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Abstract approved:

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Theresa M. Filtz

Adverse effects of anti-epileptic (AED) medications on bone density have been reported since the 1960s. Phenytoin and carbamazepine, two commonly prescribed AED, are frequently associated with osteomalacia including fractures and reduced bone formation. The mechanism by which AED induces bone loss is not fully explained. We sought to determine the effects of AED on calcium transport using Caco-2 cells. Our hypothesis is that carbamazepine and phenytoin would inhibit calcium transport from the apical to the basolateral side of Caco-2 cells grown on semi-permeable supports. Caco-2 cells, derived from a human colon adenocarcinoma, are a model system for study of the function of the intestinal epithelium. Our data demonstrates that phenytoin and carbamazepine dose-dependently inhibit active calcium transport from the apical to basolateral side of Caco-2 cells under physiologic calcium conditions. Vitamin D ameliorates the AED-induced decrease in calcium permeability. Patient perceptions of the retail pharmacist were studied to identify common themes and differences in Chile and Oregon and propose areas for improving patient care. Our hypothesis is that patient perceptions will depend on population size served by a pharmacy. With better patient care pharmacists can improve medication outcomes for patients and avoid some side effects.

Key Words: anti-epileptic drugs, osteomalacia, permeability, pharmacist, vitamin D
Corresponding e-mail address: vborstel@yahoo.com

Effects of Phenytoin and Carbamazepine on Calcium Transport in Caco-2 Cells

And

Pilot Study to Compare the Role of the Chilean and United States Pharmacist from the Patient Perspective

by

Melinda von Borstel

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APPROVED:

Mentor, representing Pharmacy

Committee Member, representing Nutrition and Food Management

Committee Member, representing Pharmacy

Committee Member, representing Pharmacy

Department Chair, Nutrition and Food Management

Academic Coordinator, International Degree Program

Dean, University Honors College

I understand that my project will become part of the permanent collection of Oregon State University, University Honors College. My signature below authorizes release of my project to any reader upon request.

Melinda von Borstel, Author

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TABLE OF CONTENTS

LIST OF FIGURES AND TABLES

DEDICATION

This thesis is dedicated with love to

my mom, for always being my number one cheerleader,

my dad, who has always told me to do my best,

no matter what I am doing,

my brother Donald, for being the best lil' brother

a big "sithy" could ever ask for and my fiancé

for carrying me through the home stretch to the finish line.

SECTION I

Effects of Phenytoin and Carbamazepine on Calcium Transport in Caco-2 Cells

INTRODUCTION

In the United States epilepsy affects nearly 2.5 million people and many more worldwide (Brunton 2006). Lifestyle, health, and cognitive ability can be greatly affected by the occurrence of seizures resulting from disordered firing of groups of neurons in the brain. The unpredictable occurrence of seizures is characteristic of epilepsy.

Currently there are no drugs available to cure epilepsy, but simply to inhibit seizures; thus, long-term therapy is necessary (Brunton 2006). Two drugs commonly used to prevent seizures and used as drugs of choice to treat partial or tonic-clonic seizures are phenytoin and carbamazepine, used since 1938 and 1974, respectively. There are more than 20 drugs currently available for anti-seizure treatment. Commonly prescribed AED are clonazepam, ethosuximide, carbamazepine, phenytoin, phenobarbital, primidone, and valproate (ehealthMD 2004; Brodie 1997), all of which were introduced before 1993.

Medical treatment of epilepsy began in 1857 with the use of potassium bromide (Scott 1992). Fifty-five years later Alfred Hauptmann was credited with the introduction of phenobarbital as an anti-epileptic agent, with its use beginning in 1912. Phenytoin and carbamazepine were the next drugs to be introduced, with the importance of phenytoin

being that anti-epileptic and hypnotic activity could be separated. In 1937 Putnam began work with phenytoin on cat and human models and one year later the drug was being used clinically. Since the introduction of phenytoin, such quick development and initiation of clinical use of a drug has been unheard of. In the 1990's a number of new drugs were developed, but they are often used in combination with older medications because the older drugs remain the therapeutic of choice.

The mechanism of action of phenytoin and carbamazepine involves prolonged depolarization of voltage-activated sodium channels leading to inhibition of seizures (Brunton 2006). In order for an action potential to occur, Na^+ channels in the axon membrane of a neuron must open. Opening of the channels is a result of depolarization of the membrane. Once opened, the Na^+ channel spontaneously closes and is in a state known as inactivation. Phenytoin and carbamazepine, among other anti-epileptic drugs, prolong inactivation. While Na^+ channels are inactivated, the neuron cannot produce another action potential, thereby slowing firing which leads to prevention of seizures in patients.

Long-term therapy with AEDs is necessary because the underlying cause of the seizures remains in most patients. Compliance by the epileptic patient is paramount in the prevention of seizures. Unfortunately, long-term use of anti-epileptic drugs such as phenytoin and carbamazepine has known consequences.

Adverse effects of anti-seizure medications/anti-epileptic drugs (AED) on bone density have been observed and reported since the early 1960s. A study evaluating hip fractures in women over 65 years of age found that women taking AED were at twice the risk of developing a hip fracture (Cummings 1995). With anti-epileptic drug (AED)

therapy, AED-induced osteomalacia, or a decrease in bone density, is observed (Stephen 1999). Phenytoin and carbamazepine are two commonly prescribed AED frequently associated with osteomalacia including fractures, bone demineralization, and reduced bone formation (Pack 2003). However, reports regarding carbamazepine-induced osteomalacia reach varying conclusions on the extent of risk imposed by carbamazepine. Some studies report changes in bone metabolism as a result of carbamazepine treatment (Feldkamp 2000; O'Hare 1980) while another reports none (Tjellesen 1983). In a population-based pharmacoepidemiologic case-control study, Danish central databases possessing records of total fractures reported and medicine prescribed, were used to assess the associated risk of AED use and fracture incidence (Vestergaard 2004). Carbamazepine use resulted in a significant increase in risk of fracture at the hip and an overall increase in risk. Phenytoin did not show a significant increase in risk of fracture, however the power of the phenytoin treatment group was low. Newer agents (e.g. topiramate, lamotrigine, gabapentin) appear to be less causative of osteomalacia but long-term studies are not as complete as for the older agents (Stephen 1999).

Long-term treatment with AED is associated with greater adverse effects on bone density which is especially problematic as AED are commonly prescribed for chronic anti-seizure effects and patients are often treated for multiple decades with an effective agent. Children are especially sensitive to the bone density-depleting effects of AED. Risk factors for osteoporosis including advanced age, low pre-menopausal bone density, and institutionalization exacerbate the AED-associated decreases in bone density (Stephen 1999; Dent 1970)

The mechanism by which AED induces bone loss is not fully explained. Hypocalcemia is associated with AED treatment and with osteomalacia. Levels of hypocalcemia with chronic AED treatment vary between 3 and 30 percent, and higher incidences are associated with poly-therapy (Gouch 1986). Decreased serum calcium will stimulate parathyroid hormone (PTH) to increase bone breakdown or resorption. Increases in serum markers of bone resorption including increased osteocalcin and increased ICTP (cross-linked carboxy terminal telopeptide I of type I collagen) levels are positively correlated with AED treatment (Valimaki 1994; Verrotti 2000). Plausible mechanisms for AED-induced hypocalcemia include induction of vitamin D catabolism, inhibition of PTH-induced calcium mobilization, or decreased dietary calcium absorption. Several studies have failed to find a consistent correlation between altered vitamin D levels and AED treatment; but induction of vitamin D catabolism may be involved in combination with other mechanisms (Verrotti 2000; Gouch 1986). Studies in rats have suggested that both impaired intestinal calcium absorption and inhibition of PTH response are associated with phenytoin treatment (Pack 2001).

Caco-2 cells are cultured human colon adenocarcinoma cells and a model system for study of the intestinal epithelium. At critical density, Caco-2 cells form a polar monolayer with apical (normally adjacent to the intestinal lumina) and basolateral (normally adjacent to the blood supply) sides, tight junctions, and the selective permeability of intact intestinal epithelium. Quantitation of drug transport across Caco-2 cells and calculation of drug permeability is commonly used to estimate oral bioavailability of pharmaceutical agents (Mandagere 2002). Caco-2 cells have also been

used to study intestinal calcium transport, including the effect of vitamin D to enhance intestinal calcium absorption (Jovani 2001; Surendran 1995; Guiliano 1991).

Calcium plasma concentrations are tightly regulated in the body. Calcium is not only important in bone structure, but also in maintenance of tooth enamel, muscle contraction, and blood coagulation. Enhanced calcium uptake from the small intestine involves 3 key hormones, 1,25-dihydroxyvitamin D (calcitriol), parathyroid hormone (PTH) and calcitonin (Groff 2000). Normal plasma calcium concentrations are approximately 9-12 mg/dL (2.5 mM). Low levels of plasma calcium are detected by the parathyroid gland (PTG), resulting in secretion of PTH. PTH activates 25-hydroxyvitamin D-1 hydroxylase to convert 25-hydroxyvitamin D to calcitriol. Calcitriol acts as a hormone to increase sensitivity of the GI tract to calcium and increase the velocity of absorption by an active transport mechanism.

Calcium is transported from the gastrointestinal tract (GI) by two different pathways (Groff 2000). One path is transcellular. It requires energy, is saturable, and is up-regulated by calcitriol. Calcitriol acts to influence the production of calcium binding protein in the intestinal cell and increase calcium uptake at the brush border (Mahan 2004). Calcium binding protein stores calcium in the cytosol and ferries it to the basolateral memberane for absorption. Calcitriol promotes synthesis of calcium binding protein over a period of about 2 days, making more transporters available for calcium movement from the GI into the plasma, thereby increasing the rate of calcium transport (Guyton 2000). The second pathway for calcium absorption from the GI is paracellular transport by passive diffusion. Passive diffusion is a non-saturable route and plays a secondary role in calcium transport. Absorption by this route occurs when calcium intake

increases above a threshold. At low concentrations of luminal calcium, transport is predominantly mediated by the vitamin D-regulated transcellular mechanism.

In a study conducted by Sheikh et al., healthy subjects on a low calcium diet (120 mg/meal) were found to absorb nearly equal amounts of calcium by vitamin D-dependent calcium transport as healthy subjects on a normal calcium diet (300 mg/meal) (Sheikh 1988). The low calcium diet (360 mg/day) nearly saturated the vitamin D-dependent transport and increased calcium absorption from a normal calcium diet (900 mg/day) was largely due to increases in vitamin D-independent calcium transport.

Adults absorb approximately 30% of ingested calcium (Mahan 2004). Calcium absorption appears to range from 10-60% dependent on a number of factors. 1997 recommendations for calcium intake are given as adequate intakes (AI) at 1300 mg/day for children 9-18 years old, 1000 mg/day from 19-50 years of age, and 1200 mg above 50 years old. Females typically do not meet the AI and although men ingest more calcium than women, often their intake falls below the AI as well. Factors affecting calcium absorption are numerous. Intestinal acidity promotes calcium absorption, as is found in the duodenum. Stomach acidity is also important for increased calcium bioavailability. As stomach acidity in older adults decrease calcium absorption can be negatively affected. Increased dietary protein consumption and caffeine lead to an increase in urinary excretion of calcium (Groff 2000). Several other dietary factors such as fiber, phytate, and oxalate found in foods that are good sources of calcium, such as whole wheat breads and green leafy vegetables like spinach, can decrease intestinal calcium absorption by binding calcium which results in decreased absorption in the small intestine and greater fecal loss of calcium.

The optimal sources of calcium are from milk and other dairy products; however if insufficient amounts are consumed then a dietary supplement is recommended. Absorption of calcium from dietary supplements is comparable to that from foods at approximately 30% bioavailability (Mahan 2004). Food sources are preferred because intake of other nutrients will occur with calcium intake. Potentially, with supplementation, too much calcium can be ingested decreasing absorption of other important minerals such as iron, zinc, and magnesium. Meeting the AI for calcium intake is important in maintaining healthy bones and teeth, but excessive supplementation can hurt the intricate balance of the body as much as sub intake of calcium.

In the body, when plasma calcium concentrations are low, calcitriol and PTH act in concert on amorphous calcium in the bone to move it into the plasma and on the kidney to increase calcium resorption (Guyton 2000). PTG will sense a rise in plasma calcium concentrations and block release of PTH. Decreased PTH will cause thyroid cells to release calcitonin, promoting deposition of calcium into bone and increasing production of 24, 25dihydroxyvitamin D, which also aids in replacement of calcium into the bone.

Given the body's intricate regulatory mechanism for maintenance of plasma calcium concentrations, it is important to understand how drugs may affect the balance of calcium absorption and storage. Better understanding may allow for development of interventions to decrease toxic side effects of AED, improving patient health and compliance. In this study, we used Caco-2 cells to assess inhibitory effects of two common AED, carbamazepine and phenytoin, on calcium transport by modeling intestinal cells in culture. Our hypothesis was that phenytoin and carbamazepine would

inhibit calcium transport from the apical to the basolateral side of Caco-2 cells grown on semi-permeable supports.

MATERIALS

Caco-2 cells, a human colon epithelial carcinoma cell line, were received from ATCC (Bethesda, MD). Penicillin/Streptomycin antibiotic mix (10,000 I.U. Pencillin, 10,000 ug/mL Streptomycin) and Dulbecco's modified Eagle's medium (DMEM) were purchased from Cellgro/Mediatech (Herndon, VA). Fetal bovine serum was purchased from Hyclone (Logan, UT). Nonessential amino acids (10 mM at 100X) were obtained from Gibco-BRL (Gaithersburg, MD). Transwell plates (3.0 um pore size) were purchased from Corning-Costar (Corning, NY). Phenytoin (5,5-diphenylhydantoin), carbamazepine (5H-dibenzazepine-5-carboxamide) and Scintisafe® gel were obtained from Fisher Scientific (Pittsburgh, PA). Active vitamin D (1,25-dihydroxycholecalciferol) was purchased from Sigma-Aldrich (St. Louis, MO).

METHODS

Caco-2 cell culture.

Frozen Caco-2 cells were routinely revived, cultured and used between the 26th and 30th passages. Cells were grown in 150 cm^2 tissue culture flasks with 30 mL DMEM supplemented with 10% fetal bovine serum (FBS), 4 mL/L antibiotic mix, 25 mg/L Amphotericin B, and 100 uM nonessential amino acids. Medium was replaced every 2-3 days and cells grew to approximately 80% confluency before subculturing. Cells used in calcium transport experiments were seeded in Transwell dishes at a density of 2.5 X 10^5 cells per Transwell insert (4.71 cm^2) and grown for 21-22 days in supplemented DMEM, described above, to allow for development of tight junctions. Medium (1.5 mL apical, 2.5 mL basolateral) was changed every other day for 14 days and then daily for another 7 days prior to use for transport studies on day 21. All cells were maintained in an atmosphere of 5% CO_2-95% air at 37˚C.

Trans-epithelial calcium transport studies.

Caco-2 cells were grown to confluency and maintained in transwell culture for 21 days until tight junctions formed. At 21 days, cells were rinsed with Hanks Balanced Salt Solution (HBSS; 0.4 g/L KCl, 0.06 g/L KH_2PO_4, 0.1 g/L $MgCl_2 \cdot 6H_2O$, 0.1 g/L $MgSO_4 \cdot 7H_2O$, 8 g/L NaCl, 0.35 g/L $NaHCO_3$, 0.09 g/L $Na_2HPO_4 \cdot 7H_2O$, 4.5 g/L D-glucose, and 2.383 g/L Hepes). HBSS was made to pH 6.8 for apical application and to pH 7.4 for basolateral application to mimic the in vivo intestinal cell environments.

Phenytoin and carbamazepine were dissolved in DMSO (vehicle) at 100X final concentration. Cells were then incubated with the indicated concentrations of vehicle, phenytoin or carbamazepine, in HBSS of appropriate pH.

After 24 hour drug pretreatment, the maintenance of tight junctions in the Caco-2 cells was confirmed by a trans-epithelial electrical resistance (TEER) reading and selective permeability calculated as follows: (resistance of transwell containing cells – resistance of transwell without cells) X 4.71. Only cultures meeting criteria of selective permeability of greater than 500 were used for transport studies.

After 24-hour drug pre-incubation, fresh drug containing apical and basolateral HBSS medium was added. Calcium transport from apical to basolateral sides of the polar cell monolayer was monitored by spiking the apical HBSS medium with 5 uCi/mL radioactive [45]$CaCl_2$ at time zero after 24 hour pretreatment. Calcium transport was quantified by sampling the basolateral medium at time zero and 20 minute intervals over a 3 hour time period following addition of radioactive calcium. Basolateral samples (50 uL) in duplicate were withdrawn and replaced with 100 uL of fresh drug-containing medium after each collection. Samples were transferred to 7 mL scintillation vials, 5 mL of scintillant was added, and a liquid scintillation counter was used to quantitate the amount of [45]$CaCl_2$ radioactivity present in the basolateral samples. Calcium permeability as a function of calcium transport over time was calculated under conditions of calcium homeostasis (equimolar 7.5 mM calcium in apical and basolateral buffers). The effects of various concentrations of phenytoin and carbamazepine on calcium transport were quantitated according to the formula:

Pe = % transport x V/(A x t)

Where Pe = permeability

V = volume of apical medium (1.5 mL)

A = area of transwell surface (4.71 cm^2)

t = time (in hours) post [45]Ca^{++} addition

A dose response relationship between drug concentration and calcium permeability was established. EC50 values for inhibition of calcium permeability were calculated by non-linear regression analysis of dose response curves using the one-site inhibition curve equation with GraphPad Prism 4 software. For all figures significance is indicated and corresponds to $p<0.05$ by non-paired t-test compared to control (vehicle-treated) samples or by ANOVA for multiple sample comparisons with a Neuman-Keuls post-test.

Determination of optimum calcium concentrations for maximum [45]Calcium transport.

Caco-2 cells were grown and incubated on transwell plates and treated 24 hours prior to experiment as described above. One half hour prior to addition of [45]$CaCl_2$, cells were pretreated with vehicle or 5 ug/mL phenytoin under 3 different concentrations of $CaCl_2$: 1 mM, 7.5 mM, and 15 mM. Calcium transport and caco-2 cell permeability were quantitated as described above.

Trans-epithelium calcium transport studies with vitamin D and modified calcium concentration.

Calcium transport studies were conducted as described above, with the inclusion of the active form of vitamin D, 1,25 dihydroxyvitamin D. Vitamin D (101 nM) or EtOH vehicle (0.04%) was added to both the apical and basolateral sides 48 hours prior to transport studies and then replenished 24 hours later. At experimental time zero, fresh medium containing vitamin D and either phenytoin (5 µg/mL), carbamazepine (2.36 µg/mL) or DMSO vehicle (10 µl/mL) were added to the cells and the medium spiked with [45]$CaCl_2$ on the apical side only in the context of equilateral medium concentrations of 2.5 mM $CaCl_2$. Basolateral samples were taken at varying times after [45]$CaCl_2$ addition, and transport and permeability data calculated as described above.

RESULTS

Calcium transport following phenytoin treatment.

The effects of the anti-epileptic drug, phenytoin, on calcium transport are shown in Figure 1. Calcium transport was linear over 160 minutes in the absence and presence of phenytoin at all concentrations tested (Fig 1A and data not shown). Concentrations of phenytoin included the physiological dose range of 200 to 600 mg/day corresponding to 50-150 ug/mL fluid. Phenytoin significantly inhibited calcium transport at all concentrations tested from 0.05 to 50 ug/mL with an EC_{50} value for inhibition of calcium permeability of 119 ± 4 ng/ml. The apical to basolateral permeability of the Caco-2 cells to calcium was decreased by a maximum of 41% at the maximally effective concentrations of 5-50 ug/mL (Fig. 1B).

Calcium transport following carbamazepine treatment.

The effects of the anti-epileptic drug, carbamazepine, on calcium transport are shown in Figure 2. Calcium transport was linear over 140 minutes in the absence and presence of carbamazepine at all concentrations tested (Fig. 2A and data not shown). Carbamazepine significantly inhibited calcium transport at all concentrations from 2.4 to 2400 ug/mL, which includes the physiological dose range from 400 to 1600 mg/day corresponding to 100-400 ug/mL fluid. The apical to basolateral permeability of the Caco-2 cells to calcium was decreased by a maximum of 54% at 236 ug/mL carbamazepine (Fig. 2B). Because of solubility issues, a maximally effective dose is

difficult to achieve. The EC_{50} value for inhibition of calcium permeability is 1.17 ± 0.11 ug/ml for carbamazepine

Calcium permeability in Caco-2 cells as a function of equilateral calcium concentration.

The effects of various extracellular calcium concentrations on [45]$CaCl_2$ permeability in Caco-2 cells is shown in Figure 3a. Maximum permeability of Caco-2 cells to calcium occurs at a concentration of 1 mM, with permeability approximately twice that found at 7.5 mM calcium concentrations. At 1 mM calcium concentration, calcium permeability was decreased 44% with the addition of 5 µg/mL phenytoin. Permeability was decreased by 29% and 22% with the addition of 5 µg/mL phenytoin at 7.5 and 15 mM calcium concentrations, respectively. Phenytoin has greatest effects on Ca^{++} transport at low Ca^{++} concentrations when transport is most actively stimulated.

Calcium transport in Caco-2 cells pre-treated with vitamin D and phenytoin.

As previously reported and expected, vitamin D affects Caco-2 cell permeability to calcium (Fleet 1999 and Fig. 4A). However, ethanol, used as vehicle for vitamin D, has significant effects on Caco-2 cell permeability to calcium in the absence of vitamin D. Permeability of cells treated with DMSO vehicle alone are approximately half as permeable to calcium as cells treated with DMSO and EtOH in combination. However, vitamin D increases permeability 20% above ethanol vehicle-treated cells. 5 ug/mL phenytoin decreased permeability in the presence of vitamin D, ethanol vehicle, or no vehicle.

Calcium transport in Caco-2 cells pretreated with vitamin D and carbamazepine.

Caco-2 cells treated with vitamin D alone or in conjunction with carbamazepine appear to have heightened permeability to calcium (Fig. 4B). Taking into account the vehicle effects of EtOH, permeability of cells treated with vitamin D was nearly 30% greater than that of cells treated with vehicle. Cell permeability was approximately double when treated with carbamazepine and vitamin D in conjuction, as opposed to carbamazepine and ethanol vehicle. Although carbamazepine has a statistically significant effect on calcium permeability in the presence of vitamin D, clearly, carbamazepine has less effect than phenytoin to inhibit calcium permeability in the presence of vitamin D. Carbamazepine reduced permeability by only 8% in the presence of vitamin D.

DISCUSSION

We determined that carbamazepine and phenytoin at therapeutic drug concentrations have significant effects on calcium transport in our model system at physiological concentrations of calcium. In our system, calcium transport was assessed under conditions of equimolar apical and basolateral calcium concentrations. This condition of equimolar, equilateral calcium concentration limits our studies to effects on active, vitamin D-regulated, transcellular transport. Passive, paracellular calcium transport should not be a factor. We quantitated calcium permeability across Caco-2 cell monolayers as a function of rate of transport in the absence and presence of varying concentrations of carbamazepine and phenytoin. We also quantitated the effects of phenytoin on calcium transport under conditions of varying calcium concentration.

The mechanism by which anti-epileptic drugs (AED) decrease bone density and lead to increased risk of fracture and AED-induced osteomalacia has been studied, but no definitive mechanism reported. Our results support an effect of AED on intestinal epithelium calcium transport. Decreased transport of calcium across intestinal epithelial cells would lead to decreased serum concentrations of calcium *in vivo*. Decreased serum calcium initiates a cascade of events to move calcium from bone into plasma. Maintenance of plasma calcium concentration is the most important site of calcium regulation in the body. When serum calcium levels are sufficient, a cascade of signals leads to deposition of calcium back into bone. Decreased bone density may then be the result of reduced dietary calcium uptake from the intestine due to AED blocking intestinal transport.

At lower calcium concentrations, it appears that the effect of AED on epithelial calcium permeability might be greatest. Calcium transport and permeability was significantly stimulated at low calcium concentrations. The data most likely reflect the importance of the active transport mechanism at lower concentrations of calcium as a way to increase calcium uptake. Phenytoin and carbamazepine may block active calcium transporters in epithelial cells. Phenytoin's affects to block calcium transport at the low calcium concentration highlights the importance of maintaining sufficient dietary calcium intake.

Increases in AED transport seen at high doses of phenytoin and carbamazepine may be a result of cytotoxicity, but cytotoxicity was not reflected in TEER readings taken prior to adding [45]$CaCl_2$.

Active vitamin D, as expected, was shown to be an important regulator of transport and permeability of calcium in Caco-2 cells (Fleet 1999). Calcium transport is enhanced through the vitamin D-dependent transport mechanism. Vitamin D approximately doubled Caco-2 cell permeability to calcium in the absence of AED treatment. With AED treatment, calcium permeability was lowered in the presence and absence of vitamin D. The importance of maintaining sufficient vitamin D levels either through the diet or exposure to sunlight is highlighted by these studies. Effects of AEDs to reduce vitamin D levels, as suggested by some studies, may compound the AED effect to reduce calcium absorption (Verrotti 2000; Gouch 1986).

Serum parathyroid hormone (PTH) levels are an important factor in bone health as a result of increased PTH levels leading to movement of calcium from bone into serum. Steingrimsdottir et al studied the relationship between calcium, 25-

hydroxyvitamin D, and PTH levels (Steingrimsdottir 2005). Using PTH as a marker of optimal calcium and vitamin D levels, they found that lowest serum PTH was achieved with vitamin D intakes sufficient to maintain serum vitamin D levels greater than 18 ng/mL. Serum vitamin D levels of less than 10 ng/mL resulted in significantly increased PTH levels. They found that high calcium intake (greater than 1200 mg) did help to lower PTH levels, but was not as effective as vitamin D in maintaining low serum PTH.

Maintenance of adequate dietary calcium and vitamin D levels is of greater concern in patients on long-term AED therapy. Drezner et al. recommend use of calcium supplements and vitamin D for prophylaxis as well as treatment of osteoporosis or osteomalacia (Drezner 2004). Recommendations include 400-2000 IU/day vitamin D for prophylaxis, 2000-4000 IU/day for osteoporosis, and 5000-15,000 IU/day for 3-4 weeks for osteomalacia. While emphasizing vitamin D intake, Drezner acknowledges the importance of sufficient calcium intake and additionally recommends an intake of 600-1000 mg/day. A study by Steingrimsdottir supports this finding, concluding that calcium intake levels of more than 800 mg/day may be unnecessary with proper vitamin D supplementation (Steingrimsdottir 2004). There may not be complete agreement on vitamin D supplementation in a healthy population (Sheikh 1988), but these issues are of greater importance with AED therapy.

A supplement is an effective way to maintain vitamin D levels despite sun exposure. A study investigating the biosynthesis of vitamin D at latitudes of 42 and 52 degrees north found that no biosynthesis occurred from December to February and November to March, respectively (Webb 1988). It was found that vitamin D levels in subjects living at 64 degrees north decreased to 11.5 ng/mL in February and March and

increased to 18.3 ng/mL in the summer months, whereas subjects on a vitamin D supplement had serum levels of 18.7 ng/mL and 22.4 ng/mL during those same months (Steingrimsdottir 2005). Throughout the year 500 IU/d of vitamin D achieved a serum level of 18 ng/mL, except for during the winter months in which 700 IU/d was required.

There are several factors that a pharmacist should be aware of besides dietary intake that have a significant affect on calcium and vitamin D levels. With increased age patients have decreased gastrointestinal acidity. Calcium absorption from the gastrointestinal tract is best in acidic conditions and with age acidity decreases leading to a need for increased calcium intake, possibly by use of a supplement. Exposure to sunlight is an important factor in serum vitamin D levels, with a lack of exposure leading to decreased vitamin D levels.

Without a better understanding of the mechanism by which phenytoin and carbamazepine appear to be blocking the transport of calcium in Caco-2 cells, it is important to compensate for decreased serum calcium seen in patients receiving AED treatment with dietary supplementation that enhances calcium uptake. However, in the future, increased understanding of the mechanism by which phenytoin and carbamazepine decrease calcium permeability in the Caco-2 model will be important.

SECTION II

Pilot Study to Compare the Role of the Chilean and United States Pharmacist from the Patient Perspective

INTRODUCTION

Pharmacists have the potential to significantly impact patient outcomes by providing patient counseling and follow up. In several studies it has been demonstrated that pharmacists positively affect outcomes of patients suffering from a wide variety of disease states including asthma and hyperlipidemia, as well as aiding in smoking cessation and medication compliance (Charrois 2005; Smith 1995). Pharmacists were shown to have an impact not only by intervening early on but also by providing follow up.

Our research described in Part 1 provides evidence that anti-epileptic drugs lead to decreased calcium transport in the Caco-2 cell model. As a result further research will need to be conducted to gain an understanding of the mechanism for decreased calcium transport, but without a better understanding the role of the pharmacist is imperative. When a pharmacist is counseling a patient on anti-epileptic drugs, particularly either phenytoin or carbamazepine, a recommendation should be made that they be on both a vitamin D and calcium supplement. The recommendation to be supplementing a healthy diet with vitamin D and calcium will be essential to maintain good bone health in

epileptic patients. Recommendations from a pharmacist may be more likely received if the patient trusts the pharmacist and regularly interacts with him or her.

To better understand the needs of patients and determine ways in which the pharmacist can better reach patients we felt it was important to develop a pilot survey study that would highlight the reasons why patients may or may not talk to their pharmacist. Gaining a feel for patient perceptions both in Oregon and in another country such as Chile helps to evaluate if there are cultural differences in patient expectations and perceptions of their pharmacist or if there are underlying factors that are important to people regardless of ethnicity or location in the world. The United States is very ethnically diverse, with approximately 25% of the population describing themselves as an ethnicity other than Caucasian in the 2000 United States Census (U.S. Census Bureau 2004). Of the total population in the U.S., approximately 14% of the population is Hispanic or Latino. Chileans are a very small subset of the total Hispanic population, 0.26 % of the 14 % total, in the United States, but as a pilot study this project aims to begin looking at expectations and perceptions the patient may have as they walk into their local retail pharmacy and serves as a starting point for future work to evaluate ways to best meet the needs of our patients as pharmacists in the community retail pharmacy setting.

The importance of good interactions and trusting relationships to effectively counsel are imperative. Foppe van Mil and colleagues speak of changes in pharmaceutical care and highlight the importance of pharmacists needing to move from behind the counter where they have always counted pills to acting as health care professionals for the public (van Mil 2004). They speak of the European Association of

the Faculties of Pharmacy (EAFP) who suggests a change in the curriculum for pharmacy schools from laboratory-based sciences to clinical sciences and practice. If pharmacists are going to gain the recognition as health care professionals they need to be trained as such.

In Chile and Oregon, the two locations where patients were interviewed, the education necessary to become a pharmacist was compared to evaluate if sheer ability and knowledge of medications might play a role in the patients trust or lack there of. In Chile there are 9 universities that offer a pharmacy title, which is earned after successful completion of the coursework and a university specific exam. The program is only 5 years from start to finish with a 6^{th} year possible if a student would like to earn a masters in pharmacy. The doctor of pharmacy degree is the standard in the United States and does not exist in Chile. An admissions counselor at University of Andres Bello in Santiago discussed the pharmacy program, providing materials that showed the curriculum for that university. As a graduate of another Chilean pharmacy school, the counselor spoke of the similarities and differences between the two programs (Campora 2005). The students take all of the sciences that are required as prerequisites in the United States including chemistry, physics, microbiology, physiology, biology, cell and molecular biology, economics, biochemistry, etc, but because the program is 2 years shorter than the Pharm D. curriculum found at Oregon State University, students do not receive as much training in pharmacy practice patient care (Oregon State University 2006). However, internships are becoming a more common part of the pharmacy curriculum in Chile, increasing the patient contact that a pharmacy student receives before becoming licensed. An article in The American Journal of Pharmaceutical Care

highlights the internship that has been instituted as part of the curriculum stating that, "the purpose of these modifications was to include and develop the concept of pharmaceutical care" (Ruiz 2002). The implementation and modifications of the internship experience for pharmacy students began at The University of Chile in 1997 and is still developing at universities such as Andres Bello University. Pharmacists are well equipped in the sciences to face the challenges of pharmacy, but patient care education is continuing to improve at schools around the world as the need for pharmacists to be part of the health care team is realized.

Currently, unlike pharmacists in the United States who are required to counsel patients on new prescriptions, as stated in Division 41 855-041-0100 of the Oregon Administrative Rules, Chilean pharmacists are not required to provide patient counseling (Oregon Board of Pharmacy 2006).

A qualitative pilot study of Chileans and Oregonians in pharmacies was conducted in Chile and Oregon. Rural, town, and metropolitan pharmacies were included to provide better insight into the patient perceptions of their pharmacist and factors that might influence patient-pharmacist interactions. Our hypothesis was that patient perspectives of their pharmacist would be similar in the two countries with differences presenting themselves as a result of the size of community the pharmacy was serving, with small town patients being more content with their experience than the city patient. Ultimately the purpose of this pilot study is to evaluate factors that are important as patients consider going to the pharmacist for advice. If a patient talks with the pharmacist before taking a medication they will be more compliant and they may be more likely to receive quality health care leading to improved outcomes. Factors affecting

patient perceptions, including customer service, convenience, and cost were discussed in each interview.

METHODS

Location

Patients were recruited at retail pharmacies in three locations based on population size. The three communities selected included the rural towns of Puerto Montt, Chile and Stayton, Oregon, small cities/towns of Temuco, Chile and Corvallis, Oregon, and Metropolitan areas of Santiago, Chile and Portland, Oregon. Pharmacies in which the interviews were conducted were prominent chains in the retail pharmacy setting, each typical of the normal pharmacy setting in its respective country. In Chile typical pharmacies make up the entire store and follow the typical drug store model with beauty care and drug products for sale whereas the pharmacies in Oregon followed the typical chain model of a drug store within a larger store that sells food and household items and the drugs are contained within a small portion of the larger store. All stores had a pharmacist, pharmacy technicians and clerks.

Survey

Interviews were conducted using a questionnaire (Appendix A), which received IRB approval at Oregon State University for use as described in Chile and Oregon. A certified court translator in the state of Oregon translated the English version of both the questionnaire and consent form from English to Spanish (Appendix B). The survey tool was used in the native language of the participants, Spanish in Chile and English in Oregon. The questionnaire consisted of 12 open-ended questions used as a starting point

to encourage conversation. Questions one through six were designed to establish a patient profile. Questions seven through 11 were used to gather information on the patient's perspective of their pharmacist. Question 12 provided the patient the opportunity to expound on any previous question as well as add anything that we may not have included in our questionnaire. The interviews in all locations were conducted in the patient's native language, either Spanish or English, by the same bilingual interviewer for consistency.

Participants

Our focus was on regular patients in the pharmacy where the interviews were being conducted. Regular was defined as a patient that had filled a minimum of three prescriptions in the last six months at that pharmacy. The pharmacist or pharmacy technician recruited regular patients as they filled prescriptions in the pharmacy. Interviews were conducted with the first five patients that were eligible and willing to participate at each location. If time allowed more participants were interviewed, with a total of 32 patients being interviewed, five at each location listed and an additional two in Temuco, Chile. Patient identity was confidential and participation was voluntary. There were no extra criteria for participation other than having filled three prescriptions and willingness to participate, resulting in a heterogeneous population ranging in age from approximately 40 years old to 80 years old and consisting of both males and females. Age was not determined exactly by questioning because in Chile asking a person their age may have been considered offensive. Patients may have been offended and have chosen not to be interviewed, thus the interviewer estimated the patient age and

eliminated question number 3 from the survey. Question number 2 regarding level of study was also not included due to advice from a Chilean pharmacists suggesting that it would be inappropriate to ask patients either their age or level of education.

Data Collection

Interviews were conducted orally and data was captured via notes. One researcher conducted all interviews to ensure consistency. Interviews were conducted at the counter in Chile and at a table outside of the pharmacy in Oregon.

RESULTS

Patients were interviewed at each of the locations, interviewing 5 patients at each location except for Temuco, Chile where time allowed for interviews with 7 patients. Patient groups were compared based on cultural, age, gender, or pharmacy population size differences to look for trends and identify themes in patient perceptions of their community pharmacist (Table 1 and 2).

In response to the question, "What else could your pharmacist do to improve your health" Oregonian patients (OP) tended to be more likely to list speed of service as something that could be improved upon to improve their health care or enhance their pharmacy experience, whereas Chilean patients (CP), particularly in the Santiago, but also in the smaller communities cited the need for pharmacist presence in the front of the store as the improvement they viewed as necessary.

> OP: They could be faster!
> OP: I see them standing back there doing what appears to be nothing and I'm standing around waiting… they should make it a priority to wait on those that are in the store.
> CP: They just sit in the back of the store and don't offer help.
> CP: The pharmacist is knowledgeable, but you have to ask for him to help. He should be out front helping.

Older patients (P), both Chileans and Oregonians, were more likely to say that service was the most important reason for them going to that pharmacy and everyone who listed service as a positive part of their experience at the pharmacy knew the pharmacist.

> P: Customer service is excellent which is why I come to the pharmacy.
> P: I love the pharmacy and come often to talk with the ladies and also with the pharmacist.
> P: The most important thing is service and I can talk to the pharmacist whenever I need to.

P: Excellent service and always able to answer my questions… that's why I come.

Many patients ranging in age from 40 to 60 years old, and particularly males within that population, reported not knowing who the pharmacist was, or not talking to the pharmacist because they did not need to.

P: Happy to come and leave without talking to the pharmacist.
P: Would describe my interactions as professional, short, business-like.
P: Don't talk to the pharmacist at all except when I have to.

Chileans tended to speak highly of the technician and clerks and some had never spoken with a pharmacist. All Oregonians were able to speak to their pharmacist, regardless of whether the experience was positive or short and business-like. Regardless of age or community size patients in each pharmacy spoke positively of their pharmacist and interaction they had with the pharmacist. The exception was the Metropolitan Chilean pharmacy in Santiago where the common response to the question was, " I've been coming to this pharmacy for several years due to location and I do not know the pharmacist or if they are even always here."

Patients most often stated that the purpose of their visit to the pharmacy was to receive information about their medication or to pick up medicine. Very few had been to the pharmacy to receive any other service. The exception was a couple of patients in Oregon who had received their flu shot at the pharmacy.

Based on responses to the question, "What services does your pharmacist provide for you?" patients may misunderstand the role of the pharmacist, both in Oregon and in Chile. There were still a couple of patients, particularly in the older age range of 70 and

80 years old, that did not understand why the pharmacist needed to go to school for so long.

> OP: “All the pharmacist does is count pills and then put the blue ones in one bottle and the red ones in another, why do they have to go to school to do that?”
> CP: “I’m not sure what the pharmacist does or why they’re necessary.”

See Table 1 and Table 2 for complete interview data and responses.

DISCUSSION

The pharmacist has a responsibility and ability to impact the medication and health outcomes of their patients. The foundation for improved patient outcomes must be built during counseling sessions between the pharmacist and patient where the patient walks away feeling that the interaction was important and worthwhile, and therefore, necessary. Pharmacists are receiving more education revolving around patient care in order to be more prepared for patient interactions and for the most part the changes are being noticed by the patient, however some patients may misunderstand the role of the pharmacist, both in Oregon and in Chile, based on the comment from the patient in Stayton, OR, "All the pharmacist does is count pills and then put the blue ones in one bottle and the red ones in another, why do they have to go to school to do that?" His response however, was an example or only a couple of responses during the interview process of a patient that felt that the pharmacist was unnecessary. All others, even though they may not talk to the pharmacist, understood that the pharmacist was available to offer information about the medication. The profession has come a long ways from just "counting pills" as that customer put it, but there are still patients out there that believe that the role of the pharmacist is to count pills. Until there is a shift in patient perceptions, patients may not take advantage of their pharmacists training to optimize their medication therapy.

In a survey conducted by Harris Interactive for the National Association of Chain Drug Stores 1001 adults were surveyed in the United States and 771 adults of those surveyed had purchased prescription medications within the past two years. One

question asked for the frequency of consulting with the pharmacist over a three month period and 36% said zero, 30% said once, 10% said twice, 13% said three times, 7% said four to six times, and 3% said seven or more (NACDS 2005). If this same survey were conducted in Chile far fewer respondents may have talked with the pharmacists in each category and the number that said zero or one time may have been much higher. In Chile pharmacists are not required to talk with each patient when the prescription is new like they are in the United States. As a result, the pharmacist often sits in the back of the pharmacy and only comes out if the patient requests them. Some of the Chilean patients recognize the need for the pharmacists to be more involved, such as the woman that commented that if she could change one thing it would be to have the pharmacist be up front and talking with patients (Puerto Montt Chile patient Dec 2005).

As previously discussed, pharmacists have been shown to enhance patient care and outcomes in several disease states including hypertension, asthma, and smoking (Charrois 2005). In a study looking at the effect of pharmacist intervention and patient education on lipid-lowering medication, it was shown that the baseline intervention by the pharmacist was the most meaningful in affecting patient outcomes (Ali 2003). This would suggest that the initial contact a pharmacist has with a patient when first giving a new medication would be very important not only for building trust, but also to have an impact on the ability of the medication to positively affect the patients disease state.

Limitations of this study include the sample size of the population. This project serves as a starting point from which further research could build in order to further explore cultural differences and to support conclusions in this document with larger numbers of participating patients. An area of possible bias in this project could have

come from pharmacists recruiting the patients. Pharmacists would be more likely to recruit patients with whom they have a good relationship thus influencing results. For this project, as a pilot study, the aim was to keep the project simple to identify and explore differences between pharmacists of different countries and patient perceptions of their pharmacist. The results appeared fairly similar between patients despite the small sample size, but perhaps with more interviews new issues would have arisen and more could be learned from the patients about ways in which the pharmacist could better meet the needs of their patients.

Pharmacists have a great responsibility as drug experts on the health care team. They need to be able to communicate their knowledge to the patient. Pharmacists may feel that they are communicating effectively, but until all patients perceive the patient pharmacist interaction as beneficial, the job is not complete. Continuing to educate the public on the role of the pharmacist and assuring that pharmacists are accessible and approachable is important. Also making the most of the time spent with the patients is crucial so that they walk away with information that is useful to them, rather than feeling overwhelmed. Not only is a description of how to take the medicine important, but also being aware of side effects such as the decrease in calcium transport with anti-epileptic drugs in which counseling accordingly is important. All patients, regardless of community pharmacy size, age, or gender, who talked with their pharmacist, both in Chile and Oregon, did so because they trusted their pharmacist and they perceived their pharmacist as friendly, knowledgeable, and positively impacting their health.

FIGURES

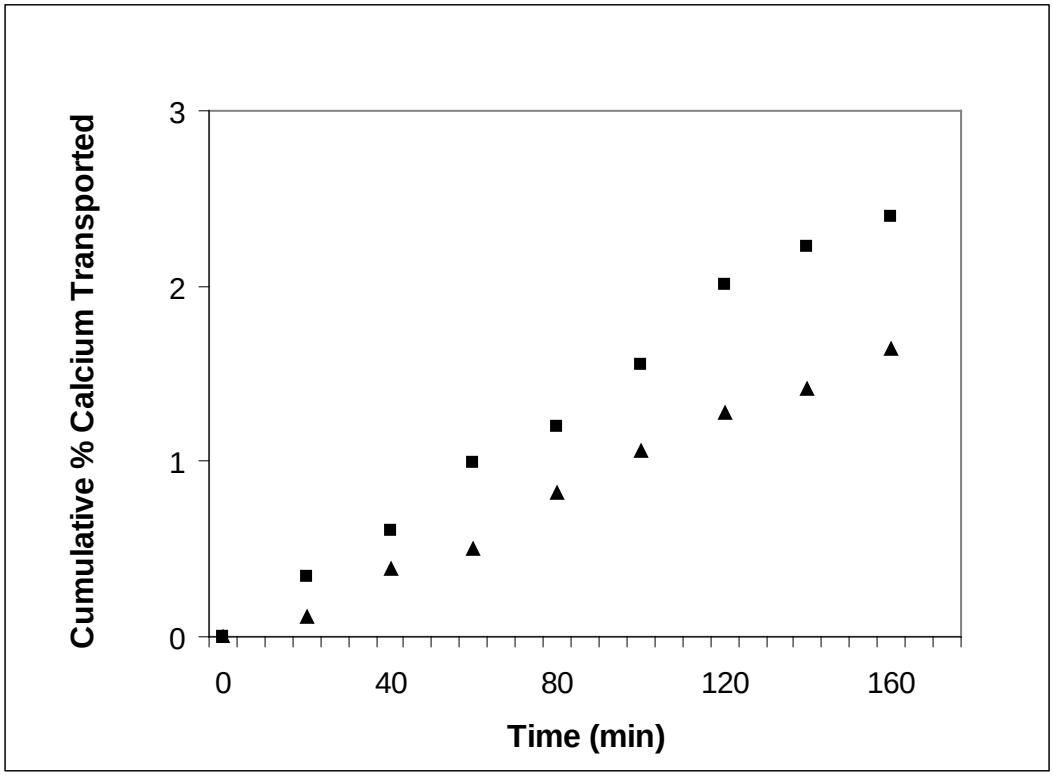

Figure 1a. Concentration-dependent effect of 24 hr phenytoin pretreatment on calcium permeability in Caco-2 cells. Effect of 24 hr pretreatment with 5 µg/ml phenytoin (▲) or vehicle (■) on apical to basolateral calcium transport over 160 min.

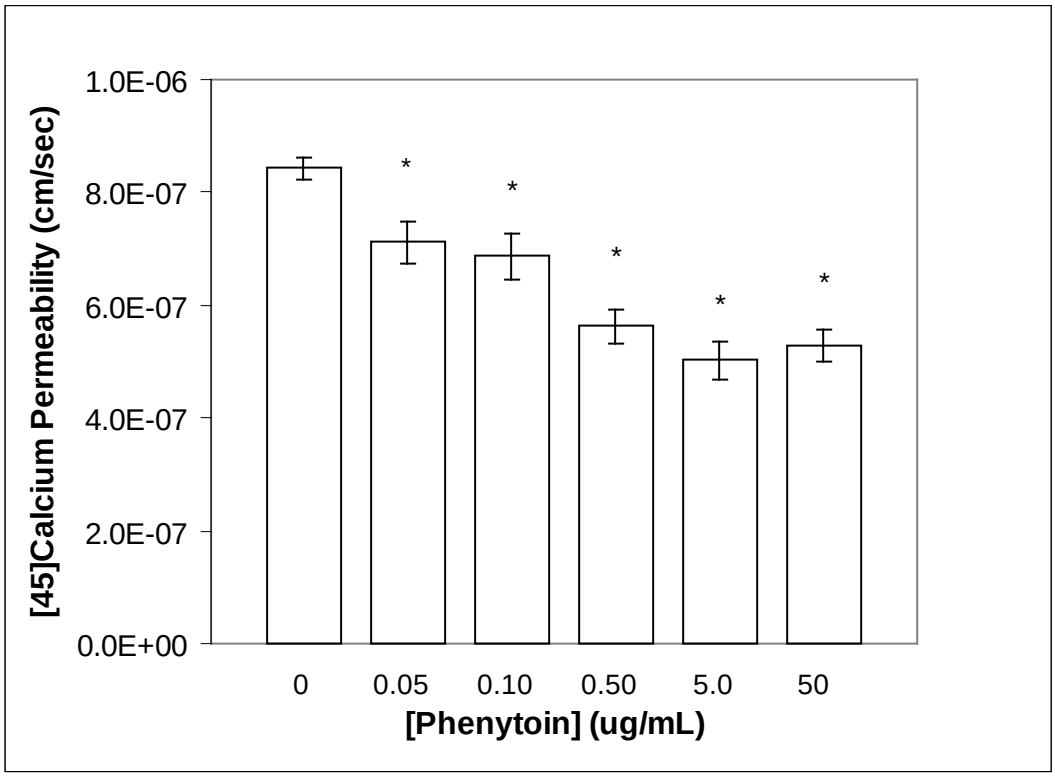

Figure 1b. Concentration-dependent effect of 24 hr phenytoin pretreatment on calcium permeability in Caco-2 cells. Effect of varying phenytoin concentration on Caco-2 cell calcium permeability calculated from transport data similar to that shown in part A. Active transport of 2.5 mM $CaCl_2$ was quantitated and permeability calculated as described in methods. Permeability data shown is averaged from 3 independent experiments conducted in duplicate. *$p < 0.05$ compared to vehicle pretreated controls.

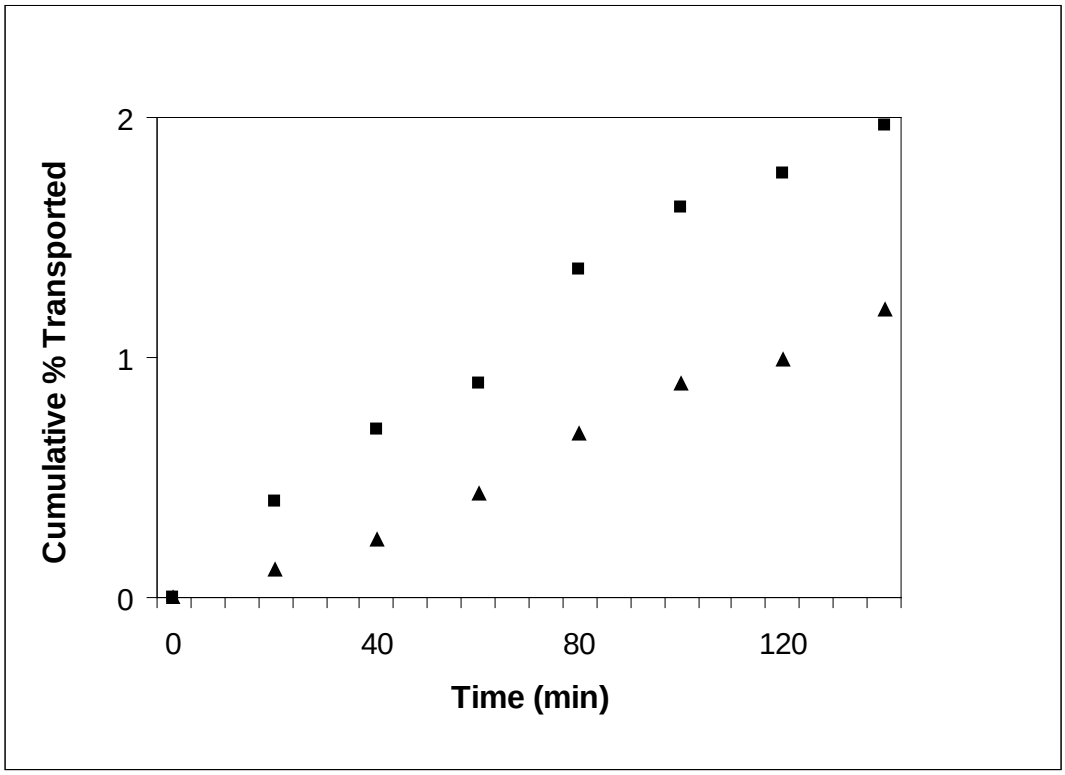

Figure 2a. Concentration-dependent effect of 24 hr carbamazepine pretreatment on calcium permeability in Caco-2 cells. Effect of 24 hr pretreatment with 236 µg/ml carbamazepine (▲) or vehicle (■) on apical to basolateral calcium transport over 140 min. Transport data shown is averaged from 3 independent experiments conducted in duplicate.

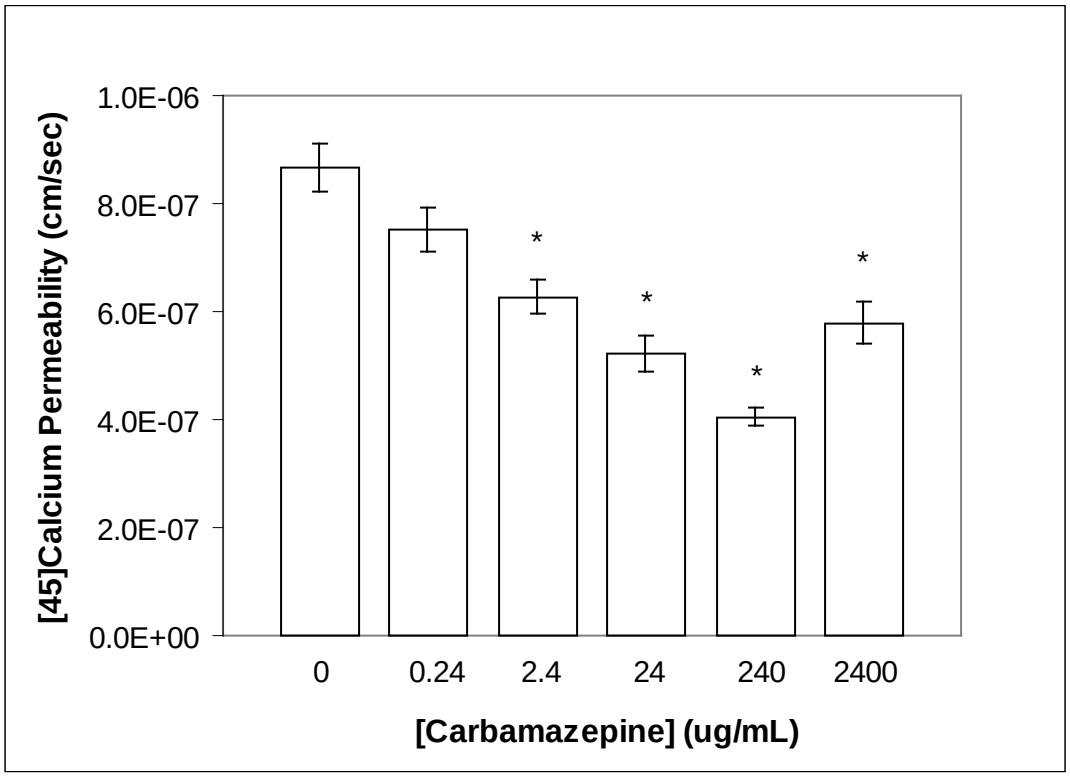

Figure 2b. Concentration-dependent effect of 24 hr carbamazepine pretreatment on calcium permeability in Caco-2 cells. Effect of varying carbamazepine concentration on Caco-2 cell permeability calculated from data similar to that shown in part A. Active transport of 2.5 mM CaCl was quantitated over 140 min and permeability calculated as described in methods. Permeability data shown is averaged from 3 independent experiments conducted in duplicate. *$p < 0.05$ compared to vehicle pretreated controls.

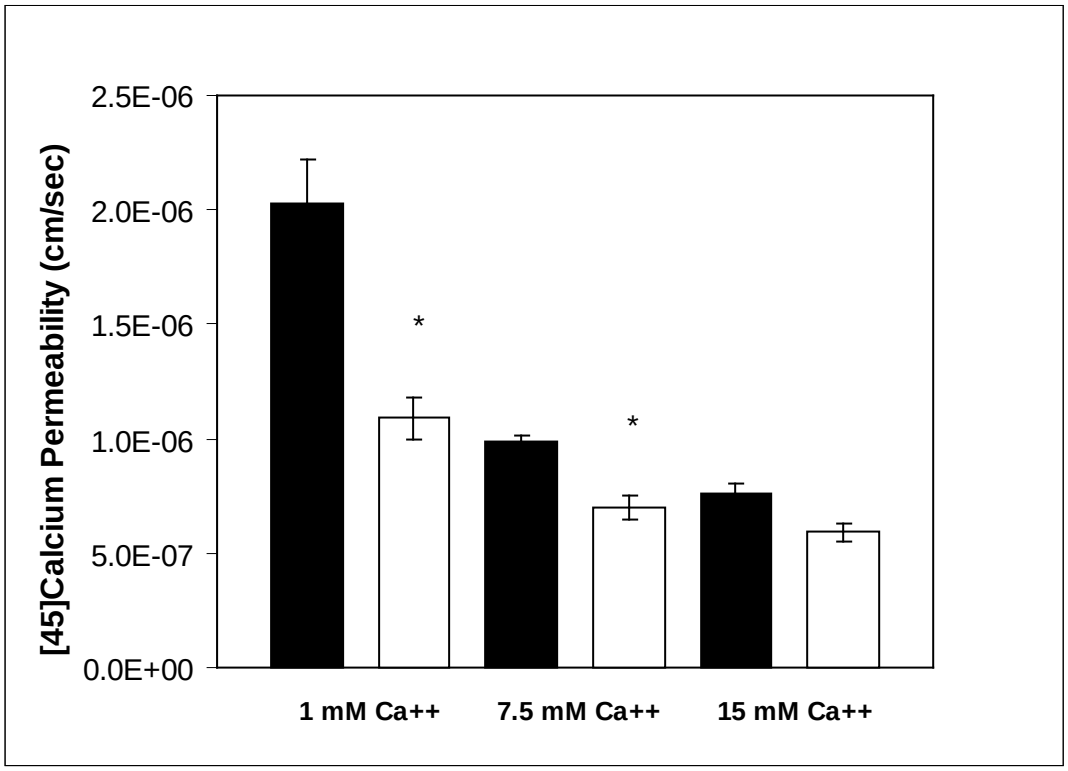

Figure 3. Effect of extracellular calcium concentration on Caco-2 cell permeability to calcium with or without phenytoin pretreatment. Caco-2 cells were pretreated for 24 hr with 5 ug/mL phenytoin (white bars) or vehicle (black bars) treatment. Apical and basolateral medium, in the continuing presence of phenytoin or vehicle were changed 30 min prior to experimentation to include the indicated concentrations of calcium. Active transport of CaCl was quantitated over 140 min and permeability calculated as described in methods. Data shown is averaged from 2 independent experiments conducted in duplicate. *$p < 0.05$ compared to vehicle treated controls.

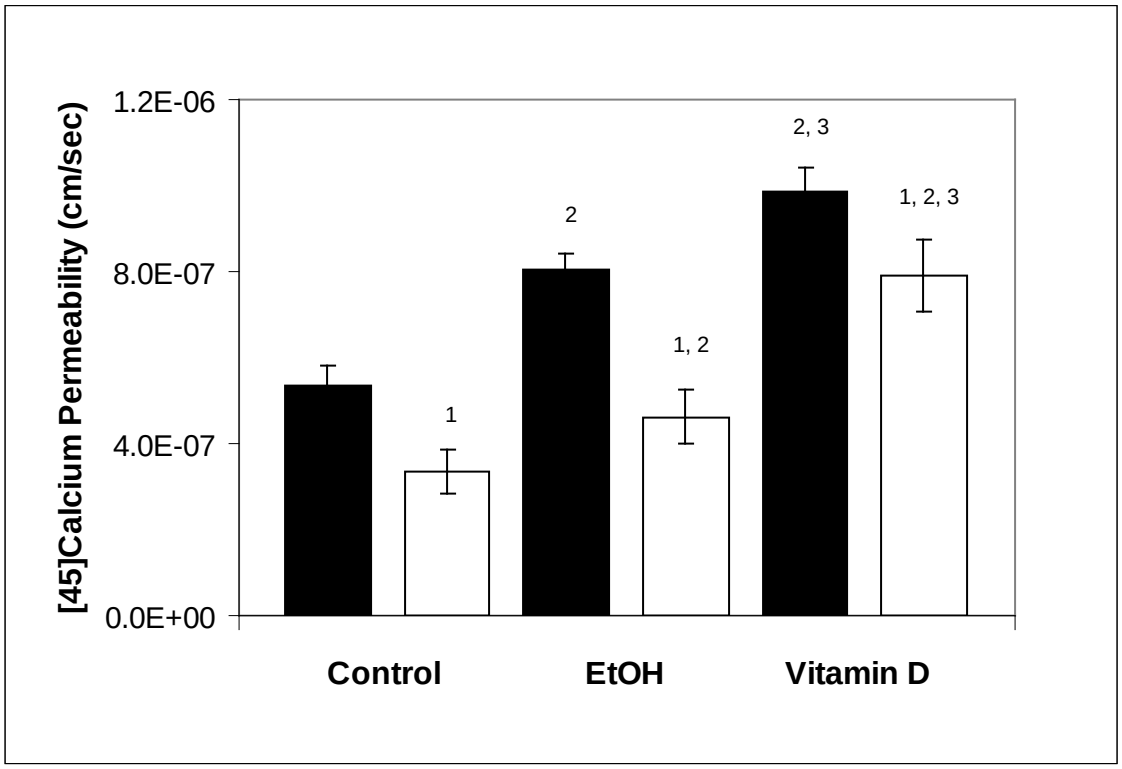

Figure 4a. Effect of vitamin D on Caco-2 cell permeability to calcium in the presence of phenytoin. Cells were pretreated with 101 nM active vitamin D (Vit D) or (EtOH) for 24 hr followed by addition of 5 ug/mL phenytoin (white bars) or vehicle (black bars) for another 24 hr in the continuing presence of vitamin D. Active transport of 2.5 mM CaCl was quantitated over 120 min and permeability relative to drug treatment was calculated as described in methods. Data shown is averaged from 3 independent experiments conducted in duplicate. $^{1}p < 0.05$ for phenytoin treated samples compared to vehicle treated controls. $^{2}p < 0.05$ compared to control treated samples with or without AED. $^{3}p < 0.05$ for vitamin D treated samples compared to EtOH treated samples with or without AED.

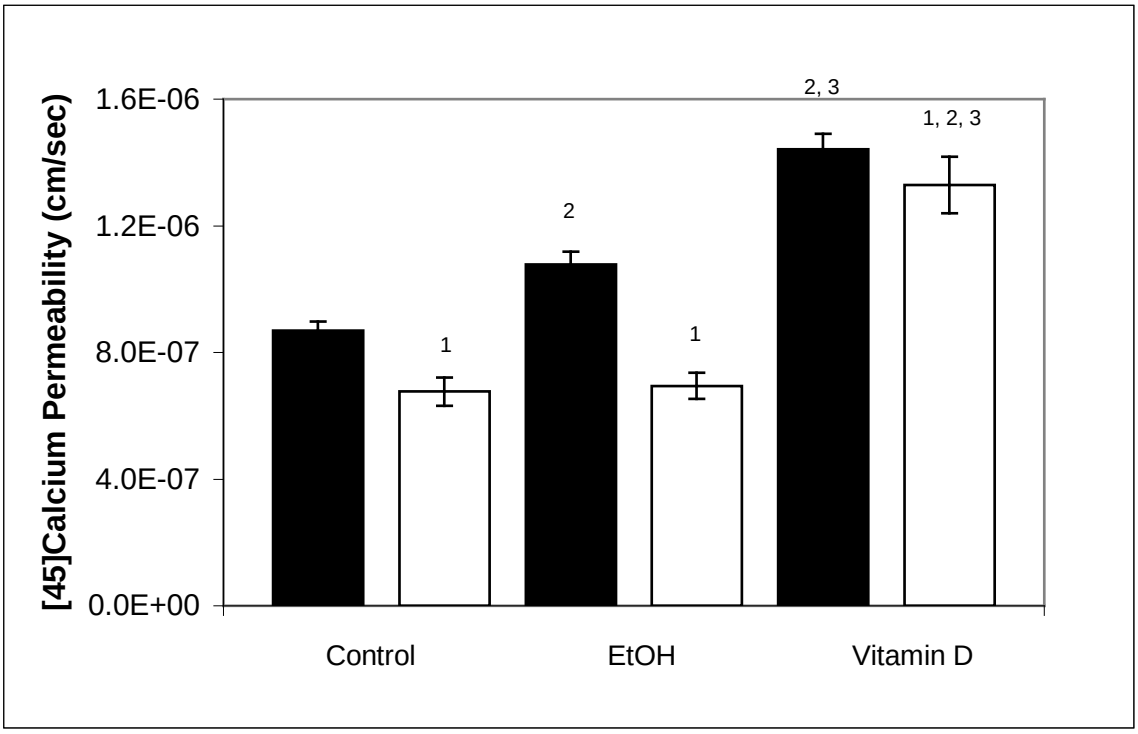

Figure 4b. ***Effect of vitamin D on Caco-2 cell permeability to calcium in the presence of carbamazepine.*** Cells were pretreated with 101 nM active vitamin D (Vit D) or 0.42 ug/mL vehicle (EtOH) for 24 hr followed by addition of 5 ug/mL carbamazepine (white bars) or vehicle (black bars) for another 24 hr in the continuing presence of vitamin D. Active transport of 2.5 mM CaCl was quantitated over 120 min and permeability relative to drug treatment was calculated as described in methods. Data shown is averaged from 3 independent experiments conducted in duplicate. $^{1}p < 0.05$ for carbamazepine treated samples compared to vehicle treated controls. $^{2}p < 0.05$ compared to control treated samples with or without AED. $^{3}p < 0.05$ for vitamin D treated samples compared to EtOH treated samples with or without AED.

TABLES

TABLE 1
Patient responses to quantitative questions 1-3 at pharmacies in Chile and Oregon

	Chile average (range) n=17	Oregon average (range) n=15
How many prescriptions have you had filled in the last 6 months at this pharmacy	27.6 (6-60)	26.6 (3-102)
How old are you?	*M: 63 (50-70) F: 57 (40-70)	M: 65 (45-75) F: 55 (45-80)
How often do you come to this pharmacy?	1.4/month (1-3)	1.6/month (0.5-4)

**estimated only because of cultural considerations; M male; F female*

TABLE 2
Patient responses to qualitative questions 4-12 at pharmacies in Chile and Oregon

How often do you take over the counter (OTC) medication?	
Chile: • Often	Oregon • Occasionally
How often do you ask for advice from your pharmacist before taking OTC medication?	
Chile: • Once in a while	Oregon • Rarely
How long have you been taking prescription medications?	
Chile: • "For years" • For 5-7 years	Oregon • "For a long time" • For 10-15 years
How often does your pharmacist offer counseling on prescription medications? On over the counter medications?	
Chile: • "If I ask they are very willing to help." • "Never, I don't even know if a pharmacist is back there."	Oregon • Whenever I have a new prescription or a change in manufacturer • More often than I would like
Do you talk to your pharmacist when you come to the pharmacy? If so, for how long and what about?	
Chile: • "Always, he's my angel" • "Never" • "I don't know who the pharmacist is"	Oregon • "Usually" • "For as long as I need to, to get my questions answered"

How would you describe your interactions with your pharmacist (friendly, uncomfortable, thorough, rushed, valuable, helpful, caring, etc)?	
Chile: • "I don't interact with the pharmacist" • Helpful, friendly, caring	Oregon • Business-like • Meaningful, useful, helpful
What services would you come to the pharmacy for (blood pressure, medication advice, shots, etc)?	
Chile: • To pick up medications • For advice on medications • To use the blood pressure machine • "I wouldn't want to bother the pharmacist with taking my blood pressure"	Oregon • To pick up medications • To get a flu shot • For advice on medications
What services does your pharmacist provide for you (medication advice, blood pressure, shots, etc)?	
Chile: • Advice on medications	Oregon • Advice on medications • Flu shots
What else could your pharmacist do to improve your health?	
Chile: • Be more available and in the front of the store • Keep track of my medications • They are doing a great job	Oregon • "Nothing I can think of" • "Fill prescriptions for waiting customers faster" • "Have more knowledge of herbal and natural products"

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APPENDIX A

PATIENT CONSENT FORM (ENGLISH)

Dear Prospective Participant:

The pharmacist in the community pharmacy setting interacts with patients on a daily basis. Cultural diversity is increasing around the world. A better understanding of cultural differences in perceptions and expectations of the pharmacist will help pharmacists to better serve their community. As a student pharmacist, community pharmacist, and professor, we are asking for your help in determining the perceptions that a patient has of their pharmacist in Chile based on past interactions.

We would appreciate you taking about 15 minutes to answer a series of questions. **Your participation in this study is voluntary and you may refuse to answer any question(s) for any reason.** Your responses will be added together with others and recorded as a group. If the results of this project are published your identity will not be made public. Only a small sample of patients will be asked to participate and answer questions regarding personal perceptions, so your participation is important to this study.

The answers you provide will be kept confidential to the extent permitted by law. Special precautions have been established to protect the confidentiality of your responses. There are no foreseeable risks to you as a participant in this project; nor are there any direct benefits. However, your participation is extremely valued.

If you have any questions about the survey, please contact me, Melinda von Borstel, at 011-541-740-1844 or by email at vborstel@yahoo.com. Ann Zweber, the principal investigator, can be reached at 011-541-737-5798 or by email at ann.zweber@oregonstate.edu. If you have questions about your rights as a participant in this research project, please contact Oregon State University Institutional Review Board (IRB) Human Protections Administrator at 011-541-737-3437 or by email at IRB@oregonstate.edu.

Thank you for your help. We appreciate your cooperation.

Sincerely,

Melinda von Borstel
Student Researcher

Ann Zweber
Principal Investigator

PATIENT QUESTIONNAIRE (ENGLISH)

1. How many prescriptions have you had filled in the last 6 months at this pharmacy?
2. How old are you?
3. How often do you come to this pharmacy?
4. How long have you been taking prescription medications?
5. How often do you take over the counter medication?
6. How often do you ask for advice from your pharmacist before taking over the counter medication?
7. How would you describe your interactions with your pharmacist (friendly, uncomfortable, thorough, rushed, valuable, helpful, caring, etc)?
8. How often does your pharmacist offer counseling on prescription medications? On over-the-counter medications?
9. Do you talk to your pharmacist when you come to the pharmacy? If so, for how long and what about?
10. What services would you come to the pharmacy for (blood pressure, medication advice, shots, etc)?
11. What services does your pharmacist provide for you (medication advice, blood pressure, shots, etc)?
12. What else could your pharmacist do to improve your health?

APPENDIX B

PATIENT CONSENT FORM (SPANISH)

Estimado Posible Encuestado:

En una farmacia, el químico farmacéutico interactua con los pacientes diariamente. La diversidad cultural está creciendo a nivel mundial. Un mejor entendimineto de las diferencias culturales en cuanto a la percepción y a las expectativas de los pacientes en relación al químico farmacéutico, les ayudará a éstos servir mejor a la comunidad. Como estudiante de química y farmacia, como química farmacéutica en una comunidad, y como profesor, estamos pidiendo su ayuda, para determinar la percepción que los pacientes tienen de los químicos farmacéuticos en Chile, basada en pasadas experiencias.

Le agradeceríamos si se pudiera tomar 15 minutos para responde una serie de preguntas. **Su participación en este estudio es voluntaria y Ud. Se puede negar a contestar cualquier pregunta, por cualquier motivo.** Sus respuestas seran agregadas a otras y seran registradas como de un grupo. Si el resultado de este proyecto es publicado, su identidad no será dada a conocer. Su participación en este estudio es muy importante, ya que solamente se tomará una muestra muy pequeña de pacientes para que participen y contesten las preguntas en cuanto a la percepción personal.

Las respuestas que Ud. Dé, seran confidenciales en cuanto la ley lo permita. Especial cuidado se ha establecido para proteger la confiabilidad de sus respuestas. No hay riesgos para Ud. En cuanto a participar en este proyecto; ni tampoco hay beneficios directos. Sin embargo su participación es extremadamente valiosa.

Si Ud tiene cualquiera pregunta acerca de esta encuesta, por favor comuníquese conmigo, Melinda von Borstel, al teléfono 1-541-740-1844 o por correo electrónico vborstel@yahoo.com. o con Ann Zweber, directora de la investigación, al teléfono 1-541-737-5798 o al correo electrónico ann.zweber@oregonstate.edu. Si tiene alguna pregunta en cuanto a sus derechos como participante de este proyecto de investigación, por favor contáctese con: Oregon State University Institutional Review Board (IRB) Human Protection Administrator al teléfono 1-541-737-3437 o por correo electrónico a IRB@oregonstate.edu.

Muchas gracias por su ayuda. Agradecemos su cooperación.

Sinceramente,

Melinda von Borstel
Estudiante Investigadora

Ann Zweber
Directora de la Investigación

PATIENT QUESTIONNAIRE (SPANISH)

1.-¿Cuantas recetas médicas ha despachado Ud. en esta farmacia, en los últimos 6 meses?
2.-¿Qué edad tiene Ud.?
3.-¿Con que frecuencia viene Ud, a esta farmacia?
4.-¿Desde cuándo ha estado Ud. tomando medicamentos recetados por su médico?
5.-¿Cuan a menudo toma Ud. medicamentos sin receta médica?
6.-¿Cuan a menudo consulta Ud. al químico farmacéutico antes de tomar medicamentos sin receta médica?
7.-¿Cómo describiria Ud. la relación entre Ud. y el químico farmaéutico(amistosa, incómoda,minuciosa, apurada, valiosa, beneficiosa, atenta, etc.)?
8.-¿Cuan a menudo el qúimico farmacéutico le ofrece ayuda cuando Ud. despacha una receta médica ?¿O cuando Ud. compra un medicamento sin receta médica?
9.-¿Conversa Ud. con el químico farmacéutico cuándo viene a la farmacia? Si es asi ¿Por cuánto tiempo y de qué conversan?
10.-¿A qué viene Ud. a la farmacia? (¿ a tomarse la presión, preguntar sobre medicamentos , a ponerse inyecciones,etc.?).
11.-¿Qué servicios le ofrece su farmacia (consejos sobre medicamentos, toma de presión, ginyecciones,etc.)
12.-¿Qué otra cosa podría hacer su farmacia para mejorar su salud?