% and 0.5% in low, mid and high dose solutions that corresponded to 2.4, 4.3 and 3.2 µg/plate respectively. Budesonide, incubated for 4 days in an aqueous solution at concentrations up to 636 µg/plate, did not cause a significant increase in the number of revertant colonies. The 21-aldehyde of budesonide caused an increase in the number of revertant colonies in TA100 and TA104 both in the absence and presence of metabolic activation. The 21-aldehydes of prednisolone was mutagenic in all three strains while the aldehyde of triamcinolone acetonide was not mutagenic. The effect of the 21-aldehyde of budesonide on the number of revertant colonies in TA100 and TA104 tester strains is shown in the Table below.

Table: Ames test of 21-dehydrobudesonide without and with metabolic activation

Metabolic Activation	21-dehydrobudesonide (µg/plate)	Salmonella Strain	
		TA100	TA104
-S9	Solvent control	133±11	513±18
	20.6	136±5	554±11
	103	169±21	762±4
	206	199±17	909±11
	309	266±20	1144±1
	412	291±19	1282±9
	1030	388±30	1529±40
	2060	218±21	1199±47
	3090	260±44	0
	Positive Control	393±26	1369±25
	+S9	Solvent Control	114±20
20.6		135±6	476±16
103		152±7	647±10
206		173±20	827±5
309		239±8	1139±27
412		270±1	1257±34
1030		389±22	1821±16
2060		309±2	1854±65
3090		0	0
Positive Control		308±7	1404±84

In the preincubation method, the sponsor examined the mutagenic potential of 21-aldehydes of budesonide and triamcinolone acetonide in TA104 strain. The 21-aldehyde of budesonide was mutagenic

under the test conditions while the 21-aldehyde of triamcinolone acetonide was not mutagenic under similar conditions. There were degradations of both aldehydes during the 60-minute pre-incubation period; the aldehyde of budesonide was more degraded (49, 59, 64 and 88% unchanged drug in incubates containing 43.1, 215, 431 and 2150 $\mu\text{g}/\text{plate}$ respectively) than 21-dehydrotriamcinolone acetonide (52, 75, 92 and 100% unchanged drug at 43.2, 216, 432 and 2160 $\mu\text{g}/\text{plate}$ respectively). The number of revertant colonies in the absence of metabolic activation in the preincubation method is shown in the Table below.

Table: Ames test of 21-dehydrobudesonide in Salmonella strain TA104 in the absence of metabolic activation.

Metabolic Activation	21-Dehydrobudesonide ($\mu\text{g}/\text{plate}$)	TA-104: Number of revertant colonies
-S9	Solvent Control	442 \pm 23
	4.32	473 \pm 52
	43.2	634 \pm 22
	108	596 \pm 28
	216	643 \pm 150
	324	670 \pm 71
	432	797 \pm 45
	864	956 \pm 16
	2160	~400
	Positive Control	797 \pm 76

The mutagenic potential of the S- and R-epimer of 21-dehydrobudesonide in the three Salmonella tester strains TA100, TA102 and TA104 were examined. Similar to the diastereomer, the S-epimer of 21-dehydrobudesonide was mutagenic in TA100 and TA104 strains, and the lowest effective dose and the observed highest number of revertant colonies were similar. The R-epimer was negative in all strains tested.

Table: Ames test of the S-epimer of 21-dehydrobudesonide without metabolic activation

Metabolic Activation	21-dehydrobudesonide ($\mu\text{g}/\text{plate}$)	Salmonella Strain	
		TA100	TA104
-S9	Solvent control	101 \pm 27	399 \pm 32
	10.4	126 \pm 6	452 \pm 16
	104	182 \pm 4	852 \pm 27
	208	282 \pm 16	1111 \pm 57
	312	354 \pm 18	1307 \pm 3
	417	470 \pm 27	1641 \pm 35
	1040	699 \pm 53	2080 \pm 136
	2080	596 \pm 49	~1200
	3120	876 \pm 7	~1200
	Positive Control	276 \pm 13	929 \pm 11

In another experiment, the sponsor examined the potential mutagenic activity of the 21-aldehydes of prednisolone, budesonide and hydrocortisone after incubation for 60 minutes with glyoxylase I and II (plus co-factor) using strain TA104. The enzymes are involved in the metabolism of small

molecular weight keto aldehydes. The 21-dehydrobudesonide and 21-dehydrocortisol showed positive mutagenic response in the Ames test after incubation with the enzymes. Thus, the mutagenic activity of the 21-aldehydes was not affected by pre-incubation with the enzymes.

In summary, the Ames tests were conducted with 21-aldehydes of budesonide and related glucocorticoids (prednisolone and triamcinolone acetonide). The 21-aldehydes of budesonide (positive in TA100 and TA104) and prednisolone (positive in TA100, TA102 and TA104) were mutagenic both in the absence and presence of metabolic activation, while the 21-aldehyde of triamcinolone was not. Budesonide, incubated for 4 days in an aqueous solution, was not mutagenic in the Ames tests. In the pre-incubation method, the 21-aldehyde of budesonide was positive in TA104; there was significant degradation of budesonide during the 60 minutes pre-incubation period. The S-epimer of 21-dehydrobudesonide was positive in the Ames tests, while the R-epimer was negative.

Mutagenicity Evaluation of 21-Dehydrobudesonide using the Ames Test (SR99289-01)

Testing Laboratory: AstraGeneca R&D
Sodertalje, Sweden.

Date Started: September 30, 1999

Date Completed: October 22, 1999

GLP Compliance: A statement of compliance was included.

Drug Batch: The 21-dehydrobudesonide (21-DHB) does not exist in the solid form and therefore, ethanolhemiacetal was synthesized. AR-D113515XX is the ethanolhemiacetal of 21-dehydrobudesonide and generates 21-dehydrobudesonide in the presence of water. Batch No. 202/99.

Methods: _____

all
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Results: The 21-DHB was toxic to all the strains both in the presence and absence of metabolic activation at 150 µg/plate and above in the presence and absence of metabolic activation. The negative and additional control (methylene chloride) was negative while the positive controls showed marked increases in the number of revertant colonies. Like 21-dehydrocortisol, 21-DHB was also found to increase the number of revertant colonies in strain TA100 both in the presence and absence of metabolic activation. This increase was reproducible and concentration-dependent. Approximately two-fold increases were also seen in strain TA98 in both the tests in the presence and absence of metabolic activation. It is to be mentioned here that 21-DHC also showed positive response in TA98. The following table shows the results of mean number of revertant colonies per plate in Test 1 and Test 2:

Strain	S9	Solvent Control		Positive Control		Meth. Chloride		21-DHB (µg/plate)									
								45.1		150		451		1500		4510	
								1	2	1	2	1	2	1	2	1	2
TA1535	-	12	9	317	314	14	8	11	11	11	12	7	7	0	1	0	0
	+	10	7	253	304	17	14	9	13	14	12	10	8	8	2	0	0
TA100	-	105	98	391	353	96	83	126	130	165	159	194	191	342	337	545	484
	+	118	117	1042	972	108	103	134	128	161	154	172	178	361	332	473	472
TA102	-	405	460	1039	1157	418	402	501	463	483	530	330	354	250	320	0	0
	+	416	488	3218	3073	494	451	474	505	498	554	408	435	483	433	56	0
TA98	-	23	22	120	125	24	26	27	27	44	29	36	36	46	44	0	57
	+	43	44	1524	1690	36	34	39	35	44	54	48	41	69	67	61	62
TA1537	-	9	9	1070	1869	6	7	5	6	8	6	5	7	1	3	0	0
	+	9	16	76	85	8	14	11	13	12	14	9	7	2	4	0	0

The 21-DHB possessed mutagenic potential in the Salmonella mutagenicity test under the conditions of the experiment described above.

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SUMMARY AND EVALUATION:

Budesonide is a glucocorticoid, structurally similar to 16α -hydroxyprednisolone. It is a mixture of two epimers 22R and 22S in a 1:1 ratio. It has negligible mineralocorticoid activity. Budesonide had 17 times more affinity for glucocorticoid receptors of rat hepatocyte cytosolic fraction as compared with that of prednisolone. It caused an inhibition of T-cell proliferation and a decrease in the expression of interleukin-2 (IL-2) receptors. Budesonide also caused an inhibition of lipopolysaccharide-induced TNF- α release from rat alveolar macrophages and it was more potent than prednisolone, hydrocortisone or dexamethasone. It was found to cause a reduction of the allergen challenge-induced plasma exudation in rat ileum *in vitro* (EC_{50} , 2.45 nM) and was effective in reducing the lipopolysaccharide-induced TNF release from rat alveolar macrophages (ED_{50} , 0.16 nM for budesonide as compared with 5.0 nM for prednisolone). Budesonide was 30 times more potent than prednisolone in reducing the ileal inflammation *in vivo*. Thus, budesonide may exert its anti-inflammatory effect by down regulating IL-2 receptors and allergen specific immune responses. Budesonide caused a dose-dependent decrease in the stress-induced plasma cortisol levels in guinea pigs and it was $1/10^{\text{th}}$ as potent as beclomethasone dipropionate. The antiinflammatory effect of budesonide was examined in oxazolone-induced colitis in rats. Oral and intrarectal budesonide caused a suppression of the oxazolone-induced colonic inflammation in rats and decreased the myeloperoxidase activity of the colonic tissue. Budesonide CIR formulation caused reductions in colonic inflammation and decreased the number of mast cells in trinitrobenzene sulfonic acid induced colitis in hamsters. Intra-rectal administration of budesonide was effective in reducing the inflammatory scores and epithelial permeability in oxazolone-or acetic acid- induced colitis in rats. Budesonide was found to cause an inhibition of the DNA binding activity of NF- κ B and cell proliferation

in an intestinal epithelial cell line and pro-inflammatory cytokines secretion by IBD mononuclear phagocytes.

The biological activities of budesonide and its two major metabolites were assessed in the rat ear edema test. The ratios of potencies for budesonide, 6 β -hydroxybudesonide and 16 α -hydroxyprednisolone were 1: 0.007: 0.0005. The relative affinities of budesonide, 6 β -hydroxybudesonide and 16 α -hydroxyprednisolone for the glucocorticoid receptors were 1, 0.008 and 0.004 respectively.

In vivo pharmacokinetic studies were conducted in mice, rats, dogs and monkeys. In mice, rats and dogs, the drug was absorbed rapidly after oral administration of an ethanol/saline solution (T_{max} in mice 5-10 min, in rats 10-20 min and in dogs 1 hr). The oral absolute bioavailabilities of the drug were 35%, 32.4% and 9-19% in mice, rats and dogs respectively. Following oral administration in rats, maximum plasma concentration of the parent compound was reached rapidly (T_{max} , 0.50 hour); absorption following intrathecal administration was faster (T_{max} , 0.083 hour). Peak plasma radioactivity (4.079 ng.eq/ml) was attained 2.5 hours after dosing and slowly declined over time. In monkeys, oral bioavailability of budesonide from both plain and CIR capsules were limited. From the plain capsules, the bioavailability was 1.7 – 2.3% with T_{max} of 1.5 - 3.0 hours and $T_{1/2}$ of 2.44 – 3.37 hours in monkeys. From the CIR capsules, the bioavailability was 0.9 – 1.4% with a T_{max} of 3.0 hours and $T_{1/2}$ of 2.91 – 3.49 hours. The extremely low oral bioavailability of budesonide after oral dosing in monkeys could be related to its extensive first pass metabolism and/or low absorption. In dogs, absorption of inhaled budesonide powder was rapid, T_{max} was reached at 6 minutes after dosing. Following a single intrathecal dose, radioactivity was detected in the following tissues: thyroid, choroid plexus, gall bladder, lung, liver, trachea, nasal and gastric mucosa, salivary and lacrimal glands, pancreas, pituitary, pineal body, adrenal cortex, stomach and stomach contents. The maximum radioactivity was detected in the trachea and the stomach. The two major metabolites detected after intrathecal administration were 16 α -hydroxyprednisolone and 6 β -hydroxybudesonide. In the human liver, budesonide was specifically metabolized by the Cytochrome P4503A (CYP3A) enzyme isoform. In rats, the major part of the radioactivity was eliminated by the fecal route (67.8%) followed by the urinary route (~17%). In dogs, after i.v. or oral administration, 55-60% of the administered dose was excreted in feces and 12-20% was excreted in the urine. In rabbits, about 40% of the i.v. dose was excreted in the feces and about 46% of the administered dose were excreted in the urine.

The metabolism of the 22S and 22R epimers by the S9 fractions of rat liver and brain was examined *in vitro*. For each epimer, 5-6 metabolites were identified; three of the metabolites were identified as 16 α -hydroxyprednisolone and R- and S- epimers of 6 β -hydroxybudesonide. The degradation of budesonide by the rat S9 fractions was 6-8 times faster in the male rats as compared with the females.

In vitro, budesonide was found to form fatty acid conjugates with human lung and liver microsomes. Plasma protein binding of budesonide was very low; 12.8-14.55, 7.7-8.4%, 10.4-11.2%, 12.5-15.3% and 14.0-14.2% of drug was protein bound in human, rat, dog, rabbit and mouse respectively.

The formation and degradation of 21-aldehydes of budesonide and cortisol by human, mouse and rat liver were assessed after incubation of the tritium-labeled steroids with the mitochondrial and cytosolic fractions of the liver. There was 21-aldehyde formation when budesonide was incubated with human, rat and mouse liver mitochondria. The concentration of the 21-aldehyde was the highest in the human liver mitochondria followed by mouse and rat. The 21-aldehyde was also formed in human

microsomes, in concentrations higher than mitochondria. Incubation of the 21-aldehydes of budesonide and cortisol with human liver cytosol or mitochondria resulted in a rapid conversion to the parent compound; a small part of it was oxidized to the carboxylic acid.

To investigate whether budesonide is metabolized to reactive products in the rat liver and brain, the co-valent binding of the compound to rat tissue proteins was examined. Budesonide was bound to liver tissue proteins suggesting that budesonide may be converted to reactive metabolites that bind co-valently to liver tissues. In another study, the extent of reactive metabolites formed was assessed by measuring the binding of budesonide to tissue macromolecules of the rat liver and brain S9 fractions. In the liver, the 22R epimer was bound more than the 22S epimer; the binding of 22S epimer was higher in males as compared with females. No such differences in the binding were observed in brain fractions.

The acute toxicity studies of budesonide were conducted in mice, rats and dogs. When given to mice by s.c and oral routes, the MLDs for male mice were 25 mg/kg and 400 mg/kg by s.c. and oral routes respectively and the MLD for the female mice was 200 mg/kg by the oral route. In male mice the MLDs for budesonide (S1320), its S-epimer (S1321) and its R-epimer (S1322) were 50, 140 and 64 mg/kg respectively by the s.c. route. The three compounds produced similar clinical signs such as decreased motor activity, piloerection and generalized edema. In rats, the MLDs were 7 mg/kg and 20 mg/kg in males and females respectively when administered by the s.c. route. The clinical signs observed in rats were not reported.

In the 1-month oral gavage toxicity study in Sprague-Dawley rats, budesonide doses of 0, 0.05, 0.5, 5.0 and 50 mg/kg/day were used. Mortalities were observed at 5 mg/kg and higher doses, and were related to the toxicological effects of the drug such as gastric ulcerations, atrophy of the thymus, spleen, lymph nodes and adrenal glands. The body weight gains were decreased in both males and females receiving the drug. There were dose-related decreases in WBC in both sexes (up to 61% in males and 64% in females). Histopathology showed atrophy of adrenal glands and lymphoid organs and gastric ulcerations in most treated animals. There was increased fat deposition in the liver at 5 mg/kg and higher doses. The 'no effect dose' was not established in this 1-month oral toxicity study; however, the toxic effects observed are the typical glucocorticoid effects.

In the 3-month s.c. toxicity study in young (6-7 days old) SD rats, 0, 0.2, 2 and 20 µg/kg doses of budesonide were used. At 2 µg/kg, there were 7% decreases in weight gains; at 20 µg/kg, there were 18% and 16% decreases in weight gains in the males and females respectively. There were deaths in the high dose groups (2/15M and 2/15F) and were related to the toxic effects of the drug (gastric ulcerations, atrophy of the thymus, spleen, lymph nodes and adrenal glands). WBC counts were decreased by 22-27% in the high dose males and females. Histopathological examinations were done only in the control and the high dose groups and revealed hypoplasia of the mesenteric lymph nodes, thymus atrophy and slight changes in the growth plates of long tubular bones (distal femur, proximal tibia) with cartilage retention and sclerosis of the primary spongiosa, extramedullary hematopoiesis in the spleen, slight bone marrow hypoplasia, and lipid depletion of zona fasciculata of the adrenal in some of the high dose animals. The 2 µg/kg/day dose can be considered as the well tolerated dose in this study.

Two 26-week toxicity studies were conducted in rats. In the first 26-week s.c toxicity study in Wistar rats, doses of 0, 5, 20 and 80 µg/kg/day were used. The body weight gains in the males were reduced by 8%, 23% and 40% at low, mid and high doses respectively; in the females, the body weight

gains were reduced by 16% and 34% at mid and high doses respectively. There were significant reductions of the WBC (30 to 39%) and lymphocyte (41 to 47%) counts in both sexes receiving the high dose. Histopathological examinations revealed atrophy of lymphoid organs in most of the mid- and high-dose rats. In addition, there were acinar hyperplasia and secretion in mammary gland in mid and high dose animals of both sexes. There were moderate panacinar hepatocytic fine vacuolation in most of the high dose females and dilatation of the uterus (0/15, 1/15, 3/15 and 5/15 in control, low, mid and high doses respectively) at all doses. The 5 µg/kg/day dose was the well tolerated dose in this study as it produced only 14% reduction of the relative adrenal weights and moderate panacinar hepatocytic fine vacuolation and uterine dilatation (each in 1 of 15 animals).

In the second 26-week s.c. toxicity study in Wistar rats, 0, 0.01, 0.1 and 5.0 µg/kg/day doses of budesonide were used. The high dose produced 14-17% reduction in relative adrenal weight in the males. The 'no effect dose' was 0.1 µg/kg/day in this 26-week study.

In the one-month s.c. toxicity study in New Zealand White rabbits, 0, 25 and 100 µg/kg/day doses of budesonide were used. There were decreased body weight gains in females (23% and 42% at 25 and 100 µg/kg/day respectively). Histopathological changes included atrophy of the adrenal glands (4/6 at low and 6/6 at high dose), regression of the thymus (3/6 at low and 6/6 at high dose) and minimal endometrial hyperplasia in females (1/3 at low and 3/3 at high dose). The 'no effect dose' was not established in this study.

In the one-month oral gavage toxicity study in dogs (1/sex/group), budesonide doses of 0.01, 0.1 and 1.0 mg/kg/day were used. The high dose males and females had significantly higher serum alkaline phosphatase (males- 622% and females- 70%) and serum alanine aminotransferase (males-172% and females- 60%) levels. Plasma cortisol levels were significantly lower in the mid- and high- dose animals. Histopathological changes included atrophy of the adrenal glands and lymphoid organs and increased glycogen contents of the liver in the mid- and high- dose animals. The target organs of toxicity were the liver, adrenal glands and the lymphoid organs. The 0.01 mg/kg/day dose was the 'no effect dose' in this study and the 1.0 mg/kg/day can be considered as a well tolerated dose.

In the 4-week preliminary oral toxicity study with controlled ileal release budesonide capsules (CIR) in monkeys, doses of 100, 330 and 1000 µg/kg/day were used. No treatment-related changes were observed in any groups. The plasma drug levels increased with increasing doses and there was no accumulation of the drug with repeated dosing. No sex differences in the plasma drug concentrations were observed. No target organs of toxicity were identified and the 'no effect dose' was 1000 µg/kg/day.

In the 26-week toxicity study with budesonide CIR capsules in monkeys, oral doses of 0, 0.5, 2.0 and 5.0 mg/kg/day were used. The body weight gains were reduced by 52% and 44% in the males at mid and high doses respectively; in females, the body weight gains were reduced by 59%, 45% and 99% at low, mid and high doses respectively. Typical glucocorticoid toxicities such as decreased serum cortisol (up to 38%), increased serum glucose (18%), decreased thymus and adrenal weights were observed in the high dose animals. In addition, reduced cellularity of the thymus was observed in the high dose males (2 of 4). The highest tested dose (5.0 mg/kg/day) can be considered as a well-tolerated dose in this study, as the adverse effects were the typical glucocorticoid effects.

In the s.c. Segment I fertility and general reproductive performance study in rats, budesonide doses of 0, 5, 20 and 80 $\mu\text{g}/\text{kg}/\text{day}$ were used. At the low dose, there were no abnormal effects on the fertility and mating performance of the animals. However, at 20 $\mu\text{g}/\text{kg}/\text{day}$ and higher doses, budesonide was materno- and fetotoxic (decreased body weight gains in the dams and decreased prenatal viability and viability of the young at birth and during lactation).

In the s.c. Segment II teratology study in rats, budesonide doses of 0, 20, 100 and 500 $\mu\text{g}/\text{kg}/\text{day}$ were used. Budesonide was materno-toxic (decreased body weight gains, decreased food intake, piloerection and drowsiness), fetotoxic (increased resorptions, decreased pup viability, decreased litter size and decreased pup weights) and teratogenic (umbilical eventration [control: 0%, low: 0%, mid: 1.5%, high: 19.7% on the basis of fetuses], cleft palate [control: 0%, low: 0%, mid: 1.2% and high: 6.3% on the basis of fetuses] and reduced ossification of the skull bones in combination with multiple skeletal defects).

In the s.c. Segment II teratology study in rabbits, budesonide doses of 0, 5, 25 and 125 $\mu\text{g}/\text{kg}/\text{day}$ were used. Budesonide was materno-toxic (decreased body weight gains, decreased food consumptions, diarrhea, vaginal bleeding and abortion [two does in the mid dose group and all does in the high dose group had abortion]), fetotoxic (fetal weights at the low- and mid- dose groups were 16% and 27% lower than controls respectively) and teratogenic (1/117 fetus from the mid dose group had brachygnathia superior in combination with fusion of the frontal and nasal bones). The frequency of skeletal abnormalities were increased markedly in the high dose group (control: 14.3%, low: 18.3% and high: 52.7%).

In the s.c. Segment III pre- and post- natal developmental study in rats, budesonide doses of 0, 5, 20 and 80 $\mu\text{g}/\text{kg}/\text{day}$ were used. From treatment Day 15 to 21, the body weight gains were decreased by 10%, 35% and 59% at low, mid and high doses respectively. There were significant decreases in litter size, pup weights and pup viabilities at Days 7 and 21 in the high dose group. The mid dose group had similar effects but of lower magnitude. Physical development of the pups was also affected in the high dose group.

In the 91-week oral (via drinking water) carcinogenicity study in CD1 mice, budesonide doses of 0, 10, 50 and 200 $\mu\text{g}/\text{kg}/\text{day}$ were used. The doses were selected on the basis of a 3-month drinking water dose range-finding study in which doses of 0, 0.01, 0.05, 0.2 and 0.7 $\text{mg}/\text{kg}/\text{day}$ were used. The highest dose tested (200 $\mu\text{g}/\text{kg}/\text{day}$) was close to MTD. The body weight gains of the males were reduced by 9%, 16% and 38% at 0.05, 0.2 and 0.7 $\text{mg}/\text{kg}/\text{day}$ doses respectively and the body weight gains of the females were reduced by 23% and 33% at 0.2 and 0.7 $\text{mg}/\text{kg}/\text{day}$ respectively. So, the sponsor selected 0.2 $\text{mg}/\text{kg}/\text{day}$ as the highest dose in this 91-week carcinogenicity study. Budesonide was not carcinogenic to mice in this 91-week carcinogenicity study.

The sponsor has conducted 3 separate carcinogenicity studies with budesonide in rats.

In the first 104-week oral (drinking water) carcinogenicity study in SD rats, 0, 10, 25 and 50 $\mu\text{g}/\text{kg}/\text{day}$ doses were used. The doses were selected on the basis of a 3-month oral dose range-finding study in which doses of 0, 0.01, 0.05, 0.2 and 0.7 $\text{mg}/\text{kg}/\text{day}$ were used. The body weight gains were reduced by 14%, 28% and 54% in males at 0.05, 0.2 and 0.7 $\text{mg}/\text{kg}/\text{day}$ and by 10%, 22%, 50% and 82% in females at 0.01, 0.05, 0.2 and 0.7 $\text{mg}/\text{kg}/\text{day}$ doses respectively. So, the sponsor selected 10, 25 and 50 $\mu\text{g}/\text{kg}/\text{day}$ doses for the carcinogenicity study. The highest tested dose was close to MTD. There were significant increase in the incidences of gliomas in the male rats receiving budesonide (control: 2%, low dose: 0%,

mid dose: 6%, high dose: 14%; $p=0.0007$). In addition, primary hepatocellular neoplasms were also increased in the treated males (control: 4%, low dose: 6.1%, mid dose: 18% and high dose: 16%; $p=0.004$).

To confirm the above findings, the sponsor conducted a second carcinogenicity study using only the high dose (50 $\mu\text{g}/\text{kg}/\text{day}$) in the male Sprague-Dawley rats. In addition, two reference corticosteroids (prednisolone, 400 $\mu\text{g}/\text{kg}/\text{day}$, and triamcinolone acetonide, 15/10/5 $\mu\text{g}/\text{kg}/\text{day}$) were included in the study and only the brain and the liver were examined histologically. In this repeat study, there were no significant increases in the incidences of glioma; thus the findings of the first study was not reproducible. However, the combined incidences of hepatocellular adenomas and carcinomas were significantly increased in all treated groups.

In the third 104-week oral (drinking water) carcinogenicity study with budesonide in male Fischer-344 rats, the vehicle, budesonide (50 $\mu\text{g}/\text{kg}/\text{day}$), prednisolone (400 $\mu\text{g}/\text{kg}/\text{day}$) or triamcinolone acetonide (15 $\mu\text{g}/\text{kg}/\text{day}$) were used. This study had numerous GLP problems and was decided not to be used for safety assessment.

The findings of the rat carcinogenicity studies were submitted to the CAC for advice and recommendations. The CAC recommendations were included in the labeling of budesonide (Rhinocort) nasal inhaler which are as follows:

There was no evidence of a carcinogenic effect when budesonide was administered orally for 91 weeks to mice at doses up to 200 $\mu\text{g}/\text{kg}/\text{day}$ (600 $\mu\text{g}/\text{m}^2/\text{day}$).

In a 104-week carcinogenicity study in Sprague-Dawley rats a statistically significant increase in the incidence of gliomas was observed in male rats receiving 50 $\mu\text{g}/\text{kg}/\text{day}$ (300 $\mu\text{g}/\text{m}^2/\text{day}$) orally; no such changes were seen in male rats receiving doses of 10 and 25 $\mu\text{g}/\text{kg}/\text{day}$ (60 and 150 $\mu\text{g}/\text{m}^2/\text{day}$) or in female rats at any dose. Two additional 104-week carcinogenicity studies have been performed with oral budesonide at doses of 50 $\mu\text{g}/\text{kg}/\text{day}$ (300 $\mu\text{g}/\text{m}^2/\text{day}$) in male Sprague-Dawley and Fischer rats. These studies did not demonstrate an increased glioma incidence in budesonide treated animals as compared with concurrent controls or reference glucocorticosteroid treated groups (prednisolone and triamcinolone acetonide).

Compared with concurrent control male Sprague-Dawley rats there was a statistically significant increase in the incidence of hepatocellular tumors. This finding was confirmed in all three steroid groups (budesonide, prednisolone, triamcinolone acetonide) in the second study in male Sprague-Dawley rats.

The genotoxic potential of budesonide was tested in six different tests that include the Ames tests, L 5178Y/TK mouse lymphoma chromosomal aberration assay, *in vitro* UDS assay, *in vitro* human lymphocytes chromosomal assay, mouse micronucleus test and sex-linked recessive lethal test in *Drosophila melanogaster*. Budesonide had no mutagenic potential in any of the above mentioned tests.

The genotoxic potential of 21-dehydrobudesonide (21-DHB) was examined in the Ames tests, *in vitro* human peripheral blood lymphocytes chromosomal aberration assay, and *in vivo* mouse micronucleus assay by the i.v. route. 21-DHB was found to be positive in the Ames tests (in strains TA100 and TA98 both in the presence and absence of metabolic activation). In the human peripheral blood lymphocytes chromosomal aberration assay, the first test was positive in the absence of metabolic activation; however, the two repeated tests were negative. The *in vivo* mouse bone marrow micronucleus

assay was negative at i.v doses up to 47 µg/kg. The 21-aldehyde of cortisol (21-dehydrocortisol) was also positive in the Ames tests on 3 tester strains (TA100, TA102 and TA98).

The sponsor submitted NDA 21-324 for budesonide controlled ileal release (Budesonide CIR) capsules (Entocort Capsules) for the treatment of mild to moderate Crohn's disease affecting the ileum and/or the ascending colon. Budesonide is available in the US (Rhinocort Nasal Inhaler and Pulmicort Turbuhaler) for the treatment of allergic rhinitis and for the maintenance therapy of asthma. Oral bioavailability of budesonide from the CIR capsules is very limited; only 0.9% to 1.4% of the administered dose is bioavailable in monkeys. Most of the adverse effects observed in preclinical toxicology studies (such as gastric ulcerations, atrophy of the thymus, spleen, lymph nodes and adrenal glands, decreased WBC levels and decreased plasma cortisol levels) are related to the glucocorticoid activity of the compound. Although, budesonide had typical glucocorticoid side effects in experimental animals, the effects were milder as compared with other glucocorticoids. In rats, budesonide was compared with prednisolone and dexamethasone for their effects on the thymus; the two glucocorticoids caused reductions of the thymus weights or the number of T-cells in the thymus, while budesonide had no effect on the thymus. Budesonide was less effective (0.1 times) than beclomethasone in suppressing the stress-induced increase in the plasma cortisol levels in guinea pigs. The sponsor has adequately characterized budesonide and conducted sufficient preclinical oral and subcutaneous toxicity studies in different species.

RECOMMENDATION:

From a preclinical standpoint, the NDA application is approvable.

Sushanta Chakder, Ph. D.

Date

Comments:

Jasti B. Choudary, B.V.Sc., Ph.D.

Date

cc:
Original NDA