Serial blood serotonin levels in a randomized controlled trial comparing the efficacy of low-dose amitriptyline, amitriptyline with pindolol and surrogate placebo in patients with chronic tension-type facial pain*

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Summary

Background: Patients often present with chronic facial pain despite normal nasal endoscopy and sinus computerized tomography. Such pain has increasingly been recognized as being of neurological origin with one of the commonest underlying causes being mid-facial segmental tension-type pain (MFP) which is a version of tension headache in the face. Descending serotonergic neuronal projections are known to modulate pain and intra-platelet serotonin levels are an accepted model reflecting central intra-neuronal serotonin.

Objectives: 1.To determine whether low-dose amitriptyline significantly changes whole blood serotonin compared to a surrogate placebo in patients with chronic MFP 2. To determine whether the addition of pindolol, a beta blocker with serotonin receptor blocking properties further alters blood serotonin.

Methodology: Sixty-two patients were randomized to three treatment groups a) amitriptyline , b) amitriptyline with pindolol, and c) loratadine as surrogate placebo. Whole blood serotonin was taken before and after 8 weeks of treatment. Serotonin was also measured in 40 age-matched healthy controls.

Results: There was a significant reduction in blood serotonin levels in the amitriptyline with pindolol group. A non-significant reduction was seen in the amitriptyline group, with no change in serotonin levels in the surrogate placebo group. A comparison of change in serotonin with change in pain frequency and intensity scores is presented. Women in the control group had significantly higher serotonin levels than men. Women with tension-type facial pain who failed to respond to treatment had significantly lower blood serotonin than women in the control group.

Conclusion: When linked to the clinical response this study provides evidence that the serotonergic system is involved in the modulation of chronic MFP. Serotonin levels are sex-dependent and related to treatment response.

Key words: serotonin, chronic facial pain, tension-type headache, tension-type facial pain, amitriptyline, pindolol, randomized controlled study, sinus pain

Introduction

Approximately 25% of 7500 patients with rhinological symptoms in Malta complained of significant mid-facial pain (personal data). Although this pain is generally interpreted by patients as being of 'sinus' origin because of anatomical proximity, the CT is normal in up to 60% of cases [1,2]. Diagnosis of chronic rhinosinusitis now includes a correlation of CT and nasal endoscopic findings [3,4] and facial pain in the presence of normal

CT and endoscopy is likely to be of neurological origin [5].
Jones described mid-facial segmental pain (MFP) [6] as a tension-type pain, pressing or aching in quality with a bilateral distribution, involving any combination of the nasion, periorbital regions, cheeks or paranasal areas. MFP was frequently associated with tension-type headache, involving the frontal, parietal and occipital regions [7].
Tension-type facial pain is thought to be due to sensitization of the ascending second order neuron at the level of the trigeminal nucleus [8]. Descending pain modulation is mediated through serotonergic projections from the midbrain [9]. Cell bodies of serotonergic neurons in the dorsal raphe brainstem nuclei possess a concentration of presynaptic 5-HT1A auto-receptors which play a crucial self-regulatory role in the function of the nociceptive system [10,11]. Pindolol, a β-adrenergic antagonist, binds to these somatodendritic 5-HT1A receptors and potentiates serotonergic effects in projection areas [12]. It has been shown to accelerate the action of selective serotonin reuptake inhibitors (SSRI) [13,14] and has also been successfully used in the treatment of fibromyalgia [15].

Platelets are considered as a model for serotonergic neurons in humans since they share the same serotonin uptake transport protein [16,17]. In the central nervous system, once released by the presynaptic neuron terminal, serotonin is taken up from the synaptic cleft by the serotonin transporter (5-hydroxy tryptamine transporter or 5-HTT). Since 5-HTT clears the neurotransmitter, any reduction in its function would augment the effect of serotonin, including its pain-modulating action, over a wider synaptic field [18].

Jones suggested low-dose amitriptyline as effective treatment for MFP [6]. Tricylic antidepressants have been shown to be effective in the prophylaxis of tension-type headache and are thought to reduce the sensitivity of the second order neurone [19].

Classification criteria of chronic tension-type facial pain were extrapolated and applied from the International Headache Society 2.3 classification, with patients suffering at least 15 headache days or more per month [20].

In this study peripheral whole blood serotonin was measured before and after treatment in three patient groups: a) amitriptyline b) amitriptyline with pindolol , and c) loratadine as a surrogate placebo. The changes in pain scores occurring during this clinical trial have already been reported in detail elsewhere [21]. Demonstration of a low platelet serotonin in tension-type facial pain would show a similarity to tension-type headache. Differences in blood serotonin levels before and after treatment would be compared to the clinical effect in these three groups.

If serotonergic projections are involved in modulation of facial pain, changes in blood serotonin may reflect intraneuronal serotonin changes during these courses of treatment. The extent of these changes in serotonin levels were compared to the efficacy of clinical response to treatment in the different groups.

Materials and methods

Patients

A cohort of 240 consecutive patients with chronic mid-facial pain with or without tension headache for more than 15 days per month for at least three months was prospectively followed up for 36 months to determine long-term patient outcomes. Detailed results of the cohort follow-up are to be reported (personal data) and 156 of the 240 patients had chronic mid-facial tension-type pain (MFP). The process of recruitment and follow-up was carried out in accordance with CONSORT guidelines [22] and the inclusion and exclusion criteria, and standard patient history have been previously reported [21].

Facial migraine without aura is another cause of chronic facial pain. Patients with mixed tension-type pain with migraine were also included as long as they did not have more than one bout of migraine monthly, according to criteria established by previous studies on tension headache [23,24]. Patients with more frequent migraine were excluded since they may have variations in blood serotonin [25] which affect the outcome of the study.

Patients had an ENT and cranial nerve examination, fundoscopy and their blood pressure was measured. Nasal endoscopy was carried out, and patients with intranasal pus or polyps were excluded. Oedema of the middle meatal mucusa was not an exclusion criterion since in previous local studies nasal mucosal oedema was a non-specific finding not associated with sinusitis [1,26].

A computed tomogram of their brain and sinuses with coronal and axial cuts to exclude sinusitis and intracranial pathology was carried out. Patients with sinus mucosal thickening of over 3 mm on the CT were excluded.

The first group of patients was treated with low-dose (10 mg) amitriptyline at bedtime for 8 weeks. The second group was treated with 10 mg daily amitriptyline at bedtime and pindolol 5 mg twice daily (half the normal dose) combined for 8 weeks. The third (surrogate placebo) group was treated with loratadine 10 mg daily at bedtime for 8 weeks. Loratadine was selected as it was a well-established antihistamine with a good safety profile, not known to have any effect upon platelet activation, as seen with the newer antihistamines such as rupatadine.
Patients were blind as to which treatment arm they were in. Whole blood serotonin was estimated in the patients joining

the clinical trial before starting treatment and at completion of 8 weeks of treatment. Over ninety percent of blood serotonin is stored within platelets [27-29], with the remainder in the plasma. For 48 hours prior to the test, patients were instructed to avoid foods high in tryptophan (the metabolic precursor of serotonin) such as tea, coffee, nuts, avocado, pineapple, tomatoes, plums, eggplant and chocolate. This was done to enable maximal uptake of plasma serotonin into the platelets prior to the test so that when taken, the whole blood serotonin would more closely reflect intra-platelet serotonin. The diet was also intended to avoid the variation possible in high- tryptophan diets, which have the potential to increase whole blood serotonin by up to 16% [27]. Those patients taking drugs that alter platelet activation, such as aspirin or clopidrogel, were excluded because activation releases serotonin into the plasma.

Serotonin levels of 40 matched healthy controls (twenty men and twenty women) having less than one episode of facial pain/headache monthly and no nasal complaints were recruited and consented from the corresponding author's general ENT practice. Statistically, this was the minimum number of patients calculated to be necessary in order to expose any gender difference in serotonin using standard sample size estimation methods for comparing means [30]. Healthy controls also had to avoid foods high in tryptophan for 2 days before their blood test. Serotonin levels were repeated after a few weeks in every third control to check the validity of the laboratory results by determining 95% confidence intervals.

Whole venous blood was collected at the St James Hospital laboratory, Malta using a pre-cooled heparinized plastic bottle and immediately frozen since serotonin levels are very sensitive to temperature. Samples were packed on dry ice in an insulated transport box, transported by courier to Biomnis laboratories in Lyon, France and analysed using High Performance Liquid Chromatography (HPLC) with electrochemical detection. Each HPLC analysis was preceded by internal standard calibration using standard solutions. Laboratory analysis was carried out by an observer not having any knowledge of the headache condition of the patients.

To estimate intra-platelet serotonin a blood sample requires that it undergo immediate centrifugation to separate the plasma, which is then decanted off, from the platelet pellet. Centrifugation may disrupt platelets leading to release of serotonin into the plasma compartment while reducing platelet volume. Therefore measurement of whole blood serotonin is preferable and more reliable [27] even if in this way one could not differentiate between plasma and platelet values.

An information sheet was supplied to the patients and the corresponding author (AA) was on hand for guidance or questions. Patients were individually consented for entry into the study and were given the option to withdraw at any time. The study was approved by the Malta Health Ethics Committee.

Statistical methods

Statistical analyses were carried out using SPSS for Windows version 16.0. Results were presented as mean ± SD. The normality of pre and post treatment serotonin was determined using Q-Q plots. Normality for pre and post treatment serotonin values for each of the three treatment groups was similarly determined. Differences in mean age of the three groups were tested using analysis of variance (ANOVA). Differences in gender between the three groups were tested using Fisher's exact test. For calculations comparing groups of 20 individuals or more, a t test was used while with groups of less than 20 individuals, a Mann-Whitney test was used.
Serotonin estimation was deemed a labile test, and Cook's distance was calculated to identify outlying post-treatment values.

Results

Forty six women and 16 men with a mean age of 36.6 ± 12.2 and 32.8 ± 7.7 years, respectively, took part in the trial. Twenty two patients received amitriptyline 10 mg at night, 20 patients received amitriptyline 10 mg at night with pindolol 5 mg twice a day and 20 patients received loratadine 10 mg daily at night as a surrogate placebo. All patients were treated for 8 weeks. These 62 patients had a history of pain prior to the study for a mean of 33 ± 42.2 months. Their pain typically lasted a few hours per day and was described as bilateral pressure in the nasion, cheek or periorbital areas with additional involvement of the frontal, occipital or parietal regions.

Q-Q plots confirmed the normality of pre- and post- treatment

Table 1. Changes in whole blood serotonin in the treatment and placebo groups after 8 weeks. The drop in the treatment groups was compared to the placebo and p value calculated (t test two sample assuming unequal variances).

	Pre-treatment values (µg/L ± SD)	Change in blood serotonin over 8 weeks: mean (µg/L) ±SD	p value (t test compared to placebo)
Amitriptyline (n = 22)	166 ± 12	-11 ± 36	p = 0.27
Amitriptyline with pindolol (n = 20)	174 ± 10	-25 ± 39	p = 0.019
Placebo (n = 20)	174 ± 10	5 ± 42	--

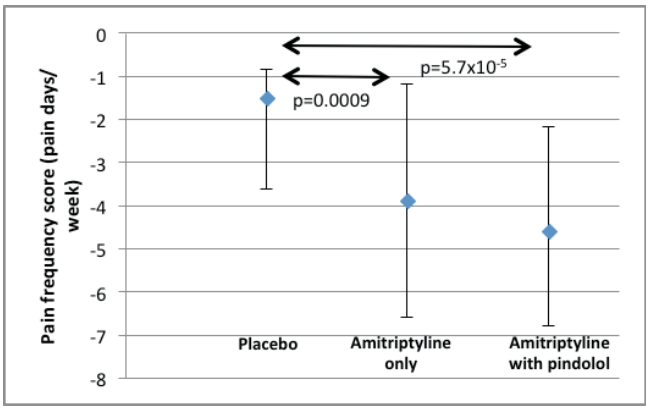

Figure 1. Reduction in pain frequency scores after 8 weeks treatment in amitriptyline, amitriptyline with pindolol and surrogate placebo groups. The points represent the mean value ± SD. P value is calculated using t test two sample assuming unequal variances (two tailed). There is no significant difference between amitriptyline and amitriptyline with pindolol groups, p = 0.58.

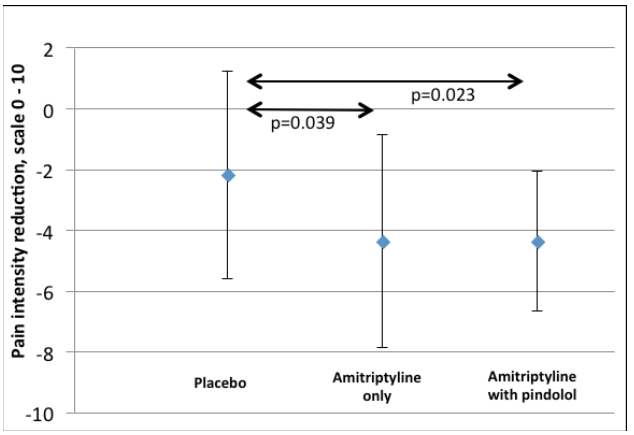

Figure 2. Reduction in pain intensity scores over 8 weeks treatment in two treatment groups and surrogate placebo. The points respresent Mean score ± SD. P values are calculated from t test two sample assuming unequal variances (two tailed). There is no significant difference between amitriptyline and amitriptyline with pindolol groups (p = 0.98).

blood serotonin levels in the 62 patients. Q-Q plots also confirmed the normality of pre-and post- treatment serotonin blood levels in each of the three treatment groups. There was no significant difference in mean age and gender between the three treatment groups [21]. Using Cook's distance to identify outlying post-treatment serotonin values, one individual in the surrogate placebo group was excluded from statistical analysis.
There was a statistically significant drop in blood serotonin after treatment with combined amitriptyline and pindolol (p = 0.019, t test two sample for means), while there was a non-significant drop in the amitriptyline group (p = 0.27, t test two sample for means) and negligible change in the surrogate placebo group (Table 1). For comparison, Figures 1 and 2 show the mean ± SD of the reduction in pain frequency (pain days per week) and pain intensity (scale of 0 to 10) in the three patient groups. Combination treatment was consistently more successful than monotherapy.

The difference between pre- and post- treatment serotonin levels was obtained by subtracting pre-treatment from post-treatment values. Patients receiving amitriptyline with pindolol had a significant drop in their serotonin levels compared to those on the surrogate placebo (Table 1). Patients receiving amitriptyline also had a reduction in their serotonin but it was not significant. Those patients who continued having facial pain despite their treatment were subsequently given a second course of amitriptyline. Patients with continuing symptoms despite the second course were classed as having 'persistent pain' and there were twelve of these amongst the initial 62 patients (19%). Patients with persistent pain developed their symptoms much earlier in life compared to those who responded to low-dose amitriptyline, and may represent a different subgroup of MFP [21].
Most patients with chronic MFP were women. There were no significant differences between pre- and post-treatment blood serotonin levels in women on amitriptyline compared to women receiving surrogate placebo (n = 14) (p = 0.54, two tailed Mann-Whitney test).

However, the serotonin level dropped significantly in women (n = 16) taking the combination treatment compared to women on surrogate placebo (p = 0.031, Mann-Whitney test) (Figure 3). The box plots in Figure 3 represent the median, 25th and 75th percentiles with maximum and minimum values. The 46 women with chronic MFP had a lower serotonin than female controls but this was of marginal significance (p=0.07, t test two tailed assuming unequal variances). In the subgroup of 8 women who did not respond to treatment their initial serotonin level was significantly lower that than the whole group with chronic pain prior to treatment (p=0.026, Mann-Whitney two tailed test (Figure 4).
Comparison of serotonin differences in men in the control group with the men in the combination treatment group showed that in men treated with pindolol there was no serotonin drop during their 8 week treatment (p = 0.11, two tailed Mann-Whitney test). This may indicate an innate gender difference in platelet serotonin uptake, which came to light after the administration of pindolol (Figure 4). However, the numbers of men were limited as was the inference that could be drawn from these results. Indeed when comparing the serotonin difference after treatment in those patients receiving surrogate placebo (n = 20)

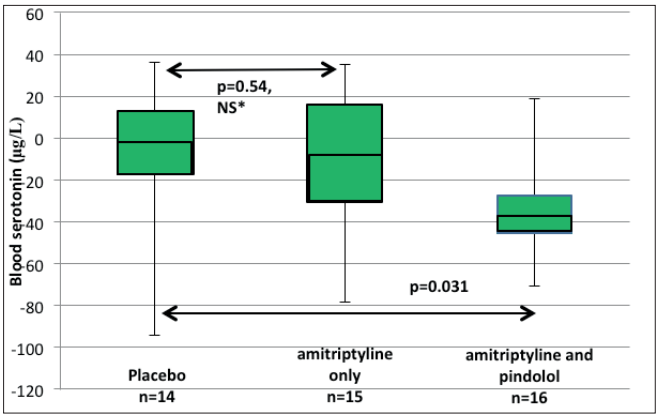

Figure 3. The change in whole blood serotonin in women after 8 weeks of treatment in the three treatment groups. When comparing amitriptyline to placebo, the difference in this reduction is not significant (* NS, Mann-Whitney). The reduction with amitriptyline combined with pindolol compared to placebo is significant. The box plots show 25th to 75th percentile with median values and range of minimum and maximum values.

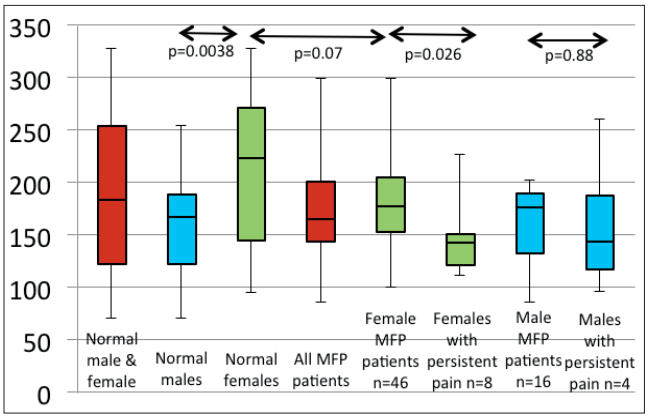

Figure 4. Whole blood serotonin levels in the various patient groups and the statistical significance when comparing them. The box plots show 25th to 75th percentile with median values and range of minimum and maximum values.

to the difference in men and women taking the combination treatment (n = 20), the difference is still statistically significant (p = 0.026, two tailed t test for unequal variances).
Out of the 8 women with persistent pain four received amitriptyline, two received combination treatment and two had surrogate placebo. The mean serotonin level in these 8 individuals was initially low before treatment, at 145 ± 36 µg/L but rose to 172 ± 60 µg/L after treatment. This difference was not statistically significant (Mann-Whitney test, p = 0.28). In the four men with persistent pain their mean serotonin level went down from 168 ± 73µg/L to 127 ± 28 µg/L (Mann-Whitney, p = 0.56, NS). Two men received amitriptyline, one received the combination treatment and one received surrogate placebo.

Whole blood serotonin was also measured in 20 men and 20 women without facial pain. The mean age in men was 33.4 ±10 years while that in women was 31.3 ± 12.3 years. The mean blood serotonin in 40 normal individuals was 181 ± 69 µg/L. The mean blood serotonin level in normal men (149 ± 42µg/L) was significantly lower than in normal women (210 ± 77µg/L) (p = 0.0038, two tailed t test assuming unequal variances). Normality of serotonin levels in these individuals was confirmed using Q-Q plots.

In 11 of these 40 normal individuals, the serotonin was re-estimated after 6 weeks to check the validity of serotonin laboratory measurements. The 95% confidence intervals for the first test in these normal individuals showed a mean of 162.1 µg/L (95% CI: 125.8, 198.3) while the second test showed a mean of 167.3 µg/L (95% CI: 130.8, 203.7). A two-tailed Mann Whitney test comparing the second set of measurements in these normal individuals to the first set was non-significant (p = 0.87). This demonstrated good concordance between the sets of data and confirmed the reliability of the laboratory results.

Cigarette smoking has been considered as a confounding variable since nicotine was reported to release serotonin from platelets [31]. From 62 patients with MFP seven women and two men were smokers while from 40 pain-free individuals, five women and two men smoked. Serotonin levels in 62 MFP patients were 170 ± 49 µg/L, while in the 9 MFP smokers, levels were 178 ± 53 µg/L. There was no significant difference between serotonin levels in MFP smokers and MFP non-smokers.
Mean serotonin level in 33 pain-free non-smokers was 184 ± 66 µg/L, and 165 ± 85 µg/L in seven pain-free smokers. There was no significant difference either between the serotonin levels in these groups (Mann-Whitney, two tailed test, p = 0.37).
In this study cigarette smoking did not have any significant effect upon the blood serotonin.

Discussion

One drawback of this study was that it was not fully blinded and that an antihistamine was used as surrogate placebo which could have affected the outcome in patients with food allergy or histamine intolerance. Loratadine was chosen as a surrogate placebo as it does not affect platelet activation and is not known to alter the nociceptive pathway.

The few studies that have investigated serotonin levels in peripheral blood in chronic tension-type pain have given mixed

results due to different methodology and patient recruitment criteria. Platelet serotonin levels in tension-type headache have been found to be relatively low [32], possibly due to a decrease in the uptake of serotonin from plasma by the 5-HT transporter [33] although one radioactive uptake study differed on this finding [34]. One single study of serial serotonin in 13 patients with tension-type headache pointed to the release of serotonin from activated platelets to the plasma compartment during attacks of pain [23].

The main finding in this study was that patients given amitriptyline with pindolol had a significant reduction in their blood serotonin. Patients taking amitriptyline alone had a non-significant reduction and patients on surrogate placebo had, as expected, no change in their serotonin levels. Clinically, both amitriptyline and amitriptyline with pindolol significantly reduced pain scores, with the combination being significantly more effective (Figures 1 and 2) [21]. As platelet serotonin is a model for central pre-synaptic serotonergic neurons this result implies that combination treatment reduced re-uptake of synaptic serotonin in the central nervous system with perpetuation of its postsynaptic activity in projection neurons to the trigeminal nucleus and a reduction in facial pain [18].

It is proposed that besides pindolol's central action on the 5-HT1A receptor, it may also have an inhibitory action on the serotonin uptake transporter protein, 5-HTT. Inhibiting the serotonin transporter centrally would increase net serotonin in the brain extracellular spaces and peripherally reduce serotonin uptake by platelets, leaving it to be metabolised in the blood with a net decrease in levels.
Beta-blockers such as propranolol may promote release of stored platelet serotonin through their non-specific lipid-solubility properties acting upon platelet membranes [35] thus theoretically increasing blood serotonin levels. Pindolol, however, has never been shown to have any measurable membrane activity [36,37] while the principal serotonin receptor in the platelet membrane is the 5-HT2 receptor to which pindolol in any case has not been shown to bind [38].

The 5-HTT in the central nervous system and platelets has an identical amino acid structure [39] and the human 5-HTT gene has been successfully cloned [40]. However, the 5-HTT gene has been shown to exhibit various polymorphisms. The most studied genetic variant, called 5-HTTLPR, occurs as two prevalent alleles, the long and short varieties. Patients with the short allele have lower 5-HTT activity [41].
Women with the short allele of the 5-HTTLPR polymorphism have an increased susceptibility to depression and anxiety [42] and a reduced response to serotonin reuptake inhibitor antidepressants [43]. Ethnic differences in allele distribution have been found within various European populations [44].
It is possible that patients with tension type MFP may have an abnormality in their 5-HTT. Genotyping of such individuals may be carried out. If, as in depression, patients with tension-type pain are shown to have low 5-HTT activity, genetic variation may explain why this condition is seen primarily in women. Moreover, if pindolol further suppresses the 5-HTT, it would accentuate the drop in platelet serotonin seen in women but not in men, as in this study (Figure 3).

It would also be of interest to investigate the proportion of patients with chronic MFP that develop depressive disorders in the future, and whether this proportion is higher than the normal population.
The subgroup of women with chronic non-responsive pain may already have an innately low serotonin re-uptake transporter activity with central serotonin depletion, reflected in decreased anti-nociceptive output to the periphery. They seem to have a much longer history of pain than those women who respond immediately to amitriptyline.
The significant reductions in pain scores in patients with chronic MFP receiving amitriptyline alone was not reflected in statistically significant reductions in serotonin levels, as seen in those who also received pindolol.

Conclusion

This study provides evidence that supports the role of the serotonergic system in the modulation of chronic mid-facial tension-type pain. The addition of pindolol to low-dose amitriptyline appears to inhibit serotonin re-uptake into platelets. Pindolol may also mediate a central effect by means of its 5-HT1A receptor binding properties on brainstem serotonergic projection neurons with decreased serotonin re-uptake and persistence in synapses that enhance pain attenuation.

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Authorship contribution

AMA: original concept of study and main author who carried out the study; RM: supervised trial and contributed to study design; NJS: contribution to concept and study design.

Conflict of interest

The authors do not have any conflicts of interest to declare.

References

1. Agius AM. Chronic sinusitis in Malta-correlation between symptoms and CT scan. Rhinology. 2010; 48: 59-64.
2. Kieff DA, Busaba NY. Negative predictive value of normal nasal endoscopy for sinus disease as a cause of isolated facial pain. J Laryngol Otol. 2011, 125: 1038-1041.
3. Fokkens WJ, Lund VJ, Mullol J, et al. European Position Paper on Rhinosinusitis and Nasal Polyps 2007; Rhinology. 45 (Suppl 20): 1-139.
4. Fokkens WJ, Lund VJ, Mullol J, et al. European Position Paper on Rhinosinusitis and Nasal Polyps 2012; Rhinology. 50 (Suppl 23): 1-298.
5. West B, Jones NS. Endoscopy-negative, computed tomography-negative facial pain in a nasal clinic. Laryngoscope. 2001; 111: 581-586.
6. Jones NS. Midfacial segmental pain:implications for rhinitis and sinusitis. Curr All Asthma Rep. 2004; 4: 187-192.
7. Jones NS. Midfacial Segment Pain: Implications for rhinitis and rhinosinusitis. Clin Allergy Immunol. 2007; 19: 323-333.
8. Bendtsen L. Sensitisation: its role in primary headache. Curr Opin Invest Drugs. 2001; 3: 449-453.
9. Fields HL, Basbaum AI, Heinrich MM. Central nervous system mechanisms of pain modulation. In: McMahon S, Koltzenburg M, eds. Textbook of Pain. 5th ed. Burlington, Massachusetts, USA: Elsevier Health Sciences, 2005: 125-142.
10. Miquel MC, Doucet E, Boni C et al. Central serotonin1A receptors: respective distributions of encoding mRNA, receptor protein and binding sites by in situ hybridization histochemistry, radioimmunohistochemistry and autoradiographic mapping in the rat brain. Neurochem Int. 1991; 19: 453-465.
11. Kia HK, Brisorgueil MJ, Daval G, et al. Serotonin 5-HT1A receptors expressed by a subpopulation of cholinergic neurons in the rat medial septum and diagonal band of Broca-a double immunocytochemical study. Neuroscience 1996; 74: 143-154.
12. Clifford EM, Gartside SE, Umbers V, et al. Electrophysiological and neurochemical evidence that pindolol has agonist properties at the 5-HT1A autoreceptor in vivo. Br J Pharmacol. 1998; 124: 206-212.
13. Artigas F, Perez V, Alvarez E. Pindolol induces a rapid improvement of depressed patients treated with serotonin reuptake inhibitors. Arch Gen Psychol. 1994; 51: 248-251.
14. Blier P, Bergeron R. Effectiveness of pindolol with selected antidepressant drugs in the treatment of major depression. J Clin Psychopharmacol. 1995; 15: 217-222.
15. Wood PB, Kablinger AS, Caldito GS. Open trial of pindolol in the treatment of fibromyalgia. Ann Pharmacother. 2005; 39: 1812-1816.
16. Pletscher A, Laubscher A. Blood platelets as models for neurons: uses and limitations. J Neural Transmission. 1980 (Suppl 1980): 7-16.
17. Lesch KP, Wolozin BL, Murphy DL, et al. Primary structure of the human platelet serotonin uptake site: identity with the brain serotonin transporter. J Neurochem. 1993; 60: 2319-2322.
18. Barker EL, Blakely RD. Norepinephrine and Serotonin transporters, molecular targets of antidepressant drugs. In Neuropsychopharmacology: The fourth generation of progress, Raven Press, New York, 2000, Eds Floyd E Bloom and David J Kupfer, Part I Preclinical section, Transmitter systems, Chap 28, Pp 321-334.
19. Bendtsen L, Jensen R, Olesen J. A non-selective (amitriptyline) but not a selective (citalopram) serotonin reuptake inhibitor is effective in the prophylactic treatment of chronic tension-type headache. J Neurol Neuorsurg Psychiatry. 1996; 61:285-290.
20. Headache Classification Committee of the International Headache Society. The International Classification of Headache disorders 2nd Edition. Cephalalgia. 2004, 24 (Suppl 1): 9-160.
21. Agius AM, Jones NS, Muscat R. A Randomized controlled trial comparing the efficacy of low-dose amitriptyline, amitriptyline with pindolol and placebo in the treatment of chronic tension-type facial pain Rhinology. 2013; 51: 143-153.
22. Moher D, Hopewell S, Schulz KF, et al. CONSORT 2010 explanation and elaboration: updated guidelines for reporting parallel group randomized trials. BMJ. 2010; 340: c869.
23. Jensen R, Hindberg I. Plasma serotonin increase during episodes of tension-type headache. Cephalalgia 1994; 14: 219-222.
24. Bendtsen L, Jensen R. Hindberg I, et al. Serotonin metabolism in chronic tension-type headache. Cephalalgia. 1997; 17: 843-848.
25. Ribeiro CAF, Cotrim MD, Morgadinho MT, et al. Migraine, serum serotonin and platelet 5-HT2 receptors. Cephalalgia. 1990; 10: 213-219.
26. Agius AM. Long-term follow-up of patients with facial pain in chronic rhinosinusitis-correlation with nasal endoscopy and CT. Rhinology. 2010; 48; 65-70.
27. Xiao R, Beck O, Hjemdahl P. On the accurate measurement of serotonin in whole blood. Scan J Clin Lab Invest. 1998; 58: 505-510.
28. Hergovich N, Aigner M, Eichler H, et al. Paroxetine decreases platelet serotonin storage and platelet function in human beings. Clin Pharmacol Therapeutics. 2000; 68: 435-442.
29. Aymard N, Honore P, Carbaccia I. Determination of 5-hydroxytryptamine and tryptophan by liquid chromatography in whole blood. Its interest for exploration of mental disorders. Prog Neuro-Psychopharmacol & Biol Psychiat. 1994; 18: 77-86.
30. Piface, http://www.cs.uiowa.edu/~rlenth/Power/
31. Rausch JL, Fefferman M, Ladisch-Rogers DG et al. Effect of nicotine on human blood platelet serotonin uptake and efflux. Prog Neuropsychopharmacol Biol Psychiatry. 1989; 13, 907-916.
32. Anthony M, Lance JW. Plasma serotonin in patients with chronic tension headaches. J Neurol Neurosurg Psychiat.1989; 52: 182-184.
33. Bendtsen L, Jensen R, Hindberg I, et al. Serotonin metabolism in chronic tension-type headache. Cephalalgia 1997; 17: 843-848.
34. Shukla R, Shanker K, Nag D, et al. Serotonin in tension headache. J Neurol Neurosurg Psychiatry. 1987; 50: 1682-1684.
35. Nathan I, Dvilansky A, Sage J, et al. Effects of propranolol and pindolol on platelet aggregation and serotonin release. Life Sci. 1977; 20, 407-412.
36. Grobecker H, Lemmer B, Hellenbrecht D, et al. Inhibition by antiarrythmic and β-sympatholytic drugs of serotonin uptake by human platelets: experiments in vitro and in vivo. Eur J Clin Pharmacol. 1973; 5, 145-151.
37. Lemmer B, Wiethold G, Hellenbrecht D, et al. Human blood platelets as cellular models for investigation of membrane active drugs: beta-adrenergic blocking agents. Naunyn-Schmiedeberg's Arch Pharmacol. 1972; 275, 299-313.
38. Cook N, Nahorski SR, Barnett DB. Pindolol binding to the human platelet β-adrenoceptor: characterisation and agonist interactions. Eur J Pharmacol. 1995; 113, 247-254.
39. Lesch KP, Balling U, Gross J, et al. Organization of human serotonin transporter gene. J Neural Transm. 1994, 95: 157-162.
40. Lesch KP, Wolozin BL, Estler HC et al. Isolation of a cDNA encoding the human brain serotonin transporter J Neural Transm. 1993, 91: 67-72.
41. Lesch KP, Bengel D, Heils A et al. Association of anxiety-related traits with a polymorphism in the serotonin transporter regulatory region. Science. 1996; 274: 1527-1531.
42. Maurex L, Zaboli G, Ohman A, et al. The serotonin transporter gene polymorphism (5-HTTLPR) and affective symptoms amoung women diagnosed with borderline personality disorder. Eur Psych. 2010, 25: 19-25.
43. Lesch KP, Gutknecht L. Pharmacogenetics of the serotonin transporter. Prog Neuro-Psychopharmacology & Biol Psych. 2005; 29: 1062-1073.
44. Noskova T, Pivac N, Nedic G et al. Ethnic differences in the serotonin transporter polymorphism (5-HYYLPR) in several European populations. Prog Neuropsychopharmacol Biol Psychiatry 2008, 32: 1735-1739.

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