Long-term Effects of Opioids in the Treatment of Chronic Pain

Investigation of Problems and Hazards on Clinical, Biochemical, Cellular and Genetic Levels

ANNICA RHODIN
Dissertation presented at Uppsala University to be publicly examined in Auditorium minus, Gustavianum, Akademigatan 3, 753 10 Uppsala, Saturday, September 25, 2010 at 13:00 for the degree of Doctor of Philosophy (Faculty of Medicine). The examination will be conducted in Swedish.

Abstract

After two decades of liberal prescribing of opioids, there has been an increasing recognition of problems connected to the prolonged use of opioids for chronic pain.

The aim of my thesis was to explore some consequences of long-term opioid treatment for chronic pain such as problematic opioid use, endocrine disorders, tolerance and genetic variations in pain and opioid response.

Sixty patients with severe pain and problematic opioid use were treated with a structured methadone programme. Risk factors were musculoskeletal pain, psychiatric co-morbidity and previous addiction. Treatment resulted in good pain relief and improved quality of life, but function was impaired by side effects indicating endocrine dysregulation.

The possibility of opioid-induced endocrine dysfunction was explored in the second paper, where 40 pain patients treated with strong opioids and 20 pain patients without treatment of strong opioids were investigated. The opioid-treated patients had significantly higher incidence of endocrine disturbance affecting gonadal and adrenal function and prolactin levels.

The functionality of the µ-receptor after long-term treatment with morphine, saline and naloxone was explored in a cell-line expressing the µ-receptor. After one and four weeks of treatment the binding was tested with morphine, methadone, fentanyl and DAMGO and function measured by GTP γ-assay. The binding of DAMGO was significantly diminished after 4 weeks in cells treated with morphine compared with saline and naloxone.

Genetic variation in three genes with functional impact on opioid response and pain sensitivity was investigated in 80 patients with chronic low-back pain and differential opioid sensitivity and in 56 healthy controls. The results indicated a higher incidence of opioid-related side effects and gender differences in patients with the minor allele of the ABCB1 gene, a correlation between increased opioid sensitivity and the major CACNA2D2 allele and a possible relationship between intrinsic protection against chronic pain and the minor allele of OPRM1.

Keywords: opioid, chonic pain, long-term opioid treatment

Annica Rhodin, Faculty of Medicine, Box 256, Uppsala University, SE-75105 Uppsala, Sweden

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Dedication

To my grandchildren for they give hope for the future
This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


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### Abbreviations

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<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>ACTH</td>
<td>Adrenocorticotropic hormone</td>
</tr>
<tr>
<td>ADH</td>
<td>Antidiuretic hormone</td>
</tr>
<tr>
<td>BDNF</td>
<td>Brain derived neurotrophic factor</td>
</tr>
<tr>
<td>BH4</td>
<td>Tetrahydrobiopterin</td>
</tr>
<tr>
<td>COMT</td>
<td>Catechol-O-methyl transferase</td>
</tr>
<tr>
<td>CRH</td>
<td>Corticotropin releasing hormone</td>
</tr>
<tr>
<td>DAMGO</td>
<td>D-Ala²N-MePhe⁴Glyol-enkephalin</td>
</tr>
<tr>
<td>DHEA(S)</td>
<td>Dehydroepiandrosteron(sulfate)</td>
</tr>
<tr>
<td>DSM IV</td>
<td>Diagnostic Statistic Manual of Mental disorders IV</td>
</tr>
<tr>
<td>EORTC-QLQ</td>
<td>European Organization for Research and Treatment of Cancer Quality of Life Instrument</td>
</tr>
<tr>
<td>FSH</td>
<td>Follicle stimulating hormone</td>
</tr>
<tr>
<td>GDP</td>
<td>Guanosine diphosphate</td>
</tr>
<tr>
<td>GH</td>
<td>Growth hormone</td>
</tr>
<tr>
<td>GHRH</td>
<td>Growth hormone releasing factor</td>
</tr>
<tr>
<td>GnRH</td>
<td>Gonadotropin releasing factor</td>
</tr>
<tr>
<td>GPCR</td>
<td>G-protein coupled receptor</td>
</tr>
<tr>
<td>GTP</td>
<td>Guanosine triphosphate</td>
</tr>
<tr>
<td>HPA</td>
<td>Hypothalamic-pituitary-adrenal</td>
</tr>
<tr>
<td>HPG</td>
<td>Hypothalamic-pituitary-gonadal</td>
</tr>
<tr>
<td>HPGH</td>
<td>Hypothalamic-pituitary-growth-hormone</td>
</tr>
<tr>
<td>HPT</td>
<td>Hypothalamic-pituitary-thyroid</td>
</tr>
<tr>
<td>IGF-1</td>
<td>Insulin-like growth factor -1</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>LH</td>
<td>Luteinizing hormone</td>
</tr>
<tr>
<td>MAPK</td>
<td>Mitogen-activated protein kinase</td>
</tr>
<tr>
<td>NMDA</td>
<td>N-methyl D-aspartate</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>PKC</td>
<td>Protein kinase C</td>
</tr>
<tr>
<td>QoL</td>
<td>Quality of Life</td>
</tr>
<tr>
<td>SNP</td>
<td>Single nucleotide polymorphism</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical Package for the Social Sciences</td>
</tr>
<tr>
<td>TRH</td>
<td>Thyrotropin releasing hormone</td>
</tr>
<tr>
<td>TSH</td>
<td>Thyroid stimulating hormone</td>
</tr>
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</table>
Introduction

The scourge of pain has followed mankind through history, presenting the existential question about the meaning of suffering in the human life. Through the ages attempts have been done to explain the meaning of pain through philosophy, religion and science. In the Iliad, Homer describes the physical, moral and emotional sufferings of the warriors on the battlefield of Troy as πῆμα pema, ἀλγός algos and οδύνη odyne signifying pain, grief and suffering of both body and soul. (Rey J. The history of Pain 1993). The modern phenomena pain, analgesia and dolour are derived from these words.

The meaning of the pain for the old Greeks was the punishment of the gods for the hubris and revolt of the humans against the order of the cosmos. Also in the Christian and Jewish religions there are thoughts about pain and illness as consequences for breaking the divine law and that pain and suffering will be means for atonement and redemption.

In modern times pain is seen as result of a pathological process with psychosocial consequences, that causes suffering and impairment of function and quality of life. The aetiologies are more a twist of nature from genetic and constitutional reasons or bad luck from trauma and diseases, rather than divine intervention for breaking the rules of the cosmic order.

Historical background of the medical use and misuse of opium

During history pain has had the imperative not only for interpretation of the existential question but also initiative for treatment of painful diseases and relief for body and soul from the stresses and harshness of life. For that purpose one substance – opium- has followed mankind from dawn of civilization.

The use of opium, *papaver somniferum*, is as old as the origins of Western Civilization. Some 5000 years ago the opium poppy was grown on the highlands of the Anatolian plateau and on the slopes of the Taurus Mountains, origins of the two rivers Euphrates and Tigris (*Tschiisch A Pharmacogosie 1923*). Mesopotamia, the country between these two rivers, was the cradle of the Sumerian, Babylonian and Assyrian cultures. Archaeological evidence in the form of clay tablets dated 3000 BC from Nippur, the spiritual centre of the Sumerians, witness about the medical and ritual use of opium.
(Sonndecker, Glenn. Emergence of the concept of opioid addiction. Journal Mondial de Pharmacie, 3,1962, 277). From old Sumer the cultivation of the opium poppy spread to the Egyptian and the Greek cultures, travelling eastwards to India with the exploits of Alexander the Great in 300 BC and further east to China with the Arabians and the spread of Islam (Kritikos KG. The History of the poppy and opium and their expansion in antiquity in eastern Mediterranean area. Bull on Narc 3 (19) 1967). In fact, the word opium derives from the Greek word `οπος -opos´, meaning juice. In Egypt opium has been found in the coffins of mummies from 1500 BC (Manniche L. An Ancient Egyptian Herbal 1999).

The first authentic written reference to the milky juice of poppy is found by Theophrastus at the beginning of the third century BC, when he describes it as `μηκώνειον - mekonion´. Hippocrates refers to a substance called mecon used as a narcotic and a purgative, which may be opium, but it can also be attributed to another plant. It seems though that Hippocrates found little use of that substance.

In the Hebrew culture the use of a bitter-tasting plant called rosh, meaning head, could be identical with opium. There is a theory that the gall mixed with wine in the first drink offered to the Christ on arriving to Golgotha according to Matthew 27:34, actually was opium (Kritikos 1967, Tschirch 1926). The word `gall´, in Greek χολη chole in the Septuagint, is translated rosh in the Hebrew Bible. Both rosh and chole are mentioned in the Old Testament in circumstances with bitter tasting herbs or poisons Dt 29:17, Ps 68:22. The poisons are especially mentioned in conjunction with snake venom. An interesting reference to the healing effects of snake poison is the symbol of physicians: the copper snake on the pole, fabricated by Moses to salvage the plague ridden Israeli people in the Sinai desert as told in Num 21:4. Poison in Greek is pharmacon-φαρμακον. Thus, there are references in Jewish-Christian cultures to opium, which can infer the idea of a poisonous pharmaceutical that used in the proper circumstance can have salubrious and healing effects but also a dangerous aspect, like the killing effects of a snake venom.

Dioscorides, a physician in the Roman army, wrote one of the first Pharmacopoeias in the first century AD. He explained that the seeds of poppy could be used against sleeplessness and as a pain reliever, but warns that `in greater doses it will make men lethargic and may kill the patient´(Dioscorides, Gunther T ed 1996). The mixture of alkaloids in the poppy juice was not exact and the effects were often unpredictable. Indeed, the famous Persian physician Avicenna, alive during the eleventh century, died from an overdose of opium. During the Middle Ages, laudanum, a tincture of alcohol and opium, was a commonly used remedy introduced and used by among others Paracelsus.
The seventeenth century English doctor, Thomas Sydenham, described a mixture of opium, sherry, and powder of saffron and cloves as `the most valuable drug in the world´. However his colleague and contemporary, John Jones of Oxford declared in `The mysteries of opium revealed´(1770), that `long-term use of laudanum can cause `intolerable anxiety, and depression and a miserable death´.

The development of opioid misuse in United States and Western Europe

Despite these warnings its use became widespread in the nineteenth century, since opium was one of the few efficient drugs available to the medical community before the event of modern medicine. In 1805 the young German pharmacist Friedrich Sertürner published his studies on opium, where he discovered an acid that he called `Opium-Säure´ or meconic acid, but also an alkaline base, which he named morphium after Morpheus, the god of sleep. He tested morphium or morphone on himself, but this substance proved so potent that he almost lost his life in the process. Together with the introduction of the syringe and needle for medical use, morphine injections became a potent drug for treating pain and other ailments in a more dose dependent way.

During the second half of the nineteenth century considerable amounts of opium and cocaine were imported from South East Asia and South America to Western Europe and the United States. Opium and cocaine were ingredients of patent and propriety medicines, which became readily available to the general public. These products were used for recreation, to round the hard edges of life and to treat diverse ailments, as well as for soothing crying babies. Until 1903 Coca Cola contained cocaine. In addition, alcoholism was often treated with morphine substitution. The pharmaceutical and medical professionals were not organized at that time, and they could not control the increasing use of opioids in the population. By the end of the nineteenth century as many as 10 % of the population, including 10% of the doctors in the USA, and a substantial part of the population on Great Britain were dependent on opioids, as described by David Musto in his book `The American Disease´ (Musto 1979) and by Victoria Berridge in `Opium and The People´(Berridge 1978). By this point of time, there were serious concerns about the increasing and regular opium use of the population and it started to be considered as a major health disorder and a disease in its own right. Dr William Osler of John Hopkins Hospital describes in the beginning of his book `The Principles and Practice of Medicine´ 1892 opium as `God’s own medicine´, but later in the book he comments that people dependent on opium are `inveterate liars and no reliance whatever can be placed on their statements´.
and that the opium habit is `extremely difficult to treat.´ He adds: `under no circumstances whatever should a patient with neuralgia or sciatica be allowed to use a hypodermic syringe´.

Restriction of opioid use and the evolvement of opiophobia

The overuse of opium was the background for strict legal and regulatory interventions that began with the Poisons and Pharmacy Act in Great Britain 1908 and the Harrison Act in the USA in 1914 (Musto 1979). This was the turning point from the liberal use to extreme restriction and fear of opium and its derivatives opioids, `opiophobia´. Furthermore, medical reports were being published describing `street addicts´ of whom a high percentage began their drug misuse as medical patients treated with opioids for pain (1).

The greatest part of the twentieth century is characterized by legal and medical restriction concerning the use of opioids in most countries. The conception that use of opioids and morphine led to tolerance, addiction and severe side effects was a commonly accepted truth shared both by the medical community, legislators and the general public.

The liberalisation of medical opioid use in acute and cancer pain

As a consequence of the restrictions and barriers for the medical use of opioids for relieving severe pain, attention to the suffering of patients in terminal cancer, acute trauma and after operations induced an advocacy for adequate relief of severe pain with the use of strong opioids. The favourable experience of the Hospice movement added to the argument for the adequate medical use of opioids. In an emotional inaugural speech to the International Association for the Study of Pain (IASP) World Congress of Pain in Hamburg 1988, Ronald Melzack made a plea to the world to relieve the `the tragedy of needless pain´, and for that reason undo the legal, medical and moral restrictions to the use of strong opioids for treatment of severe pain. These events and new research indicating that proper medical use of opioids for pain did not lead to addiction induced a new era of liberal opioid prescription in many countries. Soon good evidence was showing that opioids could effectively be used to treat acute and cancer pain conditions. The unique analgesic efficiency of opioids providing relief from severe pain is increasingly recognized as indispensible for relieving suffering. The next step would be to extend the use of opioid to treat also chronic pain.
The use of opioids in chronic pain: Problems and hazards in clinical, biochemical, cellular and genetic aspects

Chronic pain is recognized as a common health problem causing suffering and disability with socio-economic losses for the individual and society (2) (3). Increasingly, since the 1980s strong opioids have been used for relieving severe pain not only in acute or cancer pain, but also for long-lasting painful disorders. Reports of low addiction rates in hospitalized patients (4) and the suggestion that a significant painful disease protects against opioid addiction were arguments supporting the use of strong opioids also in chronic pain disorders (5, 6). However, the efficacy and side effects of long-term opioid treatment are not fully elucidated, and prospective randomised controlled studies for longer periods than a few months are still lacking (7) (8). Clinical experience reported in surveys and uncontrolled case series are contradictory. Satisfactory and sustained analgesia with moderate dosing of opioids including improved function and quality of life is reported (9-12). Other investigators describe insufficient analgesia and poor quality of life in patients treated with opioids (13) (14).

With experience of two decades of liberal prescribing of opioids, there has been an increasing recognition of problems connected to the prolonged use of opioids for chronic pain.

The development of tolerance, problematic opioid use and opioid dependence and iatrogenic opioid addiction are important issues that have affected the attitudes and clinical practices of medical practitioners. The fact that opioid substances are used and abused illegally contributes to the controversy. Cocaine and heroin are the most abused substances causing suffering, criminal behaviour and destruction of the individual and immense costs for the society. Persons addicted to heroin and cocaine are also affected by acute and chronic pain conditions and the treatment of these is more than problematic (15). Prescription opioid abuse and diversion are increasingly recognized problems causing significant morbidity and mortality (16).

The legal, appropriate and medical use and the illegal and criminal use of the same substance are reasons for the problems and stigmatisation associated with prescription and use of opioids for chronic pain. The stigma of
opioid use is still hampering the proper use of opioids to relieve severe pain conditions and many pain patients suffer from being considered as ‘addicts’ in their contacts with healthcare staff.
The aims of my thesis

In general to investigate some consequences of long-term use of strong opioids in chronic pain in order to obtain a better understanding of the limitations, complications and variations of treatment outcome. The ultimate goal is to deepen the insight and knowledge for guiding the clinician to proper use of strong opioids to the benefit of the suffering chronic pain patient.

1) To address the clinical problem of problematic opioid use and iatrogenic opioid addiction by investigation of background and risk factors and evaluating a structured methadone treatment model.

2) To investigate neuroendocrine side effects evolving in the circumstances of long-term oral strong opioids contributing to loss of function and quality of life.

3) To find explanation for the loss of opioid effect over time by focusing on the level of the opioid receptor and cellular mechanisms.

4) To penetrate the genetic variation of opioid sensitivity, side effects and pain experience in patients with chronic pain.
Study I: Background, cause and treatment of problematic opioid use and iatrogenic opioid addiction in chronic pain-the clinical level


Background

Long-term treatment with strong opioids may improve the lives of some patients with severe non-malignant pain. However, problematic opioid use and misuse are aberrant behaviours that have been increasingly recognized along with the liberal opioid use for chronic pain.

There are patients that do not get adequate pain relief even with high doses of opioids. Often doses escalate, tolerance develops, side effects emerge and health care consumption is high. The treatment with strong opioids, intended to improve the patients’ situation, has indeed become an additional problem (17) (13, 18, 19).

Many of these patients have long histories of repeated and failed surgery and other iatrogenic trauma as an additional cause of their pain. Others do not have opioid responsive pain but use the opioid for relief of anxiety and depression or a destitute social situation (20-22), (23, 24).

Since 1994 the Uppsala Methadone Programme for Pain Treatment has been used for treating iatrogenic opioid dependence in patients with chronic non-malignant pain. It was modelled after and inspired by the positive experience of the long-term beneficial effects of structured methadone maintenance programmes for treatment of opioid addiction, and how this method improved the lives of the participants (24, 25). The rationale is the pharmacokinetic and pharmacodynamic properties of methadone to relieve complicated pain disorders and protect from withdrawal.

This study was initiated a clinical follow-up of the treatment programme in order to evaluate this method to treat patients with severe pain conditions, problematic opioid use and iatrogenic opioid dependence and to penetrate background factors, pain mechanisms and co-morbidities.
Terminology

The appearance of problems connected to opioid use in chronic pain has raised the question of terminology. The use of the DSM IV criteria for addiction or substance dependence do not directly lend themselves for use on pain patients, who get their opioids legally prescribed. Therefore new definitions applicable to the situation of medical use of opioids for pain treatment are discussed (26) (27).

Problematic opioid use is aberrant behaviour that causes annoyance and nuisance, but does not adhere to the DSM IV criteria for abuse or addiction. A well designed list of factors describes problematic opioid-seeking behaviour such as requests for early refills, lost or stolen prescriptions, multiple telephone calls or visits, multiple prescribers, sometimes from illegal sources, and frequent emergency room visits and unauthorized dose escalation (28).

However, these behaviours do not necessarily indicate addiction problems but can be the result of a chaotic lifestyle, fear of withdrawal or uncontrolled pain (7).

Pseudoaddiction is reversible opioid-seeking behaviour induced by uncontrolled pain (29).

Opioid abuse is when the individual uses opioids in a harmful way or for other purposes than pain relief.

Physical dependence and tolerance are consequences of neuroadaptation resulting from physiologic changes after use not only of opioids but also other drugs such as benzodiazepins, tricyclic antidepressants and α-2-agonists. Tolerance implies the need to increase dose over time to maintain the same effect. Physical dependence is induced by continuous or repeated drug use and manifest as withdrawal upon stopping or reducing the dose, or when an antagonist is administrated. Chronic opioid treatment is often accompanied by physical dependence and this should be regarded as physiologic and pharmacologic phenomena. It could develop as early as 3 days of continuous opioid use and implies careful tapering as indicated (30).

Psychological dependence is manifested with behavioural and motivational changes resulting from the unpleasant effects of withdrawal. The background is drug-induced long-term changes and dysregulation in brain chemistry and circuitry affecting the mesolimbic reward systems and areas in prefrontal brain (31). Symptoms are anhedonia, dysphoria, craving and compulsive drug-seeking behaviour and loss of control of drug intake.

Opioid addiction/ opioid dependence are a constellation of maladaptive behaviours with adverse consequences such as compulsive overuse and preoccupation of obtaining opioids, use of street drugs and preference for short acting or parenteral preparations. In these circumstances learned and reinforced behaviours involving memory and conditioning causes long-term or irreversible changes in neuronal circuits (32).
Epidemiology
The incidence of drug dependence in chronic pain patients has been described as ranging between 3-23% in different studies (33) and (34), so the problem is of some magnitude. The lifetime prevalence of addiction has been judged as 23-54% in chronic pain patients, which is higher than that of 16% of the general population (35). Thus, it has become apparent that pain in itself does not protect from opioid addiction and dependence.

Methods
Patients
Inclusion criteria: Age over 20, severe non-malignant pain, dependence of opioids for more than one year, insufficient pain control/or side effects, and low quality of life.

The first consecutive 60 pain patients recruited for the methadone programme for pain patients filling inclusion criteria were evaluated in the study.

Investigations
Medical records and psychosocial investigation of all 60 patients were studied for pain problem, duration of pain, pain mechanism, psychiatric comorbidity, drug abuse and maximum daily dose of opioids before methadone treatment. Duration of methadone treatment and daily methadone doses was also registered.

An interview was done with 48 of the patients as an in-treatment survey after at least 3 months of methadone treatment. These 48 patients were monitored for degree of pain relief, quality of life and side effects using the EORTC-QLQ 30C modular approach (36-38) including specific items for side effects of methadone. Twelve of the 60 patients were not available for the interview, since five of them had died from their underlying diseases, two left the program recovered and pain free and 4 of them left because of side effects and one patient was excluded because of diversion.

Treatment
All of the patients were stabilised on oral methadone mixture in daily doses ranging from 10-350mg (mean 99.5mg) as in-patients during 2-6 weeks. After optimising methadone dose and treatment for mean 34 months (range 3-94 months), the patients were asked about the present degree of pain relief in three steps: ‘none’, ‘moderate’ and ‘good’. A multi-professional team of
addition and pain specialists, nurses and counsellors was performing the in-patient treatment and the follow-up.

Findings

Pain relief

All subjects were suffering from severe pain and a dismal quality of life including the problems of opioid dependence. After a minimum of 3 months of methadone treatment 75% of the patients with a mean dose of methadone of 81.5mg rated their pain relief as good and 25% with a mean dose of 187.5mg as moderate. Thus all patients obtained some pain relief. It is notable that the group of patients with the lower mean dose of methadone rated better pain relief than the group with the higher mean dose.

Quality of life (QoL)

The global health and quality of life rating of our patients was mean of 50.8 (range 0-100), where 0 indicates poor and 100 is excellent. This compares with the ratings of 73.7 in the general population in a Norwegian study of 1965 persons (37) and 33.0 in a Swedish study of 2100 chronic pain patients (38). Five of our patients could return to work.

Risk factors and co-morbidities

An interesting finding was that 41 (68%) of the patients had a background of psychiatric disease, which often was pre-existent to the pain disorder and 19 (32%) of the patients had addiction problems before development of the pain disorder.

The most common pain syndromes were: Low back pain (20%), other musculoskeletal problems including arthritis (20%). Neuropathic pain was the primary pain disorder in 13%, visceral pain disorder such as inflammatory bowel disease and pancreatitis 15% and idiopathic pain (13%) (Table 1, Fig 2).
Table 1

1. BACKGROUND FACTORS

1.1 Patient characteristics

<table>
<thead>
<tr>
<th>Description</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male/female)</td>
<td>31/29</td>
</tr>
<tr>
<td>Age</td>
<td>42/44 years (26-68)</td>
</tr>
<tr>
<td>Duration of pain before methadone</td>
<td>13 years (1-20)</td>
</tr>
<tr>
<td>Duration of opioid treatment before methadone</td>
<td>7.1 years (1-20)</td>
</tr>
</tbody>
</table>

1.2 Psychiatric co-morbidity

<table>
<thead>
<tr>
<th>Condition</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depression</td>
<td>22</td>
</tr>
<tr>
<td>Alcohol/drug addiction</td>
<td>19</td>
</tr>
<tr>
<td>Anxiety</td>
<td>10</td>
</tr>
<tr>
<td>Personality disorder</td>
<td>10</td>
</tr>
<tr>
<td>Anorexia/bulimia</td>
<td>4</td>
</tr>
<tr>
<td>Psychotic episodes</td>
<td>4</td>
</tr>
<tr>
<td>Posttraumatic stress disorder</td>
<td>3</td>
</tr>
</tbody>
</table>

(41 patients with 72 psychiatric disorders)

1.3 Main pain problem

<table>
<thead>
<tr>
<th>Condition</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low back pain</td>
<td>12</td>
</tr>
<tr>
<td>Neuralgia/neuropathia</td>
<td>8</td>
</tr>
<tr>
<td>Idiopathic</td>
<td>8</td>
</tr>
<tr>
<td>Musculoskeletal</td>
<td>7</td>
</tr>
<tr>
<td>Inflammatory bowel disease</td>
<td>6</td>
</tr>
<tr>
<td>Arthritis</td>
<td>6</td>
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<tr>
<td>Pancreatitis</td>
<td>3</td>
</tr>
<tr>
<td>Kidney disease</td>
<td>3</td>
</tr>
<tr>
<td>Vascular disease</td>
<td>2</td>
</tr>
<tr>
<td>Phantom limb pain</td>
<td>2</td>
</tr>
<tr>
<td>Osteoporosis</td>
<td>1</td>
</tr>
<tr>
<td>Posttraumatic brain damage</td>
<td>1</td>
</tr>
<tr>
<td>Atypical facial pain</td>
<td>1</td>
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</table>

(One diagnosis per patient) \( \Sigma \ 60 \)
Table 2

<table>
<thead>
<tr>
<th>OPIOID</th>
<th>ADMINISTRATION</th>
<th>DAILY DOSE RANGE</th>
<th>NUMBER OF PATIENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>ketobemidone</td>
<td>iv/ im</td>
<td>20 – 700 mg</td>
<td>13</td>
</tr>
<tr>
<td>morphine</td>
<td>iv/ im</td>
<td>60 – 750 mg</td>
<td>4</td>
</tr>
<tr>
<td>hydromorphone</td>
<td>iv/im</td>
<td>20 – 48 mg</td>
<td>4</td>
</tr>
<tr>
<td>meperidine</td>
<td>iv/im</td>
<td>600 – 1200 mg</td>
<td>3</td>
</tr>
<tr>
<td>heroine</td>
<td>iv</td>
<td>variable</td>
<td>3</td>
</tr>
<tr>
<td>fentanyl</td>
<td>transdermal/oral</td>
<td>2400 – 7200 ug</td>
<td>2</td>
</tr>
<tr>
<td>buprenorphine</td>
<td>im</td>
<td>3,6 mg</td>
<td>1</td>
</tr>
<tr>
<td>pentazocine</td>
<td>im</td>
<td>540 mg</td>
<td>1</td>
</tr>
<tr>
<td>ketobemidone</td>
<td>oral</td>
<td>30 – 500 mg</td>
<td>13</td>
</tr>
<tr>
<td>codeine</td>
<td>oral</td>
<td>300 – 720 mg</td>
<td>13</td>
</tr>
<tr>
<td>dextropropoxifen</td>
<td>oral</td>
<td>800 – 3000 mg</td>
<td>6</td>
</tr>
<tr>
<td>morphine</td>
<td>oral</td>
<td>100 – 600 mg</td>
<td>4</td>
</tr>
<tr>
<td>buprenorphine</td>
<td>oral</td>
<td>8 mg</td>
<td>2</td>
</tr>
<tr>
<td>tramadol</td>
<td>oral</td>
<td>3000 mg</td>
<td>1</td>
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</table>
Problematic drug use and dependence

The most commonly abused oral opioids were ketobemidone and codeine. Ketobemidone is an opioid, mostly used in Scandinavia, with potency equivalent to that of morphine. For parenteral use ketobemidone was again the most frequently abused drug. Ten patients were using more than one opioid at the time of inclusion in the methadone programme. All other weak and strong opioids prescribed in Sweden were represented as well (Table 2).

The daily doses were substantial in some patients; the maximum was 700 mg ketobemidone in a female patient and 750 mg morphine intravenously or intramuscularly in a male patient. These two patients were injecting the drugs themselves at home. One patient used transdermal fentanyl dissolved in water, which he drank. One patient used tramadol in extremely high dose-3000 mg, which means 60 tablets per day and another 3000mg of dextropropoxyphene.

The most frequent type of dependence was psychological (40%), which means that it was associated with aberrant behaviour, such as loss of control of dosing and craving for the opioid to maintain an optimal state of well being, according to DSM IV. Drug abuse with illegal activities such as using illicit drugs and harmful use was prevalent in 32% of the patients (Fig 1).

Side effects

Between 40% and 60% of the interviewed patients complained of a combination of symptoms such as sedation, loss of energy, weakness, weight increase, sweating and sexual dysfunction. This is in contrast with the classic opioid side effects such as constipation, somnolence, nausea and pruritus, which occurred in less than 20%. These side effects do compromise function to a substantial degree and can account for the low quality of life in as many as 50% of the patients.

Statistical and methodological considerations

This study is a consecutive descriptive case follow-up. It was not possible to obtain prospective data before the patients started the methadone treatment, since the collection of data started first early year 2000. All patients have been investigated and treated personally by the first author. Since inclusion criteria for the programme was low QoL and unrelieved severe pain, improvement in that respect can be recognized. The quality of life was measured by using the EORTC QLQ-30 form, originally modelled for cancer pain. However, since it is applied for patients with pain and opioid treatment,
it has been used frequently also for chronic non-cancer pain patients in Scandinavia. Our results can be therefore favourably compared with another investigation of Scandinavian chronic pain patients (38).

Discussion

This study illustrates that this group of very difficult and suffering pain patients with failed opioid therapy and problematic opioid use could be successfully treated with a structured treatment modelled from methadone maintenance programmes for opioid addiction. Pain relief can be obtained, some patients can return to work and the lives of most part of the others can be substantially improved. An interesting point is the fact that the group of patients that rated their pain relief as ‘good’ had lower mean methadone dosing than those who rated pain relief as ‘moderate’.

Key learning points are that musculoskeletal pain, psychiatric and addictive co-morbidities are risk factors for developing problematic opioid use, that side effects of methadone limit improvement in many of the patients and that higher methadone doses not necessarily give better pain relief.
Study II: Investigation of endocrinological side effects in long-term opioid treatment of chronic pain- the biochemical level


Background

The beneficial effects of opioids in long-term use for chronic pain can be offset by the development of side effects that compromise quality of life. Some of these side effects such as sedation, sweating, emotional disorders, fatigue and sexual disturbance can be caused by pituitary inhibition by the opioid use.

The reason postulated for these changes has been the effect of opioid drugs on gonadal and adrenal function as described by Abs and co-workers in their work with intrathecal opioids (39). Oltmanns et al describes a case with adrenal insufficiency caused by transdermal fentanyl (40).

An additional and less observed consequence is that endocrine disorders as hypothyroidism and hypogonadism also can cause and contribute to pain disorders.

At the Pain Clinic of Uppsala University Hospital, Sweden, an increasing number of patients taking moderate to high doses of oral opioids suffer from symptoms raising the question whether endocrine dysregulation could be induced by the opioid treatment. The systematic study of side effects of the patients of the methadone programme in study I, revealed a combination of symptoms that could be related to endocrine dysregulation in 40-60% of the patients.

The aim of this study was to investigate symptoms and signs of endocrine function in chronic pain patients treated long-term with opioids and compare these with a similar control group of pain patients not treated with opioids. The secondary goal was to evaluate quality of life in both groups.
Hypothalamic-pituitary-organ axes for control of hormone secretion and symptoms of dysfunction

The hypothalamus and the pituitary gland control the function of several endocrine glands: the thyroid, the adrenal and gonads, as well as a wide range of physiologic activities to integrate organ function. The hypothalamus influences the pituitary by secretion of release factors to the pituitary, which then releases the appropriate stimulator hormone that in turn regulates the function of the endocrine end organ. The pituitary gland bridges and integrates the neural and endocrine mechanisms of homeostasis. Opioids have an influence on hormonal release at the hypothalamic-pituitary level, observed both in laboratory animals and in humans (41) (42).

The hypothalamic-pituitary –thyroid axis (HPT)

Thyrotropin-releasing hormone (TRH) from the hypothalamus stimulates the release of thyroid-stimulating hormone (TSH) from the pituitary. TSH stimulates the thyroid to secrete thyroxin and triiodothyronin. The thyroid hormones increase the metabolic turnover to promote growth and regulate energy and heat production. Symptoms of hypothyroidism are mostly unspecific including weakness, apathy, neurasthenia, oedema, constipation and anovulation in women including pain states as headache, joint aches and muscle cramps.

The hypothalamic-pituitary –adrenal axis (HPA)

Adrenocorticotropic hormone (ACTH) is released by corticotropin-releasing hormone (CRH), which is mediating the response to different stressors. ACTH stimulates the adrenal cortex to release cortisol, aldosterone and adrenal androgens. Cortisol secretion is controlled by episodic circadian rhythm, at maximum in early morning, by stress via HPA-influence and feedback inhibition by cortisol and ACTH. Cortisol increases plasma glucose and has potent immunosuppressive and anti-inflammatory effects. Cortisol deficiency causes weakness, fatigue, anorexia, nausea, hyponatriemia and hypoglycemia and amenorrhea.

Another adrenal hormone is dehydroepiandrosterone (DHEA), which is a sensitive marker for adrenal insufficiency. DHEA diminishes more predictably and profoundly than cortisol (43). Moreover, DHEA is produced in larger quantities than cortisol and the diurnal variation is not as great as for cortisol. DHEA is converted to the sulfate, DHEAS by enzymes in peripheral tissues. Symptoms of DHEA deficiency are decreased libido, fatigue and depression in both men and women.
The hypothalamic-pituitary-growth hormon axis (HPGH)

The growth hormone releasing hormone (GHRH) stimulates the release of growth hormone (GH). GH acts via insulin-like growth factor (IGF-1) to enhance protein synthesis. However, the secretion of GH is also controlled and by other factors as ADH and ACTH, sleep, exercise, stress and neurotransmitters. GH-release is inhibited by somatostatin and obesity. IGF-1 is produced mainly in the liver.

Acquired GH-deficiency causes decrease in lean body mass, increase in body fat, depression, fatigue and decrease in aerobic capacity.

The hypothalamic-pituitary-gonadal axis (HPG)

The secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) is controlled by gonadotropin stimulating hormone (GnRH).

LH and FSH then stimulate the production of gonadal hormones as testosterone, estradiol and progesterone. Symptoms of hypogonadism are decreased libido, emotional instability, fatigue, sweating, aches and pains in both sexes.

Prolactin secretion

Prolactin stimulates breast development and lactation in the postpartum period. Both physiologic factors as stress and exercise, and pharmacologic factors as opioids, estrogen, dopamine antagonists increase prolactin. Dopamine agonists decrease prolactin secretion.

Hyperprolactinemia leads to hypogonadism with the symptoms of decreased libido, anovulation and decreased spermatogenesis, and disturbing symptoms as gynecomastia and galactorrhea.

Methods

Patients

A study group of 40 chronic pain patients treated with strong opioids for more than one year was compared with a control group of 20 chronic pain patients without opioid treatment. One patient had to be excluded during the study due to evolving renal insufficiency.
Hormone analyses

Hormonal products of endocrine organs were measured in the form of basal levels of testosterone and estradiol (gonads), thyroxin (thyroid), IGF-1 (liver) and DHEAS (adrenal). Basal levels of prolactin were also taken.

Pituitary function was evaluated by simultaneous stimulation with the release hormones CRH, GnRH and TRH in order to achieve function curves of the hypothalamic-pituitary-thyroid (HPT)-, hypothalamic-pituitary-adrenal (HPA)-, hypothalamic-pituitary-growth-hormone (HPGH)- and hypothalamic-pituitary-gonadal (HPG)- axes. Thus, function curves of luteinising hormone (LH), follicle stimulating hormone (FSH), thyrotropin (TSH), and corticotropin (ACTH) and cortisol were obtained. See individual function curves for TSH, ACTH and cortisol on page 35.

Pain, side effects and QoL

Quality of life (QoL), symptoms including pain and side effects were estimated with EORTC-QLQ-C30. Background factors such as concomitant diseases and the cause of pain were recorded from the patients’ journals.

Statistical and methodological considerations

SPSS 14·0 and GRETL 1·7·0 were used for the data analysis and statistics. Median differences for ordinal data were measured by Mann-Whitney rank sum test and Student’s t-test for equality of means for continuous data. Results were judged significant if $p<0.05$ in a two-sided test. The area under curve of the hormonal changes in the pituitary function test was computed in the SPSS system by the trapezoid rule.

One weakness in the statistical comparison is the age-dependent hormonal values for sex hormones. This could partly be offset by dividing the women in two groups, over and below the age of 50. This age was chosen, since most women had menstrual irregularities or amenorrhea, making it impossible to judge whether they had gone into menopause. The comparable age distribution in the opioid-treated and control group is also a factor, that can make the comparison valid.

It is also obvious that the groups differ regarding thyroid and adrenal function as presented by the function curves, even if statistical significance cannot be proven. Regarding thyroid function, there are individuals below normal and over normal secretion of TSH in the opioid treated group, but all the individuals have normal function curves in the control group. But comparing means does not give any difference.

Regarding multiple comparisons, see arguments against Bonferroni corrections in the methodological part in study IV.
Results

Low back and musculoskeletal pain, failed back surgery, arthritis, visceral and neuropathic pain were diagnosed in both groups in similar proportions. Age and gender distribution, as well as duration of the pain disorder, were the same in both groups. The opioid group was treated with a mean dose of 116 mg (range 30-320 mg) of methadone equivalents, mean dose 133mg (range 40-320 mg) in males and mean 111mg range (30-230 mg) in females. Most patients had been converted to methadone from other opioids, except for four patients that were treated with 30 mg, 120 mg, 140 mg slow-release morphine and 120 mg slow-release oxycodone.

<table>
<thead>
<tr>
<th>Pituitary-gonadal axis</th>
<th>Opioid group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference ranges</td>
<td>N=39 SEM</td>
<td>N=20 SEM</td>
</tr>
<tr>
<td>LH AUC after GnRh</td>
<td>17.7 4.57</td>
<td>50.5 15.1</td>
</tr>
<tr>
<td>FSH AUC after GnRh</td>
<td>13.8 3.93</td>
<td>32.1 8.7</td>
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<table>
<thead>
<tr>
<th>Males</th>
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<th>N=8</th>
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<tr>
<td>Testosterone (nmol/L)</td>
<td>5.56</td>
<td>1.16</td>
<td>15.5</td>
</tr>
<tr>
<td>LH 0 (IE/L)</td>
<td>1.11</td>
<td>0.27</td>
<td>6.42</td>
</tr>
<tr>
<td>LH peak after GnRH (IE/L)</td>
<td>7.91</td>
<td>1.47</td>
<td>25.5</td>
</tr>
<tr>
<td>FSH 0 (IE/L)</td>
<td>2.08</td>
<td>0.51</td>
<td>8.16</td>
</tr>
<tr>
<td>FSH peak after GnRH (IE/L)</td>
<td>4.31</td>
<td>0.89</td>
<td>13.1</td>
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</table>

<table>
<thead>
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<th>Females ≤50 years</th>
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<th>N=6</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol (pmol/L)</td>
<td>208</td>
<td>73.2</td>
<td>510</td>
</tr>
<tr>
<td>LH 0 (IE/L)</td>
<td>3.71</td>
<td>0.88</td>
<td>6.74</td>
</tr>
<tr>
<td>LH peak after GnRH (IE/L)</td>
<td>17.6</td>
<td>4.85</td>
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<td>FSH 0 (IE/L)</td>
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<td>FSH peak after GnRH (IE/L)</td>
<td>12.2</td>
<td>2.6</td>
<td>16.0</td>
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<th>N=6</th>
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<tr>
<td>Estradiol (pmol/L)</td>
<td>49.0</td>
<td>6.81</td>
<td>60.5</td>
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<tr>
<td>LH0 (IE/L)</td>
<td>14.0</td>
<td>5.58</td>
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<tr>
<td>LH peak (IE/L)</td>
<td>54.9</td>
<td>20.8</td>
<td>154</td>
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<tr>
<td>FSH 0 (IE/L)</td>
<td>25.7</td>
<td>9.8</td>
<td>60.7</td>
</tr>
<tr>
<td>FSH peak (IE/L)</td>
<td>49.2</td>
<td>17.1</td>
<td>94.2</td>
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</tbody>
</table>

Table 2.
The opioid-treated group suffered from endocrine dysfunction mainly in the form of hypofunction of the pituitary-gonadal axis with sexual disturbance and menstrual irregularities. Low values of LH and FSH were found in the opioid group suggesting an inhibitory effect of the opioids on the hypothalamic-pituitary levels, with secondary effects on estradiol and testosterone levels. Both men and women in the opioid-treated group had sexual dysfunction, significantly more so than the patients in the control group (Table 2). The area under curve for LH was significantly lower in the opioid treated group (Fig 1).

The opioid-treated group had signs of stimulation of the pituitary-adrenal axis with higher levels of ACTH than the control group (Fig 2), which appeared to have a suppressed ACTH and cortisol response. We found lower levels of DHEAS in the opioid-treated group of women indicating hypoadrenalism induced by opioids in concordance with other studies (43)(Table 3).

All patients in the control group had normal prolactin levels; contrasting with the opioid-treated group, where 14 of 38 patients (42%) had supernormal values of prolactin (Table 3).

There were no significant differences regarding thyroid function comparing the opioid-treated and control groups. However, several individuals had signs of primary hypothyroidism or hypothalamic hypothyroidism in the opioid-treated group. The primary hypothyroidism could be an incidental finding, but with four patients with insufficient response to TRH, the opioids could be causing an inhibiting effect.

The somatotrop factor IGF-1 was low in both groups, indicating decreased GH function. Another study has also given evidence of an impact of opioid treatment on the GH-IGF1 axis with risk of developing loss of energy, decreased muscle strength and abnormal body composition: this is in agreement with our findings (39).
Figure 1  Area under curve of LH after GnRH-stimulation

Figure 2  Area under curve of ACTH after CRH-stimulation
<table>
<thead>
<tr>
<th></th>
<th>Opioid group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Reference ranges</td>
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</tr>
<tr>
<td>Pituitary-adrenal axis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACTH 0 (ng/L)</td>
<td>&lt;46</td>
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<tr>
<td>ACTH peak after CRH</td>
<td>73.7</td>
<td>8.46</td>
</tr>
<tr>
<td>Cortisol 0 (nmol/L)</td>
<td>250-750</td>
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<tr>
<td>Cortisol peak after CRH</td>
<td>610</td>
<td>35.2</td>
</tr>
<tr>
<td>ACTH AUC after CRH</td>
<td>54.8</td>
<td>5.94</td>
</tr>
<tr>
<td>Cortisol AUC after CRH</td>
<td>550</td>
<td>34.4</td>
</tr>
<tr>
<td>DHEAS males (µmol/L)</td>
<td>0.6-10</td>
<td>3.52</td>
</tr>
<tr>
<td>DHEAS females (µmol/L)</td>
<td>0.6-10</td>
<td>1.56</td>
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<tr>
<td>GH-IGF1-axis</td>
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<td></td>
</tr>
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<td>GH 0 (mIE/L)</td>
<td>1.83</td>
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<td>GH peak</td>
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<tr>
<td>IGF1 (µg/L)</td>
<td>81-307</td>
<td>118</td>
</tr>
<tr>
<td>Prolactin (µg/L)</td>
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<td>Pituitary-thyroid axis</td>
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<td>TSH 0 (mIE/L)</td>
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</tr>
<tr>
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</tr>
<tr>
<td>TSH AUC after TRH</td>
<td>12.2</td>
<td>3.09</td>
</tr>
<tr>
<td>Free thyroxin (pmol/L)</td>
<td>10-18</td>
<td>13.3</td>
</tr>
</tbody>
</table>

Table 3.
TSH by TRH stimulation opioid

TSH by TRH stimulation controls
The degree of pain did not differ between the two groups, but side effects such as nausea, constipation, sedation, sweating, pruritus, dry mouth, depression and sexual dysfunction were more prevalent in the opioid treated group (Fig 3). In the opioid-treated group QoL was lower than in the control group for physical (p<0.001), role (p<0.01), social (p<0.01), and emotional functioning (p<0.05) (Fig 4).
Only three patients in the opioid treated group had no signs of endocrine dysfunction. These patients also had comparatively low daily doses of opioids: 30 mg of methadone, 40 mg and 120 mg of slow-release morphine.

Discussion

The main findings in earlier studies have been sexual dysfunction and low levels of sex hormones in patients treated with intrathecal opioids (44), [Abs 2000(39), (45)], high-dose oral opioids for cancer pain (46) and methadone maintenance for heroin addicts (47). Our study corroborated those findings.

Some studies provide evidence for opioid induced hypofunction of ACTH and cortisol release (39, 48), which contrasted with our results. However, in one study of former heroin addicts treated with methadone, a similar pattern was seen with higher response of ACTH after CRH-stimulation than in normal control persons (49). This is congruent with our results. Hypofunction HPA-axis, with suppression of ACTH and cortisol release, have been diagnosed in chronic pain patients with failed back surgery, as presented by Geiss and co-workers (50), and low diurnal cortisol variability in patients with pain of lumbar disc herniation and severe disability (51). Thus, the findings of hyperfunction of HPA-axis in opioid-treated individuals and hypofunction of HPA-axis in chronic pain patients in these last two studies are in concordance with the results presented here. The hyperfunction of HPA-axis was found in patients with a history of very high daily opioid doses including long-term intravenous or intramuscular use. These individuals may, at least in a pharmacological way, be compared with heroin addicts.

Low DHEAS can cause additional sexual disturbance and fatigue in both sexes (52), (53). There is evidence of inhibition of adrenal androgen production as inferred by low values of DHEAS in patients treated with sustained action opioids for pain, which is in agreement with our findings. The symptoms of fatigue, depression and sexual dysfunction respond to DHEAS replacement therapy (54). This is an important clinical finding with practical consequences for improving the quality of life of chronic pain patients.

The significantly higher prolactin values in the opioid-treated can explain the disturbing symptoms of breast enlargement, tension and lactation in some patients with prolactinemia. The reasons for high prolactin levels can be due to stress (55) or a direct stimulatory effect of the opioid drug itself (56).
Conclusion

Differences between the opioid-treated and the control groups are found regarding pituitary-gonad and pituitary-adrenal axis, including prolactin levels, indicate an opioid effect causing endocrine dysregulation. Typical symptoms are sedation, sweating, sexual dysfunction, gynecomastia and low physical and emotional function. An important factor is that function and quality of life was better in the control group of pain patients in spite of no differences in pain between the two groups.

Chronic pain appears to strain the endocrine system and opioid treatment enhances dysregulation causing side effects and lowering of quality of life. In spite of substantial doses of opioids, the opioid–treated patients had substantial pain. Substitution and replacement of relevant hormones can diminish symptoms and improve quality of life in older patients with ongoing painful diseases. For younger patients and those with chronic pain syndromes with a mechanism of sensitization the best solution, if possible, would be to taper and stop the opioid dosing altogether. The rationale behind this is that chronic pain involving neuropathic components and central sensitization and opioid tolerance and hyperalgesia seem to share the same cellular mechanisms.
Study III: The loss of opioid effect over time and functionality of the \(\mu\)-opioid receptor-cellular and molecular level

**Background**

During the last years the problems of tolerance and loss of opioid efficacy including opioid analgesic refractoriness have been apparent in the treatment of chronic pain with long-term opioids. Consequences are discontinuation of treatment or dose escalation (30). In other circumstances, it has been clear that opioids in themselves induce central sensitization, opioid hyperalgesia, aggravating or causing pain (57). Many explanations have been proposed for these phenomena. One focus of interest has been the cellular and molecular adaptation following chronic opioid use. A central agent in these processes is the behaviour of the \(\mu\)-opioid receptor in long-term opioid use.

The aim of the cell study was to explore the effects on functionality and binding of long-term opioid treatment on C-6 cells expressing the OPRM1-receptor compared with treatment with saline as placebo and the \(\mu\)-opioid antagonist naloxone.

**Proposed mechanisms**

Cellular processes as down-regulation, recycling and internalisation of the drug target, the opioid receptors, have been suggested explanations for loss of function. Other adaptive mechanisms have been described as change of second messenger systems, synaptic and network levels (58).

1. *Pharmacological tolerance*, meaning need for higher doses over time to achieve the same analgesic effects, is observed in humans (59) as well as
in animal models (60). Proposed mechanisms are uncoupling of the μ-receptor from its cognate G-protein with or without internalization, changes in the adenylyl cyclase cascade or development of partial depolarization due to down-regulation of the sodium-pump. These mechanisms can be seen as adaptive to chronic opioid exposure (61). Other mechanisms are binding and modification of the receptor by phosphorylation (62) and binding to scaffolding proteins as β-arrestins (63) and also control of receptor density by gene transcription factors (64). Microglia activation has been proposed to regulate neural plasticity including morphine tolerance. Chronic morphine administration induces glial proinflammatory response that activates the MAPK and PKC pathways, key players in the cascade leading to morphine tolerance (65).

2. **Withdrawal** is a syndrome presented both by humans and animals after abrupt removal of agonist on the opioid receptor causing hyperalgesia, anhedonia and dysphoria. The cellular and molecular mechanism is not well understood but postulated mechanisms are dysregulation in the downstream signalling involving hypertrophy of cAMP and phosphorylation-transcriptional cascades (58).

3. **Opioid induced hyperalgesia** is one neuromodulatory mechanism that has been discussed widely the last years. Opioids can induce central sensitization and thus aggravate pain. While tolerance can be overcome by increasing the dose, opioid hyperalgesia causes deterioration of pain when dosing is increased (57). Proposed mechanisms are activation of NMDA-receptors, down regulation of spinal glutamate transporters, stimulation of expression of PKC in the dorsal horn and apoptosis of dorsal horn neurons (66) (67). In addition, a facilitatory spinal loop induces increase in spinal dynorphin that has pronociceptive effects. (68, 69) The induction of opioid hyperalgesia has also been suggested due to switching the inhibitory mode of μ-receptors to a stimulatory (70, 71). The μ-receptor is attached to inhibitory G-protein (Gi/G0) that is dissociated to α-fragment and Gβγ fragment. In this process GDP is transformed to GTP that interacts with adenylyl cyclase inhibiting neuronal action. During chronic opioid exposure Gi/G0 receptors shift mode to Gs, which in turn interacts with GM1 gangliosides that promote excitatory opioid action (57, 72).

Opioids interfere with intracellular calcium levels by interacting with voltage-gate calcium channels, intracellular calcium stores and capacitive calcium entry. The opioid effects are complex and can be both inhibitory and excitatory.
Methods

C-6 cells transfected with μ-receptor were treated four weeks with morphine, saline, naloxone. Binding and functionality after that was assessed for morphine, methadone, fentanyl and D-Ala³N-MePhe⁴Glyol-enkephalin (DAMGO) with GTPγ assay.

Cell culture and membrane preparation

A C6 glioma cell line (73) transfected with the human μ-opioid receptor was used in order to test the different opioids. Cells were grown in Dubeccos modified medium containing 5% containing fetal bovine serum (FBS) and 1% penicillin- streptomycin (PEST) at 5% CO2. The cells were treated with different opioids at concentration of 0.1 µM. After one respectively 4 weeks of opioid treatment the cells were harvested and washed twice in phosphate-buffered saline (10 mM sodium phosphate pH 7.5 and 0.15 NaCl, 1mM CaCl₂, 1mM MgCl₂). Cells were then collected by centrifugation for 5 min at 1000 x g at 4°C. The pellet was suspended in 50 mM Tris-HCl buffer, pH 7.4 containing 1mM EDTA, homogenized for 1-2 s using a polytron homogenizer and centrifuged for 15 min at 35,000 x g at 4°C. The final pellet was resuspended in 10% ice cold sucrose, 10 mM Tris-HCl buffer pH 7.4 and 0.2 mM EDTA and stored in aliquots at -70 °C until needed.

[ 35 S] -GTPγS binding assay

The cell homogenates were preincubated with the selected opioid ligands for 15 minutes (30°C) in a total volume of 1 ml 50 µM Tris-HCL, 100mM NaCl, 0.2 mM EGTA and 10µM GDP (pH 7.4). [ 35S]-GTPγS (Amersham; 1000-1200 Ci/mmol) was added at concentrations of 0.05 nM. Non-specific binding was determined in the presence of 10 µM GTPγS. The samples were incubated for an additional 1 h in 30°C before terminating the reaction by a rapid filtration through GF/B filters, followed by 3 ml washes with cold 50mM Tris/ 1mM EDTA buffer. Bound reactivity was determined in a Beckman liquid scintillation counter in 5 ml Ecoscent A (National Diagnostics).

Binding reactivity was measured for DAMGO, fentanyl, oxycodone, morphine, methadone, buprenorphine and codeine in log concentration from -10 to -5 M solution for each 4 samples as baseline.

After one week and four weeks of cell pre-treatment with morphine, naloxone and NaCl in three batches, 20 mg of cell protein was analyzed in four samples of each batch.

Statistics was analyzed by t-test for equality of means of independent samples using the SPSS 16.
Results

Baseline binding and functionality as measured by mean values of [35S]-GTPγS-assay for different concentrations of codeine, buprenorphine, morphine, oxycodone, fentanyl and DAMGO is presented in figure 1. In this study fentanyl, oxycodone and DAMGO acts as strong agonists, where function is increased by rises in concentration. Methadone acts the same way in this assay. Morphine appears to have a ceiling effect and buprenorphine and codeine level at lower concentrations.
After one week of pre-treatment of cells with saline, naloxone and morphine, the binding and function of the µ-receptor is decreased in the same way for all opioids with no significant differences between the different solutions (Fig2).

Figure 2

After 4 weeks, however, the ability of DAMGO to activate the µ-receptor is significantly decreased in the cells pre-treated with morphine compared to those cells treated with either saline or naloxone ($p<0.01$) (Figure 3).

Figure 3
Conclusion

In this study it was possible to prove long-term decline function of the prototype μ-agonist DAMGO after 4 weeks of morphine treatment compared to either saline or naloxone.

This can indicate a long-term effect of morphine on the function of the μ-opioid receptor proven by the model reference μ-agonist. However, there were no differences in binding and functionality measured by $[^{35}\text{S}]\text{GTP} \gamma \text{S}$-assay after one week. It would also be expected that not only DAMGO but also the other full agonist opioids would have responded differently after being pre-treated by morphine solution. One reason could be a spontaneous and natural down-regulation of the opioid-receptor in the cell-culture already after one week and more so after four weeks. The fact that DAMGO, in spite of this, presented different functionality after 4 weeks, could be due to intense receptor phosphorylation by DAMGO and not the other ligands. Another reason could be recruitment of β-arrestins hampering the functionality of the receptors already after one week. The cells and the receptor could also, for some technical reason, stop functioning after one week in spite of meticulous culturing.

It may also be that in vivo signs of GPCR internalization and signs of decreased receptor function in transfected in vitro cell system cannot model the physiological environment and complex integrated responses in humans or animals.
Study IV: Variation of opioid sensitivity, opioid–related side effects and pain in patients with chronic low back pain— the genetic level

Manuscript for submission to Clin J Pharm Therap

Background
The treatment of moderate to severe pain is largely dependent on the use of opioids. Opioids have a narrow therapeutic index with wide variations in individual response. Significant individual differences in sensitivity to these drugs can impair effective pain treatment and increase side effects. It is assumed that individual differences in opioid sensitivity may be due in part to genetic differences in the molecular elements involved in the pharmacokinetics and pharmacodynamics of opioids. Thus, genetic variability such as polymorphisms in the genes coding for opioid-metabolizing enzymes, transporter proteins, and the μ-opioid receptor could partly explain the observed inter-individual variations in response to opioids (74-76).

Candidate genes for pain modulation and opioid sensitivity
A wide range of candidate genes relevant for pain and opioid effects have been investigated in search for explanation for variation in analgesic response, pain sensitivity and evolvement of side effects. They belong to diverse groups of molecules involved in pain processing such as neurotransmitters, their receptors and transporters, metabolic enzymes, ion channels, inflammatory mediators and their receptors, growth factors, intracellular enzymes and messengers.

The metabolic enzyme COMT, that regulates the levels of catecholamines and enkephalins, has a common SNP in the coding gene, and also several
common haplotypes shown relevant for pain sensitivity (77). Activity in COMT has been shown to be relevant to pain experience among other in fibromyalgia (78).

**Inflammatory mediators** such as polymorphism in IL 10 have been associated with chronic prostatitis (79), IL-6 with discogenic pain (80) and IL-1Ra for postoperative morphine consumption (81).

**Growth factors**: Brain-derived neurotrophic factor (BDNF) modulates pain sensitivity in the central nervous system and increased levels of BDNF have been detected in patients with fibromyalgia (82).

**Neurotransmitter receptors** of interest are the µ-opioid receptor OPRM1 and the alpha-2 adrenergic receptor. Of these the polymorphism A118G of the opioid receptor gene has been most investigated. The SNP investigated for OPRM1 was A118G, where the mutant allele in position N40D was A>G. Earlier studies implicate that the persons with the minor allele should be in the risk zone for opioid addiction, have threefold increased binding of β-endorphin (83), increase requirements of morphine in patients with malignant disease (84), decreased opioid utilization in chronic pain patients (85), decreased sensitivity to experimental pain (86).

**Ion channels**: Variations of Na, K, Ca-channels genes have been associated with migraine and neuropathic pain (87). Defective calcium-channels have altered sensitivity to G-protein coupled receptors, e.g. opioid receptors. CACNAD2 is a gene that codes for the α2δ fragment of the N-type calcium-channel (88).

**Transmitter transporters**: Serotonin transporter and the ABCB1 transporter have been studied for various disorders. The serotonin transporter has been related to analgesic response (89). The ABCB1 transporter or P-glycoprotein is one of ATP-barrier cassette. This includes 8 ATPas-powered membrane transporters that catalyse outward efflux of ions and substances across the cell membrane and the blood brain barrier (90)). ABCB-1 (multidrug resistance 1 protein, MDR-1, Pgp) has been associated with outward transport of xenobiotics and the development of multi-drug resistance. It is also the basis for transport over the blood-brain-barrier. Genetic variations in ABCB1 have been suggested as one cause of inter-individual differences in drug-response (91). The ABCB1 investigated the C3435T SNP in exon 26 where C>T. Earlier studies have implicated lesser effects of methadone in patients with the minor allele (92), (93), but increased risk of early respiratory depression with fentanyl treatment (94) and increased risk of opioid-induced side-effects (95). The minor allele of CACNAD2 has been implied with altered sensitivity of receptors mediated by G- proteins, including OPRM1 (96).
Intracellular enzymes and messengers

Variation in the gene for GTP cyclohydrolase, the enzyme that regulates the production of tetrahydrobiopterin (BH4), has shown relevance for pain sensitivity after herniated lumbar disc operation. BH4, biopterin, is an essential co-factor for catecholamine, serotonin and nitric oxide production (97).

Aim of the study

Since pain is a complex bio-psychosocial phenomenon, the task of searching for and finding single gene variations to explain individual differences could be seen as futile.

Thus, the aim of this study is to focus is on three genes with functional impact on the opioid response: OPRM1, coding for the μ-opioid receptor; ABCB1 for the glycoprotein-P-transporter enzyme; and the calcium channel complex subunit CACNA2D2. These genes encode proteins related to the pain-relieving effects of opioids, measured in a homogenous patient population suffering from the same pain syndrome combined with an analysis of plasma levels of β-endorphin.

Patients

Eighty patients with low back pain who were scheduled for lumbar fusion surgery and who had previously been investigated for opioid sensitivity and 56 healthy volunteers were recruited for the study. The patient group comprised 39 men and 41 women with a mean age of 46.2 (25-66) years. The control group consisted of 25 men and 31 women with a mean age of 40.5 (27-61) years.

All patients had received opioids, but none was receiving daily doses of strong opioids at the time of opioid testing and blood sampling. The results from the patients were compared with those from the control population of 56 healthy volunteers without pain. The patients were recruited from a waiting list for a lumbar fusion operation.
Test for opioid sensitivity with a target-controlled infusion of Remifentanil

The patients were investigated for opioid sensitivity using a target-controlled infusion of remifentanil, as described by Schraag et al. (98). This was a double-blind, placebo-controlled investigation, where a significant response was taken to be a 50% reduction in pain, using a 100 mm visual analogue scale, and a 50% increase in the pressure pain threshold at the point of maximum pain in the lower back. The pressure pain threshold was measured with an Algometer™ (Somedic AB, Sollentuna, Sweden) (Abstract ESA 2003, Glasgow). The target levels of remifentanil that were measured for 50% pain relief were 1-7 ng/ml. High responders to remifentanil had remifentanil blood levels of 0.5-1.5 ng/ml, while normal responders required 2-7 ng/ml. In non-responders to remifentanil, the infusion had to be stopped because of sedation or side effects before any pain relief was obtained. There were 16 high responders, 44 normal responders and 20 non-responders.

Pain, QoL and opioid side effects were measured in both patients and controls using the EORTC-QLQ-30 form.

Analyses

Blood samples from the patients and controls were centrifuged, and the red cells and plasma were used for DNA and β-endorphin analyses, respectively. The Magtration 12GC system (Precision System Science, Chiba, Japan) and the Magazorb® DNA Common Kit-200 (PSS, Chiba, Japan) were used for the total genomic DNA preparation from collected whole blood samples. The DNA fragments containing the SNP site were amplified using PCR and purified with the PCR Clean-Up Kit (Invitrogen, Carlsbad, CA). Handy Bio-Strand was used for the SNP genotyping. Briefly, the amplified DNA was spotted on a micro-porous nylon thread (Bio-Strand) and hybridized with allele-specific oligonucleotide competitive hybridization (ASOCH). Results were analyzed by the computer program Hy-Soft (PSS, Chiba, Japan).
Radioimmunoassay

The frozen plasma samples were thawed on ice and centrifuged at 4°C for 10 min at 3000 x g. The supernatants were collected, diluted (1:5) with 0.1 M formic acid and 0.018 M pyridine (buffer I) and separated on minicolumns (1 ml) packed with SP-Sephadex C-25 gel according to a previously outlined procedure (Glämsta et al., 1993). Prior to sample application, the columns were washed with 10 ml buffer I, and then with 5 ml of 0.1 M formic acid/0.1 M pyridine, pH 4.1 (buffer II). The peptide-containing fractions were then eluted with 4 ml of 1.6 M formic acid/1.6 M pyridine, pH 4.1 (buffer V). All buffers contained 0.01 % mercaptoethanol. The eluted samples were then evaporated in a Speed Vac centrifuge (Savant, Hicksville, NY, USA).

The EURIA-beta-Endorphin kit (EURO-DIAGNOSTICA AB, Sweden) was used for the ß-endorphin radioimmunoassay (RIA). This RIA is based on the principle of double-antibody precipitation. The evaporated samples were diluted with 220 µl diluent (0.05 M phosphate, pH 7.4, 0.25% human serum albumin, 0.05% sodium azide, 0.25% EDTA and 500 KIU Trasylol®/ml) and incubated with 100 µl of anti-beta-endorphin antiserum for 24 h at 4° C. After incubation, the labeled peptide was added to each sample and incubated for an additional 24h, at 4°C. Thereafter, the double antibody PEG was added, and the test tubes were incubated for 60 min and then centrifuged for 15 min at 3000 x g at 4°C. Finally, the supernatants were decanted and the radioactivity of the precipitates was counted in a gamma counter.

Results

The genotype frequencies for OPRM1, ABCB1 and CACNA2D2 were all in accordance with Hardy-Weinberg equilibrium.

1. ß-endorphin levels

The opioid responders had higher levels of ß-endorphin (26.6 ± 3.59 fmol/ml) than the non-responders (24.7 ± 3.20 fmol/ml; p < 0.05) and the patients had lower ß-endorphin levels (26.2 ± 3.57 fmol/ml) than the healthy controls (28.2 ± 4.63 fmol/ml; p < 0.01; (Table 1).
Table 1.

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>beta-endorphin fmol/L</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>pain patient</td>
<td>80</td>
<td>26.2(3.59)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>control</td>
<td>56</td>
<td>28.2(4.63)</td>
<td></td>
</tr>
<tr>
<td><strong>pain patients</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>responder</td>
<td>60</td>
<td>26.6(3.57)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>non-responder</td>
<td>20</td>
<td>24.7(3.20)</td>
<td></td>
</tr>
<tr>
<td><strong>gender differences</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>pain patients</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>male OPRM1 AA</td>
<td>31</td>
<td>26.8(3.25)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>male OPRM1 AG</td>
<td>8</td>
<td>24.2(2.96)</td>
<td></td>
</tr>
<tr>
<td>female OPRM1 AA</td>
<td>28</td>
<td>26.1(4.02)</td>
<td>ns</td>
</tr>
<tr>
<td>female OPRM1 AG</td>
<td>11</td>
<td>25.9 (3.50)</td>
<td></td>
</tr>
<tr>
<td><strong>controls</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>male OPRM1 AA</td>
<td>19</td>
<td>25.8(2.17)</td>
<td>ns</td>
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<tr>
<td>male OPRM1 AG</td>
<td>6</td>
<td>31.5(9.13)</td>
<td>ns</td>
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<td>female OPRM1 AA</td>
<td>23</td>
<td>29.3(4.60)</td>
<td>ns</td>
</tr>
<tr>
<td>female OPRM1 AG</td>
<td>8</td>
<td>28.2(1.63)</td>
<td>ns</td>
</tr>
</tbody>
</table>

Figure 1
2. OPRM1 receptor gene

2.1 The major and minor alleles were distributed as presented in figure 1, which shows an apparent higher incidence of the minor allele among the high responders than among the other participants. The allelic frequency of the minor allele was \( \frac{7}{16} (44\%) \) in high responders, \( \frac{8}{44} (22\%) \) in normal responders, \( \frac{5}{20} (25\%) \) in non-responders, and \( \frac{14}{56} (25\%) \) in controls (Table 2).

2.2 Male patients with the major allele of OPRM1 had higher concentrations of \( \beta \)-endorphin (26.8 ± 3.25 fmol/ml) than male patients with the minor allele (24.2 ±2.96 fmol/ml; \( p < 0.05 \)). There were no differences in that respect among female patients or in the control groups (Table 1).

Frequency of minor alleles Table 2:

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>High responder</th>
<th>Normal responder</th>
<th>Non responder</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>OPRM1</td>
<td>A&gt;G</td>
<td>44% N=7</td>
<td>22% N=8</td>
<td>25% N=5</td>
<td>25% N=14</td>
</tr>
<tr>
<td>ABCB1</td>
<td>C&gt;T</td>
<td>32% N=5</td>
<td>27% N=12</td>
<td>25% N=5</td>
<td>34% N=19</td>
</tr>
<tr>
<td>CACNA2D2</td>
<td>G&gt;A</td>
<td>0% N=0</td>
<td>27% N=12</td>
<td>30% N=6</td>
<td>25% N=14</td>
</tr>
</tbody>
</table>

Figure 2
3. ABCB1 transporter gene

3.1 ABCB1 allelic distribution was similar in all groups (Figure 2). The frequency of the minor allele TT was 5/16 (31.75%) in high responders, 12/44 (27.2%) in normal responders, 5/20 (25%) in non-responders, and 19/56 (34%) in controls (Table 2).

3.2 There was a trend for different results among male and female patients regarding ß-endorphin levels. Men with the minor allele TT tended to have higher ß-endorphin levels (27.4 ± 3.40 fmol/L) than men with the major allele CC (25.8 ± 3.20 fmol/L). Similarly, women with the major allele CC tended to have higher ß-endorphin levels (26.4 ± 3.30 fmol/L) than women with the minor TT allele (24.9 ± 3.66 fmol/L; (Figure 3).

3.3 Among patients with the minor TT allele, there was a trend for sex differences in ß-endorphin levels. Men tended to have higher levels than women in the group of TT-individuals (p = 0.057; Figure 3).

![Figure 3. Difference in ß-endorphin concentration between males and females with the minor TT-allele P=0.057](image)

3.4 Patients with the minor TT allele also had more symptoms and side effects related to opioid treatment, such as sweating, sedation, tension and stress, than other patients (p < 0.05; Figure 4). The general quality of life and emotional function results were also poorer in the patients with the minor allele than in those with the CT/CC alleles. No difference in the rating of pain was noted between these groups, however (Figure 5).
4. CACNA2D2 gene

4.1 The allelic distributions are presented by groups of high-, normal and non-responders and controls. The minor CACNA2D2 G> A allele was not found in any of the 16 high responders (0%), while 12/44 (27%) of the normal responder group, 6/20 (30%) of the non-responders, and 15/56 (25%) of the control group had this allele (Table 1). Thus, high responders to remifentanil had a higher incidence of the major CACNA2D2 allele than normal responders, non-responders or controls (p < 0.05).

Figure 4

Figure 5. Pain and symptoms in patients with CC/CT and TT alleles
Statistics and methodological considerations

The material was analyzed with SPSS 14 quantitative data using Student’s t-tests. Ordinal data and variables, not normally distributed, were analyzed by the Mann-Whitney U-test, the Kruskal Wallis test and the z-test for comparison of population proportions. Hardy-Weinberg equilibrium was assessed for each SNP by \( \chi^2 \) analyses.

It was not possible to find statistically significant differences in pain experience or opioid sensitivity for any of the gene variants, except for the connection between opioid sensitivity and the major allele of CACNA2D2. One reason can be the small sample of patients. The frequency of homozygous alleles of OPRM1 was very low in our material. The major symptom differences would be attached to the homozygous minor allele and in a lesser degree in the heterozygous allele. Also there was some ethnic variation in both the patient and the control groups with a couple of people with Middle Eastern heritage. The ideal sample is of course from a homogenous population, which is not easy to obtain in a multicultural society.

The strength of the study, however, is that the patients suffer from the same pain syndrome, chronic low back pain, and that they were classified in different groups of opioid response, done by meticulous opioid testing.

The multiple testing of side effects has also an inherent risk for finding false positives due to chance, type I error. However, we did not do the Bonferroni post hoc test, since this method often creates more problems than it solves. The method supposes that all null hypothesis are simultaneously true, which they seldom are(99). Instead the risk is a type II error, missing important information. Findings can instead be corroborated by other investigators.
Discussion

In this study, we found that there were gender differences in β-endorphin levels and an increased incidence of opioid side effects in patients with the minor allele of the ABCB1 transporter gene, that there was a relationship between high opioid sensitivity and the presence of the major allele of the CACNA2D2 gene and that the minor allele of the OPRM1 gene tended to be associated with less requirements of opioids and thus intrinsic pain protection.

It is interesting to note that the number of patients was sufficient to provide some significant correlations in the group of patients with chronic pain, a complex bio-psychosocial entity. This was probably due to the homogeneity of the patient groups, and the fact that this study focused on correlating the presence of the gene variation affecting the function of the u-opioid receptor with its ligand β-endorphin and opioid-related symptoms in a clinical population with the same chronic pain syndrome. This study also presents the gene for a calcium channel fragment as a plausible contributor to individual variations in opioid sensitivity. The gender variations were prominent, as has been demonstrated in other studies. These results take the issue of differences in opioid and pain sensitivity a step further; gender and genetic variations could explain differences in the pharmacokinetics and pharmacodynamics of opioids.
Conclusions and visions for the future

The medical use of opium is as old as our Western Civilization- 5000 years. It was born in the land between the two rivers, Mesopotamia. During the ages of history opium has relieved pain and suffering, but also caused death and destruction of the individual through misuse and abuse.

Going into the 21st century, in spite of the immense advances in science and technology, the problem remains. Opium, or its natural or synthetic derivative, is still the best drug obtainable to relieve severe pain. But opium named φαρμακον pharmacon, the greek word for a healing drug or a deadly poison, is even in modern times a paradox. Addiction, misuse and abuse of opioids are still problems to cope with also in proper medical circumstances. Side effects, both short term and long term, evolve and limit the beneficial effect of pain relief. Loss of effect over time is a problem when treating chronic pain conditions. Individual differences in opioid response and side effects make dosing difficult and call for the need of careful titration and follow-up.

This thesis addresses and reflects over these many facets of evolving problems in long-term use of opioids for chronic pain.

Iatrogenic opioid addiction and problematic opioid use are clinical problems that affect patient, doctor and health care staff. The conclusion of the study is that musculoskeletal pain, psychiatric disease and previous addiction are risk factors that have to been taken into consideration for the doctor in order to decide on the adequate pain treatment and proper follow-up. A structured methadone programme can help the most difficult pain patients with opioid dependence.

Opioid endocrinopathy is a significant clinical syndrome causing debilitating side effects and low quality of life. The symptoms are unspecific like fatigue, mood changes, sweating, sexual dysfunction, but also more pain in some cases. For younger persons without significant diseases or pathology, the choice must be to taper the opioids. For older patients with severe pain and co-morbidities, hormone replacement therapy can ameliorate the symptoms, improve function and open a possibility for reduction of the opioid dose.
The review of the long-term effects of morphine on the μ-opioid receptor function and cellular mechanisms is contributing to the knowledge of the phenomena tolerance, withdrawal and opioid hyperalgesia. The loss of opioid effect over time can be explained and hopefully treated. There are already measures and drugs that can partially reverse and ameliorate tolerance and hyperalgesia such as opioid rotation, NMDA-antagonists, clonidine and ultra-low doses of naloxone. We may hope for new and better substances tailored to these mechanisms.

The great individual differences in opioid response and development of side effects can at least partially be explained by genetic variation. We found a relation between opioid response and variation in the gene for the calcium channel fragment α2δ and that the homozygous major and minor allele for the ABCB1 gene are differing between men and women regarding concentration of β-endorphin and that the patients with the minor allele of ABCB1 had more opioid-related side effects. These results can indicate future research in the quest for personalized medicine.

My hope is that when my grandchildren are grown-up, there will be more knowledge about appropriate use of opioids and new treatment models to relieve severe chronic pain conditions that still scourge the human being.
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A doctoral dissertation from the Faculty of Medicine, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine. (Prior to January, 2005, the series was published under the title “Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine”.)