NCI, DCPC
Chemoprevention Branch and Agent Development Committee

CLINICAL DEVELOPMENT PLAN:

18β-GLYCYRRHETINIC ACID

DRUG IDENTIFICATION

CAS Registry No.: 471-53-4

CAS Name (9CI): (3β,20β)-3-Hydroxy-11-oxo-olean-12-en-29-oic Acid

Synonyms:
Biosine
Enoxolone
Glycyrrhetic Acid
Glycyrrhetin
Glycyrrhetinic Acid
Glycyrrhitinic Acid

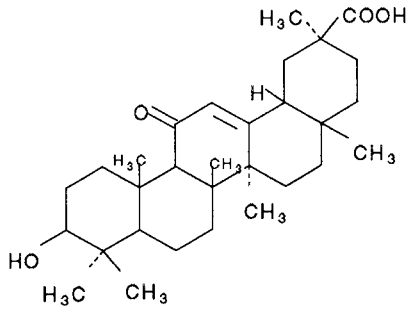
Related Compounds:

18α-Glycyrrhetinic Acid

Glycyrrhizin (CAS No. 1405-85-3)
- (3β,20β)-20-Carboxy-11-oxo-30-norolean-12-en-3-yl 2-*O*-β-D-Glucopyranuronosyl-α-D-glucopyranosiduronic Acid (9CI)
- Glycyrrhetinic Acid Glycoside
- Glycyrrhizic Acid
- Glycyrrhizinic Acid

Carbenoxolone (CAS No. 5697-56-3)
- Biogastrone® (Active Ingredient)
- (3β,20β)-3-(3-Carboxy-1-oxopropoxy)-11-oxoolean-12-en-29-oic Acid
- Bioral Gel® (Active Ingredient)
- Duogastrone® (Active Ingredient)
- Glycyrrhetinic Acid Hydrogen Succinate
- 18β-Glycyrrhetinic Acid Hydrogen Succinate
- 3β-Hydroxy-11-oxoolean-12-en-30-oic Acid Hydrogen Succinate

Structure:



EXECUTIVE SUMMARY

18β-Glycyrrhetinic acid is a triterpenoid occurring naturally in licorice root (*Glycyrrhiza* spp.) as the diglucuronide conjugate glycyrrhizin. Glycyrrhizin is metabolized to 18β-glycyrrhetinic acid by bacteria in the human gastrointestinal tract before absorption. In the U.S., licorice extract containing glycyrrhizin is a GRAS flavoring agent and enhancer with specific limits on final content in foods [1,2]; it has been used outside the U.S. as an anti-inflammatory agent. The water-soluble 3β-*O*-hemisuccinate derivative, carbenoxolone, has been used clinically in Europe, Australia and Japan for treatment of gastric ulcer and esophagitis [3–5].

The evaluation of glycyrrhetinic acid as a potential chemopreventive drug is based on its demonstrated efficacy in animal cancer inhibition studies. The chemopreventive efficacy of glycyrrhetinic acid has been attributed primarily to inhibition of many activities associated with tumor promotion and progression. The most widely observed of these is hydrocortisone-like antiinflammatory effects in animal models of edema and arthritis [6–10], which do not seem to result from inhibition of prostaglandin synthesis [9–14]. In fact, carbenoxolone has been shown to inhibit 15-hydroxyprostaglandin dehydrogenase, which increases local prostaglandins and promotes healing of ulcers by stimulation of mucus secretion and cell proliferation [reviewed in 15].

Glycyrrhetinic acid also inhibits effects induced by the tumor promoter TPA, such as stimulation of phospholipid metabolism [16,17], induction of ODC activity [18], hexose transport [19], and adhesion of HL-60 promyelocytic leukemia cells [20]. The agent appears to bind to the TPA receptor in the epidermal cell membrane and inhibits protein kinase C activity [14,21]. Other antipromoting activities of glycyrrhetinic acid include inhibition of free-radical generation and lipid peroxidation [22], and enhancement of cAMP levels by inhibition of phosphodiesterase [23,24]. It has also demonstrated antiviral activity by inactivation of particles and induction of interferon [25,26].

Glycyrrhetinic acid may also act as an anti-initiator, as demonstrated by inhibition of indirect acting mutagens (*e.g.*, B(*a*)P, 2-AAF, AFB_1) in the Ames/*Salmonella* assay [27,28]. Concomitantly, specific effects on hepatic microsomal drug metabolism (*e.g.*, AHH, 7-ethoxycoumarin-*O*-deethylase) and mutagen-DNA binding have been demonstrated *in vivo* [29–31].

Chemopreventive efficacy has been demonstrated for both 18β-glycyrrhetinic acid and carbenoxolone. Glycyrrhetinic acid was effective in rat mammary gland and mouse colon models of carcinogenesis, and it inhibited the development of premalignant lesions in rat colon (foci of aberrant crypts) and mouse liver and skin (papillomas) *in vivo*, and in mouse mammary organ culture *in vitro* [hyperplastic alveolar nodules (HAN)]. Carbenoxolone inhibited carcinogenesis in rat colon and mammary glands. These studies are adequate to support development of 18β-glycyrrhetinic acid or carbenoxolone as a chemopreventive drug.

Preclinical one-year toxicity studies on glycyrrhetinic acid contracted by the CB have been completed in rats and dogs. Preliminary results suggest that the adverse effects observed in humans were not noted, *e.g.*, hypertension, hypokalemia, sodium retention and edema. Reproductive and carcinogenicity studies will be required for long-term clinical trials.

A CB-sponsored Phase I trial of glycyrrhetinic acid found no evidence of significant toxicity after single 300–700 mg/m^2 (*ca.* 0.02–0.04 mmol/kg-bw) doses. Multiple doses of 400 and 500 mg/m^2 qd (*ca.* 0.02 and 0.03 mmol qd) led to hypertension and hypokalemia significant enough to reduce or discontinue treatment. These apparent mineralocorticoid excess symptoms have also been reported in

the literature. The mechanism is inhibition of 11β-hydroxysteroid dehydrogenase, which normally inactivates corticosterone and cortisol. The combined effects of these glucocorticoids with aldosterone at the mineralocorticoid receptor results in sodium retention, edema, and hypertension.

No further Phase I or II trials are planned for 18β-glycyrrhetinic acid or carbenoxolone at this time. The identification of an effective dosing regimen with acceptable side effects is a criterion for continued development of glycyrrhetinic acid. Because of apparent drug accumulation, a regimen with dosing on alternate days should be assessed.

The tablet formulation (Pioneer Pharmaceuticals) supply of 18β-glycyrrhetinic acid (MacAndrews and Forbes, supplier) used in the Phase I trial has expired. Carbenoxolone (Winthrop) may be purchased as tablets, capsules or gel in the U.K. as a therapeutic for gastric and mouth ulcers.

PRECLINICAL EFFICACY STUDIES

In CB-sponsored studies, both glycyrrhetinic acid and carbenoxolone inhibited tumors in the colon and mammary glands. In the MNU-induced rat mammary gland model, glycyrrhetinic acid (1 g/kg diet, or *ca.* 0.1 mmol/kg-bw/day) reduced carcinoma multiplicity at a lower dose than carbenoxolone (2 g/kg diet, or *ca.* 0.2 mmol/kg-bw/day). However, both agents inhibited premalignant lesions (HAN) in the DMBA-induced mouse mammary organ culture *in vitro* assay at the same concentration (0.001 μM). Glycyrrhetinic acid is currently on test in the DMBA-induced rat mammary gland model of carcinogenesis *in vivo*.

Carbenoxolone has been tested in combination with other agents in the MNU-induced rat mammary cancer model. At a dose which was ineffective alone (1,750 mg/kg diet, or *ca.* 0.15 mmol/kg-bw/day), the agent significantly inhibited tumor incidence when administered with DFMO, oltipraz, and DFMO + tamoxifen.

Both glycyrrhetinic acid and carbenoxolone inhibited colon carcinogenesis, but in different models: glycyrrhetinic acid in the MAM acetate-induced mouse (800 mg/kg diet, or *ca.* 0.2 mmol/kg-bw/day) and carbenoxolone in the AOM-induced rat (1,200 mg/kg diet, or *ca.* 0.1 mmol/kg-bw/day). Glycyrrhetinic acid was also effective against premalignant lesions (aberrant crypt foci) in the AOM-induced rat colon (2.5 g/kg diet, or *ca.* 0.3 mmol/kg-bw/day).

In other models used by the CB, glycyrrhetinic acid (0.1% in diet, or *ca.* 0.25 mmol/kg-bw/day) inhibited DMBA-initiated/TPA-promoted skin papilloma multiplicity in the mouse. In published reports, topical application of glycyrrhetinic acid decreased the incidence, multiplicity and size of DMBA-induced/TPA- or teleocidin-promoted mouse skin papillomas [16,28,32]. It is in mouse skin that *in vivo* inhibition of ODC activity (TPA-induced), TPA membrane binding (TPA-induced) and carcinogen-DNA binding (DMBA, B(*a*)P) has been demonstrated [18,21,28]. Carbenoxolone is currently being tested against putative premalignant lesions (GGT-positive foci) in the DMBA-induced hamster buccal pouch model.

Finally, glycyrrhetinic acid inhibited the development of 3'-Me-DAB-induced premalignant changes in mouse hepatocytes *in vivo* [33]. In a published review, glycyrrhizin has been reported to inhibit mouse lung and liver tumorigenesis [14]. The multiplicity of spontaneous hepatomas in C_3H/He mice decreased 50% with addition of 5 mg glycyrrhizin per 100 ml drinking water. The same dose of glycyrrhizin in the drinking water of 4NQO-initiated/glycerol-promoted ddY mice during promotion reduced lung tumor multiplicity by 75%.

In CB-sponsored *in vitro* assays, glycyrrhetinic acid inhibited B(*a*)P-induced morphological changes and ODC induction in rat tracheal epithelial cells. Both glycyrrhetinic acid and carbenoxolone were effective against anchorage-independent growth of human lung tumor A427 cells, another cell transformation assay.

PRECLINICAL SAFETY STUDIES

Safety One-year CB-contracted toxicology studies of glycyrrhetinic acid in rats and dogs have been completed. At doses of 100, 300 and 1,000 mg/kg-bw/day (0.2, 0.6 and 2.1 mmol/kg-bw/day, ig), no significant changes in body weight, food consumption, behavior, appearance, clinical chemistry or histology were observed in the rat. Serum potassium and chloride levels were only slightly reduced at a few intervals in the high-dose group. Thus, the NOEL is 1,000 mg/kg-bw/day in the rat under the conditions of the study. In contrast, dogs given the highest dose displayed decreased body weight gain and increased serum alkaline phosphatase and alanine aminotransferase values. Feces of dogs given the two highest doses were whitish-yellow in color, possibly indicating elimination of the agent by this route. The MTD appears to be 300 mg/kg-bw/day (0.6 mmol/kg-bw/day) for this species.

Toxicity has also been noted in the agent control group in a CB-funded efficacy study in female SENCAR mice. Deaths occurred after 3 and 10 weeks in mice receiving 0.2% glycyrrhetinic acid in the diet (*ca.* 0.6 mmol/kg-bw/day) and topical acetone. Clinical signs included weight loss, hunched posture, hypoactivity, and rough fur. Gross necropsy revealed yellow appearance of the skin, small, pale rough kidneys, distended stomach, and dark adrenal glands and lungs.

No studies specifically evaluating the toxicity of glycyrrhetinic acid were identified in the published literature. The oral 10-week MTD for the parent compound glycyrrhizin in mice was reported to be 407 mg/kg-bw/day in females and 229 mg/kg-bw/day in males [34]. The NOEL for 16-week studies in mice and rats was 90 mg/kg-bw/day [1]. The primary treatment-related effects were altered water consumption, decreased weight gain, increased blood pressure, and decreased serum potassium. At necropsy, rats fed 2,600 mg/kg-bw/day for 16 weeks had increased heart and kidney weights. A 96-week carcinogenicity study of glycyrrhizin in mice was reported in the literature [34]. At estimated doses of 0.15% in drinking water (*ca.* 229 mg/kg-bw/day) in males and 0.3% in drinking water (407 mg/kg-bw/day) in females, no changes in tumor incidence, latency or type were observed compared with controls. The only published report of a study assessing reproductive or developmental toxicity involved ammoniated glycyrrhizin. Following administration of 21.3–679.9 mg/kg-bw/day in drinking water to female rats on gestation days 7–17, a dose-related increase in embryolethality and minor anomalies was observed [35]. The latter included external hemorrhage, sternebral variants, and renal ectopy.

The CB is not evaluating the toxicity of carbenoxolone at present. A published review stated that no adverse effects or tumorigenicity were obtained in rodent, dog or monkey studies [5]. The phenomenon of sodium retention was not observed; however, data were not available for review.

ADME In published studies, bolus iv administration of 18β-glycyrrhetinic acid to rats and rabbits appeared to follow a two-compartment model with dose-dependent terminal disposition [reviewed in 36,37]. In rats, plasma drug concentration increased with increasing dose, and the elimination $t_{½}$ ranged from 15 min to 2 hrs. In contrast, plasma Cl decreased with increasing dose, and elimination was saturable. This suggests that the pharmacokinetics of glycyrrhetinic acid is dose-dependent due to Michaelis-Menten elimination. Biliary excretion of unchanged drug and its metabolites has been reported as 17–50% of the dose, with negligible enterohepatic circulation [37,38]. Excretion of intact glycyrrhetinic acid in urine was also negligible. The agent appears to be extensively metabolized, including microsomal hydroxylation [39]. This should increase excretion; however, the identity of the saturating elimination mechanism is unknown.

Little data are available on the preclinical pharmacokinetics of orally administered glycyrrhetinic acid. An ig dose of the glycone glycyrrhizin appears to be well absorbed [40], but requires hydrolysis by gastrointestinal bacteria [41].

Plasma drug levels were obtained in the CB-contracted one-year chronic toxicity study. They did not show a dose relationship in both rats and dogs, nor did plasma levels increase with length of administration. For example, in rats, the mean plasma glycyrrhetinic acid concentrations in the 1,000 mg/kg-bw/day group were 1.9-fold higher than the 300 mg/kg-bw/day group and 3.8-fold higher than the 100 mg/kg-bw/day group.

In a CB-funded efficacy study of carbenoxolone in combination with other agents, serum levels were measured in female rats for 25 weeks. In the agent control group given 3,500 mg carbenoxolone/kg diet (*ca.* 0.3 mmol/kg-bw/day), serum levels were highest at one week (9 μg/ml), but fell by two-thirds at 25 weeks. At a lower dose of 1,750 mg/kg diet (*ca.* 0.15 mmol/kg-bw/day), serum levels remained at <1 μg/ml for the entire 25 weeks. Although drug levels were not evaluated in rats given carbenoxolone alone, the three different combinations did not appear to alter the pharmacokinetics. This suggests that the drug does not accumulate in the rat with chronic intake, which may be the reason that adverse effects were not seen. However, these data may not be representative of the human response. The gastrointestinal microflora of rats, mice and rabbits hydrolyze carbenoxolone to glycyrrhetinic acid and succinate before absorption; in dogs, ferrets, monkeys and humans, it is absorbed intact, ex-creted into the bile after conjugation with glucuronic acid, and undergoes extensive enterohepatic circulation.

CLINICAL SAFETY: PHASE I STUDIES

A CB-sponsored Phase I clinical trial (Dr. V. Vogel, University of Texas, M.D. Anderson Cancer Center) of glycyrrhetinic acid in patients with previous breast or colon cancer has ended. Single-dose

pharmacokinetics and toxicity data were obtained.

Drug Effect Measurement A drug effect measurement is still being sought. In rats, inhibition of 11β-hydroxysteroid dehydrogenase has been measured in liver, testes and lung [42]. It is unknown if more accessible tissues (*e.g.*, skin) have high enzyme activities in humans. Alternate measurements which are feasible for clinical trials include prostaglandin levels or ODC activity in skin punch biopsies.

Safety In the single-dose portion of the Phase I trial, patients with previous breast cancer received 300–700 mg glycyrrhetinic acid/m^2 (*ca.* 0.02–0.04 mmol/kg-bw) without evidence of significant toxicity [43]. Serum potassium levels were decreased from baseline at 4–6 hrs post-dosing; however, they began to return to baseline at 8 hrs. In the multidose study, patients (n=6) receiving 400 and 500 mg/m^2 qd (*ca.* 0.02 and 0.03 mmol/kg-bw qd) experienced hypertension or hypokalemia that necessitated dose reduction or discontinuance; only 2 patients completed the 16-week treatment schedule.

In the published literature, licorice extract and carbenoxolone have been reported to have similar effects, including hypertension, sodium retention with edema, hypokalemia, and decreased plasma renin and angiotensin II and urinary aldosterone levels [44–46]. In studies with normal subjects, daily doses of at least 100 mg glycyrrhizin or its ammonium salt for periods of 3 days to 4 weeks were required [45]. Although recovery from the hypertension and electrolyte imbalance caused by licorice may occur within several days to 2 weeks after intake is discontinued, several case reports describe patients in which the effects were prolonged for 1.5–4 months.

These adverse effects were also typical in a double-blind study evaluating the efficacy of Duogastrone® in the treatment of duodenal ulcers [47]. Duogastrone® is a formulation of carbenoxolone sodium in which the capsule is designed to resist degradation by gastric juice. Seventy-one patients with duodenal ulcer received the drug at 200 mg qd for 6 weeks. Significant side effects included edema, hypokalemia, and increased blood pressure; the incidence of side effects was low and none was severe except for 2 patients. However, in a second study of gastric ulcer patients, doses of 300 mg carbenoxolone sodium qd for one week, then 150 mg qd for 5 weeks, produced hypokalemia or edema in 44% of subjects [3]. Adverse effects were more frequent in the elderly due to decreased elimination. Individual cases of severe side effects from carbenoxolone, especially due to hypokalemia, have also been reported [48]. Possible sequelae of hypokalemia include headache, edema, cardiac failure, angina, hypertension and seizures [3]. No studies of longer duration were found in the literature.

The effects of glycyrrhetinic acid and related compounds resemble those of the mineralocorticoid aldosterone. The mechanism appears to be inhibition of 11β-hydroxysteroid dehydrogenase [49], which normally inactivates corticosterone and cortisol. These glucocorticoids and aldosterone share affinity for the mineralocorticoid receptor that regulates sodium and potassium transport in the kidneys; thus, their combined effect at the receptor results in sodium retention, edema, and hypertension [12].

ADME In the Phase I trial, plasma levels of glycyrrhetinic acid were detectable (0.9 μg/ml) after single doses <500 mg/m^2 (*ca.* <0.03 mmol/kg-bw); at 500 mg/m^2, plasma levels peaked at 4.6 μg/ml after 4 hours [43]. Published data suggest that elimination time, C_{max}, and AUC increase with dose, with biphasic decay of the concentration-time curve at doses >500 mg [37,50]. For example, AUC values in healthy volunteers were 19.8, 40, and 65.2 mg·hr/L after single doses of 500, 1,000 and 1,500 mg (*ca.* 0.02, 0.03, and 0.05 mmol/kg-bw), respectively [50]. The second elimination phase $t_{½}$s at the two higher doses were 11.5 and 38.7 hr, respectively. Twenty-four hour urinary elimination of glycyrrhetinic acid and its glucuronides was less than 1% of the administered dose. In contrast to rats, enterohepatic circulation appears to be more extensive in humans.

Limited multidose pharmacokinetics data from the Phase I trial suggested approximately 2-fold drug accumulation over 2–4 months of treatment with 18β-glycyrrhetinic acid based on AUC values. Published multidose studies of carbenoxolone also demonstrated accumulation of the drug at the therapeutic dose of 300 mg qd (*ca.* 0.008 mmol/kg-bw qd), especially in the elderly. The plasma $t_{½}$ was 19 hr in subjects <40 years of age and 56 hr in subjects >65 years of age [reviewed in 5]. A combination of enterohepatic circulation, slow clearance, and long plasma $t_{½}$ led to accumulation in the latter population. Reduction of the dose to 150 mg qd (*ca.* 0.004 mmol/kg-bw qd) was reported to overcome the problem.

CLINICAL EFFICACY: PHASE II STUDIES

No Phase II efficacy trials of 18β-glycyrrhetinic

acid or carbenoxolone have been sponsored by the CB. A published review stated, however, that the efficacy of glycyrrhizin in reducing hepatoma incidence is being evaluated in a clinical trial using liver cirrhosis patients [14]. No further information is available.

PHARMACODYNAMICS

The minimal effective chemopreventive doses in rat studies were 0.1 mmol/kg-bw/day for glycyrrhetinic acid and 0.08 mmol/kg-bw/day for carbenoxolone. These doses are approximately 20-fold lower than the one-year NOEL in the same species (1,000 mg/kg-bw/day, or 2.1 mmol/kg-bw/day). Based on this ratio, it would appear that an effective human dose without hypokalemia could be attained. However, the therapeutic dose of carbenoxolone (300 mg qd, or *ca.* 0.008 mmol/kg-bw/day), which is 10-fold lower than the dose that inhibits rat colon carcinogenesis, produces appreciable incidences of hypokalemia. The rat may not be the best model for human pharmacokinetics and safety of either agent. In the rat, plasma glycyrrhetinic acid shows a slow second elimination phase as in humans, but hypokalemia was not observed in preclinical toxicity assays. In contrast to humans, enterohepatic circulation is negligible. Hepatic metabolism and excretion may differ between species, but further information is needed. In addition, carbenoxolone is metabolized differently. In humans, it is absorbed intact, excreted into bile as the glucuronide, and undergoes enterohepatic circulation. In the rat, carbenoxolone is hydrolyzed to glycyrrhetinic acid before absorption, which does not appear to accumulate.

PROPOSED STRATEGY FOR CLINICAL DEVELOPMENT

Drug Effect Measurement Issues

No obvious drug effect measurements exist for glycyrrhetinic acid and related compounds, except for those related to toxicity (*e.g.*, serum potassium). If clinical development of glycyrrhetinic acid continues, assessment of protein kinase C, 15-hydroxyprostaglandin dehydrogenase, prostaglandins, or ODC activity could be used as endpoints. In the case of DFMO, for example, ODC activity in skin punch biopsies shows potential as a drug effect measurement.

Safety Issues

The toxicity of glycyrrhetinic acid may become significant with chronic intake and accumulation. If the drug is developed further, multidose pharmacokinetics should be characterized more fully. In addition, the effect of concomitant treatment with potassium salts or a diuretic should be assessed.

Since the adverse effects of glycyrrhetinic acid in humans were not reproduced in the CB-funded preclinical toxicity studies, additional special animal studies may be needed to assess effects on sodium/potassium balance. The species of animal should be carefully chosen, since rodents may metabolize glycyrrhetinic acid and carbenoxolone differently.

If carbenoxolone is developed further, information on dosing and post-marketing adverse events would be available.

Pharmacodynamics Issues

Titration of the effects of 11β-hydroxysteroid dehydrogenase inhibition against chronic intake of glycyrrhetinic acid should be performed if the drug is developed further. The associated toxicities can be significant in some subjects, such as hypokalemia and hypertension. In the Phase I trial of glycyrrhetinic acid, these effects occurred at doses of 400 and 500 mg/m^2 qd (*ca.* 0.02 and 0.03 mmol/kg-bw); the therapeutic dose for carbenoxolone in the treatment of peptic ulcers is 300 mg qd (*ca.* 0.008 mmol/kg-bw), or 25-fold lower. The dose used in clinical trials should avoid significant accumulation of glycyrrhetinic acid with chronic treatment, perhaps by using a qod dosing regimen.

Regulatory Issues

The full complement of preclinical toxicity studies on glycyrrhetinic acid have not been performed by the CB; however, the in-life phase of the one-year study in two species has been completed. If the final report demonstrates that a NOEL has been achieved in rats, a Phase II clinical trial of six months could be undertaken. For longer clinical trials, carcinogenicity and reproductive/developmental studies will need to be contracted.

Winthrop initiated an NDA submission for carbenoxolone as a gastric ulcer therapeutic in the 1970's. This was abandoned, however, due to the approval of cimetidine for this use. If clinical development of carbenoxolone as a cancer chemopreventive drug is undertaken, preclinical toxicol-

ogy studies performed for submission of the NDA may be adequate to allow Phase II and III trials.

Supply and Formulation Issues

The tablet formulation of 18β-glycyrrhetinic acid was prepared from bulk drug (MacAndrews and Forbes) by Pioneer Pharmaceuticals (Irvington, NJ). The expiration date for the 55 and 110 mg tablets has been reached. Patents for use of this agent as a metastasis inhibitor and as a cancer therapeutic have been issued in the U.S. and Japan, respectively.

Carbenoxolone may be purchased directly from Winthrop as a capsule (50 mg), tablet (20, 50 mg) or gel (2%) [3]. A placebo would need to be prepared for Phase II trials. No current U.S. patents exist for use of carbenoxolone except as an antiviral treatment for blood products.

Intermediate Biomarker Issues

In preclinical studies, glycyrrhetinic acid inhibited the development of premalignant lesions in skin (ig, top), colon and liver *in vivo* and in mammary glands *in vitro*. It also decreased ODC activity in skin *in vivo* and in tracheal epithelial cells *in vitro*. Carbenoxolone inhibited premalignant mammary lesions *in vitro* and is on test against GGT-positive foci in the hamster buccal pouch model *in vivo*. In future preclinical testing, the effect of this agent on other classes of biomarkers (*e.g.*, proliferative, differentiation, genetic) in these lesions should be ascertained [51]. This may also help to clarify its mechanism of action, especially in models without TPA promotion.

Clinical Studies Issues

No trial for either agent is planned at this time. Several protocol issues for investigation in future trials, if development is pursued, have already been suggested, such as the pharmacodynamics of the adverse effects, a reliable drug effect measurement, and the effect of concomitant potassium or diuretic treatment. In addition, Phase II trials could assess the effect of glycyrrhetinic acid on histological/premalignant biomarkers. These would be short-term trials with lower potential for drug accumulation after oral administration. Topical absorption of glycyrrhetinic acid on premalignant lesions could also be assessed to circumvent the systemic toxicity; potential trial subjects include actinic keratosis, Barrett's esophagus, CIN with HPV, or oral leukoplakia patients. In preclinical studies, the agent inhibited the development of skin papillomas with either topical or intragastric administration.

Clinical information on pharmacokinetics and adverse events may be available from Winthrop if carbenoxolone is developed as a cancer chemopreventive drug. The gel formulation of carbenoxolone (Bioral Gel®) for mouth ulcers could be used in a Phase II trial in oral leukoplakia patients.

REFERENCES

1. U.S. Food and Drug Administration. GRAS status of licorice (*glycyrrhiza*), ammoniated glycyrrhizin, and monoammonium glycyrrhizinate. *Fed. Reg.* 48: 54983–54990, 1983.
2. U.S. Food and Drug Administration. GRAS status of licorice (*glycyrrhiza*), ammoniated glycyrrhizin, and monoammonium glycyrrhizinate. *Fed. Reg.* 50: 21043–21045, 1985.
3. Reynolds, J.E.F. and Prasad, A.B. (eds.), *Martindale. The Extra Pharmacopoeia*, 28th ed., London: The Pharmaceutical Press, pp. 77–78, 494, 691–692, and 1673, 1982.
4. Altman, D.F. Drugs used in gastrointestinal diseases. In: Katzung, B.G. (ed.), *Basic and Clinical Pharmacology*, 4th ed., Norwalk, Connecticut: Appleton and Lange, pp. 793–801, 1989.
5. Eason, C.T., Bonner, F.W., and Parke, D.V. The importance of pharmacokinetic and receptor studies in drug safety evaluation. *Regul. Toxicol. Pharmacol.* 11: 288–307, 1990.
6. Finney, R.S.H and Somers, G.F. The anti-inflammatory activity of glycyrrhetinic acid and derivatives. *J. Pharm. Pharmacol.* 10: 613–620, 1958.
7. Tangri, K.K., Seth, P.K., Parmar, S.S., and Bhargava, K.P. Biochemical study of anti-inflammatory and anti-arthritic properties of glycyrrhetic acid. *Biochem. Pharmacol.* 14: 1277–1281, 1965.
8. Bhargava, K.P., Nath, R., and Tangri, K.K. Effects of anti-inflammatory agents on plasma fibrinolytic activity. *Indian J. Med. Res.* 64: 1094–1098, 1976.
9. Capasso, F., Mascolo, N., Autore, G., and Duraccio, M.R. Glycyrrhetinic acid, leucocytes and prostaglandins. *J. Pharm. Pharmacol.* 35: 332–335, 1983.
10. Inoue, H., Mori, T., Shibata, S., and Koshihara, Y. Modulation by glycyrrhetinic acid derivatives of TPA-induced mouse ear oedema. *Br. J. Pharmacol.* 96: 204–210, 1989.
11. Inoue, H., Saito, H., Koshihara, Y., and Murota, S.-I. Inhibitory effect of glycyrrhetinic acid derivatives on lipoxygenase and prostaglandin synthetase. *Chem. Pharm. Bull.* 34: 897–901, 1986.
12. Baker, M.E. and Fanestil, D.D. Licorice, computer-based analyses of dehydrogenase sequences, and the regulation of steroid and prostaglandin action. *Mol. Cell. Endocrinol.* 78: C99–C102, 1991.

13. Franco, L., Manara, P., Erbetti, I., and Velo, G.P. Anti-ulcer activity of carbenoxolone and ISF 3401 on PGE_2 release in rat gastric mucosa. *Pharmacol. Res.* 27: 141–150, 1993.
14. Nishino, H. Antitumor-promoting activity of glycyrrhetinic acid and its related compounds. In: Wattenberg, L., Lipkin, M., Boone, C.W., and Kelloff, G.J. (eds.), *Cancer Chemoprevention*, Boca Raton, LA: CRC Press, pp. 457–467, 1992.
15. Baker, M.E. Licorice and enzymes other than 11β-hydroxysteroid dehydrogenase: An evolutionary perspective. *Steroids* 59: 136–141, 1994.
16. Nishino, H., Kitagawa, K., and Iwashima, A. Antitumor-promoting activity of glycyrrhetic acid in mouse skin tumor formation induced by 7,12-dimethylbenz[*a*]anthracene plus teleocidin. *Carcinogenesis* 5: 1529–1530, 1984.
17. Nishino, H., Yoshioka, K., Iwashima, A., Takizawa, H., Konishi, S., Okamoto, H., Okabe, H., Shibata, S., Fujiki, H., and Sugimura, T. Glycyrrhetic acid inhibits tumor-promoting activity of teleocidin and 12-*O*-tetradecanoylphorbol-13-acetate in two-stage mouse skin carcinogenesis. *Jpn. J. Cancer Res.* 77: 33–38, 1986.
18. Okamoto, H., Yoshida, D., Saito, Y., and Mizusaki, S. Inhibition of 12-*O*-tetradecanoylphorbol-13-acetate-induced ornithine decarboxylase activity in mouse epidermis by sweetening agents and related compounds. *Cancer Lett.* 21: 29–35, 1983.
19. Kitagawa, K., Nishino, H., and Iwashima, A. Inhibition of 12-*O*-tetradecanoylphorbol-13-acetate-stimulated 3-*O*-methyl-glucose transport in mouse Swiss 3T3 fibroblasts by glycyrrhetic acid. *Cancer Lett.* 24: 157–163, 1984.
20. Nakayasu, M., Fujiki, H., and Sugimura, T. Possible anti-tumor promoters screened by the method of HL-60 cell adhesion. *Proc. Annu. Meet. Jpn. Cancer Assoc.* 44: 185, 1985.
21. Kitagawa, K., Nishino, H., and Iwashima, A. Inhibition of the specific binding of 12-*O*-tetradecanoylphorbol-13-acetate to mouse epidermal membrane fractions by glycyrrhetic acid. *Oncology* 43: 127–130, 1986.
22. Kiso, Y., Tohkin, M., Hikino, H., Hattori, M., Sakamoto, T., and Namba, T. Mechanism of antihepatotoxic activity of glycyrrhizin. I: Effect on free radical generation and lipid peroxidation. *Planta Med.* 5: 298–302, 1984.
23. Amer, M.S., McKinney, G.R., and Akcasu, A. Effect of glycyrrhetinic acid on the cyclic nucleotide system of the rat stomach. *Biochem. Pharmacol.* 23: 3085–3092, 1974.
24. Birnbaum, J.E., Sapp, T.M., and Tolman, E.L. Cyclic AMP-phosphodiesterase and epidermal mitosis. *J. Invest. Dermatol.* 67: 235–239, 1976.
25. Abe, N., Ebina, T., and Ishida, N. Interferon induction by glycyrrhizin and glycyrrhetinic acid in mice. *Microbiol. Immunol.* 26: 535–539, 1982.
26. Okamoto, H., Yoshida, D., and Mizusaki, S. Inhibition of 12-*O*-tetradecanoylphorbol-13-acetate-induced induction in Epstein-Barr virus early antigen in Raji cells. *Cancer Lett.* 19: 47–53, 1983.
27. Yamaguchi, T. and Watanabe, T. Effects of triterpenes on the mutagenicities of various mutagens toward *Salmonella*. *Agric. Biol. Chem.* 48: 3137–3139, 1984.
28. Wang, Z.Y., Agarwal, R., Zhou, Z.C., Bickers, D.R., and Mukhtar, H. Inhibition of mutagenicity in *Salmonella typhimurium* and skin tumor initiating and tumor promoting activities in SENCAR mice by glycyrrhetinic acid: Comparison of 18α- and 18β-stereoisomers. *Carcinogenesis* 12: 187–192, 1991.
29. Ngo, H.N., Teel, R.W., and Lau, B.H.S. Modulation of mutagenesis, DNA binding, and metabolism of aflatoxin B_1 by licorice compounds. *Nutr. Res.* 12: 247–257, 1992.
30. Leibman, K.C. Studies on modifiers of microsomal drug oxidation. *Chem. Biol. Interact.* 3: 289–290, 1971.
31. Abdugafurova, M.A., Li, V.S., Sherstnev, M.P., Atanaev, T.B., Isamukhamedov, A.Sh., and Bachmanova, G.I. Antioxidative properties of glycyrrhyzic acid salts and their effect on the liver monooxygenase system. *Vopr. Med. Khim.* 36: 29–31, 1990.
32. Takizawa, H., Konishi, S. and Nishino, H. Glycyrrhetic acid: A potent inhibitor of tumor promotion in two-stage carcinogenesis of mouse skin. *Kyoto-furitsu Ika Daigaku Zasshi.* 94: 999–1004, 1985.
33. Watari, N., Torizawa, K., Kanai, M., Mabuchi, Y., and Suzuki, Y. Ultrastructural studies on the protective effect of glycyrrhizin for liver injury induced by a carcinogen (3'-Me-DAB) (The second report). *J. Clin. Electron. Microsc.* 9: 394–395, 1976.
34. Kobuke, T., Inai, K., Nambu, S., Ohe, K., Takemoto, T., Matsuki, K., Nishina, H., Huang, I.-B., and Tokuoka, S. Tumorigenicity study of disodium glycyrrhizinate administered orally to mice. *Fd. Chem. Toxicol.* 23: 979–983, 1985.
35. Mantovani, A., Ricciardi, C., Stazi, A.V., Macri, C., Piccioni, A., Badellino, E., De Vincenzi, M., Caiola, S., and Patriarca, M. Teratogenicity study of ammonium glycyrrhizinate in the Sprague-Dawley rat. *Fd. Chem. Toxicol.* 26: 435–440, 1988.
36. Ishida, S., Sakiya, Y., Ichikawa, T., and Awazu, S. Pharmacokinetics of glycyrrhetic acid, a major metabolite of glycyrrhizin, in rats. *Chem. Pharm. Bull.* 37: 2509–2513, 1989.
37. Krähenbühl, S., Hasler, F., and Krapf, R. Analysis and pharmacokinetics of glycyrrhizic acid and glycyrrhetinic acid in humans and experimental animals. *Steroids* 59: 121–126, 1994.
38. Kawakami, J., Yamamura, Y., Santa, T., Kotaki, H., Uchino, K., Sawada, Y., and Iga, T. Kinetic analysis of glycyrrhetic acid, an active metabolite of glycyrrhizin, in rats: Role of enterohepatic circulation. *J. Pharm. Sci.* 82: 301–305, 1993.
39. Akao, T., Aoyama, M., Akao, T., Hattori, M., Imai, Y., Namba, T., Tezuka, Y., Kikuchi, T., and Kobashi, K. Metabolism of glycyrrhetic acid by rat liver microsomes—II. 22α- and 24-Hydroxylation. *Biochem. Pharmacol.* 40: 291–296, 1990.
40. Raggi, M.A., Amffei, F., Bugamelli, F., and Forti, G.C.

Bioavailabity of gycyrrhizin and licorice extract in rat and human plasma as detected by a HPLC method. *Pharmazie* 42: 269–271, 1994.

41. Akao, O., Hayashi, T., Kobashi, K., Kanaoka, M., Kato, H., Kobayashi, M., Takeda, S., and Oyama, T. Intestinal bacterial hydrolysis is indispensable to absorption of 18β-glycyrrhetic acid after oral administration of glycyrrhizin in rats. *J. Pharm. Pharmacol.* 46: 135–137, 1994.
42. Marandici, A. and Monder, C. Inhibition by glycyrrhetinic acid of rat tissue 11β-hydroxysteroid dehydrogenase *in vivo*. *Steroids* 58: 153–155, 1993.
43. Vogel, V.G., Newman, R.A., Ainslie, N., and Winn, R.J. Phase I pharmacology and toxicity study of glycyrrhetinic acid as a chemopreventive drug. *Proc. Am. Assoc. Cancer Res.* 33: 208, abstract no. 1245, 1992.
44. Epstein, M.T., Espiner, E.A., Donald, R.A., and Hughes, H. Liquorice toxicity and the renin-angiotensin-aldosterone axis in man. *Br. Med. J.* 1: 209–210, 1977.
45. Epstein, M.T., Espiner, E.A., Donald, R.A., and Hughes, H. Effect of eating liquorice on the renin-angiotensin aldosterone axis in normal subjects. *Br. Med. J.* 1: 488–490, 1977.
46. Henry, D.A. and Langman, M.J.S. Adverse effects of anti-ulcer drugs. *Drugs* 21: 444–459, 1981.
47. Archambault, A., Farley, A., Gosselin, D., Martin, F., and Birkett, J.P. Evaluation of Duogastrone (carbenoxolone sodium) for the treatment of duodenal ulcer: A multicentre study. *Can. Med. Assoc. J.* 117: 1155–1159, 1977.
48. Metcalfe, M.J. and Entrican, J.H. Carbenoxolone and hypokalaemia. *Lancet* 2: 1525–1526, 1987.
49. Stewart, P.M., Wallace, A.M., Atherden, S.M., Shearing, C.H., and Edwards, C.R.W. Mineralocorticoid activity of carbenoxolone: Contrasting effects of carbenoxolone and liquorice on 11β-hydroxysteroid dehydrogenase activity in man. *Clin. Sci.* 78: 49–54, 1990.
50. Krähenbühl, S., Hasler, F., Frey, B.M., Frey, F.J., Brenneisen, R., and Krapf, R. Kinetics and dynamics of orally administered 18β-glycyrrhetinic acid in humans. *J. Clin. Endocrinol. Metab.* 78: 581–585, 1994.
51. Kelloff, G.J., Malone, W.F., Boone, C.W., Steele, V.E., and Doody, L.A. Intermediate biomarkers of precancer and their application in chemoprevention. *J. Cell. Biochem.* 16G (Suppl.): 23–26, 1992.

Table I. Clinical Trials of Glycyrrhetinic Acid Sponsored/Funded by NCI, DCPC

Study No. Title (PI) Period of Performance IND No.	Cancer Target	Study Population No. of Subjects	Dose(s) Study Duration	Endpoints	Remarks
Phase I (Safety, ADME)					
NO1-CN-85108-01 Phase I Pharmacokinetic Study in High-Risk Breast and Colon Cancer Patients (Dr. Victor Vogel, Univ. of Texas, M.D. Anderson Cancer Center) 3/89–3/94 IND 33,132	----	Men and women with inactive colon or breast cancer not undergoing treatment or previously resected Single dose: 15 patients Multidose: 6 patients	Single dose: Oral 300–700 mg/m^2 (50 mg/m^2 increments) Multidose: 400–500 mg/m^2 qod for 16 wks	Single and chronic dose pharmacokinetics; toxicity (hemogram, liver and kidney function, electrolytes, albumin, LDH, protein, prothrombin time, glucose, uric acid, urinary Na^+, K^+, aldosterone)	Nonrandomized pharmacokinetics and safety study cancelled because not technically feasible to continue. No toxicity observed at maximum single dose. Multidose protocol demonstrated accumulation and toxicity (hypertension, hypokalemia) Published report: [43]