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EUROMED PHARMACY

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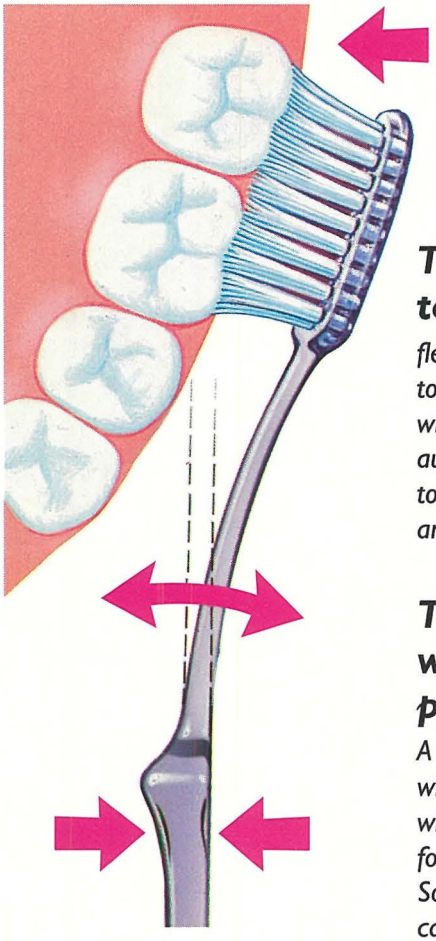

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EDITORIAL

Perceptopharmacology may be defined as the effect of perceptions such as smell, environment (a hospital ward or a disco) and distress on drug action. The philosophy of perception as it influences life has amply been investigated as has its influences on diseases such as cancer development and prognosis.

The care exerted today in providing the 'right' drug at the 'correct' time to the 'real' patient is justified and accepted as an indispensable professional service. We have also shown through studies carried out by our department that 'talking' to the patient also reduces side effects. Investigating a group of 32 breast cancer patients we found that there was significantly less ($p < 0.05$) nausea in patients who were advised verbally and through the distribution of specially prepared literature.

The investigation of chronopharmacological response independent of perception of sensory features such as alternating sunlight and darkness requires further studies. The cyclic occurrence of epilepsy is associated with menstruation and referral to the cyclic moon changes is sometimes made as an indication of the 'time' when an attack of epilepsy could be predicted. In Malta epilepsy is often referred to as *'tal-qamar'* - disease of the moon. Methods of treatment of epilepsy could be perhaps improved by applying chronopharmacological knowledge.

William J.M. Hrushesky, Professor of Medicine and Oncologist at Albany Medical college in New York, addressing the New York Academy of Sciences in 1994 stated that "Time is Everything - in sickness, as in health, rhythm is a critical factor. Effective treatment must work with the body's clocks, not against them." Methods and examples to determine the 'therapeutically wise' time of the day when a drug should be administered are given for areas such as cardiovascular, allergy, asthma and cancer treatment.

The benefits of timing can now be better understood with the advancements in Molecular Biology. The level of DNA synthesis in people is highest in the first half of the working day, the reverse of its level in mice ! This may yet be another explanation in addition to that of pharmacogenetics to justify inter-patient variation which is actually then an intra-patient variation of drug action.

This idea of chronopharmacology was brewing in the mind and thoughts of a number of scientists for the last twenty years. The development of biotechnology leads to further possible practical applications of this science. The concern for the adverse effects of glucocorticosteroids and the benefits of taking into consideration the cyclic diurnal differences, and the influence of shift work on the variations of hormone concentration and other biogenic substances is a classical example of the importance of chronopharmacological knowledge in clinical applications. It is the time to extend the chronopharmacological investigations to the molecular level especially in such cases as chemotherapy. No longer can the convenience of the staff be the deciding factor when a drug is given - the pharmacist has a new compliance exercise to do - an important and definite one in planning dosage regimens.

Anthony Serracino Inglott

STEREOISOMERIC PHARMACOVIGILANCE AND RACEMIC DRUGS IN HOSPITAL FORMULARIES

Janet Mifsud

Summary

The rapid emergence of published data regarding drug enantiomers should be reflected in the use of racemic drugs in the clinical setting. The Department of Pharmacy, University of Malta, becoming aware of the problems created by the unknowledgeable use of racemic drugs has conducted two studies. The first study data focused on quantifying the number of chiral drugs in the Maltese National Formulary, being administered as racemic mixtures, in order to provide a better basis for purchase specifications when procuring drugs. The second study analyzed attitudes by physicians towards drug chirality.

Keywords

Enantiomers, chirality, stereoisomerism, pharmacovigilance, racemic drugs.

Introduction

The realization of the importance of stereochemistry in pharmacology and therapeutic action has a valid contribution to make in the development of safer and more effective medicine[1]. Such new knowledge should be reflected in the use of racemic drugs. Both the scientific and popular press have focused their attention on the upsurge in stereoisomeric interest - some with 'alarmist' headlines[2,3,4,5,6,7]. This should provide a better basis for purchase specifications of drugs and the promotion of stereoisomeric awareness amongst authorities and members of the health care team.

Stereoisomeric Drugs

Racemates are 50/50 mixtures of the left and right-handed forms or enantiomers of compounds, whose molecular structure lack symmetry because the central carbon atom is surrounded by four different atoms or groups of atoms. This phenomenon is referred to as chirality - derived from the Greek word *'cherios'* which signifies handedness i.e. being left or right handed. Enantiomers are mirror images and can be distinguished from the way they rotate polarized light, hence the (+)/(-) or (d)/(l) nomenclature. The accepted nomenclature today is the (R)/(S) system which is related to the Cahn-Ingold-Prelog Convention[7]. For example labetolol is only one of a number of chiral drugs with two chiral centers (figure 1). Often one isomer is therapeutically active but this does not mean that the other is inactive. The therapeutically nonactive form in a racemate may be regarded as an impurity but it may not be a passive component of the drug mixture. It may be an agonist, or an antagonist, or it may have actions on other receptors, resulting in either unwanted side effects or contributing to overall drug efficacy. In addition, its metabolites may also be active or toxic[2].

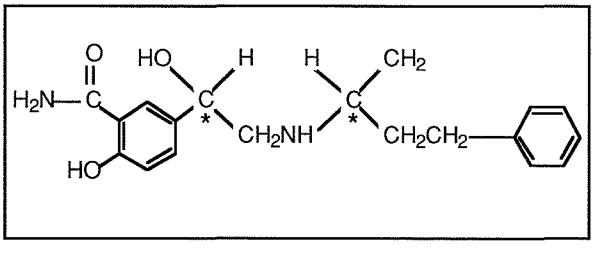

Figure 1: The chemical structure of labetolol. (*chiral centres).

Perhaps the most dramatic example is highlighted by the thalidomide tragedy. This drug was marketed during the 1960's as a sedative, and it was widely used by pregnant women, many of whom later gave birth to deformed children. Thalidomide was administered as a racemic mixture of its two optical isomers. Too late, did subsequent research show that it was only the sinistral (-)-isomer, and not the (+)-isomer, which had a teratogenic effect on rat embryos, producing the same birth defect as those of the thalidomide children in the early 1960's [8].

About 40% of synthetic drugs are chiral. Mason[9] has calculated that more than 80% of the synthetic chiral pharmaceuticals which appear in the United States Pharmacopoeia are administered as their racemates i.e. as equal mixtures of relatively 'active' and 'inactive' isomers. Terms such as isomeric ballast[2] and composite chiral drugs (CCDs)[10] have now become commonplace. But unfortunately medical curricula still deal with stereochemistry as an area to be investigated only when referring to the biochemistry of amino acids and sugars. Few teaching and/or reference medical books ever mention stereochemistry and its significance[1].

Methods

Study 1

A comprehensive database was compiled of all drugs in the updated version of the Maltese National Formulary, subclassified as synthetic, semisynthetic and natural, chiral and achiral and including stereochemical properties, efficacy, risk benefit, and cost effectiveness of single isomers of drug racemates (if available). Standard textbooks such as *Martindale*[11], *British Pharmacopoeia*[12] and *Therapeutic Drugs*[13] were used to determine the structure of each drug and whether it was available as single enantiomer or not.

Study 2

A survey was conducted on a randomized sample of registered physicians in Malta in order to review their attitudes towards inherent problems associated with the use of racemic drugs. The thesis behind the study expounded on Tucker's presupposition that most clinicians are unlikely to 'know their right hand from their left'[3].

Results

Study 1

From an in-depth scrutiny, it was revealed that approximately 47% of drugs in the Maltese National Formulary classified as semisynthetic/natural; 99% of these had at least one chiral center but nearly all were available as one isomer, while more than 60% of synthetic chiral drugs were purchased as racemates (figure 2, figure 3). The distribution of racemic drugs amongst different pharmacological categories was found to be highly uneven. The appearance of a racemate in the field of peptides or hormones was exceptional.

The study also included a separate review of geometric isomers, such as tamoxifen and phytomenadione, which had previously been pooled with chiral isomers. These made up about 3% of the total number of drugs (figure 4). Problems associated with the use of the wrong isomer of tamoxifen had already been described in our hospital[14].

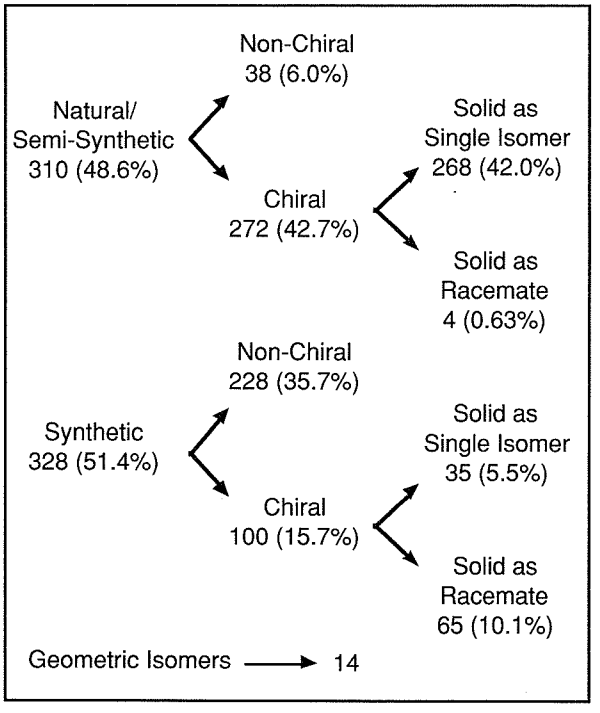

Figure 2: The chirality of drugs in the Maltese Formulary.

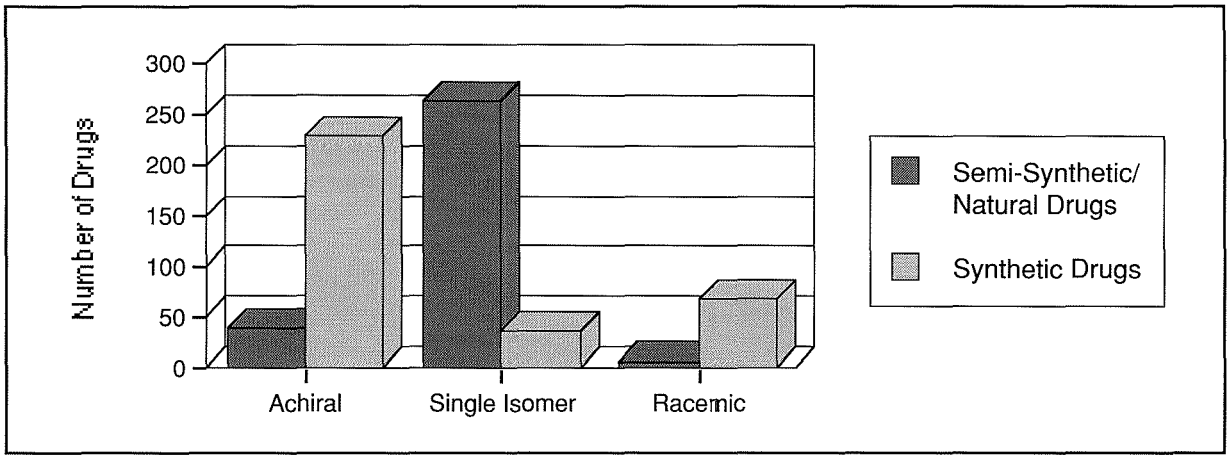

Figure 3: A comparison of the stereoisomeric configuration of organic drugs in the Maltese Formulary.

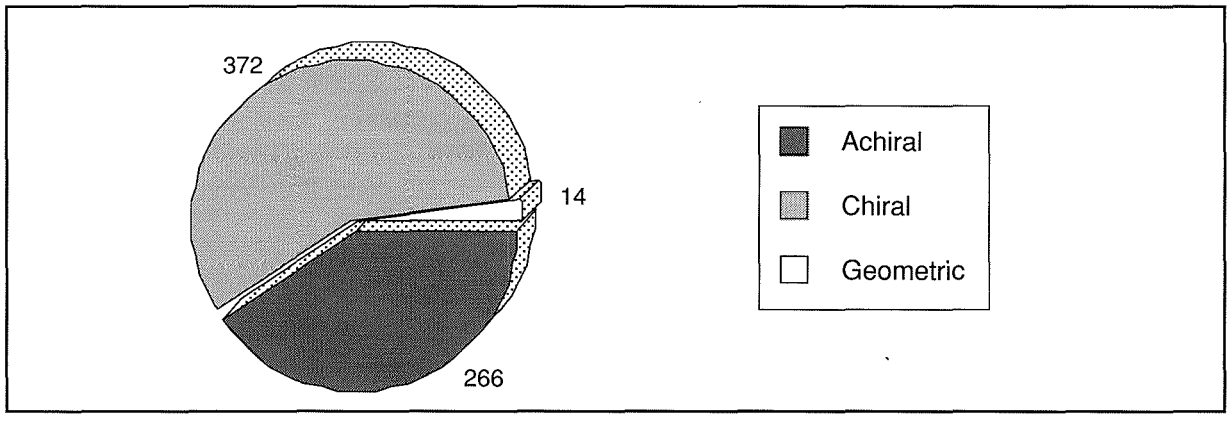

Figure 4: Subdivision of organic drugs found in the Maltese Formulary.

These results were consistent with previously published data [15,16,17]. Most racemic drugs are now under investigation and the pharmacokinetic and pharmacodynamic differences studied. Only a few synthetic chiral drugs e.g. naprosyn, timolol, methyldopa, and dextromethorphan are already available as a single enantiomer[1,18,19].

A review of published literature was undertaken to evaluate when the use of single isomers is recommended. References were found to indicate large pharmacological differences in quite a large number of chiral drugs[19]. It has been reported that it is (d)-propranolol which acts as the beta-adrenoreceptor agent, but both stereoisomers contribute to its local anesthetic and histamine releasing action[20]. (d)-Ketamine is predominantly a hypnotic and an analgesic, whereas the (l)-isomer is the main source of its unwanted side-effects [21]. The therapeutic index of disopyramide could be improved by eliminating the (-)-isomer. This enantiomer is a less potent anti-arrhythmic then the (+)-form, and it is mostly responsible for the heart failure precipitated by administration of the racemic drug, through its marked negative inotropic effect [22].

Care must be exerted if decisions are taken to change from a racemate to an isomer. Although the (S)-isomer of warfarin is about 3-5 times as potent as its antipode, switching from the racemate to the (S)-isomer, would necessitate a reduction in the dose. There would be no change in therapeutic index and this would precipitate a specific set of potential drug interactions[23]. On the contrary, the greater intrinsic anti-inflammatory potency of (S)-ibuprofen is compensated to some extent when using the racemate by metabolic inversion of the less active (R)-form and in fact approximately 60% of an oral dose of (R)-ibuprufen is inverted to the (S)-enantiomer [24]. Drugs available as single isomers were often prohibitively expensive compared to their racemic counterparts. Cost effectiveness of the use of single isomers is still to be determined.

The results and recommendations of this study are to be handed over to the Maltese Drugs and Therapeutic Committee, which is responsible for updating the Maltese National formulary and also to the Drug Information Unit, at St. Luke's Hospital, the major teaching hospital in Malta.

Study 2

A survey was made among a randomized sample of registered physicians in Malta. Over 320 questionnaires were sent by post. There was a 33% response, consistent with previous surveys of this type and it reflected the actual population of Maltese physicians. Nearly 40% of these graduated 5-10 years ago (figure 5). The respondents were mostly housemen (21%) or consultants (23.1%). 28% worked in the community, while 10% were retired (Figure 6); 66.2% regularly attended continuing education courses.

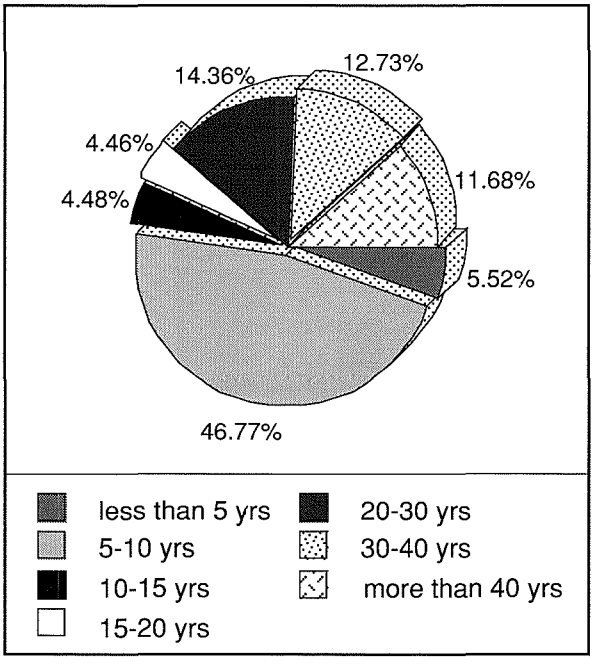

Figure 5: Registration year of physicians who responded.

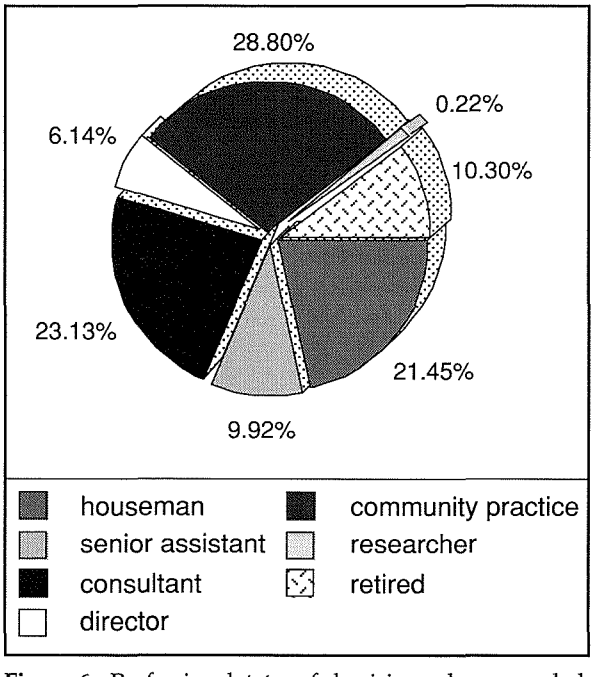

Figure 6: Professional status of physicians who responded.

When questioned about the terms stereoisomerism and chirality, 52% said that this topic had only been mentioned briefly in their undergraduate years, mostly in their biochemistry and pharmacology courses. Less than 10% had heard the terms in postgraduate training or continuing education courses (Figure 7). Two doctors referred to the use of (-) folic acid and importance of stereoisomerism in legal definitions of drugs of abuse. 83% had never read any information about drug chirality while 11% had read at least one article in the medical literature. Only in 2 cases, had the subject been mentioned by a drug medical representative (Figure 8).

Were the terms *Stereoisomerism* or *Chirality* ever mentioned ?

	Yes	No
Undergraduate Years	52.00%	48.00%
Postgraduate Years	9.88%	90.12%
Continuing Education	5.82%	94.18%

Figure 7: Physicians' knowledge of chirality.

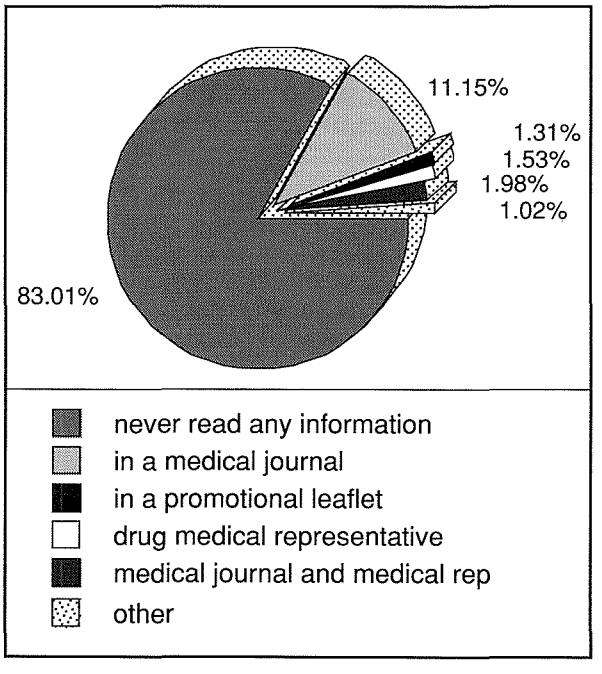

Figure 8: Physicians' sources of information on chirality.

43% were of the opinion that these problems should be tackled by physicians, pharmacists and biochemists, and some even suggested that it should be tackled by the local authorities and the pharmaceutical industry (Figure 9). It was interesting to note that nearly all were grateful for the information we had given them and 92% wanted to learn more about the subject, especially by receiving more information at home or attending a lecture. Some respondents were keen to participate in continuing education sessions on the subject (Figure 10). Only a small percent of physicians (<13%) questioned were actually aware of the problems associated with administering drug racemates (Figure 11). Nearly all the doctors found the subject very interesting and even showed their professional gratitude for the study.

Interested in learning more about drug chirality	**92.26%**
More literature at home	62.06%
Read more about it in medical journals	27.52%
Attend organised lectures	15.72%
Part of continuing education course	33.31%

Figure 10: Respondents' interest to further knowledge.

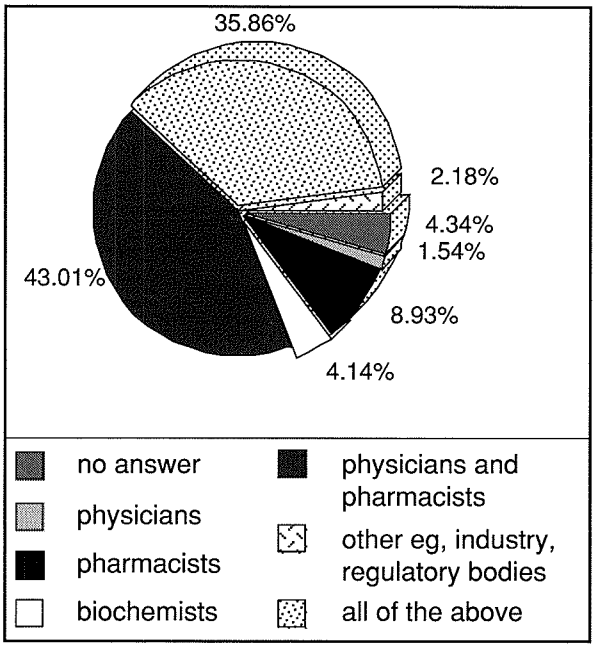

Figure 9: Members of the health care team who, according to physicians, should tackle the problem.

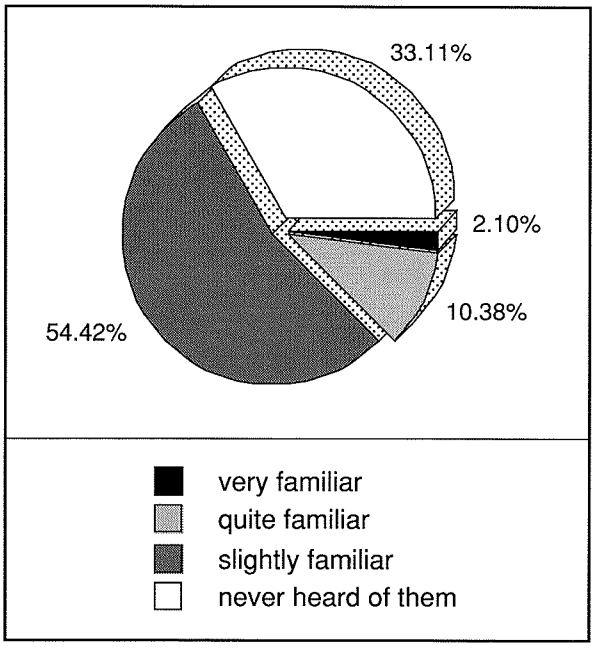

Figure 11: Physicians' familiarity with the terms *stereoisomerism* and *chirality*.

Conclusion

Most chiral synthetic drugs are still widely used as racemic mixtures in the clinical setting even if published literature suggests pharmacological differences. It can be argued that the majority of physicians are still unaware of problems associated with administering racemic drugs, but it appears from this study that most are willing to learn more. Several regulatory agencies have issued guidelines[25,26,27,28]. Perhaps it is wrong to start a witch hunt for racemic drugs which are already marketed[29], but it can be predicted that a full clinical study of the pharmacological and toxicological effects of both enantiomers of any new chiral drug, may be required soon, in order to obtain authorization for marketing the drug as a racemic mixture [30](Figure 12). Only 5 of the 12 leading chiral drugs in the USA are being sold as single isomers. Some drugs are already being developed as the pure enantiomers from existing composite chiral drugs with still valid patents. These include S-atenolol, R-salbutamol, S-terfenadine, R-ketoprofen, RR-formoterol, S-fluoxetine, S-ondansteron, R-salmeterol, S-fluriprofen and S-ibuprofen. Some drugs such as fenfluramine, are being sold simultaneously as a single isomer (Adifex^{R}) and as a racemate (Ponderax^{R}). The less

cardiotoxic R-isomer of verapamil is being investigated for the prevention of MDR in cancer chemotherapy.

Stereoisomeric pharmacovigilance on the use of racemic drugs should be encouraged and the new EU and FDA regulations translated to the clinical setting[31] but it appears that, so far, little has been done. In very few of the recommended textbooks of pharmacology, pharmacokinetics and toxicology, are the principles of stereoselectivity properly described or even mentioned in the index, although some specialized pharmacy journals have included the subject in continuing education articles for their readers[32,33]. Financial restraints often dictate health policies and procurement procedures. However, stereoisomeric pharmacovigilance on the use of racemic drugs in hospital formularies must be encouraged. Appropriate education is needed if choices between racemates and single isomers are to be presented. Drug therapy can only gain from the fruitful discussions on the continual assessment of **'looking glass drugs'**, and to Tucker's challenge 'Does the left hand know what the right hand is doing, we ask ' Does the left hand want to know what the right hand is doing'. The answer from Malta is an unequivocal grateful 'yes'.

Arguments in favour of marketing drugs as pure enantiomers
• Improved less complex and more selective pharmacological profile • Better therapeutic index • Less complex pharmacokinetics • Less complex concentration-response relationships particularly in relation to therapeutic drug monitoring
Arguments against marketing drugs as pure enantiomers
• Cost of development • Cost of production

Figure 12: Enantiomers or Racemates?[30]

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Ms. Janet Mifsud is a lecturer at the Department of Pharmacy, University of Malta and is currently reading for a Ph.D. at Queen's University of Belfast. This paper was presented at the European Medicines Research: Perspectives in Pharmacotoxicology and Pharmacovigilance Conference, Brussels, December 1993, held under the auspices of DGXII of the European Union. The author wishes to express her thanks to Dr. J. S. Millership from the School of Pharmacy, Queen's University of Belfast for his encouragement and insights into this subject, to Prof. A Serracino Inglott, Head of Department of Pharmacy, University of Malta, for his help in obtaining funds to attend the conference and to Mr. T. Ebejer B. Pharm (Hons.) who collected most of the data.

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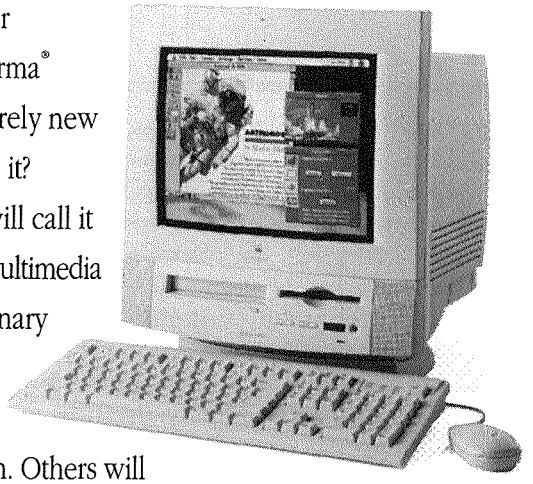

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THE INFLUENCE OF pH ON THE SOLUBILITY OF MICONAZOLE AND ITS EFFECT ON SURVIVAL OF *C. ALBICANS*

Anna McElhatton

Summary

The in-vitro activity of miconazole against *Candida albicans* (NCPF 3262) was investigated using Time-survival curves. An HPLC assay was also developed to produce a pH-solubility profile which showed that miconazole solubility varied from (2.5 ± 0.3) mg/L at pH 12 to (28.9 ± 0.6) mg/L at pH 5. Time survival curve determinations demonstrated a relationship between miconazole concentration and the area under the curve (AUC). In controlled conditions using buffered aqueous solutions of miconazole (8 mg/L), a change in pH from 8 to 5 resulted in an increase in the antifungal activity of miconazole. Experiments performed in buffered YNB using the same concentration of miconazole showed that a change in pH from 7 to 5 resulted in a decrease in miconazole antifungal activity. Such results indicate that apart from the well known inhibitory effects that the growth media have on azole activity, pH at which tests were performed may also influence growth of C. albicans in batch culture.

Keywords

Candida, miconazole, pH, solubility, viable counts, area-under-the-curve (AUC), high performance liquid chromatography (HPLC).

Introduction

Time-survival curves have recently been used to evaluate the effect of variation of the test conditions on antimicrobial susceptibility of microorganisms to antimicrobial agents. Interpretation of this kind of data entails the comparison of the pattern of survival, rates of kill or reduced growth and viable count at 24h[1,2].

Every organism has a range of pH over which growth is possible and an optimal pH at which growth is most favoured, different microorganisms are known to have different tolerances to environmental change including pH. Fungi are reported to be more tolerant to pH changes than bacteria[3]. Miconazole is an azole antifungal agent with a pK_a of 6.7[4],that is used in the treatment of candidosis[5]. Reports in literature indicate that the unprotonated form of the drug is the one that is responsible for antifungal activity[6].

A series of experiments to investigate the effect that pH has on *in vitro* activity against *Candida albicans* in a defined growing medium were designed. To complement this *in vitro* microbiological work a simple reversed phase High Performance Liquid Chromatography (HPLC) assay was developed. Reports in the literature suggest that the chromatographic techniques represent the favourite approach for quantitation of azoles such as miconazole[7]. The assay was a simple chromatographic separation involving a binary mixture on a reversed phase column. From the data obtained a pH solubility profile of miconazole in aqueous medium constructed. The data obtained was used to investigate the possibility of a correlation between miconazole activity and pH.

Method

Materials required for the HPLC assay:
The apparatus used throughout the HPLC assay for the determination of the solubility of miconazole in aqueous solution (at pH values that range from 5 to 8) consisted of: An Ultracentrifuge (MSE® Superspeed 50) fitted with a 30° angle set to reach an average RCF of 100,000 **g** and a rotor speed of 35,000 rpm, a Shimadzu® L.C. 6A chromatograph fitted with a rheodyne injector and a 20 ml injection loop, a Shimadzu® UV-visible variable wavelength detector, a 25 cm x 4.6 mm i.d., 5 µm particle size reverse phase column (Spherisorb® ODS 2. Phase Separations, UK), and a single channel strip chart recorder. (Gallencamp®, UK). Reference materials, chemicals and solvents used were, miconazole (Janssen®, Belgium), ketoconazole (Janssen®, Belgium), dimethyl formamide (DMF) (Sigma®, UK), ammonium phosphate (BDH®, UK) deionised water, methanol HPLC grade (Labscan®, UK). The mobile phase selected for this assay consisted of a 95% methanol and 5% 0.05M ammonium dihydrogen phosphate mixture. After mixing, the mobile phase was filtered through 0.45 µm PTFE filters to degas and remove any residual particulate matter.

Materials required for the microbiology experimentation:
C. albicans (NCPF 3262), maintained on Bacto Yeast Morphology Agar (BYMA, Difco, USA) slopes at 4°C., Yeast Nitrogen Base (Bacto® YNB, Difco, USA) prepared according to the Wickerham Formula[8], Sorensen's Buffer, filtration apparatus (Millipore®, USA), 0.22 µm and 0.45 µm filters and stock solutions of miconazole (1000mg/L) which were allowed to self sterilise[9].

Methods used in the HPLC assay:
Samples used in the HPLC assay were prepared from a stock solution of miconazole (1000 mg/L) in methanol (100% $^{v}/_{v}$, HPLC grade). A series of standard solutions that ranged from 0.5 to 10 mg/L were used to construct calibration curves of miconazole in Sorensen's buffer (pH 7). The solutions were vortexed for 1 min and a 2 ml aliquot taken and shaken, on a rotary mixer, for one hour with a volume (2 ml) of chloroform. The extraction process was repeated a second time with a further 2 ml volume of chloroform. The chloroform layers were removed, dried under a stream of nitrogen at a constant temperature of 35°C and reconstituted in a total volume of 1 ml of a 2.5 mg/L solution of ketoconazole (internal standard) in mobile phase, previously filtered through 0.45µm PTFE filter. Triplicate injections (20 µl) were made for each solution, peak heights for miconazole (m) and ketoconazole (k) were measured manually and the mean peak height ratio (m/k) and standard deviation (sd) were calculated for each concentration. Samples for analysis consisted of saturated solutions of miconazole in a 1% solution of methanol in Sorensen's buffer, prepared at pH 5, 5.5, 6.0, 7.0 and 8.0 respectively. The saturated solutions were left for 24h in a shaking water bath set to 25°C and 100 strokes/min. About 20 ml of each solution was placed in ultracentrifuge tubes that were balanced to the nearest 10 mg. The solutions were spun down for 90 min at an average RCF of 100,000 **g**. An aliquot (2 ml) of supernatant was taken from each tube and subjected to a similar extraction procedure and then finally reconstituted in 1 ml of mobile phase containing 2.5 mg/L ketoconazole internal standard. This process was carried out over forty eight hours, during which a total of three sets of test solutions for each pH value were prepared. On each test day, the calibration standards and the test solutions were run. Reversed phase chromatography using an octadecylsilane (ODS) column together with a mobile phase that consisted of 95% methanol and 5% 0.05M ammonium dihydrogen phosphate was used throughout the experiment. The mobile phase was pumped through the chromatograph at ambient temperature, at a rate of 2 ml/min and the peaks detected at a wavelength of 230 nm with 0.02 attenuation value. Traces obtained from the manually injected samples (Figure 1) were recorded on a strip chart recorder set at a chart speed of 5 mm/min. The miconazole concentration in each sample and therefore solubility was determined from the measurement of miconazole and ketoconazole peak heights.

Figure 1: HPLC Chromatogram of miconazole (m) and ketoconazole (k).

Methods used in the microbiology experimentation:

MIC and MFC determinations were carried out using a (macro) broth dilution technique, using a two-fold dilution series.

The experiments designed to investigate the survival of *Candida albicans* challenged with miconazole in YNB and aqueous medium required the same materials needed for the MIC and MBC determinations, the procedure adopted was as follows:

One loopful of *C. albicans* from a YMA slope was inoculated into buffered YNB (100 ml) and placed overnight in a shaking water bath (37°C, 150 strokes/min).The experimental challenge was prepared by addition of 10 ml overnight culture into warmed buffered YNB and grown until an $O.D._{.540}$ value of 0.5 was obtained. This culture (100 ml) was collected on a 0.45 µm filter and rinsed with warmed media. The culture was then resuspended in 10 ml of warmed media.

Sterile solutions of miconazole, 8 mg/L and 16 mg/L in buffered YNB and 1% DMF, were prepared by hundred-fold dilution of the appropriate sterile stock solutions (in 100% DMF) with sterile buffered YNB.

A volume (1 ml) of culture was used to inoculate the test media that consisted of miconazole in buffered YNB and 1% DMF. A control flask that did not contain miconazole was also inoculated with this culture The flasks were then incubated for a total of 24h during which samples were taken for viable count determinations. Throughout the exercise, the pour plate technique was used exclusively for viable count determinations.

In this set of experiments replicate cultures exposed to concentrations of miconazole were used for each pH and concentration.

Results

The results of the theoretical solubilities of miconazole in buffer, obtained with the use of the Henderson-Hasselbalch equation[10], and those determined by HPLC varied with changes of pH. Both sets of data show that the solubilities of miconazole at pH values above 6 are low (Table 1) and therefore media that contained more than 3.99 mg/L miconazole in solution could not be prepared at pH 6 and above. Due to these results, the process of double membrane filtration, as a means of sterilisation, could not be directly and safely applied to aqueous growing media (with 1% $^{v}/_{v}$ organic solvent) that contained more than 3.99 mg/L miconazole.

pH	Mean solubility* (mg/L)	Theoretical solubility† (mg/L)
5.0	28.90	129.84
5.5	10.28	42.79
6.0	3.99	15.27
7.0	2.79	3.81
8.0	2.37	2.67

*(HPLC determination)
†(Henderson-Hasselbalch equation calculation)

Table 1: Mean solubility profile determined by HPLC and the theoretical pH solubility profile for miconazole estimated by the Henderson-Hasselbalch equation as functions of pH.

Data from MIC and MFC determinations at pH 6 and 7 showed that not more than a two-fold difference was obtained with determinations carried out at pH 6 and 7. Time survival experiments were performed with the MFC values obtained at these two pH values (Table 2).

The data obtained from the time-survival experiments, carried out in YNB, was used to calculate the Area Under the Curve (AUC) with the use of the Trapezoidal rule (Table 3). In YNB, at pH 6 and 7, at both 8 and 16 mg/L, the algebraic sum of the AUC obtained is negative thus indicating that the deleterious effect exceeded that of growth. At pH 5 and 5.5 the algebraic sum of the AUC is positive. It should be noted that the AUCs obtained for both 8 and 16 mg/L have been estimated to have the same area.

The results obtained from experiments carried out in an aqueous medium (Tables 4), indicate a relationship between the extent of the killing process of the antifungal agent and pH of the medium.

pH	MIC (mg/L)	MFC (mg/L)
6.0	4.00	16.00
7.0	4.00	8.00

Table 2: MIC and MFC value for miconazole (mg/L) against *C. albicans* (NCPF 3262).

pH	AUC Conc. Miconazole (Mean, ± sd) 8mg/L	16mg/L
5.0	+ (11.90 ± 0.23)	+ (11.22 ± 0.61)
5.5	+ (3.36 ± 0.42)	—
6.0	- (2.25 ± 8.85)	- (43.92 ± 2.40)
7.0	- (18.62 ± 16.47)	- (32.61 ± 26.10)

Table 3: The relationship between AUC, miconazole concentration and pH of growing medium for cultures of *C. albicans* growing in buffered YNB.

pH	AUC (Mean, ± sd)
5.0	+ (17.53 ± 1.09)
5.5	+ (12.95 ± 0.91)
6.0	- (11.18 ± 3.69)
7.0	- (4.70 ± 1.86)
8.0	- (3.22 ± 1.81)

Table 4: The relationship between AUC and pH of cultures of *C. albicans* growing in aqueous solutions of miconazole (8mg/L) containing 1% DMF.

Discussion

The sensitivity of *C. albicans* (NCPF 3262) to miconazole was assessed by MIC and MFC determinations. This isolate was sensitive to miconazole and the values were within the expected sensitivity range according to previous in-vitro determinations[11]. Results at pH 6 and 7 were similar with no result differing by more than one dilution.

The inocula used throughout were obtained from cultures that were in log phase It has been suggested that the most effective killing occurs with organisms in mid-log phase. Less effective killing was reported with late log phase organisms with the least effective killing occurring with organisms in the stationary phase; which factors could influence the outcome of tests[12].

Time-survival determinations. give a better understanding of the changes in viability that can occur over the test period and measurement of the AUC permits an overall view of the time-survival curve and has recently been used to examine the activity of antibiotics against *Ps. aeruginosa* [5]. The results of the HPLC assay show that there is a direct relationship between pH and solubility of miconazole in a liquid medium. In other microbiological experimentation it has been shown that at pH 7, that there is a relationship between miconazole concentration and both rate of kill and AUC. A rapid increase in AUC was noted in the lower concentration range, but as concentration increased, the effect was considerably reduced[13].

These effects may be due to the fact that at pH 7 the solubility of miconazole in an aqueous medium is low. The actual solubility ranges between 2.8 and 3.8 mg/L, depending on whether the solubility is taken as that previously determined by HPLC or that determined by application of the Henderson-Hasselbalch equation. Above pH 6, solubility of miconazole in an aqueous medium is thus a problem when experimental procedures are designed to investigate the effect that specific concentrations of miconazole have on the behaviour of batch cultures of *Candida albicans*. Low solubility of miconazole in aqueous media, therefore did not permit the standard sterilisation by filtration of solutions, or rather suspensions, of miconazole in aqueous buffer solution or in aqueous buffered solutions of YNB. The two constituents had to be prepared separately and sterilised and then mixed together under aseptic conditions[9].

Apart from solubility, the deleterious effect exerted by miconazole may also be dependent on the degree of ionisation of the molecule. With a pK_a of 6.7 miconazole exists predominantly in its unionised form at pH 7 ($\alpha_{pH\ 7}$ = 33.4%), which moiety is reported to be required for antimicrobial activity; in this case, direct lethal action that causes damage to *C. albicans* cells. This has led to the opinion that pH regulation of the direct lethal action of miconazole against *C. albicans* reflects the influence of the H^+ ion on drug molecules, rather than on yeast cells. In fact non-protonated miconazole appears to be required for direct lethal action (DLA)[6].

The effect of pH on solutions of miconazole (8 and 16 mg/L in 1% DMF as solvent) in buffered aqueous solutions or in YNB (buffered to the required pH with Sorensen's buffer) were markedly different. In YNB, the efficacy of miconazole increased with increasing pH, in which conditions solubility decreased and a the degree of ionisation decreased. In contrast, the efficacy of miconazole in aqueous solution, at lower pH was greater.

In a growth medium such as YNB, components of the growth medium itself may exert some effect on the drug. As an example, the medium may adsorb the drug thus reducing the active concentration in solution. A further factor associated with assessment of time-survival in growth medium compared to the aqueous solution, is the behaviour of the organism. The growth medium may protect the organism and aid its survival.

The influence of media on the activity of azoles is well known[11]. However, it has not been previously demonstrated that this is complicated by the pH of the test conditions and this may account for some of the variability in the results reported.

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The Author

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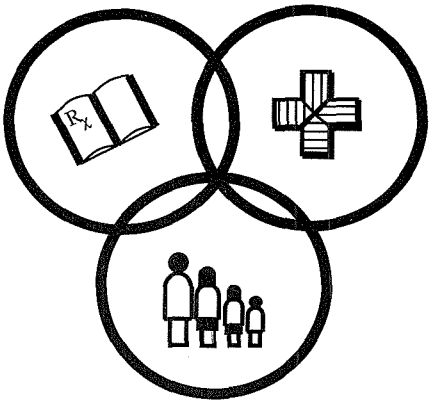
The Malta College of Pharmacy Practice



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Providencia

Escherichia

Klebsiella

BAYER **Ciproxin®**
Cip:ɔfloxacin

Moraxella

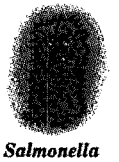
Haemophilus

Proteus

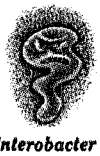
Neisseria

Staphylococcus

Pseudomonas



Salmonella



Enterobacter

Legionella

Streptococcus

Haemophilus

intelligent, fast, bactericidal

Neisseria

Diplococcus

ACUTE VIGABATRIN-PHENOBARBITONE-INTERACTION ON EXPLORATORY BEHAVIOUR OF RATS

Fathi M. Sherif Abudalla S. El-Hwuegi Eva Kumlien

Summary

Vigabatrin (gamma-vinyl GABA) is an irreversible inhibitor of the enzyme GABA-transaminase (GABA-T) which is responsible for the catabolism of the major inhibitory neurotransmitter gamma-aminobutyric acid (GABA) in the brain. Vigabatrin causes a several fold increase in the levels of brain GABA. The current study investigated further the effects of acute treatment with vigabatrin (100 mg/kg, i.p.) & phenobarbitone sodium (20 mg/kg, i.p.), alone and in combination, in two rat behavioural models of exploratory activity: the elevated plus-maze model of anxiety and the open field test of locomotor activity. A single injection of vigabatrin or phenobarbitone alone, produced anxiolytic effects in the elevated plus-maze test and increased locomotor activity in the open field test. In contrast, after the concomitant administration of both drugs, the anxiolytic effects were no longer produced in the elevated plus-maze. The increased locomotor activity was also diminished in both tests of exploratory behaviour. These results shed light on the GABA hypothesis of anxiety, insofar as the increased availability of GABA, resulting from either GABA-T inhibition (vigabatrin) or facilitation of GABA-mediated chloride channels (phenobarbitone), seems to result in an increased emotional reactivity which, however, subsequently disappears during combined treatment.

Keywords

Anxiety, elevated plus-maze test, exploratory behaviour, GABA-T, interaction, phenobarbitone sodium, open-field test, vigabatrin.

Introduction

In the mammalian central nervous system (CNS), gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter[1]. Modulation of GABA-ergic activity can influence CNS events in a number of ways. Thus a reduction in GABA availability has been implicated in a variety of neurological and psychiatric disorders[2] including epilepsy[3], anxiety and depression[4]. In the brain, GABA is metabolised by a transamination reaction catalysed by the enzyme GABA-transaminase (E.C. 2.6.1.19; GABA-T) (for review, see Sherif, ~). Thus, agents capable of enhancing GABA-ergic inhibition via the $GABA_A$ receptor have therapeutic uses as anxiolytics and anti-convulsants[6]. Vigabatrin (gamma-vinyl GABA) is a selective irreversible inhibitor of GABA-T[7], causing a rapid dose-dependent increase in brain GABA levels[8]. Effective central inhibition of GABA-T was demonstrated following peripheral administration of vigabatrin[7,9]. Clinically, vigabatrin is demonstrated to have a consistent anticonvulsant effect in patients with chronic drug-resistant epilepsy[10].

Barbiturates belong to another class of drugs that are still used in the management of grand-mal epilepsy[11]. Until the discovery of benzodiazepines in 1960s, barbiturates were the drugs of choice in the treatment of anxiety[12]. However, some barbiturates (phenobarbitone) have specific anticonvulsant and anxiolytic effects, which do not seem to be a reflection of non-specific central nervous system depression, and these have a continuing clinical use [12]. There is a substantial evidence of a specific interaction of barbiturates and the GABA system. Thus, barbiturates bind onto a specific recognition site on the $GABA_A$

receptor chloride complex[13]. Stimulation of the barbiturate receptors leads to an increase in the binding of GABA to its receptor with a consequent increase in the inhibitory effect[12].

Previously, Sayin *et al.*[14], Sherif and Oreland[15] and Sherif *et al.*[16] reported that vigabatrin has an anxiolytic-like effect in the elevated plus-maze model of anxiety in rats. The elevated plus-maze model is based upon an unconditioned aversion to heights and open spaces[17], and was validated for rodents[18,19]. The validation of this test has also been reported in Pellow and File[20], showing that it can detect the activity of non-benzodiazepines anxiolytics, and of several putative anxiogenic compounds. In a recent communication, pre-treatment with vigabatrin significantly potentiated the sleeping time of barbiturates in rats[21]. Therefore, the aim of this study was to further explore the interaction between vigabatrin and phenobarbitone on rat exploratory behaviour using the elevated plus-maze test and open-field behaviour. Doses of the compounds chosen for the present investigation have been selected on the basis of the previous Studies[16,21].

Method

Adult male albino Wistar rats weighing 302.0±22.9 gram (Mean ± S.D.) at the beginning of the experiments were used in this study. They were inbred at the Central Animal Laboratory at the Medical University, Tripoli, Libya. The animals were housed in a standard laboratory cages (55 x 33 x 20 cm), in a group of four per cage. They were maintained under a 12 h reversed light cycle (lights off 7.00 a.m.) in a temperature-controlled environment (22 ± 2°C). Rats were housed in these conditions for one week before behavioural testing. Food (pellets containing essential nutrients) and tap-water were freely allowed. Measurements of exploratory behaviour have been conducted during the dark phase of the light cycle in a dimly illuminated laboratory between 10.00 a.m. and 1.00 p.m.

Vigabatrin (gamma-vinyl GABA) has been obtained from Astra-Pharmacia Pharmaceutical Company, Uppsala, Sweden, as a gift and phenobarbitone sodium was purchased from (BDH Chemicals Ltd Poole England).

The elevated plus-maze apparatus was made of wood, consisted of two opposite open arms (50 x 10 cm) without side walls, and two opposite enclosed arms of the same size, with side walls and end wall (40 cm in height) The arms were connected from the central platform (10 x 10 cm), forming the shape of a plus sign, and each arm was divided by lines into three equal squares[15]. The maze was elevated to a height of 50 cm from the ground.

The open-field apparatus was a simple square arena (100 x 100 x 40 cm). The arena has been divided into 16 equal squares, thus allowing the observer to record: number of squares visited and number of rearings, during a 4 minute time period[15].

The rats were randomly assigned into four groups as follows: the control group received only physiological saline (i.p., $n = 8$), the phenobarbitone group received phenobarbitone sodium in a dose of 20 mg/kg (i.p., $n = 8$), 30 minutes prior to testing, and the vigabatrin group received 100 mg/kg vigabatrin (i.p., $n = 8$), 3 hours prior to the testing, and the fourth group ($n = 8$), was treated with 20 mg/kg phenobarbitone sodium, 2.5 hours after vigabatrin pre-treatment (1~0 mg/kg, i.p.). Thirty minutes. later, the activity was recorded in the elevated plus-maze and open field apparatus.

For measuring anxiolytic effects of the drugs in the elevated plus-maze, the animal was gently taken from the home cage and placed in the center of the plus-maze apparatus, with its head facing the closed arm, and observed for a period of 4 min. As traditionally employed, the key indices of anxiety in this test are the proportion of open arm entries and proportion of time spent on the open arms[19]. The following parameters were recorded: time spent, number of squares crossed and number of entries into open and closed arms. The behavioural parameter was considered on a certain arm when the animal had placed all of its four feet on that arm.

For measuring the locomotor activity in the open-field test, the rat was gently placed (after the plus-maze testing) in the center of the apparatus and observed for a period of 4 minutes. The apparatus can differentiate the vertical locomotor activity - rearing activity from the horizontal locomotor activity - ambulatory activity (number of squares visited with all of its four feet on the square).

Statistical analysis

Analysis of variance (one-way ANOVA) and subsequent *post-hoc* analysis with the Fisher's PLSD test were performed in order to detect any difference between means of the control and individual groups.

Results

In Table 1, analysis of the data by ANOVA revealed significant differences between the groups of rats, treated with phenobarbitone and vigabatrin alone, and in combination, in time spent on ($F = 7.96$; $P < 0.0001$) and number of entries into ($F = 3.45$, $P < 0.05$) open arms of the elevated plus-maze model of anxiety. Further analysis with the Fisher test indicated that treatment with a single dose of phenobarbitone sodium (20 mg/kg) produces a significant increase in the time spent on the open arms ($P < 0.005$) and a significant decrease in the time spent on the closed arms ($P < 0.01$) of the maze in comparison with control rats (Table 1). The single dose of vigabatrin (100 mg/kg) produced effects similar to that of phenobarbitone in increasing the time spent on open arms ($P < 0.01$) and in decreasing the time spent on closed arms ($P < 0.005$) of the maze. Similar observations have also been found in the number of open arm entries (Table 1). However, pre-treatment with vigabatrin completely abolished the effect of phenobarbitone on the open and closed arms of the plus-maze. Thus, rats receiving both treatments showed no difference from the control group with regards to the time spent and number of entries into open and closed arms of the elevated plus-maze test (Table 1). The analysis of the data with ANOVA has also revealed significant changes in the percentage of time spent on open arms per total time ($F = 7.51$: $P < 0.005$) and in the number of open arm entries per total arm entries ($F = 4.33$; $P < 0.01$) between the groups (Table 1). Further analysis with Fisher test indicated that the percentage of time spent on the open arms per total arms is significantly increased in the groups of rats treated with phenobarbitone ($P < 0.005$) and vigabatrin ($P < 0.01$) alone, however, with no change in the rats receiving both treatments. With regard to the percentage of open arm entries per total entries, similar increases were observed after phenobarbitone ($P < 0.005$) and after vigabatrin ($P < 0.005$) treatments, however, without a change in rats which received both treatments in comparison with control rats.

The effects of the treatment with phenobarbitone and vigabatrin alone, and in combination, on line crossings on the arms of the maze are also shown in Table 1. Thus, significant changes in the line crossings on the open ($F = 3.62$; $P < 0.05$) and closed ($F = 3.42$; $P < 0.05$) arms of the elevated plus-maze test were found after the treatments. Further statistical analysis indicated a significant increase of these measures after treatment with phenobarbitone or vigabatrin in comparison with control group. However, pre-treatment with vigabatrin has abolished the effect of phenobarbitone (Table 1). The locomotor activities of rats, as calculated from the plus-maze data, for groups of phenobarbitone and vigabatrin alone and in combination are also given. Thus, a significant difference in the total number of arm entries ($F = 3.44$, $P < 0.05$) was observed. Treatment with phenobarbitone and vigabatrin alone significantly increased the total number of arm entries ($P \sim 0.01$ and $P < 0.01$, respectively), however, rats which received combined treatment did not show any significant difference from the control rats.

Group	Control	Vigabatrin	Phenobarbitone	Vig. + Phenobarb.
Time spent on				
open arms	11.3 ± 6.16	29.88 ± 5.76**	39.38 ± 5.52***	10.25 ± 4.42
closed arms	200.87 ± 12.87	113.88 ± 12.57***	145.75 ± 14.84**	192.63 ± 15.82
Lines crossed on				
open arms	1.50 ± 0.96	4.5 ± 0.43**	6.38 ± 1.5**	2.38 ± 1.02
closed arms	9.63 ± 2.61	15.88 ± 1.19*	19.50 ± 4.36*	9.13 ± 2.52
No. of entries into				
open arms	1.63 ± 0.82	4.25 ± 0.53**	5.13 ± 0.74***	2.25 ± 1.03
closed arms	3.63 ± 0.82	5.25 ± 0.82	6.38 ± 1.73	3.25 ± 0.82
Total no. of arm entries				
	5.0 ± 1.65	9.5 ± 0.98**	12.3 ± 2.72**	5.5 ± 1.68
% time spent on open arms/total time				
	5.9 ± 3.6	21.7 ± 4.0**	23.0 ± 3.5***	5.9 ± 2.8
% open arm entries/total arm entries				
	20.0 ± 6.2	44.2 ± 2.8***	45.6 ± 4.2***	27.3 ± 9.2

Data shown are means ± S.E.M. *P < 0.05, **P < 0.01, ***P < 0.005 significantly different from the control.
No statistically significant differences were found between the Vig. + Phenobarb.-treated rats and control rats.

Table 1: Effects of treatment with Phenobarbitone Sodium (20mg/kg, i.p.) and Vigabatrin (100mg/kg, i.p.) alone, and in combination on rat behaviour in the elevated plus-maze model of anxiety.

In Figures 1 and 2, an analysis of the data with ANOVA has indicated significance differences between the treated groups in the number of squares visited (horizontal activity, $F = 4.12$, $P < 0.01$) and number of rearings (vertical activity, $F = 7.45$, $P < 0.005$) in the open-field test. Phenobarbitone alone (20 mg/kg) was able to significantly increase the horizontal ($P < 0.01$) and vertical ($P < 0.005$) activities in comparison with control rats and rats treated only with vigabatrin (100 mg/kg). There were, however, no significance differences between the control rats and rats treated with vigabatrin, with regard to the horizontal and vertical activities (Figures. 1 and 2). Pre-treatment of rats with vigabatrin has completely abolished the stimulatory effect of phenobarbitone on the locomotor activity in the open field behaviour.

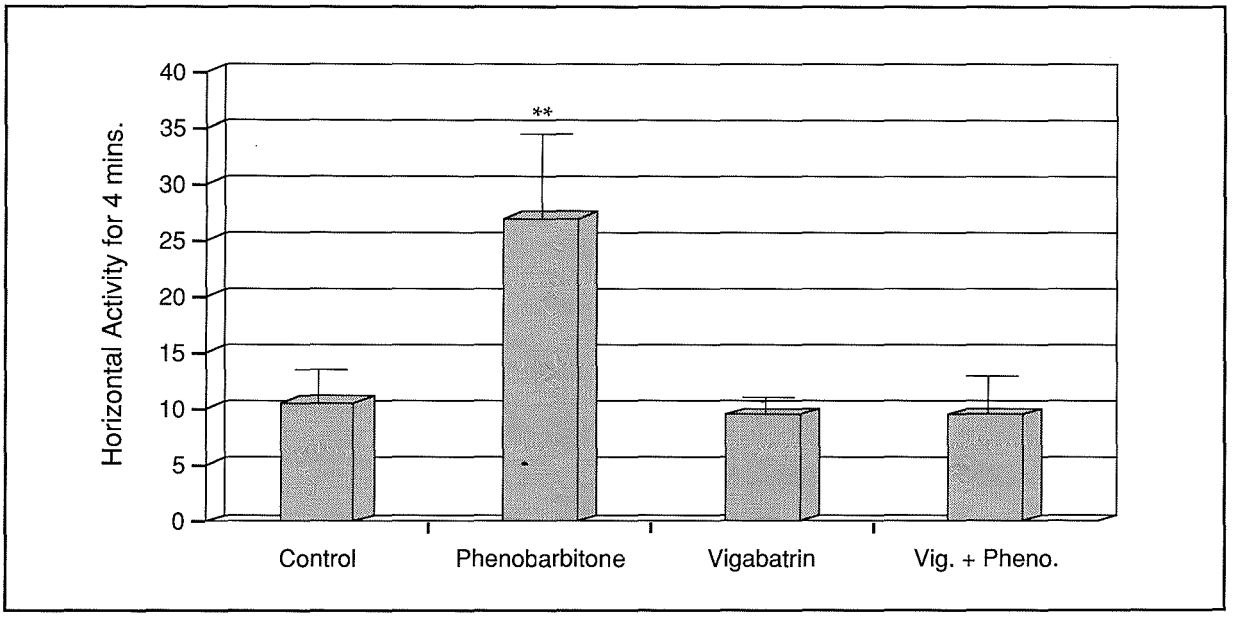


Figure 1: Effects of administration of phenobarbitone sodium (20mg/kg, i.p.) and vigabatrin (100mg/kg, i.p.) alone, and in combination on rat horizontal activity in the open-field test. Data are means ± S.E.M., **P < 0.01 significantly different from the control group by Fisher's PLSD test.

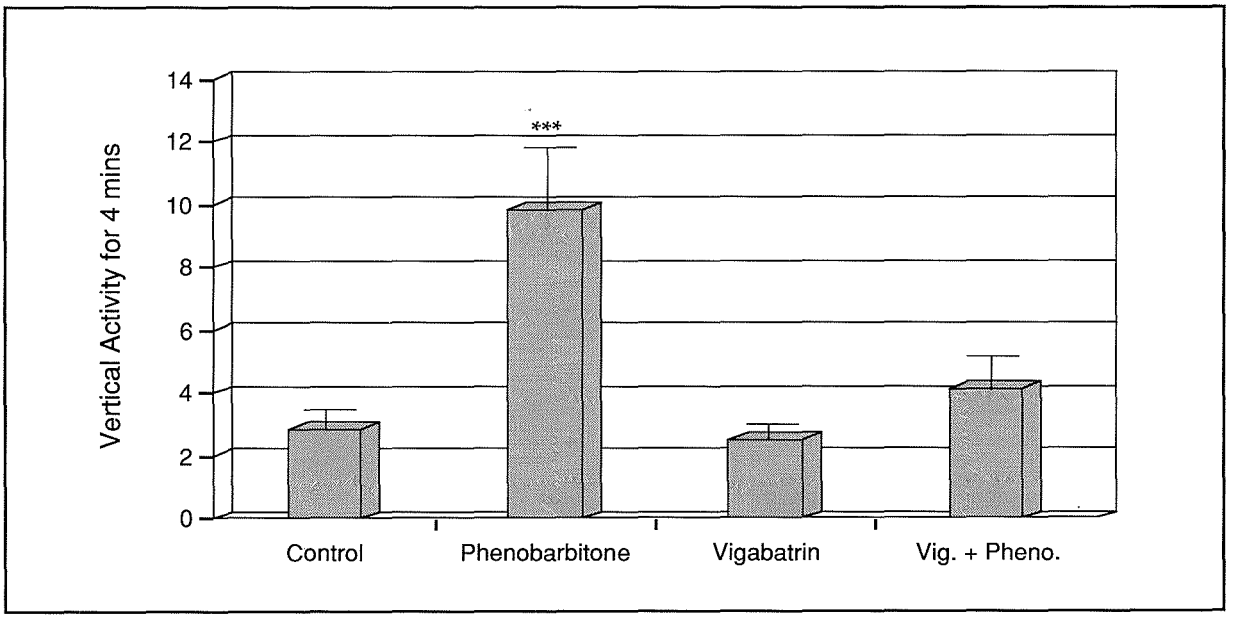


Figure 2: Effects of administration of phenobarbitone sodium (20mg/kg, i.p.) and vigabatrin (100mg/kg, i.p.) alone, and in combination on rat vertical activity in the open-field test. Data are means ± S.E.M., ***P < 0.005 significantly different from the control group by Fisher's PLSD test.

Discussion

Our present results are consistent with a number of reports[12,14,15,16] showing that vigabatrin and phenobarbitone alone produced a release of the suppressed behaviour induced by the aversive stimuli of the open arms in the elevated plus-maze model of anxiety, as indicated by the increase in time spent and in the number of entries into open arms of the maze. Recently, the elevated plus-maze test and open-field behaviour have extensively been used to investigate the exploratory behaviour in rodents[22]. The role of GABA in anxiety has been suggested by findings that anxiolytics agents such as the benzodiazepines and barbiturates bind to specific recognition sites on the GABA-chloride complex (see Introduction). Because barbiturates and GABA-T inhibitors act postsynaptically to enhance GABA-ergic neurotransmission, cross generalization between these classes can be taken as evidence that GABA-ergic mechanisms are involved in the transduction of their anxiolytic and exploratory behaviour. However, the major finding presented in this study was that pre-treatment of rats with vigabatrin abolished the anxiolytic action of phenobarbitone sodium in the elevated plus-maze model of anxiety (Table I) and the increased locomotor activity in the open-field test (Figs 1 and 2). The anxiolytic effect of vigabatrin has also disappeared in phenobarbitone-treated rats. This behavioural result suggests that another neurotransmitter system may be involved or GABA has a dual effect against anxiety and exploratory behaviour. At low levels of GABA-ergic activity (under the effect of either vigabatrin or phenobarbitone) GABA would inhibit the anxiogenic pathways, while at high levels of the activity (under the effect of combined treatment), GABA inhibits anxiogenic and anxiolytic pathways in the brain resulting in masking of the anxiolytic effects. Previously, indirect role of GABA in anxiety by inhibiting the anxiogenic noradrenergic pathway to the *locus coeruleus* and serotonergic neurons in the dorsal raphe has also been suggested[23]. It is possible, therefore, that the dual effect of GABA reported in this study might have come from a non-specific effect of GABA.

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"SPECIALS" MANUFACTURE IN THE N.H.S.

WE'VE BEEN DOING IT FOR YEARS...

Sandra Millership

Summary

Over the past 30 years there has been a vast change in the manufacture of "specials" within the National Health Service. From being primitive, badly equipped units situated in the basement of nearly all hospitals, N.H.S. production units have changed to purpose built, hi-tech units licensed by the Medicines Control Agency, providing a fast, efficient and validated "specials" service. Although non-profit making organisations, N.H.S. Production units have to be self-financing. Thus staff costs, overheads and replacement of equipment must be financed by sales. Production pharmacists in the N.H.S. are now working together to rationalise the product list within their units. Certain units have taken over the roel of national supplier for items and instead of duplicating effort, other units will buy from them.

Keywords

"Specials", N.H.S., Medicines Control Agency.

Introduction

In the mid to late 60's, virtually every hospital pharmacy department had its own small production unit. These were usually situated in the basement of the hospital, in close proximity to the pharmacy and conditions were often primitive. All types of sterile and non-sterile products were manufactured, often using very old machinery, and much of the tablet pre-packing was done by hand, using dispensing triangles. Sterile production units in those days generally produced little more that Normal Saline and Dextrose infusions in 500 ml glass bottles together with a few ampoules and eye drops.

There was no proper costing carried out on these exercises so nobody knew if the products could have been bought more cheaply from industry. Controls and testing procedures were often rudimentary. Production was just another facet of pharmacy and every hospital that did not have a production unit, however primitive, was thought to be missing a vital area of post-registration training. Professional expertise alone was considered to provide sufficient safeguards for what was literally dispensing on a large scale and no outside regulatory body was concerned.

In 1968 the government produced a piece of legislation which was designated : "An act to make new provisions with respect to medicinal products and related matters, and for purposes connected therewith."[1] This legislation was called The Medicines Act 1968 and its publication has had a profound effect on the Pharmaceutical profession in the United Kingdom ever since. The purpose of the act is : "To control, by a licensing system, the safety, quality and efficacy of medicinal products."[1]

At this point manufacture of pharmaceuticals in hospital units was covered by what is known as "crown immunity" and did not require official licensing, however, as there could be no justification for lower standards applying in hospital units than in commercial undertakings the spirit of the act was applied to hospital units and they were expected to conform to the new standards.

The desirability of this course was emphasised by the Medicines Commission in their report on the preparation of infusion fluids following what is known as the "Devenport Incident" in 1972. This was an incident when 5 people died in a Plymouth hospital following the infusion of a contaminated batch of Dextrose 5%. This was actually a commercially prepared infusion but the hospital was not absolved of all responsibility as it was felt they should have examined the infusions before issuing them to the wards.

The Commission stated : ".....too many people believe that sterilisation of fluids is easily achieved with simple plant operated by men of little skill under the minimum of supervision, a view which is wrong in every respect."[2]

An interim report by Lord Rosenheim in 1972 concerned with the same incident recommended, in particular, that: "Control of hospital manufacture should be no less rigorous than that which applies to pharmaceutical firms who are required to be licensed under the Medicines Act."[3]

Accordingly, a system of inspection of hospital manufacturing units was implemented. At first this was on an informal basis, licenses not being required. But it had profound implications for the hospital service. Many units were closed down as being unsuitable and those which remained open, after extensive modification, went in one of two ways. Firstly, in the late 1970's the large scale hospital production unit appeared. These units were able to manufacture sterile fluids on a near commercial scale and were, in essence, in competition with industry. There were not too many of this type of unit but Parkfields in the West Midlands and Clatterbridge in the Wirral were two such. But the main type of unit to appear was what we call the "Specials" unit.

What is a special?

As a general rule, the Medicines Act 1968 requires all dealings in medicinal products to be in accordance with product licences and all manufacturers of medicinal products to hold a manufacturer's licence.

They do, however, recognise that practitioners may need to prescribe medicines that are not available as licensed products. Under such circumstances the product can be prepared in a pharmacy registered with the Pharmaceutical Society or in a hospital pharmacy under the supervision of a Pharmacist.

This is under what is called a section 10 exemption from the regulations and enables a small amount of preparation, for immediate use. There are strict guidelines on the amount of preparation permitted under this exemption, for example :

20 packs of a non-sterile liquid, antiseptic or cream, 100 capsules or suppositories,

10 packs of terminally sterilised products, this includes eye drops, injections, infusions and sterile creams;

25 packs of repackaged tablets, capsules or liquids.[4]

Such items are said to be extemporaneously dispensed.

It is generally not permitted to prepare "aseptically" manipulated products for stock at all. Unless made in a licensed unit, these should be for immediate use only.

For all items covered by a section 10 exemption there is a limit of one batch per month prepared for stock. An acceptable level of quality assurance is still required and products should be made in conditions which comply with good manufacturing and dispensing practices and should be regularly audited by quality control staff. In addition, unless substantiated by stability data, the maximum permitted shelf life for these preparations is 28 days. Alternatively, the pharmacist or practitioner may order the product from the appropriately licensed manufacturer.

"Specials" are defined as Medicinal products in respect of which a Product Licence is not in existence but which may have been made by the holder of a special manufacturer's licence, to the order of a practitioner, for administration to a particular patient.[4]

Until this year commercial manufacture, even in licensed units, had to be by, or under the supervision of a pharmacist but since the introduction of European licensing requirements this is no longer the case.[5] Manufacture under the section 10 exemption however remains under the auspices of a pharmacist.

It is licences issued under these 1994 regulations and those which these regulations superseded that are commonly referred to as "specials" licenses and the products made under there authority are termed "specials".

A "special" can be :

- An unusual formulation of a product that is only available in certain licensed forms.
- An unusual strength.
- An unusual combination of products.
- A preservative-free version of an eye-drop A discontinued product.
- An item available overseas but not marketed in Britain.
- Products made to order due to the temporary unavailability of the licensed form.

The Present Situation

Things changed again in 1990 in Great Britain and 1992 in Northern Ireland when crown exemption was removed and all units, including those situated in hospital pharmacies, were required to apply for manufacturers licences or to cease manufacture. Again more units closed leaving a nucleus of specially built, high quality units within the National Health Service.

These units were formally inspected by the Medicines Inspectors and, if the standards were met, were issued with official licences. Henceforth there would be absolutely no difference between Health Service units and commercial concerns.

It would be difficult to compare the modern day units with those of the late 60's. Most are purpose built, designed to the highest standards, and fitted with expensive, high quality air handling plants providing clean or sterile air to the working environment. The operators more resemble spacemen than pharmacists or the technicians in their one piece all encompassing suits, headgear, masks and gloves.

There are currently 212 holders of specials licenses issued by the Medicines Control Agency. Of these licenses, 123 are held by National Health Service hospitals and 99 by industrial concerns. About a dozen of these industrial concerns supply a commercial specials service.

It must be borne in mind that the onus is on the purchasing pharmacist, rather then the manufacturer, to ensure that any unlicensed medicines purchased are up to standard. Due to the increased significance of product liability over the last few years, the Quality Controllers are increasingly being asked to re-test purchased specials on receipt, unless they can be furnished with a Certificate of Analysis. This can greatly add to the cost of purchased specials. The Quality Controllers have, however, agreed between themselves to accept the testing procedures of other N.H.S. specials units without the need to re-test. We can, and do, however, provide Certificates of Analysis if required.

Some specials manufacturers offer a comprehensive service, others specialise in a particular type of product. The N.H.S. licensed units, by and large offer a very comprehensive service, many of them being licensed to provide an aseptic service. Aseptic filling is a high cost operation, very labour intensive and involving extensive validation and broth trials. Many commercial specials manufacturers do not offer this service for these reasons.

Industry is offering a commercial, profit making service, it is out to make money. The N.H.S. specials units are there to provide a service. They are able to respond rapidly to changing situations and are often in the forefront of developing new delivery systems and admixtures. Many units also undertake Total Parenteral Nutrition, Central Intravenous Additive Services or C.I.V.A.S. and Patient Controlled Analgesia services. Although these are not strictly manufacturing procedures the expertise developed in manufacturing, together with the high standards in the units make them the ideal place from which to conduct these essential services.

National Health Service units have recently introduced a comprehensive service for the relief of post-operative pain. Where Total Parenteral Nutrition was the in vogue treatment of the 70's and the Central Intra Venous Additive Service that of the 80's, Patient Controlled Analgesia is now one of the fastest growing fields in modern medicine. The ability to let a patient control their own post-operative pain allows a patient the unique opportunity to contribute to their own recovery and has been proven to increase recovery rates and the patient's feeling of well being.

P.C.A. is administered either by pre-filled syringe, which requires a syringe pump, a mini-bag, requiring an infusion pump, or, for ambulatory patients, an infusor device with a switch, worn on a wrist strap, powered by a small battery. The syringes, mini-bags, and infusors are all filled using an automatic dosing machine which acts like a mechanical thumb. This device is used for all repetitive filling in our unit. As most of the analgesics used by this method are narcotics, either morphine or fentanyl, either alone or in combination with other substances, it is important to realize that these devices are pre-set. It is not possible for the patient to exceed the required dose either by increasing the volume of the metered dose or by decreasing the interval between doses. The patients however, can control their own pain when they need it and not when the nurse or doctor has the time to give them relief.

Also, over the past 10 years the N.H.S. specials units have been working together to rationalize the product list within their units and thus reduce

duplication of effort. In the early 1980's the Regional Pharmaceutical Officers set up several sub-committees dealing with Drug Information, Quality Control, Radiopharmacy, Education and Production and asked for representatives from each region in each discipline. The Pharmaceutical Production Committee has been instrumental in rationalizing production, especially of sterile products, within the N.H.S. It is due to the work of this committee that several units have taken over the roll of national supplier for certain items.

At our sterile unit in Belfast we produce, on a national scale, various strengths of Baclofen either as ampoules or as vials for refilling implanted reservoirs associated with a miniature pumping device to give patient controlled relief from neurological spasticity. The royal Victoria Hospital was one of the first units in the country to pioneer this treatment, and, as the shelf life of Baclofen in normal saline has been found to be only 9 months and the 12ml vials will refill a pump for the period of 2 to 3 months, the demand within any single unit is not enormous and it is more economical for other hospitals to buy from us than to manufacture their own. In fact we supply these to over 2 dozen hospitals throughout the United kingdom and Eire. We are also the national supplier for Paraldehyde injection, a particularly nasty substance to work with, used to control seizures in status epilepticus.

The Production Pharmacists sub-committee, with the collaboration of the Quality Control and Radiopharmacy sub-committees have, this year, set up, in conjunction with Leeds University an MSc in Pharmaceutical Technology and Quality assurance to ensure a constant renewal of pharmacists qualified to take over the technical jobs within pharmacy. This has become vital as, with the decrease in the number of production units and their associated quality control units, it is now not every pre-registration graduate who has more than passing contact with one of these specialties and has the chance of first hand experience.

It is also hoped that this course will lead to registration as a Qualified Person. Although this is not, at the moment, necessary for a Qualified Person to be named on the application for a "Specials" license, there is no guarantee that this will continue.

The Market Economy

In addition the Market Economy has come to the N.H.S. Although they are not producing specials primarily to make money and some units see it as a form of income generation, N.H.S. units are not allowed to make a loss. Production units are required to be self-financing. Staff costs, overheads, capital charges and repair and replacement of equipment must be financed by sales. This has led to production managers having to assume a more business like approach to their enterprises. This is made more difficult by the embargo on advertising by specials units. It is a requirement of the specials license that, although the service provided can be advertised, specific products must not be advertised and can only be supplied on receipt of an unsolicited order.

It is from the need to advertise that I get the title of my paper. Due to the need to be self-financing, we need to advertise our services. The Production Pharmacists Committee have coined the logo "We've been doing it for years...." and now use it on all their promotional literature.

At first sight the embargo on advertising specific products may seem unreasonable but if you think about it logically, if you were able to advertise an unlicensed drug, then what would be the point of applying for an expensive product license.

It is however, not illegal for a specials manufacturer to make a drug for which there is a licensed alternative. Many new products start life as specials made in N.H.S. units. From these beginnings, having proved their efficacy, they go on to licensed products. Industry jumping on the N.H.S. bandwagon. In some cases a hospital specials unit has been making a preparation for years when a licensed product becomes available. The unit will only cease to manufacture when the customer no longer requires the drug. This usually only occurs when prescribing patterns change or the licensed alternative is cheaper, definitely not always the case.

In the very odd case, an N.H.S. produced drug may be more expensive but still required. It is the prerogative of the doctor to have the drug he requires and if he has been working successfully with the N.H.S. produced drug for a number of years, he may be loathe to change. Although economic restraints are now being applied, it is generally accepted that if a doctor want something badly enough, he will get it.

Where Do We Go Next?

We are definitely seeing an increase in the requirement for injections in pre-filled syringes, especially for use in emergency situations. Also, the use of mini-bags seems to be on the increase and most new autoclaves are fitted with plastic bag cycles. Having said that, the N.H.S. is one of the few services that is now able to supply large volume infusions in glass rather than plastic and this is essential for some preparations.

On the non-sterile front, casualty and take home packs are ever increasing and in mainland hospitals, pre-packs for out-patient dispensing are always required. In Northern Ireland drugs are not given from outpatient clinics, the patient is instead furnished with a prescription. We are also seeing an increase in the use of suppositories and the need for medication to be given in a liquid form. "Specials" units are developing and manufacturing more and more of these

Conclusion

National Health Service specials units will never be able to compete with industry on a commercial scale, neither should they attempt to do so. However, they can, and do offer a high quality, comprehensive service, able to respond quickly and efficiently to the needs of the health service.

They are able to do this by harnessing their expertise to those areas which it is not commercially viable for industry to tackle and, by their increasing awareness of the commercial necessity of being self-financing, they are able to provide this service at a competitive price whilst covering their costs.

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The Authors

This represents a re-print of the paper presented at the symposium on Pharmaceutical Care in Malta in April 1994. Sandra Millership is the Principal Pharmacist in charge of production in the Eastern Health & Social Services Board in Northern Ireland where she manages both a Sterile and Non-Sterile production unit.

GLUCOCORTICOID THERAPY IN ASTHMA

Erratum

*The section **'The Authors'** at the end of this paper presented by Sonia Chetcuti and which appeared in Issue No. 2 of the journal should have read as follows:*

Sonia Chetcuti carried out this work as part of the B. Pharm (Hons.) degree dissertation, "Glucocorticoid Therapy in Asthma: Effect on T-Lymphocyte Function and Immune Responses" under the supervision of Prof. M. Cauchi MQR, AM, MD, MSc, PhD, FRCPA, FRC (Path.), Head of Department of Pathology, St. Luke's Hospital G'Mangia, Malta and Dr. C. Scerri MD, Molecular Genetics Laboratory, University of Malta, Msida, Malta. She would also like to thank Prof. A. Felice MD, PHD, Head, Department of Molecular Genetics, University of Malta for providing the research facilities used during this project.

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LICENCE TO SELL AROMATIC DRUGS GRANTED TO A SHOPKEEPER IN 1764

Paul Cassar

Summary

A document of 1764 held in a manuscript at the National Library of Malta shows the role of the *Collegio di Sanità* in issuing licences and setting down regulations regarding the sale of medicines when Malta was under the rule of the Order of St. John. This, and other documents carrying an earlier date prove the existence of regulatory bodies controlling the practice of pharmacy in Malta even as far back as the 17th Century.

Keywords

Order of St. John, *Protomedicus, Collegio dei Medici, Collegio di Sanità,* licence.

Introduction

During the rule of the Order of St. John in Malta (1530-1798), the regulation of the public health and of the medical and pharmaceutical professions were entrusted to the *Protomedicus* or Physician-in-chief corresponding to the Chief Government Medical Officer of today. This official was appointed by the Grand Master and was usually one of the senior physicians of the Holy Infirmary with a long medical experience.

The duties of the *Protomedicus* were laid down in some detail on the occasion of the appointment of Dr. Nicholas Cilia to the post of *Protomedicus* on the 2nd August 1624. They were repeated on the 10th June 1634[1]. They ranged from the approval of physicians, surgeons and apothecaries regarding their competence to exercise their respective professions, to the examination of the medical preparations compounded in pharmacies and of the medicaments and 'drugs' offered for sale by licensed shopkeepers (*medicamenta et drogas vendere*)[2].

Following the death of the *Protomedicus* Pietro Paolo Azzopardi in the summer of 1764, his office was replaced by a Medical College (*Collegio dei Medici*) composed of three physicians and the senior surgeon of the Holy Infirmary[3]. This college was established by Grand Master Emmanuel Pinto de Fonseca (1741-73) on the 3rd July 1764 with the aim of providing 'for the just and good government of the very important affairs of the (public) health' until a successor to the late Dr. P. P. Azzopardi was appointed as *Protomedicus*. The members of the college were Dr Giorgio Imbert, Dr. Domenico Biagio, Dr. Giuseppe Bigeni and the Master Surgeon Michel' Angelo Grima[4]. They were bound by the same obligations previously imposed on the *Protomedicus* among which was the checking of the qualities of the drugs kept in pharmacies and in other shops authorised to sell drugs and medicaments[5].

A few weeks later, the college asked the Grand Master for permission to order, from the government printing press, a number of printed forms of the licences corresponding to each particular profession or to each 'type of shop' concerned. They also suggested the registration of these licenses at the Grand Court of the Castellania (the Grand Court of Law) to serve as evidence in any 'contraventions that might arise'. These requests were granted on the 1st August 1764[6].

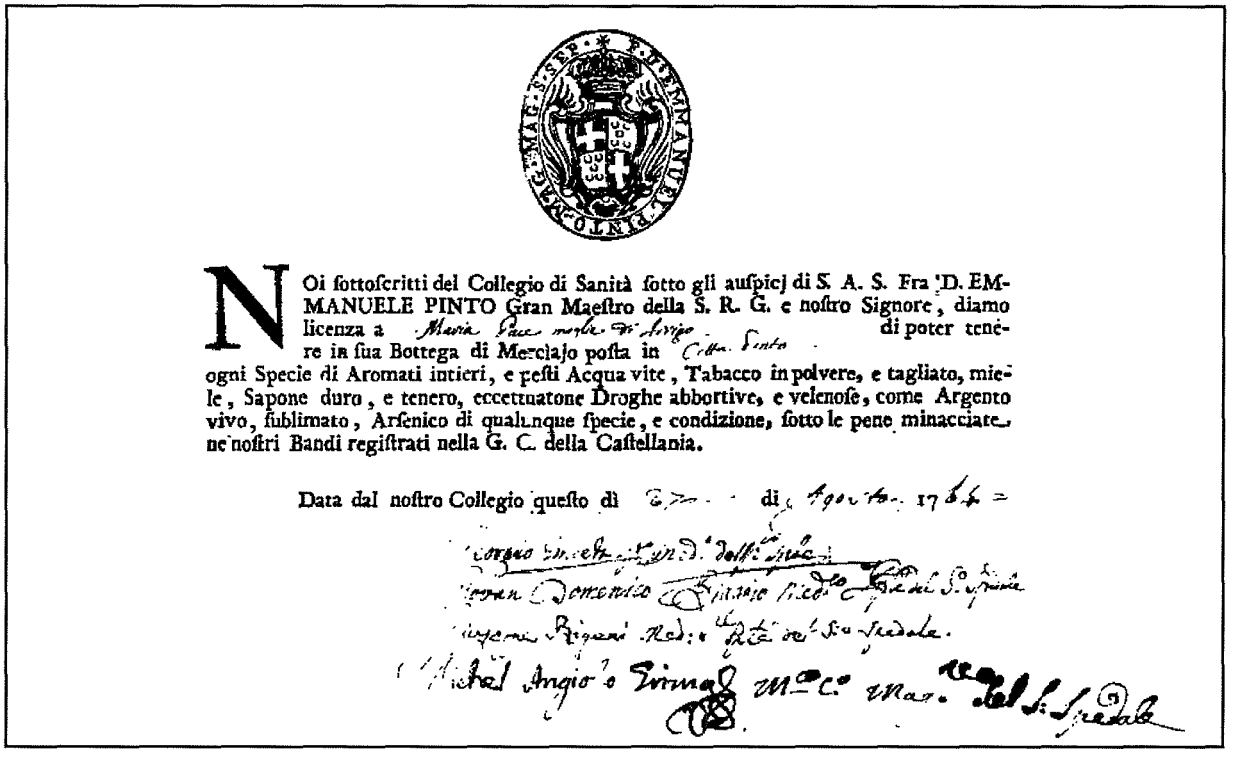
Licence Issued to Maria Pace

One of these licences had survived among a mass of unrelated papers in a manuscript volume held by the National Library of Malta. It measures 25 by 35cm and has a watermark VLS (?) in the upper right hand corner. At the top, in the center, it bears an oval stamp with the coat of arms of Grand Master Emmanuel Pinto whose name and title surround the edge of the oval.

The licence was issued to Maria Pace, wife of Arrigo, who run a retail shop (*bottega di merciajo*) at Qormi (*Città Pinto*). It is dated 27th August 1764 over the signatures of the four members of the college who refer to themselves as the *Collegio di Sanità* on this occasion. This document, written in Italian, is here published for the first time and is shown in the accompanying illustration (Figure 1). Freely translated it runs as follows: "We, the undersigned, members of the *Collegio di Sanità* under the auspices of His Most Serene Highness Fra. D. Emmanuel Pinto, Grand Master of the Sacred Order of (St. John) of Jerusalem grant a licence to Maria Pace, wife of Arrigo, to sell from her retail shop every kind of aromatic substances in the natural or compounded state, solution of wine in water (*aqua vitae*), tobacco in powder or leaf, honey, hard and soft soap but excluding abortive and poisonous drugs such as mercury, sublimate and arsenic in all its forms, under the penalties laid down in the proclamation registered at the Grand Court of the Castellania[7]. Given from our College on the 27th August 1764".

Regulations controlling the sale of medicaments and drugs by pharmacists and other shopkeepers had been enacted by the Order since at least the early 16th century. The original point of departure were the *Prammatiche* promulgated during the Grandmastership of Fra. Emeric D' Amboyse (1503-12) while the Order was still in Rhodes. These *Prammatiche* laid down the conditions under which traders (*De mercatoribus*), shopkeepers (*Bazarioti*), spice sellers (*De speciarjis*) and pharmacists (*De apotecarijia*) were given licences to carry out their business[8].

On its coming to Malta, the Order enacted similar *ordinazzioni* in 1624 and 1634. They were confirmed on the 19th June 1662 when Dr. Blazio Cazzola was raised to the protomedical office as had been done when Dr. Giuseppe Ducosso was appointed to the same office on the 31st January 1650[9]. These *ordinazzioni* also form chapters in the legal codes of laws promulgated by Grand Master Manoel Antonio de Vilhena in 1724 and Grand Master Emmanuel de Rohan in 1784[10]. We will now consider the items referred to in the licence.



NOi ſottoſcritti del Collegio di Sanità ſotto gli auſpicj di S. A. S. Fra 'D. EMMANUELE PINTO Gran Maeſtro della S. R. G. e noſtro Signore, diamo licenza a Maria Pace moglie di Arrigo di poter tenére in ſua Bottega di Merciajo poſta in Città Pinto ogni Specie di Aromati intieri, e peſti Acqua vite, Tabacco in polvere, e tagliato, miele, Sapone duro, e tenero, eccettuatone Droghe abbortive, e velenoſe, come Argento vivo, ſublimato, Arſenico di qualunque ſpecie, e condizione, ſotto le pene minacciate ne' noſtri Bandi regiſtrati nella G. C. della Caſtellania.

Data dal noſtro Collegio queſto dì 27 di Agosto 1764 =

Figure 1: Licence granted by the *Collegio di Sanità* to Maria Pace of Qormi to sell aromatic substances in the natural or compounded state. Dated 27th August 1764. Courtesy National Library of Malta.

Droghe e Drogherie.
Until the late 18th century the word 'drug' (*droga*) had no specific pharmaceutical connotation nor as yet achieved the present day notoriety of any addictive properties; but was the general name applied to medicinal therapeutic agents (medicinal drugs); to substances with a pungent smell, used as food condiments such as spices, coffee and chocolate; to colouring oleo-resins (colouring drugs) employed in various trades and manufactures for dyeing of draperies and for mixing paints and varnishes; and to the so-called 'perfuming drugs' in vogue for the disinfection of ships and merchandise[11].

An earliest official reference in Malta to the word *drogherie* occurs in the *Prammatiche Magistrali* of the 31st January 1650[12]. It recurs in the legal codes of 1724 and 1784 and lastly in the *Bando* (proclamation) of the 18th July 1797[13].

Pace's licence refers to two distinct classes of preparations i.e. abortive drugs (*droghe abortive*) and poisonous drugs (*droghe velenose*). The abortive drugs are not specified as to what drugs the licence was meant to cover although their administration had already been prohibited by the *prammatiche* of the 31st January 1650 and repeated in all subsequent legal codes. On the other hand, the poisonous drugs are specified individually i.e. *argento vivo* (mercury), *sublimato* (bichloride of mercury) and *arsenico di qualunque specie e condizione* (arsenic in all its forms and conditions).

The prohibition of selling poisons mirrors the fears of the ordinary citizen, but especially of those in authority, of the possibility that certain poisons under the cloak of medicines could be used with criminal intent. Infact this fear was such a continuous preoccupation that pharmacists were enjoined to keep such poisons under lock and key and not to sell them to 'slaves, whether Moslems or Christians, to servants , children and other suspected persons'[14].

Aromati
The *aromati intieri e pesti* were vegetable products in their natural state or compounded with an oily base i.e. roots, leaves fruits mixed or pounded with such piquant ingredients as saffron, cinnamon and pepper, all of which were used in the flavouring of food[15].

Shops Under Public Health Control
Certain shops selling organic medicinal and nutritive preparations, that were liable to decay by the passage of time, were subject to the annual inspection by the *Protomedicus* to ensure that they remained fit for human consumption. They were those of the *aromatari, droghieri* and *mercieri* sometimes referred to as *bottega di cose aromatiche* and *bottega di drogherie*. Holders of the last two kinds of shops were enjoined by law to register themselves at the Grand Court of the Castellania in Valletta with their names and surnames and address, these particulars being entered in a register kept for this purpose[16].

Maria Pace, as a seller of 'all kinds of aromatic substances', had to abide by this condition; but there is no evidence that she did so as no registers of licensed retailers has yet been found.

Modern Parallels

With what category, if any, of present-day shops may Maria Pace's *bottega di merciaio* be compared or identified ?

The current Maltese term *Hanut tal-mercja*, corresponding to the English 'grocery shop or store' immediately comes to mind but there is no clear indication in her licence that would definitely place her shop in the grocery class in the modern sense. There appears to have been a hazy area in her time between the sellers of eatables and those of pharmaceutical preparations. Infact this area comprised such preparations as sweetmeats, and preserved and candied confections made of a combination of fruits, almonds and liquorice with sugar or honey. These preparations were prescribed by physicians as medicines for the relief of constipation and stomach complaints but were also eaten by healthy persons for pleasure as foods.

Hence this crossing of the line, between the trade of the *merciaio* and that of the professional pharmacist, which appears to have lasted until the close of the 18th century (1797 at least).

Conclusion

The printed licence here illustrated is of particular interest to the historian of the economics of Maltese pharmacy because:

1. It throws light on the household items (soap, honey and aromatic ingredients) stocked by the village shopkeeper in the 18th century,
2. It refers indirectly to the importation of non-indigenous chemicals with poisonous properties and to the state control of their marketing on account of their misuse for criminal purposes;
3. It authorised a non-professional person to sell 'all sorts of aromatic substances' showing that there was some overlap between the trade of the *bottega di merciaio* and the *apoteca* of the professional pharmacist apparently without any rivalry;
4. It is the only document of its kind that has come to light so far.

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The Author

This article is an original work presented by Dr Paul Cassar M.D., Ph.C, B.Sc., D.P.M. (Eng.), F.R.Hist.S. (Lond.), D.Litt. (Hon. Causa), Hon. Fellow of the University of Malta, residing at 'St. Luke', Pope Alexander VII Junction, Balzan, Malta. Dr. Cassar, a retired physician, is well known for his interest in local medical history and is a lecturer in the subject at the University of Malta. Among his main publications are *Medical History of Malta*, London, 1965 and the latest *The Holy Infirmary of the Knights of St. John "La Sacra Infermeria"*, Malta, 1994. On the 8th March 1995 Dr Cassar was conferred with the *Croce di Commendatore dell'Ordine al Merito Melitense* by the Sovereign Military and Hospitaller Order of St. John of Jerusalem, Rhodes and Malta, based in Rome for his contributions to the history of the medical services of the Order in Malta.

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* Savin R. Clin Exp Dermatol. 1989; 14: 116–119.

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